

Impact of browsing after burning on aspen growth and litter decomposition

by

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The thesis *Impact of browsing after burning on aspen growth and litter decomposition* of candidate Kathryn Barr is arranged in a manuscript format with three manuscripts designated as Chapters two, three, and four. At the time of thesis defense, these three manuscripts have not yet been submitted for publication in the peer-reviewed literature. For these three manuscripts to subsequently be submitted for publication, the statements below outline the authorship and contribution made by each author to those manuscripts.

Co-authorship Statement

This statement concerns the following manuscript to be submitted for publication:

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Candidate: Kathryn Barr. Role: first author

Contribution of Candidate: establishment of field experiment, collection of field data, analysis and interpretation of data, and writing of the manuscript

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Contribution of co-author: design of the study, analysis and interpretation of data, and writing of the manuscript

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Determination of historic extent of grasslands in Riding Mountain National Park with soil pits to determine organic carbon with depth

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Co-author: Terence McGonigle. Role: second author

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Abstract

Manitoba has a small number of rough fescue (*Festuca hallii*) grasslands, which are commonly utilized by elk for winter grazing in upland Manitoba. Aspen encroachment currently threatens these grasslands within Riding Mountain National Park, where augmentation of grasslands is a priority. This study examined the interaction between browsing and fire on aspen growth, the effect of browser manure on aspen leaf decomposition, and examined soil profiles to identify historic grasslands. Browsing simulations were carried out within exclosures across sites with-and-without recent burns to measure the effect of browsing alone versus a combination of fire and browsing. To assess the effect of browsing on the apical shoot, a subset of trees was randomly assigned an apical shoot manual-removal. High intensity of clipping was effective to suppress aspen, but fire had no effect. Field-placed litter bags were used to measure the effect of ungulate manure on aspen litter decay. Soil fecal incubation were used to measure the release over two months of plant-available N as nitrate-N ($\text{NO}_3\text{-N}$) and ammonium-N ($\text{NH}_4\text{-N}$) in soil. The manure amendments used in both studies were bison and a wild-ungulate blend of deer, elk and moose. Aspen decomposition was seen at rates known for other studies, but manure was without impact. In addition, the use of soil organic carbon (SOC) at depth as an indicator for historic grasslands was tested. In grasslands, a large portion of the organic matter input is from fibrous root systems within the mineral soil. In forest, most of the residue input is from leaves falling to the litter layer above the mineral soil on the forest floor. Stoniness and soil texture were included with SOC to better characterize the sites. Soil pits were dug at long-term forested sites, long-term prairie sites, and recently forested sites. Samples were taken at depth intervals of 10 cm. Stoniness at depth was associated with grassland and recent forest cover, but not long-term forest. SOC patterns reflected litter inputs and suggest historic cycling of prairie and aspen cover on stony sites.

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Thesis format and accomplishments

The thesis is presented and prepared in a manuscript format and is comprised of five chapters. Chapter One introduces the study area, previous vegetation research conducted at Riding Mountain National Park, and the research questions. Chapters Two, Three and Four are written in manuscript style. Chapter Five concludes the major findings.

Chapter Two: *Impact of browsing after burning on aspen encroachment*

Chapter Three: *Impact of ungulate manure on aspen leaf breakdown using field placed litter bags*

Chapter Four: *Determination of historic extent of grasslands in Riding Mountain National Park with soil pits to determine organic carbon with depth*

In addition to the to the research presented in this thesis, Kathryn Barr presented “Irrespective of burn history, high levels of browsing are required to effectively control aspen encroachment in upland Manitoba” at the 6th Native Prairie Restoration/Reclamation Workshop, on 7-8 February 2018 in Saskatoon, SK.

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1 Chapter one: INTRODUCTION

1.1 FOREST-GRASSLAND DYNAMICS

The theoretical framework for tree-grass systems has been well studied (House et al., 2003; Li, 2002; Sankara et al., 2004); and is based around the idea that a system can be stable at equilibrium or dynamic at non-equilibrium. A system is stable as long as it remains unchanged in the face of disturbance. A system is meta-stable if it remains unchanged when subjected to only small disturbances. The extensive and persistent occurrence of savannas suggests stability, whereas woody species encroachment suggests meta-stability. Trees have a competitive advantage over grasses for light, whereas grasses can more readily withstand dry conditions than can trees (Scholes and Archer, 1997). Thereby, grasslands and forest have coexisted for centuries. In North America, encroachment of woody species is a widespread phenomenon across the prairies (Bailey and Wroe, 1973). In savanna systems, an increase in tree cover following ranching has been observed (Scholes and Archer, 1997). A similar increase in woody cover has been in seen in North American grasslands subjected to historic ranching (Brown and Archer, 1999).

The theoretical framework around tree-grass coexistence is either based on demographic dynamics resulting from vegetation-disturbance relations (Sankara et al., 2004) or on interspecies competition for light and water (Eagleson and Segarra, 1985). In the last 160 years, woody species replacing herbaceous species has been unprecedented. This shift is most likely due to a vas array of changes in abiotic or biotic factors (Van Auken, 2009).

1.2 COMPETITION AND DISTURBANCE IN GRASSLAND STRUCTURE AND DYNAMICS

1.2.1 PLANT COMPETITION

The species composition of grasslands is the result of multiple factors, such as environmental conditions, disturbance, and interactions amongst plants. Plants show intraspecific and interspecific competition for resources (Crawley, 1997). If competition occurs, the magnitude of competition may vary over time and space, it may influence the distribution and absolute abundance of species, and plant-plant interactions can influence relative abundances or species diversity within environments (Weigelt and Jolliffe, 2003). Within grasslands, the inherent characteristics that affect competition and create a patchy environment are soil depth, soil water, soil carbon-nitrogen ratio (C:N), and soil microbial N. Variation in a species ability to compete within these patches may be an important component of the ability of species to coexist in grasslands (Reynolds et al., 1997).

1.2.2 DISTURBANCE

The intensity of competition varies in systems at equilibrium compared to systems in non-equilibrium, such as disturbed sites. Disturbance will favour some species but hinder others (Sloan, 1997). The size of a disturbance-created gap will determine the species more likely to establish. Small gaps are commonly revegetated by neighbouring species through vegetative reproduction or seed production. Large gaps are usually colonized by fast-growing species (Hook et al., 1994). The intermediate disturbance hypothesis is a commonly used explanation for non-equilibrium maintenance of species diversity. The hypothesis predicts that maximized species diversity will be achieved at intermediate disturbance frequencies, where species losses

are minimized, while at the same time allowing for new species to establish (Roxburgh et al., 2004).

1.2.3 FIRE

Historically in North America, fires occurred more frequently than they do today. Occurrence of frequent fires during 1750–1900 is, in part, credited to native people lighting fires on the grasslands of Canada the United States. Part of the motivation for fire use was to control the movement of wildlife on a large scale (Nelson and England, 1971). Fire suppression was strongly encouraged during the 1900s, but the use of prescribed fires to maintain ecological health and restore habitats is now common practice (Brown et al., 2004). Understory species richness has been observed to increase with higher fire frequency, maximized with biennial fires, and with an associated decline in overstory woody species richness (Peterson and Reich, 2008). To restore areas with fire-resistant tree species, continuous active management may be a necessity (Brown et al., 2004).

1.3 ASPEN BIOLOGY AND ENCROACHMENT

Encroachment of woody species is well documented since the 1800s and is part of a global trend of increased number of woody species expanding into grasslands and savanna (Scholes and Archer, 1997). In Canada, Trembling aspen (*Populus tremuloides*) encroachment is a widespread phenomenon across the prairies (Bailey and Wroe, 1973; Blood, 1966b).

Aspen can establish sexually by seeds or vegetatively by clones. Barnes (1996) defines a clone as the sum of the ramets produced asexually from a single genetic individual. A ramet is defined as a distinct organism that has originated from a single parent plant. Aspen clones are connected underground by an intricate root system. The root systems start off as being relatively

simple, with all ramets laterally connected to the ortet. The ortet being the original tree all the ramets have sprouted from. However, as the stand ages, the root system becomes more complex. In time, some roots will die, creating ramets with independent root systems. Physical damage to above-ground portions of clones promotes suckering (Bird, 1961). Also, wounding the root systems can nearly double the rate of suckering in the vicinity of the cut. Increased suckering associated with root damage may offer a survival advantage in high disturbance areas (Fraser et al., 2004).

Trembling aspen employs several defence mechanisms against herbivory, one of which is chemical defence. These chemical defenses include phenolic glycosides and condensed tannins, which occur in leaves, bark, and roots. These chemical defences are effective against insect predators. However, effects of chemical defenses on mammalian herbivory has been less studied (Lindroth and St. Clair, 2013). Herbivore tolerance for plants with non-tannin phenolics is directly related to amount of digestible dry matter. Non-tannin phenolics are associated with toxicity when absorbed, whereas digestible dry matter is directly related to the amount of nutrition and energy an animal can take from a food source (McArthur et al., 1993). In addition, aspen is susceptible to several fungus diseases, which cause the premature deaths of many trees (Bird, 1961).

Aspen have been observed to present two different colour morphologies in their bark. In addition to the more familiar powdery-white bark, some populations present a yellow-green bark. The green colour in the bark is due to chlorophyll. A study found that in the early growing season, more chlorophyll per unit stem area was in the bark than the leaves. In the growing season, there was more chlorophyll in the leaves. Starch staining and stem grinding indicates that the photosynthates produced in the bark are easily translocated to other parts of the plant through

the phloem. The ability for mature stems to conduct photosynthesis could be an adaptation to thrive in an environment dominated by evergreen conifers (Pearson and Lawrence, 1958).

1.4 MANAGEMENT STRATEGIES

The mandate for Parks Canada dictates that ecosystems must be preserved with ecological integrity for future generations (Parks Canada, 2005). To accomplish this goal, adaptive management (AM) is often used. AM is centered around the idea of furthering the understanding of an ecosystem, and it is based on the assumption that the natural system being managed is dynamic and changes through time. The natural system is considered to be only partially predictable, and so management strategies may change over time (Williams, 2011).

AM can be either passive or active. The main difference between these AM strategies is that they put a different emphasis on the reduction of uncertainty (Williams, 2011). Active AM focuses on the reduction of uncertainty and involves direct manipulation of ecological processes. Passive AM does not have as a main goal to reduce uncertainty. Instead, reduction of uncertainty occurs as a side effect. Passive AM relies on predicting the outcome of ecological processes based on historical data (Baron, 2009). Active AM may be the only viable option when the sizes of protected areas are too small for natural process to continue without intervention. Often, the vicinity of the small protected areas has been subjected to strong anthropogenic modifications. These modifications influence immigration and emigration of animals and plants, alters the severity and frequency of fires, and changes the quality and quantity of water in the system. Scientific research is essential when making management plans for protected areas and is often a necessity to maintain some biotic communities in accordance with the general mandates of national park systems (Leopold et al. 1963).

1.5 FOREST STAND STRUCTURE AND DYNAMICS AT RIDING MOUNTAIN NATIONAL PARK

The relatively flat terrain on the west side of Riding Mountain National Park (RMNP) allows for spread of fire and promotes early succession species such as balsam poplar (*Populus balsamifera*), aspen, and beaked hazelnut (*Corylus cornuta*). This west-side forest is classified by Caners and Kenkel (2003) as a Type VII stand with canopy dominated by trembling aspen and balsam poplar. The Type VII stand type has trembling aspen as the dominant advance regeneration species and dominant shrub species of beaked hazelnut, prickly rose (*Rosa acicularis*), chokecherry (*Prunus virginiana*), and saskatoon (*Amelanchier alnifolia*). A similar understory is seen below Type VIII stands of white spruce (*Picea glauca*) and trembling aspen, which is present in other areas of RMNP. Balsam fir (*Abies balsamea*) is almost completely absent from the western regions of the park, because fires eliminate seeds. Under conditions where there are no major disturbances, balsam fir is more shade tolerant and is expected to replace not only trembling aspen, but also any associated paper birch (*Betula papyrifera*) and white spruce.

In terms of forest regeneration, the extant canopy dictates tree regeneration by seed or vegetative root suckering. The canopy composition also provides the growing conditions that dictate regeneration in terms of light, litter type, soil moisture, and understory composition. Caners and Kenkel (2003) found that the regeneration of species such as white spruce and balsam fir is limited by tall shrubs like beaked hazelnut and mountain maple (*Acer spicatum*). In contrast, the following species are capable of regenerating by basal- or root-suckering in areas with dense shrubs: trembling aspen, paper birch, and balsam poplar. Suckering below dense shrubs is possible, because these regenerating species can reach a height of two meters or taller

in a single growing season. In addition to trembling aspen and balsam poplar being self regenerating through suckering, intense ungulate herbivory on dense shrub systems results in perpetuation of the communities of shrubs such as beaked hazelnut. Stand Type VII appears to be self preserving and long lasting under the conditions present in the western part of RMNP.

1.6 HERBIVORY ON POPULUS TREMULOIDES IN RMNP

Elk (*Cervus canadensis*), moose (*Alces alces*), bison (*Bison bison*), and mule deer (*Odocoileus hemionus*) are native to RMNP and occurred in abundance before European settlers. Since then, mule deer have been outcompeted by white-tailed deer (*Odocoileus virginianus*) (Trottier et al. 1983), and bison are only present within the park in enclosures.

Deer, elk, and moose browse on aspen year-round, but more predominantly during the winter. Trottier et al. (1983) found that there is a difference in the vertical distribution of browsing amongst different species of ungulates. On average, deer have been found to browse between 58-63 cm, elk between 70-77 cm, and moose between 116-137 cm. This height discrepancy allows for all three species to utilize the same resource simultaneously. Aspen is deciduous, and herbivory occurs almost exclusively on growth of the current year. Elk utilize aspen as winter feed the greatest, followed by moose, and finally white-tailed deer. Moose summer diet is made of 95% of shrub leaves collected by stripping, of which *Populus tremuloides* and *Populus balsamifera* contribute between 3.2-6.6%. Elk summer diet is made up of 91-94% shrubs, out of which the same species of *Populus* contribute between 1.3-1.7%. Deer diet has between 92-94% shrub leaves over the summer, of which *Populus* leaves contributes 0.9-1.4%. These percentages were determined by a two-year study using microfecal analysis within RMNP.

1.7 RANGELAND HISTORY OF RMNP

Riding Mountain was first protected in 1895 as a Dominion Forest Reserve (DFR). In this reserve, timber resources were harvested, and consumption of wildlife was permitted but managed. While classified as a DFR, cattle grazing was strongly encouraged as a method to control fire by preventing fuel accumulation. Grazing started in 1914-1915, reaching its peak in 1920 when 4628 head of cattle and 118 horses grazed the range. Grazing occurred in over 50% of the grasslands in Riding Mountain, including areas in Deep Lake. Riding Mountain gained the status of National Park in 1933, which triggered studies to assess the impact of grazing on the local flora and fauna (Trottier, 1986).

Prairies in the western side of RMNP were classified as rough fescue prairies. Rough fescue (*Festuca hallii*) is considered a highly palatable species (Blood, 1966b), and selective browsing on it by cattle has a detrimental effect. Blood (1966a) found that competition for food sources by cattle had no major effect on elk, but the plant community was altered by cattle. The plant community changed from a climax fescue prairie to a community of Kentucky bluegrass (*Poa pratensis*) and dandelion (*Taraxacum officinale*), especially in overgrazed sites (Blood, 1966a). In 1969, seasonal cattle grazing was eliminated from RMNP, because changes to the plant communities are incompatible with National Park policy (Trottier, 1986).

Follow-up work (Trottier 1974) assessed the recovery of grasslands after grazing. Sites that were not grazed or lightly grazed were dominated by rough fescue, interspersed mainly with native grasses. As the intensity of grazing increased, the number of herbaceous species and percentage of rough fescue present steadily decreased, with some sites under intense grazing presenting no rough fescue at all. Trottier (1986) concluded that natural recovery of fescue in the intensely grazed prairie would be unlikely without active management. A later assessment found

that moderately to heavily grazed sites still presented low or no rough fescues, high numbers of woody species, and weeds (Dushnisky, 1981).

1.8 CONCEPTUAL AND THEORETICAL FRAMEWORK OF THE THESIS

The origin of this project lies in the need to understand methods to prevent further aspen encroachment within remnant fescue grasslands of RMNP. Historic work of Blood (1966b) and Trottier (1986) in RMNP showed extensive encroachment of aspen into what previously were grasslands.

1.8.1 STUDIES OF FESCUE GRASSLANDS OF RIDING MOUNTAIN NATIONAL PARK

Flook (1956) studied the conditions of the rangeland in Birdtail Valley, the Strathclair trail, the McCreary trail, and the area of Lake Audy. Areas that were heavily grazed showed proliferation of non-palatable shrubs, including shrubby cinquefoil (*Potentilla fruticosa*) and snowberry (*Symphoricarpos occidentalis*). Flook (1956) listed the dominant grasses following grazing as species of bluegrass in the genus *Poa*, species of wheatgrass in the genus *Agropyron*, and timber oatgrass (*Danthonia intemedia*). In his report, Flook (1956) did not refer the presence of rough fescue, although he mentioned a haying operation along Birdtail trail with good quality grass. Flook (1956) described bluegrass and wheatgrass as being good browse for elk.

Blood (1966a, b) characterized the rough fescue grassland association in Manitoba and estimated that rough fescue grasslands cover about 0.2% of RMNP, or about 600 ha. Blood (1966a, b) hypothesized that rough fescue would in the past have been widely distributed in the Park, but the total area of open prairie is now greatly reduced due to encroachment by aspen and white spruce. Based on dry matter collection amongst grasslands in RMNP, Blood (1966a, b) found that rough fescue contributed 43% of total biomass and 72% of graminoid biomass,

whereas Kentucky bluegrass contributed less than 0.3% of total biomass and less than 0.5% of graminoid biomass. Speargrass (*Hesperostipa curtiseta*) was the second most prevalent graminoid species after rough fescue. Blood (1966a, b) also found that cattle grazing affected both the species composition and range conditions of fescue grasslands. In areas of moderate to heavy cattle grazing, rough fescue was completely replaced by Kentucky bluegrass.

A detailed survey of fescue grassland conditions in RMNP was undertaken after cattle grazing was terminated. Trottier (1974) established permanent transects in 33 sites and sampled using a modified point-transect method. Rough fescue was recorded in 20 of the 33 sites. Kentucky bluegrass, which is known to tolerate heavy grazing and trampling, dominated many of the moderately to heavily grazed sites. Heavy grazing eliminated rough fescue and increased the proportion of forbs. Sites at the highest level of grazing at the time of the survey were dominated by weedy invasive species. Trottier (1974, 1986) classified grazing regimens into five categories from slight to severe. Rough fescue on average was 59% cover in slightly grazed areas and 0% in severely grazed areas. In contrast, Kentucky bluegrass had an average percent cover of 5% in slightly grazed areas and 74% cover in severely grazed areas.

Dushnisky (1981) also evaluated the status of the grasslands that were formally grazed by cattle in order to assess if it would be acceptable to graze cattle in the park in cases of extreme drought. Dushnisky (1981) found that 1875 acres of grassland had been lost since 1978 due to beaver flooding, but noted that beaver flooding is dynamic. Findings were that the areas heavily grazed were still recovering from the grazing, rough fescue was abundant in moderately grazed areas, and that weeds and shrubs were generally low and non-existent in lightly grazed areas. Moderate to heavy grazing had decreased the cover of rough fescue, and it has been replaced predominantly by wheatgrass, bluegrass, and Junegrass (*Koeleria macrantha*). Dushnisky (1981)

concluded that, due to the vulnerability of rough fescue grasslands to grazing, alternative placement of cattle should be made during drought.

Slogan (1997) determined the species composition, community structure, richness, and diversity for the vegetation of the Trottier transects. Fescue grassland soils were also assessed for nutrient status, pH, and electrical conductivity. Through the analysis of aerial photographs, Slogan (1997) determined the extent of shrub and tree invasion into grasslands between 1969 and 1994. Graminoid species composition remained consistent between 1973 and 1995, with the exception of smooth brome (*Bromus inermis*), which increased. Although both Blood (1996a, b) and Trottier (1974) noted the presence of smooth brome in RMNP, it was not encountered in the Trottier transects in 1974. In 1995, smooth brome was the fourth most abundant grass encountered in the 33 transects. In 1973, rough fescue was the second most dominant graminoid, but by 1995 it ranked third in dominance after Kentucky bluegrass and slender wheatgrass (*Elymus trachycaulus*). Most of the native graminoid species declined in abundance from 1973 to 1995, particularly sedge species in the genus *Carex*, as well as June grass, porcupine grass (*Hesperostipa spartea*), and rough fescue. Forb abundance increased between 1973 and 1995, but species composition has remained consistent. Species richness increased post-grazing.

In terms of shrub and tree expansion into the prairies, four out of the 11 sites studied by Trottier (1974) showed visible signs of encroachment between 1969 and 1994. Three sites showed declines in shrub cover, and four showed no change. The soil analyses found that potassium and sulphate were available in sufficient amounts, but that most sites were deficient in nitrate and phosphate.

Lastra (2011) evaluated aspen properties that cause aspen clone expansion and dieback. Trembling aspen can be classified as “encroaching” or “non-encroaching” based on young-plant

stem density in relation to the number of mature stems present in the stand. Aspen clones that were non-encroaching had more mature stems in the core forest. Encroaching clones had significantly fewer mature stems. However, the total number of alive and dead stems did not differ, indicating that encroaching clones had suffered greater stem mortality in recent years. Encroaching clones also showed greater stem recruitment, which could be a consequence of canopy breakup. Increase in solar radiation reaching the forest floor can elevate the temperature to over 12°C, which is required for suckering to occur.

Partial dieback of the canopy was found to result in a four-fold increase in root suckering within the aspen stands and an eight-fold increase along the boundary. In contrast to what was commonly believed, the results from this study show that a gradual breakup of the mature canopy results in ongoing root suckering at modest densities. Lastra (2011) showed that the degree of canopy dieback, or reduction in mature stem density, is a reliable predictor of encroachment, with an increased likelihood of encroachment to occur when stand density is under 1400 stems ha⁻¹.

The plant community that occurs in an encroaching stand differs from a non-encroaching stand. The determining factor may be light, where plants adapted to low light conditions, such as rice grass (*Oryzopsis asperifolia*), dominate the forest community. In encroaching stands, there is more light reaching the forest floor, allowing greater diversity in the plant community. In consequence, encroaching stands result in greater heterogeneity of the plant community.

Lastra (2011) studied the spatial and temporal pattern of stem growth and mortality for clones in the dieback stage. Clones in the dieback stage are characterised by having the majority of mature ramets dead and a dense second cohort established. Ramets that did not survive the study period were designated as dying. The results showed that dying ramets were significantly

shorter in height than surviving ramets. Thereby, it seems that ramets have an extended period of reduced growth before dying. The youngest ramets were less than 1 cm diameter and showed the greatest growth rate, which more than doubled over the study period. The growth rate declines as the ramet increases in age. Overall, the results indicated that clone demography is driven by endogenous rather than exogenous processes.

As mentioned earlier, trembling aspen grows in stands usually composed of clones. Colonial integration, meaning they are connected by a root system, as well as subsequent disintegration, referring to the breaking of connections amongst ramets, is common in colonial species. To study the effect of disintegration on ramet productivity, groups of suckers had their roots severed by trench digging, isolating them from the rest of the genet. A reduction in leaf production was seen in the subsequent year, as well as a reduction in relative growth rate. This reduction in production was most pronounced in the driest areas, indicating that colonial integration is more important for ramet productivity in stressful environments. A very large reduction in growth rate following disintegration was observed for ramets newly established after a fire, indicating that post-fire root suckers are especially dependent on this integration to obtain resources for establishment (Lastra, 2011).

Trembling aspen produce secondary phenolics such as coniferyl, alcohol benzoate, condensed tannins, and phenolic glycosides. Vigorous clones are defined as having low mortality of mature stems, smaller stems not browsed by animals, and continuous recruitment. Vigorous clones had much higher levels of phenolic glycosides than dieback clones. Dieback clones are defined as having modest-to-high mortality of mature stems, smaller ramets with animal browse, discontinuous recruitment of ramets, and vertical suppression of growth. Browsing damage was significantly higher on dieback clones, which is consistent with the strong negative correlation

between browsing and total phenolic glycoside concentration. Clones that produce high levels of phenolic glycosides regenerate successfully and show limited browsing. The amount of glycosides decreased with less soil moisture, indicating that the concentration of glycosides increases in higher stress environments. Repeated browsing creates a positive feedback loop, where the tree produces less glycosides the more heavily it is browsed, increasing the browsing pressure. This feedback leads to stunted growth with larger number of branches at the browse level. With a few exceptions, such as fire or severe drought, tree mortality appears to be the result of long-term processes instead of short-term ones (Lastra, 2011).

1.9 RESEARCH OBJECTIVES

The main objective of this study was to understand the interactive effects of herbivory in the form of browsing and fire on the growth of aspen within the fescue grasslands of RMNP. Secondary aims were to determine the influence of browser feces on aspen leaf-litter breakdown, as well as to investigate the historic extent of prairies within RMNP. The three objectives are derived from the need to understand the impacts of aspen encroachment.

IMPACT OF BROWSING TO CONTROL ASPEN RE-GROWTH WITH-OR-WITHOUT RECENT STAND BURNING

Hypothesis: A combination of browsing with a recent burn will give the greatest suppression of aspen re-growth.

IMPACT OF BROWSER MANURE AMENDMENT ON ASPEN LEAF BREAKDOWN USING FIELD-PLACED LITTER BAGS

Hypothesis: Browser manure amendment will increase the rate of loss of mass of aspen litter.

DETERMINATION OF HISTORIC EXTENT OF GRASSLANDS IN RMNP WITH SOIL
PITS TO DETERMINE ORGANIC CARBON WITH DEPTH

Hypothesis: Historic grasslands will have soil organic carbon at greater depths than historic forests.

2 Chapter two: IMPACT OF BROWSING AFTER BURNING ON ASPEN GROWTH

Kathryn Barr and Terence McGonigle

Chapter summary

Aspen (*Populus tremuloides*) encroachment is a widespread phenomenon across the Canadian prairies and results in the reduction of native grassland habitat. Manitoba has a small number of rough fescue (*Festuca hallii*) grasslands, which are commonly utilized by elk for winter grazing in upland Manitoba. Aspen encroachment currently threatens these grasslands within Riding Mountain National Park, where augmentation of grassland is a priority. Although wild ungulates readily browse on suckers, this feeding alone has not been able to suppress aspen expansion. Prescribed burning is a common practice to deter the encroachment of woody species into grasslands, but it has not always been successful. Browsing simulations were carried out within exclosures across sites with-and-without recent burns to measure the effect of browsing alone versus a combination of fire and browsing. A split-plot design was used to determine growth following different clipping treatments to simulate browsing, with 100%, 50%, or 0% of leaves removed. To assess the effect of browsing on the apical shoot, a subset of trees was randomly assigned an apical shoot manual-removal treatment within 50% leaf-removal plots. There was no difference in aspen cover between the two burn treatments. However, growth was reduced significantly in response to clipping, independent of burn history, but only when applied at 100% leaf removal. Suckers that had their apical shoot removed had significantly less vertical growth compared to the sucker with the apical shoot left intact. In terms of understory species, greater evenness was observed in burn sites, as well as greater species richness.

2.1 INTRODUCTION

Encroachment of woody species is widely recognized to have increased since the 1800s and is part of a global ecological phenomenon of woody species expanding into open grasslands and savanna (Van Auken, 2009, 2000; Brown and Archer, 1999; Browning et al., 2008; Sala and Maestre, 2014). In Canada, aspen encroachment is a widespread phenomenon across the prairies (Bailey and Wroe, 1973; Blood, 1966b, Parks Canada, 2010). Riding Mountain National Park (RMNP), Manitoba, has been identified as an area of concern of forest encroachment due to a rapid decrease in rough fescue occurrence in prairies (Blood, 1996; Trottier, 1974). The amount of herbaceous biomass present does not seem to influence the establishment of woody species (Brown and Archer, 1999).

Aspen can establish sexually by seeds or vegetatively by clones. A clone is a group of individuals propagated by asexual reproduction from a single organism that was produced by sexual reproduction. For a stand to establish, a seed must first give rise to a tree. Propagation then proceeds by suckering. In an aspen stand, the original tree established by seed is called the ortet, while the other shoots that appear from suckering are called ramets (Barnes, 1996). Old ramets have characteristic bud scales on the stems, as well as small blades, short internodes, and obtuse leaf bases. New ramets have large leaf blades, long internodes, and cordate or truncate leaf bases. Juvenile sprouts also have higher amount of resins, which make them less palatable to browsers (Romme et al 1995). Aspen clones are connected underground by an intricate root system. The root systems of a young clone begin with all ramets laterally connected to the ortet. As the stand ages, some roots die, creating ramets with independent root systems (Barnes, 1996). In a study of Barnes (1996), 70% of the suckers within a clone were found to have separate root systems. This disintegration becomes more evident as the clone ages. The root systems of trees

with diseased shoots had low vigor yet relatively healthy root systems. Roots systems of one stand may penetrate an adjacent clone. However, shoot genet mixing is infrequent unless the ramets of one of the clones are destroyed. Intergrowth between two clones does occur when multiple ortets are established from seeds germinating in close proximity.

Damaging to above-ground portions of clones promotes suckering (Bird, 1961; Frey et al., 2003), but effects of damaged root systems are less well known. Fraser et al. (2004) demonstrated that wounding the root systems can nearly double the rate of suckering within 50 cm of the injury. Higher rates of suckering were observed when the roots were completely severed, in comparison to being scraped. The suckers located near the wounds were also found to be taller and had a higher number of leaves when compared to suckers appearing from non-wounded roots. Increased suckering associated with root damage may offer a survival advantage in high-disturbance areas (Fraser et al., 2004). Aspen suckering may also be related to soil temperature, such that disturbance combined with an increase of soil temperature will favor suckering (Bailey and Wroe, 1973).

2.1.1 HERBIVORY

For herbivore forage selection, the quality of the food source plays an important role. Quality is determined by levels of nutrients, structural components such as fiber, and anti-herbivore defensive chemicals (Wooley et al., 2008). Trembling aspen employs defence mechanisms against herbivory, one of which is chemical defence. These chemical defenses include phenolic glycosides and condensed tannins, which occur in leaves, bark, and roots. Although it is well known that these chemical defences are effective against insect predators, their effect on mammalian herbivory has been less studied (Lindroth and St. Clair, 2013). Digestible dry matter is directly related to amount of nutrition and energy an animal can get from

a food source. Nontannin phenolics are associated with toxicity when absorbed (McArthur et al., 1993). Ungulates such as mule deer (*Odocoileus hemionus hemionus*) and black-tailed deer (*Odocoileus hemionus sitkensis*) select forage based on the digestible dry matter and the amount of constituent nontannin phenolics. Tolerance for plants with nontannin phenolics is directly related to amount of digestible dry matter. Foraging on species with chemical defenses, such as aspen, has been shown to alter the relative abundance of such chemicals. Browsing by elk has been shown to elevate the levels of phenolic glycosides in aspen (Bailey et al., 2007).

Increasing the pressure of herbivores on aspen may possibly stop its advancement (Hessl and Graumlich, 2002). Browsing on aspen has been shown to negatively affect its ability to grow and expand (Baker et al., 1997; Cahill et al., 2013; Fitzgerald and Bailey, 1980; Hessl and Graumlich, 2002; Kota and Bartos, 2010). Elk are one of the main predators on aspen amongst native ungulates in North America (Cahill et al., 2013). Aspen suckers browsed by elk are at a higher risk of death than not browsed trees as a result of herbivory (McArthur et al., 1993), although aspen tolerates fairly high browsing intensities in comparison to related species (Campa III et al., 1992). High levels of mortality are also associated with mechanical damage caused by bison due to trampling (Cahill et al., 2013).

By aging the trees, Kay and Bartos (2000) were able to find a relationship between aspen regrowth and a reduction in wild ungulate numbers. In years when the number of deer and elk spiked, no trees survived at the height of browsing. In contrast, aspen growth was successful when wild ungulate numbers dropped. Aspen located within total-exclusion plots was able to regrow successfully and presented a multi-age stand, whereas browsed plots showed a lower regeneration rate than the total-exclusion plots. Aspen in livestock-grazed plots was unable to regenerate successfully, or regeneration rates were very low. The understory within the plots was

also vastly modified based on the type of herbivory allowed. Plots browsed by deer presented more native grasses. However, the plots grazed by livestock had lower native grass cover but increased cover of invasive grasses and bare soil.

During a simulated browsing experiment, Carson et al. (2007) found that trees clipped earlier in the growing season presented greater dieback than trees clipped later on. This difference suggests that the longer the amount of time the tree has before winter dormancy, the longer will be the time for dieback to occur. Jones et al. (2009) showed annual sucker height growth was more negatively affected by browse on the terminal leader compared to browse on lower branches. Timing of browse also impacts growth, because less growth occurred when browse was mid-season compared to early season or late season. As well as a dieback effect, the number of lateral branches per stem were greater in the absence of herbivory (Cahill et al., 2013).

Application of a browse treatment at different times during the growing season influences the success of regrowth. Overall, growth is lowest for suckers browsed mid-season with apical bud removal of 50% or more (Jones et al., 2009). Total biomass of leaves and shoots produced from aspen stems was higher for early and late-season clipped stems compared to mid-season (Carson et al., 2009). Based on these results, to find the greatest decrease in growth, clipping treatments should be applied mid-season with 50% or more of the terminal leader removed.

2.1.2 FIRE

Historically in North America, fires occurred more frequently than they do today. Frequent fires throughout the period of 1750–1900 is, in part, attributed to man lighting fires on the grasslands of Canada the United states (Nelson and England, 1971). Prescribed burning is used in current times to control encroachment of woody species into grasslands, but it has had

varying results (Guedo and Lamb, 2013). Species diversity generally increases with burning in grasslands and shrublands, but season of burning plays an important role to determine which species are most affected. Burning in early spring has been found to reduce the proportion of woody species and increase grassland expansion. The only woody species that increased cover under this burning regiment was aspen (Anderson and Bailey, 1980). Another study found that spring burnings are more effective at controlling aspen encroachment compared to fall burnings (Guedo and Lamb, 2013). After a fire with high burn severity, above-ground biomass of aspen re-sprouts 10 times more than in sites of intermediate burn severity, with virtually no aspen regeneration without fire (Bailey and Whitham, 2002). Burning can also have a detrimental effect on the herbaceous and graminoid community if done after the plants have started to sprout, because meristematic tissues are most susceptible when actively growing (Wright and Bailey, 1982).

2.1.3 HERBIVORY AND FIRE

In Yellowstone National Park, USA, a pattern of aspen stand dieback has focussed interest on why recruitment has not been occurring (Hinds 1985; Romme et al., 1995). The dominant meristem in aspen produces auxins that suppress root suckering. However, if the tree receives any damage that could impair this apical dominance, the tree will respond by suckering (Despain 1990). Damage to the apical meristem has been reported from ungulate herbivory and from fires that kill off the mature trees. Romme et al. (1995) suggested that extensive fires would cause the production of too many aspen suckers for them all to be browsed by elk, allowing for stand regeneration. These authors suggest that elk would be attracted to recently burned areas, causing heavier browsing on sucker regenerating after a fire compared to outside burn areas. Contrary to what they suggested, Romme et al. (1995) found there was no significant difference

between the intensity of browsing in recently burned areas and areas not burnt. In addition, Romme et al. (1995) quantified browsing on two different age groups, new sprouts and old sprouts. New sprouts were classified as suckers that were less than one-year-old, whereas old sprouts were classified as suckers older than one year. Romme et al. (1995) found no difference in the intensity of browsing of new sprouts versus old sprouts. These results suggested that the density of elk is too high to allow for selective browsing. Romme et al. (1995) also found 45-55% of suckers were browsed in winter compared to only 5-10% in fall. In addition, the sprouts not browsed in the spring were under snow, suggesting they escaped browsing by being hidden. Overall, intense browsing on aspen suckers prevented the ability of a stand to regenerate successfully, independent of fires.

In Arizona (Bailey and Whitham, 2002), burn severity affected aspen regeneration, with higher numbers of ramet resprouts per area as burn severity increased. Elk also selectively browsed aspen ramets in high-severity burn sites at a rate two times more intensely than aspen ramets in intermediate-severity burn sites. Bailey and Whitham (2003) theorized that all the resources in the roots are used to re-sprout new suckers following fire-induced death of the above-ground portion of mature trees. In the case the mature trees that are not killed, resources stored in the roots are shared between sprouting suckers and repairing crown damage. In the absence of fire, few resources are used for sprouting new suckers.

2.1.4 GRAZING FESCUE GRASSLANDS

One of the objectives at RMNP is to maintain a healthy elk population within the park. Rough fescue is the preferred winter browse for elk (Knight, 1970), and so healthy rough fescue grasslands should allow for greater survival rates for elk during the winter. Reduction in rough fescue biomass in lands used for grazing is caused by trampling and stem-base removal during

feeding (Jourdonnais and Bedunah, 1990). Deterioration of grazed grasslands is related to elimination of desired species such as rough fescue (Short et al., 2003; Willms et al., 1985).

With suppression of fire, litter accumulation can inhibit grazing of rough fescue by reduction in accessibility. Jourdonnais and Bedunah (1990) found that prescribed burning decreased the amount of rough fescue in the first season after burning. However, rough fescue subsequently recovered in the second season, the amount of rough fescue being similar in the burned sites and control sites. Burn treatments were more successful at removing litter than cattle grazing. Short et al. (2003) found that fall grazing by cattle to reduce any non-palatable standing dead material improved spring and summer forage of wild ungulates (Short et al., 2003). Cattle grazing can also be used to manage aspen encroachment. A single heavy late grazing by cattle practically eliminated aspen regeneration after a burn (Fitzgerald and Bailey, 1984), but other shrubby species such as wild rose (*Rosa acicularis*) and wild raspberry (*Rubus strigosus*) are preferred over aspen. Western snowberry (*Symphoricarpos occidentalis*) is less preferred by cattle, so that aspen will be grazed preferentially when alongside this shrub. Cattle chose more readily to graze on aspen late in the season than early (Fitzgerald et al., 1982), but late-season grazing can promote greater density of the non-palatable western snowberry (Bailey et al., 1990). Historically in RMNP, cattle grazing was strongly encouraged as a method to control fire, by preventing fuel accumulation. Grazing peaked in the 1920s and occurred in over 50% of the grasslands in Riding Mountain, including areas in the vicinity of Deep Lake. Cattle grazing was later prohibited in RMNP due to conflict with the Parks Canada mandate to preserve ecological integrity (Blood 1966a).

The goal of this study was to determine the impact of three different browsing treatments to control aspen re-growth with-or-without recent stand burning. We hypothesized that a

combination of browsing with a recent burn will give the greatest suppression of aspen re-growth, because applying two growth suppression methods should be more effective than each one on their own.

2.2 MATERIALS AND METHODS

2.2.1 STUDY SITE

The study was conducted at RMNP, Manitoba. This region is in the Mixedwood Section of the Boreal Forest Region (Rowe, 1972). This region is characterized by well-drained soils, which are dominated by species like white spruce (*Picea glauca*) and aspen (Rowe, 1972). The study was conducted at the west end of the park near Deep Lake, in forest-grassland transition zones. The plots were located in grasslands burnt by Parks Canada in spring 2015. The aspen presented suckering.

Environmental conditions were taken from Wasagaming, a town located within RMNP at 50 39.300N 99 56.517W. Historical weather data of 29 years was obtained between the years 1981 and 2010. The historical daily temperature averages were 17°C for July and 15.9°C for August. Total averaged annual rainfall was 372.1 mm, with the most rainfall in June of 80.1 mm, followed by July with 66.7 mm, and then by August with 59.2 mm. Total precipitation averaged 488 mm per year. In 2016, the mean average temperature for July was 17.1°C, and total precipitation was 82.9 mm. For August 2016, averaged daily temperature was 15.7°C, with total precipitation of 79.9 mm. In 2017, the mean average temperature for July was 17.5°C, and total precipitation was 7.7 mm. For August 2017, averaged daily temperature was 15.7°C, with total precipitation of 48.8 mm (Environment Canada, 2017).

2.2.2 SELECTION OF EXCLOSURE LOCATION

Aspen clones within the selected grasslands were visually assessed to make sure they met the following criteria. Only clones that showed suckering with ramets under 2 m in height were selected for this study. Only the outer layer of the clone was considered, where encroachment into the prairie was evident. Polygons were delineated surrounding the areas that met these criteria, and a point was selected randomly within the polygon. The point corresponded to the SW corner of an enclosure, used here to mean a fenced square to exclude browsing animals. However, if the random point would have caused part of the enclosure to fall outside of the polygon, then that point was switched in sequence to correspond to the enclosure corner at the NW, NE, and SE, respectively. The random points were flagged in the field. On a later date, the enclosures were built. A total of six enclosures were built in the recently burnt areas, with six more enclosures built in the non-burnt areas. The placement of enclosures was to control for wild ungulate browsing, which would otherwise have compromised the results of the simulated browsing treatment.

2.2.3 EXPERIMENTAL DESIGN

This study is based on a split-plot design. The browsing enclosures are 12 m wide x 12 m long x 1.8 m tall, and they are subdivided with temporary ropes into three 4 m x 12 m subplots. The browsing treatments applied to the subplots were randomly assigned and comprised 100% leaf removal, 50% leaf removal, and 0% leaf removal as a control. Browsing treatments were applied by hand stripping. Hand stripping was a standardized treatment applied to all suckers under 2 m tall within a plot. For the 50% leaf-removal treatment, leaves were removed from every second branch to attain approximately 50% of the original leaf cover. Percent cover was recorded before and after the treatment was applied. Cover scoring was undertaken by placing a

two-meter metal rod in multiple positions in the subplot in the same manner as a pin frame, with pins evenly spaced along transect lines. Each plant contact to a pin was scored. Leaf and petiole were recorded in one category, while stem counts were recorded separately. Percent cover was determined for all tree and shrub species within the plot. A half metre buffer was left between the fence line and the first pin drop, to exclude possible trampling from building the exclosures. The simulated browsing treatment was applied only on trembling aspen. Leaves were stripped and placed in bags to be taken away from the vicinity of plots and discarded. To prevent any possible spread of disease, gloves were changed between plots. The treatment was applied twice, once between 4 July 2016 and 12 July 2016, and again between 1 June 2017 and 22 June 2017. Dates were selected to try and hinder the growth during the peak of the growth season. Percent cover was taken again on aspen near the end of the field season on 24 August 2016, and between 21 August 2017 and 24 August 2017 to measure regrowth.

In spring 2017, tree tags were placed on aspen suckers located in the 50% leaf removal treatment. Browsing treatments were randomly assigned to aspen suckers. For a stem to be considered an individual ramet, it was required to sprout from the soil without any visible attachment to other ramets. If the ramets was joined at any portion above the soil surface, the structured was considered a single individual. Tree tags were only used on trees in the 50% leaf removal subplot within the exclosures. Within these subplots of 4 m x 12 m, two treatments were applied. The first treatment included 50% removal of lateral growth in the form of leaves and petioles only but leaving dominant shoot intact. The second treatment included 50% removal of lateral growth and removal of the growth of the current year on the dominant shoot, that is 90% removal of apical bud. Treatments were applied in June to correspond to the midseason. Initial height and basal diameter were taken in June 2017 before the clipping treatments were applied.

At the end of August 2017, during the end of the growing season, the height of the dominant stem of each aspen sucker was re-measured.

2.2.4 ENVIRONMENTAL CHARACTERIZATION

Vegetation was identified outside each enclosure within the categories of herbaceous, graminoid, and woody plants. In 2016, one transect was taken outside each of the 12 enclosures, beginning from the center of the fence nearest to the prairie located roughly six meters between corners. From that location, a 40 m transect was placed heading out into the open prairie. Every two meters, a pin frame containing 10 pin drops were scored, each pin 10 cm apart. A total of 2400 pin drops were done along 12 transects, one at each of the 12 enclosures. Percent cover was noted by species. A count was taken of how many times each species touches the pin, to give percent cover. In July 2017, transects were done inside each of the 12 enclosures. Once again, cover was recorded by species to encompass vegetation herbaceous, graminoid, and woody. Two 12 m transects were placed inside each subplot within the enclosures. On each transect, five pin frames were placed 2 m apart, with a 1.2 m buffer around the edge of the enclosure. A total of 3600 pins were dropped along six transects within each of the 12 enclosures.

2.2.5 STATISTICAL ANALYSIS

All analyses were undertaken in Statistix 8 using $P=0.05$ to determine significance. The \log_{10} was taken on the percent covers for the simulated browsing experiment in order to normalize the frequency distribution. A split-plot analysis of variance was conducted on the logarithmic data at four different sampling times: July 2016, August 2016, June 2017, and August 2017. A total of 652 trees were tagged, of which 629 trees were included in the tree tag experiment. Tree loss was due to death during the experiment. A T-test was conducted on the difference in height between samples with apical shoot removed ($n=308$) and apical shoot left

intact (n=321). For the environmental characterization, evenness was calculated for both burned sites and not burned sites with the Shannon-Wiener biodiversity index. Percent evenness was calculated for each site by rescaling values between 1-100 using a theoretical maximum evenness and theoretical minimum evenness. Means of transformed data were compared using a T-test for species present over 20% in either burned sites or not burned sites. For transects in 2017, the substrate was also recorded as either litter, bare soil, forbs and graminoids, or bryophytes. We report p-values, and complete analysis of variance tables are provided in the Appendices.

2.3 RESULTS

The initial state of the plots in July 2016 before any treatment was applied had no significant difference ($p=0.80$) in percent cover between burn treatments, no significant difference ($p=0.92$) in percent cover between clipping treatments, and no significant interaction ($p=0.94$) between the burn treatments and clipping treatments (Figure 2-1). Following clipping applied in July 2016, percent cover in August 2016 had no significant difference ($p=0.22$) in percent cover between burn treatments, but there was a significant difference ($p<0.001$) in percent cover among clipping treatments (Figure 2-2). Also for August 2016, there was no significant interaction ($p=0.36$) between the burn treatments and clipping treatments (Figure 2-2). Cover in June 2017 was recorded before the second application of clipping treatments. For June 2017, there was no significant difference ($p=0.026$) in percent cover between burn treatments, there was a significant difference ($p<0.001$) in percent cover among clipping treatments, and no significant interaction ($p=0.52$) between the burn treatments and clipping treatments (Figure 2-3). Final percent cover in August 2017 was recorded after the second application of clipping treatment in June 2017. In August 2017, there was a significant increase

($p=0.003$) in percent cover in the burn treatment, there was a significant difference ($p<0.001$) in percent cover among clipping treatments, but there was no significant interaction ($p=0.15$) between the burn treatments and clipping treatments (Figure 2-4). Complete analysis of variance table available (Appendix 2-1). The stimulation of aspen cover in the burned plots was evident across the study (Fig. 2-5). There was a significant difference ($p=0.029$) in increase in height between trees with the apical shoot removed and trees with the apical shoot left intact (Figure 2-6).

For the environmental characterization in 2016, a total of 104 species were identified across the transition from the exclosures to the open prairie (Appendix 2-2). Species with an average percent cover of 20% or higher in at least one of the burn treatments were included in the statistical analysis. For 2016, eight species were included in this comparison, along with percentage cover for standing dead. In 2016, *Poa pratensis* had a percentage cover higher significantly ($p=0.034$) in the sites not burned than in the burned sites, whereas *Fragaria virginiana* ($p=0.002$) and *Lathyrus ochroleucus* ($p=0.005$) had greater cover at the burned sites (Table 2.1). Evenness was not significantly different between burn treatments (Table 2-3).

In 2017, a total of 84 species were identified within the exclosures (Appendix 2-3). Species with an average percent cover of 20% or higher in at least one burn treatment were included in the statistical analysis. For 2017, eight species plus standing dead were included in this comparison. In 2017, *Carex inops* ssp. *heliophila* had a significantly higher percentage cover ($p<<0.001$) in the sites not burned than in the burned sites, whereas *Fragaria virginiana* ($p<0.001$) and *Liatris ligulistylis* ($p=0.031$) had greater cover at the burned sites (Table 2.2). Evenness was significantly higher ($p=0.016$) in burned sites (Table 2.3). Basal cover below the pin in 2017 was 85.6% litter, 11.4% bare soil, 2.5% vascular vegetation, and 0.4% bryophytes.

2.4 DISCUSSION

Romme et al (1995) described old aspen sprouts as having characteristic bud scales on the stems, as well as small blades, short internodes, and obtuse leaf bases. New aspen sprouts were described as having large leaf blades, long internodes, and cordate or truncate leaf bases. This difference was evident between recently burned sites and sites not burned in this study. The saplings under 2 m high in the sites not burned presented growth characteristics of older trees, with woody stems, small leaf blades, short internodes, and obtuse leaf bases. This difference can be interpreted to mean that the suckers in the sites not burned were old sprouts that presented stunted growth, whereas the suckers under 2 m tall in the recently burned sites were young saplings with green stems, large leaf blades, long internodes, and cordate leaf bases. This discrepancy between the suckers used in this study for the different burn treatments may have an unknown influence on any responses to clipping treatments, and so the results must be interpreted with caution.

The effect of clipping seen here to reduce height of aspen suckers differs from Campa III et al (1992), who found that the mean heights of aspen on clipped plots did not differ from controls. The present study found a significant difference between 100% removal of leaves and the control. This outcome matches data of Romme et al (1995), where high intensity of browse influenced aspen growth regardless of burn history. In the present study, clipping the apical shoot decreased the mean height of the suckers compared to those with the apical shoot left intact. These results concur with Jones et al (2009), who reported greatest annual height growth on suckers with no terminal leader browse and lowest growth when 90% of terminal leader length was browsed. From the suckers with terminal leader browsed, growth was most reduced with removal of the terminal leader and 50% of branch biomass.

The natural increase in cover in the control sites not clipped was higher in burn sites than in the sites not burned (Figure 5). These results fall in line with the findings of Guedo et al (2013), which was annual burns showed a higher amount of regrowth in the long term than in plots with less frequent burns. The results of the present study are also consistent with Anderson et al (1980), who found that burning decreased cover of all woody species except aspen suckers. Bailey and Whitham (2003) also reported that the density of aspen suckers increased with severity of fire.

The meta-analysis of D'Antonio (2000) found that, in 80% of studies, prescribed burning increased invader abundance. In the present study, in contrast, percent cover of the invasive species *Poa pratensis* was lower in recently burned areas. Anderson et al (1980) found that burned areas had twice as many grass species compared to areas not burned. In the present study, a slightly higher number of grass species were observed in the burned areas. A more pronounced difference was seen in the evenness of the species, where the community had higher evenness in the burned areas following partial removal of *Poa pratensis*.

2.5 CONCLUSION AND MANAGEMENT IMPLICATIONS

Adaptive management (AM) is often used as a management strategy in national parks. AM can either be active or passive. Active AM involves directly manipulating ecological process, while passive AM aims to predict how an ecosystem will respond to changes based on historical data (Baron et al, 2009). Regardless of burn history, high levels of clipping are required to suppress aspen regrowth. In the current situation at Deep Lake, there does not seem to be high enough pressure by herbivores to successfully suppress aspen encroachment into the grasslands. A stronger impact on the aspen suckers might be produced by combined impact of higher browsing intensity, more frequent fires, and bison trampling. Bison or cattle can be

expected to cause more physical damage to the aspen suckers by tramping than ungulates such as elk, deer, and moose. RMNP comprises 2,969 km² (Parks Canada, 2007), a limiting space for large migratory mammals such as elk, moose, and bison. The boundary of RMNP is not fenced, which allows for free movement for the animals between the park and neighboring land. The nature of this movement allows for healthy populations of large mammals to reside in RMNP for part of the year, yet utilize large home ranges. Re-introducing bison to roam freely within RMNP might result in problematic management implications, such as animals damaging neighboring property. Cattle are also known to have the unfortunate outcome of encouraging *Poa pratensis* expansion. As an alternative active AM strategy, mowing the perimeter of the grassland in the actively encroaching zone might be an option to prevent further loss of the grassland. Prescribed burns increase evenness of understory species and suppress the dominant non-native species *Poa pratensis*, which in turn allows subordinate species to increase in percent cover. Based on the results of the present study, prescribed burns allow for a healthier plant community. Increasing frequency of burns may result in greater success at suppressing aspen regrowth, because repeatedly damaging the suckers could eventually cause dieback. However, weather conditions preclude use of fire in some years. Another possibility is to consider passive AM options. Intrinsic factors of aspen clones make some stands more susceptible to dieback. In the absence of fire, clone canopies will begin to break up after 80 to 100 years. Conceptual framework suggests that clones with higher concentrations of phenolic glycosides will self-perpetuate with more vigor than clones with lower concentrations (Lastra, 2011). Intrinsic levels of glycosides dictate susceptibility to herbivore attack. Clones could be tested for phenolic glycoside concentrations to determine if they are susceptible to dieback without the need for active AM.

Future studies could include aspects of soil analysis within, or in the vicinity of, browsing exclosures. Soil has a strong effect on plant growth, and there may be soil components influencing aspen re-growth at the different sites that were not taken into consideration in the current study. Tree age determination should also be addressed, because trees under 2 m tall were included in this study regardless of age. Young aspen suckers show different growth patterns than older trees, such that young saplings grow faster in early years. These differences may effect patterns of re-growth following clipping. To better understand the effect of bison trampling on aspen survival, exclosures could be placed within the bison enclosure. In this way, encroachment could be measured with-and-without bison present.

Table 2-1. Percentage cover averaged across the transects from enclosure into prairie in 2016. Species with an average percent cover of 20% or higher in at least one burn treatment are listed. Species that present a significant difference in percent cover between burn treatments are marked with an asterisk.

Species	Common name	Burn	No Burn	P-value
<i>Poa pratensis</i>	Kentucky bluegrass	100%	234%	0.034 *
<i>Festuca hallii</i>	rough fescue	61%	24%	0.754
<i>Galium boreale</i>	northern bedstraw	31%	12%	0.144
<i>Solidago canadensis</i>	Canadian goldenrod	30%	36%	0.615
<i>Carex inops</i> ssp. <i>heliophila</i>	Sun sedge	29%	15%	0.140
<i>Fragaria virginiana</i>	wild strawberry	25%	3%	0.002 *
<i>Lathyrus ochroleucus</i>	creamy peavine	23%	7%	0.005 *
<i>Hesperostipa spartea</i>	porcupine grass	8%	20%	0.435
	standing dead	85%	119%	0.058

Table 2-2. Percentage cover averaged across the transects inside the exclosures in 2017. Species with an average percent cover of 20% or higher in at least one burn treatment are listed. Species that present a significant difference in percent cover between burn treatments are marked with an asterisk.

Species	Common name	Burn	No Burn	P-value
<i>Poa pratensis</i>	Kentucky bluegrass	49%	243%	0.107
<i>Carex inops ssp. heliophila</i>	Sun sedge	49%	4%	0.002 *
<i>Fragaria virginiana</i>	wild strawberry	40%	4%	<0.001 *
<i>Festuca hallii</i>	rough fescue	12%	32%	0.281
<i>Solidago canadensis</i>	Canadian goldenrod	30%	13%	0.125
<i>Liatris ligulistylis</i>	blazing star	28%	0.2%	0.025 *
<i>Symphoricarpos occidentalis</i>	snowberry	28%	22%	0.906
<i>Galium boreale</i>	northern bedstraw	27%	12%	0.079
	standing dead	107%	230%	0.061

Table 2-3. Evenness across all burned and not burned sites for both 2016, and 2017. Sites that present a significant difference in evenness between burn treatments are marked with an asterisk.

Evenness	Burn	No Burn	P-value
2016	21%	15%	0.072
2017	23%	14%	0.016 *

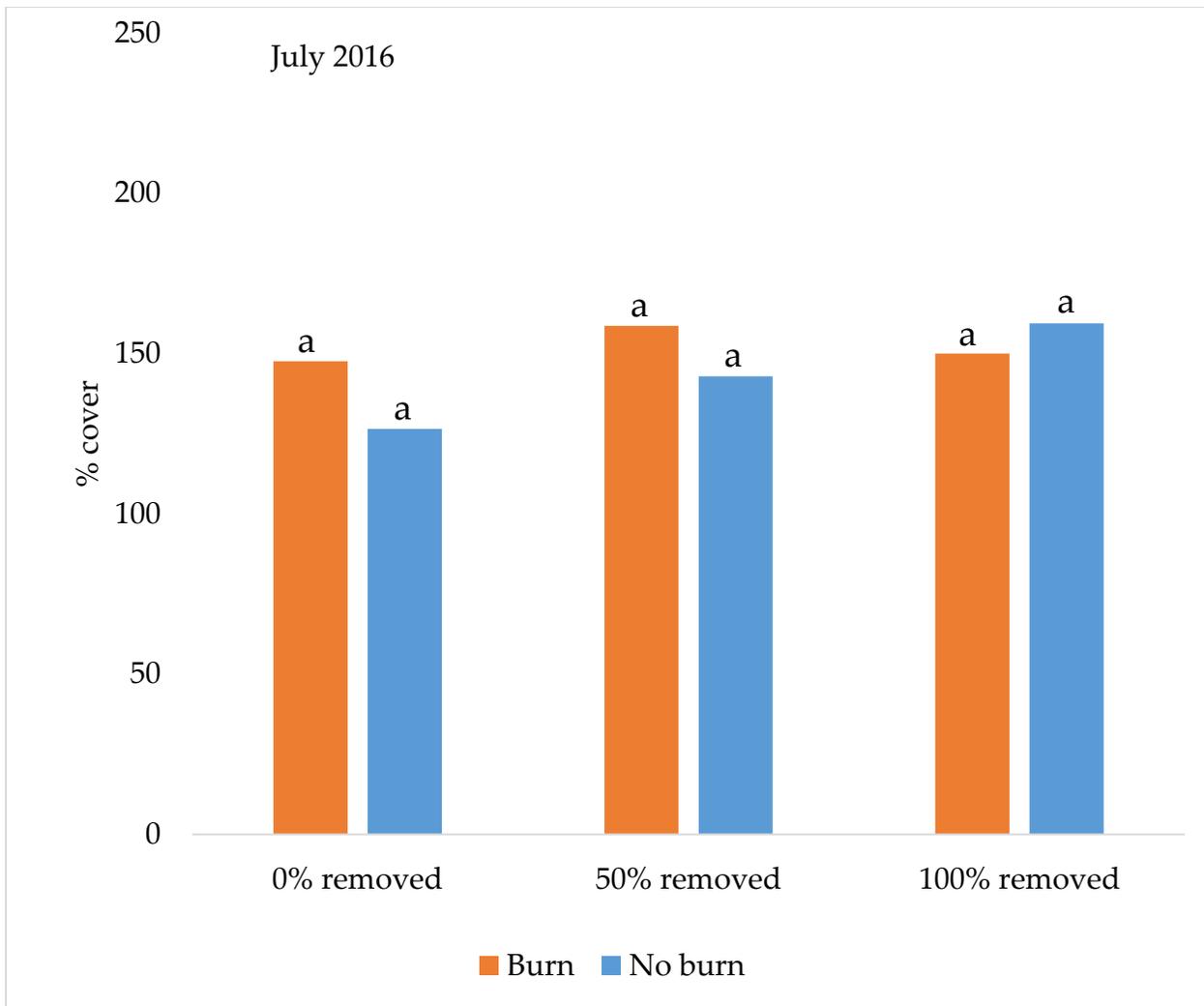


Figure 2-1. Initial state of plots in July 2016 for percentage cover of aspen before clipping treatments were applied. Means that are statistically identical are marked with same letter (a).

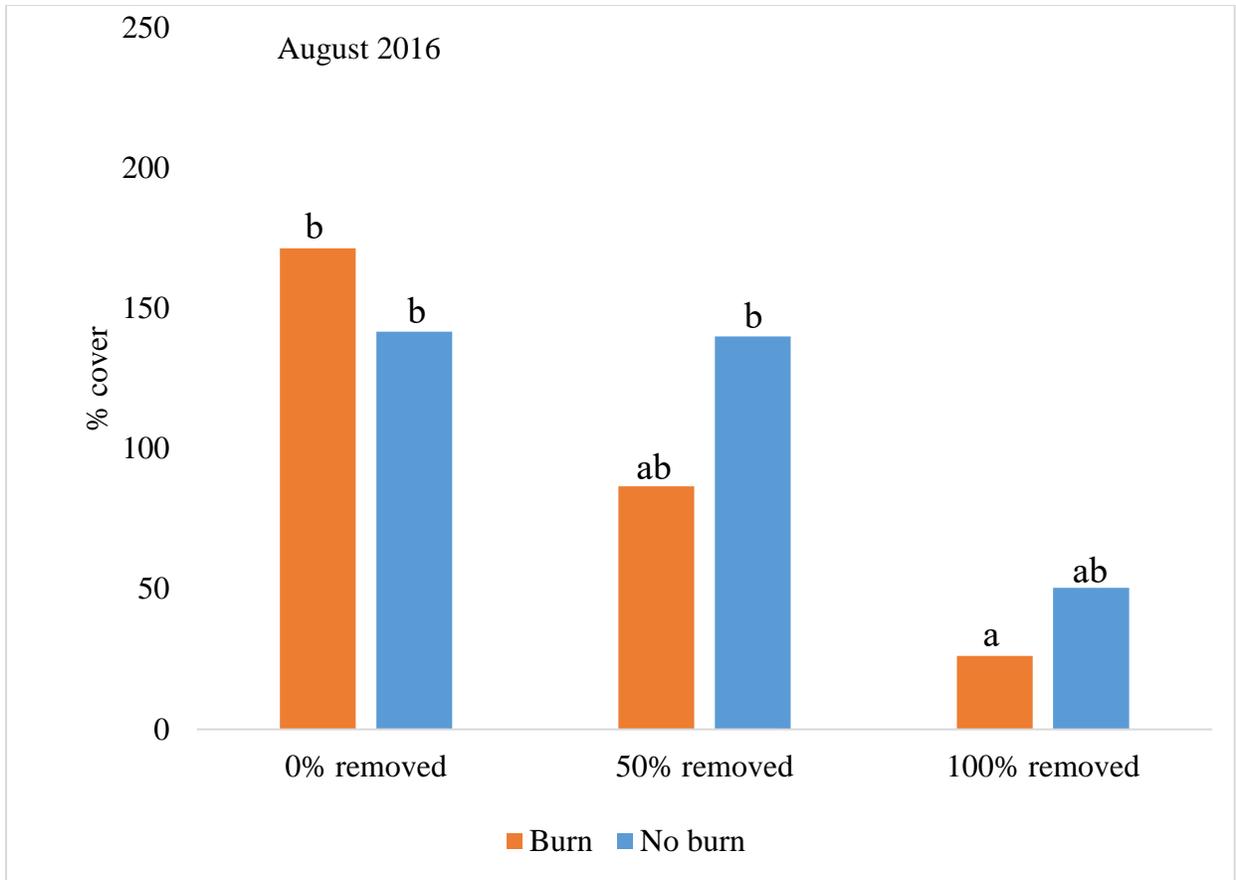


Figure 2-2. Final state of plots in August 2016 for percentage cover of aspen after treatments were applied in July 2016. Means that are statistically identical are marked with same letter (a, b, or a combination of these).

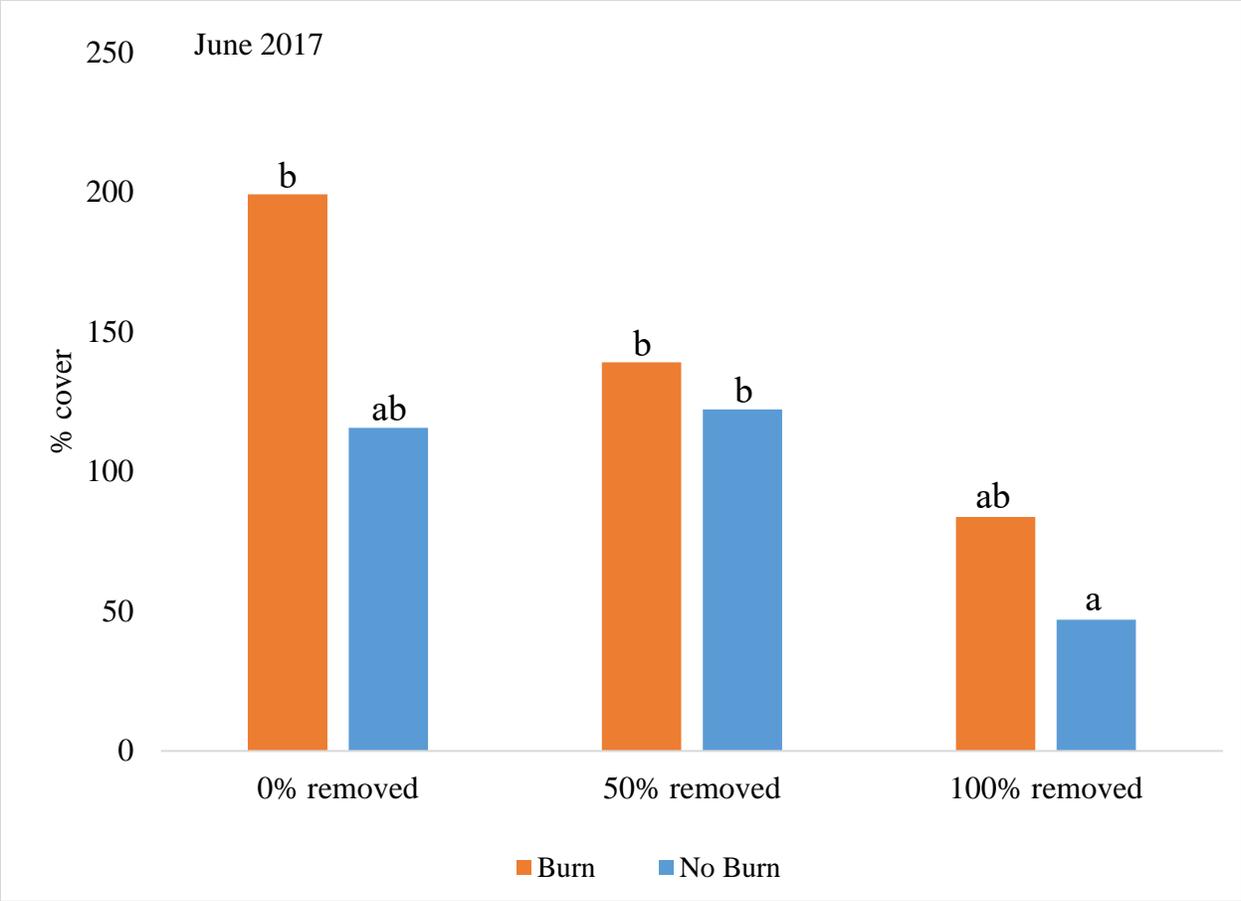


Figure 2-3. Initial state of plots in June 2017 for percentage cover of aspen before 2017 clipping treatments were applied. Means that are statistically identical are marked with same letter (a, b or a combination of these).

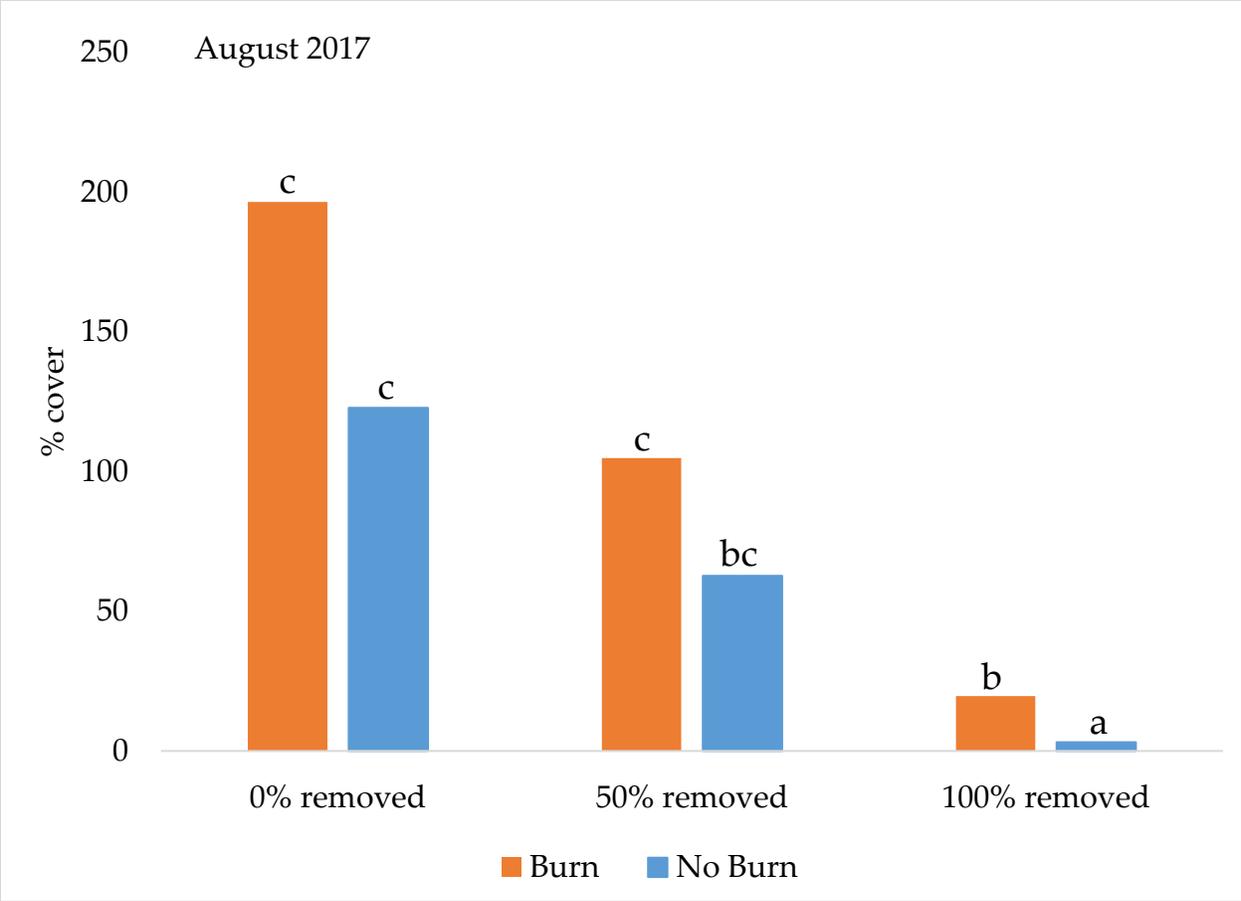


Figure 2-4. Final state of plots in August 2017 for percentage cover of aspen after two years of treatments were applied. Means that are statistically identical are marked with same letter (a, b, c, or a combination of these).

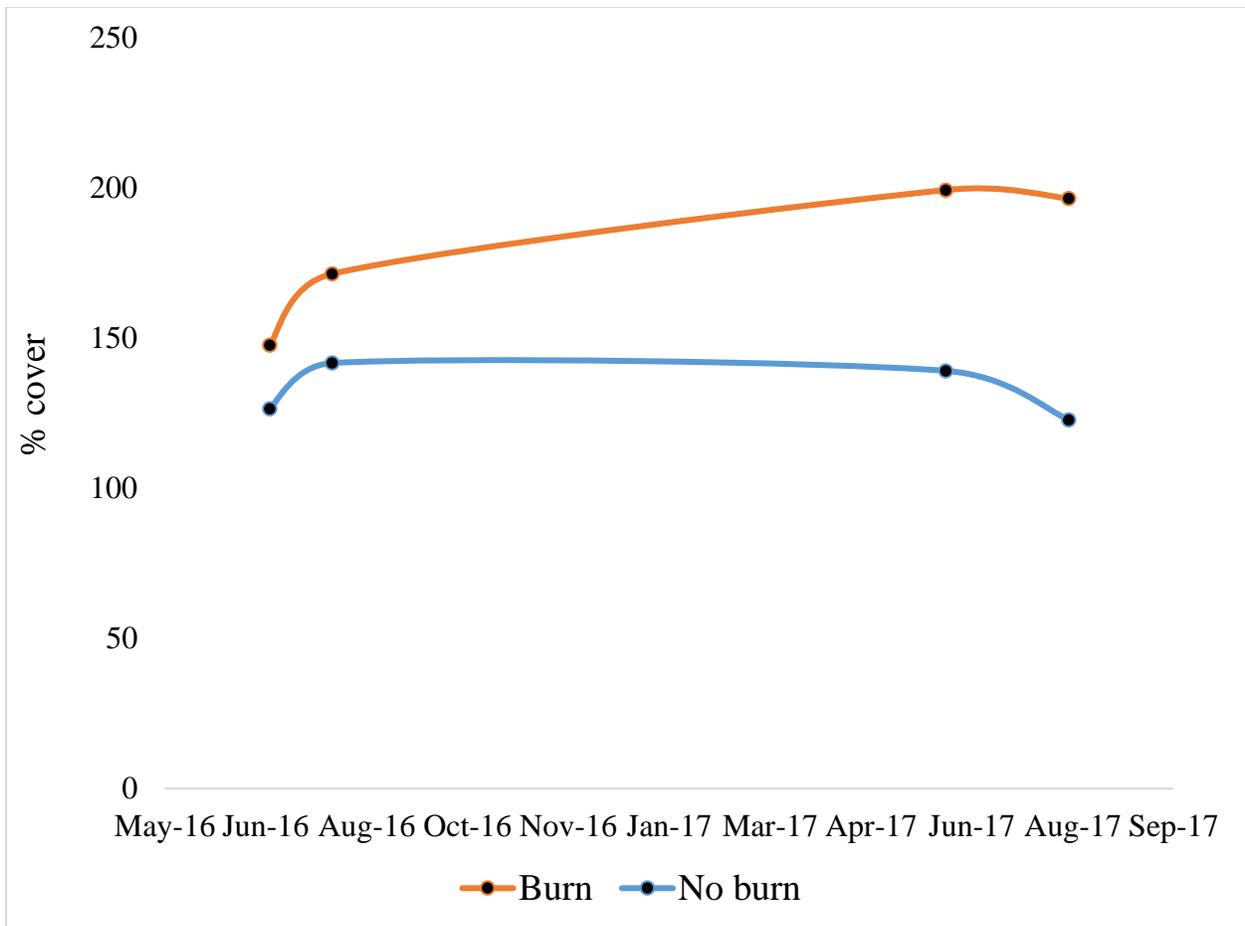


Figure 2-5. Increase in percent cover of aspen in the control pots not clipped over two field seasons. Average percent cover in the recently burned sites (Burn) are shown in blue, while average percent cover in the un burnt sites is shown in orange (No Burn).

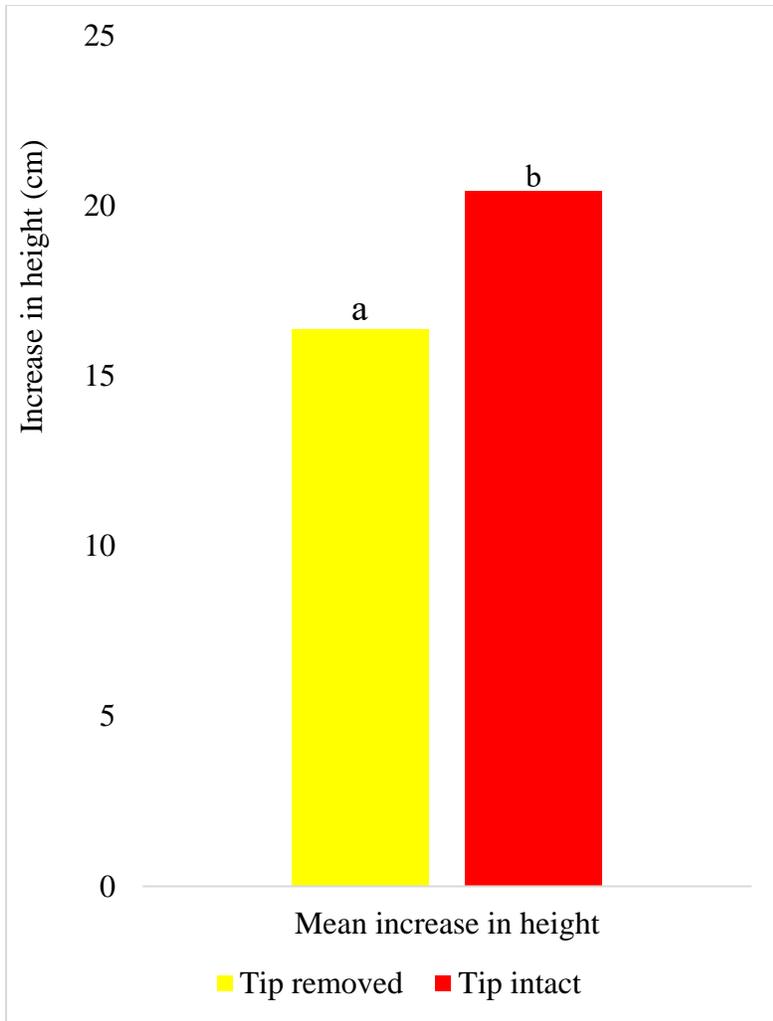


Figure 2-6. Mean increase in height in one growing season of aspen saplings with the apical shoot removed (yellow) and for saplings with the apical shoot left intact (red). Means that are statistically identical are marked with same letter (a, b).

3 Chapter Three: IMPACT OF UNGULATE MANURE ON ASPEN LEAF BREAKDOWN
USING FIELD PLACED LITTER BAGS

Kathryn Barr and Terence McGonigle

Chapter summary

Riding Mountain National Park (RMNP), Manitoba, has seen a rapid decrease in rough fescue (*Festuca hallii*) prairies, caused in part by aspen (*Populus tremuloides*) encroachment. A possible way to thwart aspen encroachment is to increase pressure by herbivory. However, more herbivores will increase the amount of feces deposited into the system. Wild ungulates present in RMNP are elk (*Cervus canadensis*), moose (*Alces alces*), white-tailed deer (*Odocoileus virginianus*). In addition, bison (*Bison bison*) are present in enclosures with RMNP. Two studies were conducted. First, field-placed litter bags were used to measure the effect of 5% ungulate manure on aspen litter decay. Second, a laboratory incubation of soil and manure were used to measure the release of plant-available N in the form of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ over two months. The manure amendments used in both studies were of two types: bison manure, and a wild-ungulate blend of deer, elk and moose. In 5 g of soil, three rates of manure were incubated: 0.1 g, 0.2 g, 0.4 g. For the field study, there was no significant effect of either type of amendment on litter decay over the course of one year. Also, there was no effect of bison manure or wild ungulate blend manure on $\text{NO}_3\text{-N}$ release in soil for any of the applied rates. However, both manure treatments stimulated an initial burst of $\text{NH}_4\text{-N}$ release in soil. There was a dose-related response for $\text{NH}_4\text{-N}$ release, which indicates that the $\text{NH}_4\text{-N}$ is coming from the manure amendment and not from mineralized organic matter in the soil. There was a slight difference between manure amendments, with the wild-ungulate blend being higher in both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ than the bison manure. The addition of ungulate manure to the system did not affect litter decay or increase plant-available N at the rates tested.

3.1 INTRODUCTION

Across the Canadian prairies, encroachment of woody species such as aspen into grasslands has been decreasing the extent of grasslands (Blood, 1966; Coupland and Maini, 1959; Widenmaier and Strong, 2010). RMNP has been identified as an area of concern, because of a rapid decrease in rough fescue prairies (Blood, 1996; Trottier, 1974). A possible way to thwart aspen encroachment is to increase pressure by herbivory (Hessl and Graumlich, 2002). In turn, more grassland is expected to increase the number of wild ungulates. In Manitoba, there are three main species of wild ungulates: elk, moose, and white-tailed deer. Historically, bison also occurred naturally on a large scale (Bird, 1961).

Multiple factors influence the growth and survival of plants. Light and water are clearly needed. Plants also require essential elements to grow and survive, which mainly are obtained as ions dissolved in the soil solution. In grassland environments, the two main limiting elements that influence plant growth are nitrogen (N) and phosphorus (P). Plants obtain mineral N by breakdown of organic forms of N from the environment, which are eventually returned in the form of plant litter. Another influencing factor on plant performance is herbivory. The impact of herbivory on plant performance depends on its timing, location, intensity, and frequency. Herbivory by ungulates can not only affect plant architecture and growth, but also the species composition on a plant community due to selective browsing of palatable species (Crawley, 1997). Selective browsing by ungulates also alters the relative abundance of N-high and N-low plants (Côté et al., 2004).

Aspen has various chemical defenses such as phenylpropanoid-derived compounds, including phenolic glycosides, also called salicinoids, and condensed tannins. Phenolic glycosides reduce feeding, growth and survival of insect herbivores, and they also deter feeding

by mammalian herbivores (Lindroth and St. Clair, 2013). Deer select plants to trade-off between the benefit derived from digestible dry matter and the cost of non-tannin phenolics, which is presumably associated with toxicity when absorbed (McArthur et al., 1993). Phenolic levels vary amongst aspen stands, and ungulates such as elk, who actively browse aspen, will selected stands with lower levels of toxins (Wooley et al., 2008).

Herbivores can have either an accelerating (Holland et al., 1992; Ritchik et al. 1998; Singer and Schoenecker, 2003; Sirotnak and Huntly, 2011) or a decelerating effect on nutrient cycling (Bryant et al., 1991; Pastor and Naiman, 1992; Sirotnak and Huntly, 2011). Deer generally prefer leaves with a low leaf C:N ratio and low lignin content, and so the leaves of palatable species are less resistant to decomposition. For example, deer will select leaves of *Ulmus laciniata* with a C:N ratio of 20 over leaves from *Quercus crispula* with a C:N ratio of 26. Furthermore, deer will choose leaves of both *Ulmus laciniata* and *Quercus crispula* over leaves of *Abies sachalinensis* with a C:N ratio of 50 (Kasahara et al., 2016). Selective browsing changes litter quality, affecting the conditions for N mineralization. For example, if ungulates selectively consume vegetation with a low C:N ratio, then litter with high C:N ratio will return to the soil. In other words, litter containing high levels of C are added to the soil following selective browsing. For litter of C:N ratio greater than 25, microbial growth becomes limited by N. Competition between microbes and plants for N intensifies due to increased immobilization of N in the microbial biomass. Immobilization reduces availability of N to plants, which negatively affects plant growth (Hobbs, 1996). Ungulates can also increase denitrification via gaseous N loss from soil if sufficient moisture is available (Frank and Groffman, 1998).

Ungulates also have a direct influence on N input into an ecosystem, because a substantial portion of plant N consumed by ungulates is returned to the soil in urine and feces

(Hobbs, 1996). More ungulates browsing in an environment will increase deposition of feces and urine, and in turn, increase fecal N and urine N delivery to soil. Excretion of N in feces and urine is important to N recycling, because it accelerates decomposition of litter and contributes to soil-N turnover (Frank et al., 1994). Manure and urine contain high levels of soluble ammonium and urea (Floate, 1970). Soils amended with ungulate feces have higher rates of N mineralization than soils alone or soils amended with leaf litter (Pastor et al. 1993; Ruess & McNaughton, 1987). Pastor et al. (1993) found that moose feces mineralized less N than soil alone, but feces combined with soil mineralized more N than the sum of the two separately. Total fecal-N may vary amongst individuals, because it is directly related to dietary-N in ungulates (Howwery and Pfister, 1990; Mould and Robbins, 1981). Ungulates may affect the decomposition of leaf litter due to physical disturbance as well. Kashara et al. (2016) found that litter decomposition was significantly faster for both leaves and roots in a deer enclosure than in a control site with deer herbivory. Physical disturbance of soil by trampling was the likely cause for this slowed decomposition. Cattle grazing was also found to increase root decomposition and N loss (Biondini et al., 1998).

Leaf litter decomposition has been extensively studied, because it has an important role in soil formation, nutrient recycling, and microbial activity (Brady and Weil, 2002; Carreiro et al., 2000; Prescott, 1995). Apart from ungulate presence, there are multiple factors that influence litter decomposition. Loss of mass from leaf litter depends on leaf species, soil conditions, moisture, and temperature (Howard & Howard, 1974). Amongst abiotic factors, soil moisture has been found to be the primary controlling factor in surface litter mass loss (Bryant et al., 1998). The location where the leaf litter decomposition occurs also plays an important role in decomposition. Decomposition rates are slower in forest than in prairie. Shade treatments

suggested that sunlight is responsible for higher decomposition rates in prairie (Kochy and Wilson, 1997). In addition, each litter type decomposes faster when placed in its ecosystem of origin. This interaction suggests that decomposers in an ecosystem are adapted to the most prevalent types of litter (Hunt et al., 1988).

Elk use rough fescue as winter browse (Knight, 1970), and the fescue grassland is noted as having desirable plant diversity. Advance of aspen into grassland with rough fescue is detrimental to maintaining open aspen parkland for elk at RMNP. Deer, elk, and moose browse on aspen year-round, but more predominantly during the winter. Trottier et al. (1983) found that there was a difference in the vertical distribution of browsing amongst different species of ungulates within RMNP. On average, deer have been found to browse between 58-63 cm, elk between 70-77 cm, and moose between 116-137 cm. This height discrepancy allows for all three species to utilize the same resource. When aspen clones mature, they most often present suckers on the outer portions of the clone (Schier, 1985). This sucker placement creates a transition zone between forest and grassland, with smaller trees within the browse zone of ungulates.

Studies of litter decay are more often conducted in forest systems (Hobbie, 2000; Howard and Howard, 1974; Kasahara et al., 2016; Magill and Aber, 1998; McClaugherty et al., 1985; Prescott, 1995; Prescott et al., 2000; Taylor et al., 1989), and grassland systems (Aerts et al., 2003; Bontti et al., 2009; Brandt et al., 2010; Seastedt et al., 1992; Smith and Bradford, 2003; Vossbrinck et al., 1979), with few in grassland-forest transition zone (Throop and Archer, 2007). Annual mineralization of N from litter is directly related to N content of residues and inversely related to soil C:N ratio (Frank et al., 1994). In terms of N influencing leaf litter decay, there have been mixed results. Several studies have reported significantly faster decay rates in response to increased external N availability (Carreiro et al., 2000; Hobbie, 2000; Hunt et al.,

1988). However, many others have reported either no significant change (Bryant et al., 1998; Carreiro et al., 2000; Knorr et al., 2005; Prescott, 1995), or a decrease in decay rates (Magill and Aber, 1998).

Air-dried leaf litter is universally used in decomposition experiments. Dry litter allows for a more accurate experimental representation of the natural condition of leaf litter decomposition. There is a difference between using fresh litter and air-dried litter. Mass loss is substantially faster from fresh litter than from air-dried litter. One study found that fresh aspen leaves decayed 62% faster than air-dried leaves (Taylor, 1998). As previously stated, leaf litter decomposition rates are also influenced by litter type (Kochy and Wilson, 1997). Depending on the potential for decomposition, litter can be classified as high-quality or low-quality. High-quality litters have low lignin content, while poor-quality litters have high lignin content (Knorr et al., 2005). N-poor litters have been found to decompose slower than N-rich leaf litter, because the lack of N limits microbial production. Another limiting factor that has not been as extensively studied is P, which also limits decomposition rates (Taylor et al., 1989b). Knorr et al. (2005) found that litter decomposition is slowed down or inhibited by N additions when litter quality was low, typically in the case of high-lignin litters. In contrast, the addition of N stimulates decomposition in high-quality litter.

There are multiple ways to predict decomposition rates of leaf litter. These methods include quantifying the concentrations in leaves of lignin, N, and P. Also relevant are C:N ratio and the lignin:N ratio. The most accurate predictors of mass-loss rate are N concentration and C:N ratio (Taylor et al., 1989a). While N concentration increases over time, the percentage of C does not show significant change in leaf litter, decreasing the C:N ratio amongst different tree species leaf litter (Howard and Howard, 1974). Taylor et al. (1989b) found that mixing nutrient-

poor litter such aspen with nitrogen-rich green alder (*Alnus viridis* (Chaix) DC.) litter accelerated the decomposition of the aspen litter.

Litter has been found to accumulate N from the atmosphere. N accumulation rates are significantly higher in aspen litter than in grass litter. In aspen litter, N accumulates steadily over the growing season (Kochy and Wilson, 1997). There are many sources which potentially contribute N to leaf litter. Microorganisms that decompose organic matter take up N and retain it until the organisms die and realise the nitrogen (Howard and Howard, 1974). There is also NH₄-N and NO₃-N in rain water, which can contribute to the litter N accumulation (Knorr et al., 2005). In field-based litter decomposition experiments, it is difficult to be certain of the source of N (Howard and Howard, 1974).

The goal of this study was to determine the impact of browser manure amendment on aspen leaf breakdown using field-placed litter bags in aspen parkland. We hypothesize that browser manure amendment will increase the rate of loss of mass of aspen litter. In addition, incubations were undertaken to quantify the mineralization of NH₄-N and NO₃-N naturally occurring when feces alone are placed in soil.

3.2 MATERIALS AND METHODS

3.2.1 STUDY SITE

The study was conducted at RMNP, Manitoba. Most of RMNP lies in the Mixedwood Section of the Boreal Forest Region (B.18a) described by Rowe (1972). This region is characterized by well-drained soils, which are dominated by species like aspen and white spruce (*Picea glauca* (Moench) Voss). The study was conducted at Deep Lake in forest-grassland transition zones. The plots were located in grasslands burnt by Parks Canada staff in spring 2015,

with aspen presenting suckering in spring 2016. Aspen clones within the selected grasslands were assessed visually to make sure they met the required criteria. Only clones that showed suckering with ramets under 2 m in height were selected for this study.

Environmental conditions were taken from records at Wasagaming, a town located within RMNP at 50 39.300N 99 56.517W. Historical weather data of 29 years was obtained between the years 1981 and 2010. The historical daily temperature averages are 17°C for July and 15.9°C for August. Total averaged annual rainfall was 372.1 mm, with the most monthly rainfall of 80.1 mm in June, followed by 66.7 mm in July and then by 59.2 mm in August. Total precipitation averaged 488 mm per year. In 2016, the mean average temperature for July was 17.1°C and total precipitation was 82.9 mm. For August 2016, the average daily temperature was 15.7°C and total precipitation was 79.9 mm. In 2017, the mean average temperature for July was 17.5°C, and total precipitation was 7.7 mm. For August 2017, averaged daily temperature was 15.7°C, with total precipitation of 48.8 mm (Environment Canada, 2017).

3.2.2 FIELD-PLACED LITTER BAGS

To measure the impact of browser manure amendment on aspen leaf breakdown, aspen leaf litter was mixed with different manure amendments. The amendments consisted of 5% bison manure, 1% urea, a control of no amendment, and 5% of a wild ungulate manure made up of a blend of deer, elk and moose. Aspen leaf litter was collected in September 2016. The litter was air-dried and passed through a one-centimetre sieve to promote mixing. A total of 3 g of air dried leaves were placed in each of 144 10-cm x 15-cm litter bags made from 50 µm nylon mesh. Urea had to be mixed with 2.5 ml of distilled water due to the small amount of amendment, and so 2.5 ml of distilled water was added to all other treatments for consistency. The manure was collected throughout the summer of 2016 at RMNP and frozen until use. Only summer feces were

collected that were visually distinguished by looseness of form. Once in the laboratory, the feces were air dried and ground with a mortar and pestle into a fine powder.

Litter bags were placed in the field on the 22 October 2016. A total of 144 litter bags were placed in the field. For litter bag placement, only the outer layer of aspen clones was considered, where encroachment into the prairie was evident. Areas were visually selected to meet the criteria of a uniform elevation and uniform aspen regrowth. Litter bags were placed at six sites located within three grasslands. Six browsing exclosures were built in spring 2016. There were 24 litter bags placed near each of the six exclosures. The placement of the litter bags was random. The bags were placed one metre apart, with sets of four bags attached by fishing line to a 20 cm nail. After field placement, bags were partially buried with soil to simulate natural conditions. Burying consisted of covering approximately 50% of the bag with soil. The GPS location of each nail was recorded, allowing the bags to be recovered at a future date. The litter bags were removed at six different times: immediately after placement on 22 October 2016, after spring melt 16 April 2017, 2 June 2017, 7 July 2017, 4 August 2017, and the final set was recovered one year after placement on 22 October 2017.

After collection, bag contents were assessed for mass, N concentration, and P concentration. For N and P, all samples ran through a potassium persulfate digestion in a Mars6 microwave digester. Bag contents were placed in a fan oven at 60°C for 24 hours to record oven-dry weight. Prior to digestion, samples were ground into a fine powder using a rock tumbler. A subsample of 0.035 g of the litter was mixed with 40 ml of the oxidizing solution, consisting of 45 g $K_2S_2O_8$ and 9.5 g $NaOH L^{-1}$. Once the sample and oxidizing solution were added to the vessel, they were shaken vigorously for 10 seconds to allow mixing. In each run, seven standards and control checks were added. Each run also included calibrant blanks and a foliage standard to

find percent recovery. The foliage standard used in this case was apple, NIST 1515, containing 1.59 mg P g⁻¹ and 22.5 mg N g⁻¹. After digestion, the samples were diluted to 50 mL with reverse osmosis (RO) water. The samples were then filtered with Whatman No. 42 filter paper into snap-cap vials, covered with Parafilm, and stored in a 5°C refrigerator until use. For N-analysis on the Shimadzu TN analysis system, samples were diluted 2:1 with RO water. TN-unit uses platinum-based catalytic conversion of all forms of N to nitric oxide at 720°C, followed by chemiluminescence detection. Samples were not diluted for P-analysis on the Astoria segmented-flow spectrophotometric analysis system. Ammonium molybdate reagent was used to develop the colour of P, which was then analyzed at a wavelength of 880 nm.

3.2.3 MANURE INCUBATION

Manures were incubated to quantify the release of N over time in soil. N concentration in the manure collected from RMNP was determined by potassium persulfate digestion, as described above. Ten replicates for the wild ungulate manure blend and six replicates for the bison manure were analyzed to quantify the initial concentration of N in the fecal samples.

For the incubations, soil collected from RMNP was partially air dried for two hours to improve friability, and then passed through a 1-cm sieve. A subsample was taken from the 1-cm sieved soil and partially air-dried for three additional hours for the same reason, before passing through a 2-mm sieve. Moisture within a sample of the 2-mm sieved soil was determined at 105°C for 24 hours. The 2-mm sieved soil was stored in a Ziploc bag in the dark until use in order to prevent growth of algae. To find soil water-holding capacity, subsamples of the 2-mm sieved soil were taken into the greenhouse and saturated with water. Four small pots were used to contain the soil, and the drainage holes in the pots were covered with a single layer of paper towel to prevent soil loss. The pots were filled with 2-mm sieved soil and moistened gradually

until the point of free drainage. This wetting was repeated once every two days, three times in all. On the last day, the pot was saturated with water once: two hours later, it was saturated with water again and left to drain until water no longer exited the pot. The samples were then weighed and placed in the oven at 105°C for 24 hours. After the oven drying, the soil was weighed again to get moisture loss and determine 100% water holding capacity.

Seven treatments were used for the incubations, with ten replicates per collection time. The mass added per incubation as follows: 0.1 g of wild ungulate blend, 0.2 g of wild ungulate blend, 0.4 g of wild ungulate blend, 0.1 g of bison manure, 0.2 g of bison manure, 0.4 g of bison manure, and no amendment. These values were selected based on field fertilization rates in agriculture; 0 g manure (0 kg ha⁻¹), 0.1 g manure (200 kg ha⁻¹), 0.2 g manure (400 kg ha⁻¹), 0.4 g manure (800 kg ha⁻¹). A total of 420 samples were used, with 60 corresponding to each treatment. The manure amendments were mixed in 30 g of oven-dry soil, equalling 37.41 g of partially air-dried 2-mm sieved soil. The samples were placed in snap-cap vials, and 2.5 mL of RO water was added to moisten the soil to 60% of moisture holding capacity. This moisture level was selected to allow for optimal microbial growth. A total of ten replicates were incubated for six different time periods. Samples were collected immediately after placement, after one-day, one-week, two-weeks, four-weeks, and eight-weeks. This schedule allowed measurement of the release of N over time from the manure amendments. A subsample of 5 g was collected at each harvest. This subsample was mixed with 50 mL of 1.0 M KCL and shaken for two hours at 200 rpm. The samples were then filtered with Whatman No. 42 filter paper into snap-cap vials, covered with Parafilm, and stored in the freezer until use. Samples were then thawed and analysed with the Astoria for plant-available N in the form of nitrate and ammonium. The

colorimetric analysis for ammonia used a wavelength 660 nm and nitrate was analyzed at a wavelength of 880 nm.

3.2.4 STATISTICAL ANALYSIS

All analyses were performed in Statistix 8 using $P=0.05$ to determine significance. Decay rates (k) for the field-placed litter bags were calculated by linear regression for natural logarithm of mass versus time. The relationships between different manure amendments and k were examined with the randomized complete block analysis of variance (ANOVA). We provide the p -values in the text, and the ANOVA for k values is provided in Appendix 3-1A. The release of N and P over time for the litter bags was examined with a two-factor ANOVA. Means were compared with the Tukey test when appropriate. We provide the p -values in the text and the ANOVA for N and P concentrations is provided in Appendix 3-1 B. The ANOVA for N and P contents is provided (Appendix 3-1 C). The relationship between manure amendments and release of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in the incubations was investigated with the Kruskal-Wallis non-parametric test, because of non-normality in the data for mineral N. The Kruskal-Wallis non-parametric analysis is provided (Appendix 3-2).

3.3 RESULTS

3.3.1 FIELD-PLACED LITTER BAGS

Eight litter bags were excluded from the decay analysis for k due to damage or loss. There was no significant difference ($p = 0.44$) between the decay constants for the different amendments and the control (Figure 3-7). The overall mean decay constant for all manure amendments was $k = 0.38\text{-year}^{-1}$, corresponding to a half life of 1.81 years.

Four litter bags were excluded from the chemical analysis, because they were not recovered from the field. There was a significant increase ($p < 0.001$) in N concentration for the Urea treatment compared to the Bison treatment and the Control, and also for the Ungulate treatment compared to the Control (Figure 3-2.A). There was a significant increase ($p = 0.036$) in P concentration for the Ungulate treatment over the control (Figure 3-2.B). There was a significant increase ($p < 0.001$) in N concentration over time (Figure 3-2.C). There was also a significant difference ($p < 0.001$) in P concentration over time, although a decline was seen at the final harvest (Figure 3-2.D). There was a significant interaction ($p = 0.024$) between the treatment factors for amendments and time for N concentration, such that the concentration in litter amended with Urea was initially higher at time one compared to all other amendments and the control (Table 3-1). There was a significant interaction ($p = 0.024$) between the treatment factors for amendments and time for P concentration (Table 3-1).

N content was significantly greater ($p < 0.001$) in the urea and wild ungulate treatments, compared to the control (Figure 3-3.A). P content was significantly greater ($p < 0.001$) in the urea and bison treatments, compared to the control (Figure 3-3.B). There was a significant difference ($p < 0.001$) in N content over time, showing an increase in mg N bag^{-1} up until time four (Figure 3-3.C). There was a significant difference ($p < 0.001$) in P content over time, showing a decrease in mg P bag^{-1} over time (Figure 3-3.D). There was a significant interaction ($p = 0.021$) between the treatment factors for amendments and time, such that the N content for litter in the urea and wild ungulate treatments was higher at time one than the control and bison treatments (Table 3-2). There was no significant interaction ($p = 0.466$) between the treatment factors for amendments and time for P content (Table 3-2).

3.3.2 FECAL INCUBATIONS

At the time of incubation set up, bison manure had 16.5 mg N g⁻¹ (n=6, s.d.=2.2) and wild ungulate manure had 29.5 mg N g⁻¹ (n=6, s.d.=2.6). There was a significant effect (p<0.001) of time on concentrations of both NH₄-N and NO₃-N in the bison manure incubation, with an initial burst of NH₄-N (Figure 3-4.A) and a steady increase with time of NO₃-N (Figure 3-4.B). There was a significant difference (p=0.027) for NH₄-N concentration among rates of bison manure amendment to soil, with the 0.4 g rate significantly greater than the control (Figure 3-4.A). One sample was lost from the nitrate analysis. There was a significant difference (p=0.004) in NO₃-N concentration in the bison manure incubation for the 0.2 g with 0.4 g rates compared to the control (Figure 3-4.B). There was a significant difference (p<0.001) in NH₄-N and NO₃-N concentrations through time in soils given ungulate manure, with an initial burst of NH₄-N (Figure 3-5.A) and an increase with time of NO₃-N (Figure 3-5.B). There was a significant difference (p<0.001) in NH₄-N concentration in soil the ungulate manure incubation, with all NH₄-N concentrations higher than the control with exception of the 0.1 g rate (Figure 3-5.A). There was no significant difference (p=0.578) in NO₃-N concentration in the ungulate manure incubation among amendment rates (Figure 3-5.A). There was a significant difference (p=0.023) in NH₄-N concentration in soil given wild ungulate manure compared to bison manure, with higher concentration following wild ungulate manure addition (Figure 3-6.A). There was no significant difference (p= 0.084) in NO₃-N in soil between the two manure types (Figure 3-6.B).

3.4 DISCUSSION

Aspen leaf litter decay rate did not vary from the control when manure amendments or urea were added. These results are consistent with Knorr et al. (2005), who found that fertilizer type, litterbag mesh size, and climate did not influence the litter decay response to N additions in

a meta-analysis of various litter decay studies. As a positive control for N addition, urea was expected to show an increase in decay rate; this was not seen in the present study. The urea amendment did show a significantly higher concentration of N compared to the control, which indicates that urea did provide N input. As litter mass was lost, N concentration increased. This increase in N concentration is expected during breakdown of organic matter by microbes.

Respiration will reduce mass and concentrate whatever N is present, and the microbes are also known to immobilize N from the environment according to their needs for growth.

Immobilization clearly occurred, because the N content of the litter increased through time.

There was on average 2.9% N in the wild ungulate manure blend used in this study, which falls within the range seen in other studies for white-tailed deer, moose, and elk. Pastor et al. (1993) found that moose pellets varied between 1.2% N in June to 1.8% N in August. Leslie et al. (1989) found that fecal N for deer varied from 1.2% in March to 3.7% in July, whereas fecal N for moose varied from 0.8% in March to 3.0% in September. Osborn & Ginnett (2001) found that white-tailed deer manure had 2.2% N. Cook et al. (1994) found that juvenile elk fecal-N was 4.2% before consumption of solid food began, but this changed to 2.2% when solid food made up 80% of total daily intake. There was on average 1.7% N in the bison manure used in the present study, which was lower than the 2.2% reported for bison by Hernandez et al. (2005). Fecal-N is closely related to N in diet, which could account for the variation in bison fecal N between the two studies.

As leaf litter breakdown occurred by respiration of microbes, the concentration of N and P increased. The concentration of N across treatments increased over time up until time four. Urea and the wild ungulate manure treatments had significantly higher levels of N and behaved in similar ways, while the bison treatment litter N was statistically similar to the control. These

finding are consistent with studies of solid cattle manure application (Qian and Schoenau, 2002). In that study, solid cattle manure negatively affected the amount of available N in the soil (Qian and Schoenau, 2002). Solid cattle manure is similar to solid bison manure and may cause a similar effect. N concentration was highest at time six after one year, but N content was highest at time four in July. The heat and humidity would make July more suitable for microbial growth, giving maximum demand for immobilization during the summer. In terms of P, there is an initial burst after Time one, where upon it remained relatively constant until time six, at which point it returned to initial levels. Although there is an initial burst of P release, that P was ultimately lost to the system by mineralization.

In the incubations, there was no effect of wild ungulate manure on $\text{NO}_3\text{-N}$ concentration in soil for any of the rates applied. The lack of a dose response for $\text{NO}_3\text{-N}$ indicates that the $\text{NO}_3\text{-N}$ was released from native organic matter already present in the soil, because the increase of $\text{NO}_3\text{-N}$ over time was seen in the control as well as in the amended soils. There was an effect of the bison manure on $\text{NO}_3\text{-N}$ concentration, but only at the highest rate of 0.4 g manure added. In this case, there was a reduction in $\text{NO}_3\text{-N}$ concentration with the higher rate of manure, indicating that something in the bison manure is making $\text{NO}_3\text{-N}$ unavailable. Both manure treatments stimulated an initial burst of $\text{NH}_4\text{-N}$, but this release was in modest amounts compared to $\text{NO}_3\text{-N}$, it is probably not biologically significant to plant growth. There was a dose response for $\text{NH}_4\text{-N}$ release, which indicates that the $\text{NH}_4\text{-N}$ is coming from the manure amendments. There was a slight difference between manure amendments, with the wild ungulate treatment having higher $\text{NH}_4\text{-N}$ than the bison treatment. Although this difference was statistically significant, it is probably not biologically significant, because the amount of N present in the soil is already substantial. The timing of the release of $\text{NH}_4\text{-N}$ is within the first week after

amendment. The quantity of $\text{NH}_4\text{-N}$ released from the added manures at the highest rate compared to the control treatment was approximately 2 mg kg^{-1} , which was small compared to the approximately 50 mg kg^{-1} released from the organic matter. Overall, the manures studied contribute little to available soil N for plants relative to mineralization from organic matter. Thereby, the lack of impact of the manure amendments on litter breakdown can be understood, with organic in soil providing sufficient mineral N to meet the needs of microbes engaged in leaf litter breakdown. Future work should address the question of why bison manure lowers the amount of available nitrate in soil, such as by immobilization.

Table 3-4. Concentration of N and P (mg g⁻¹) for litter over time for the Control and for all amendments Bison, Urea, and Ungulate. The six collection times were: 1 day (Time 1), 176 days (Time 2), 223 days (Time 3), 258 days (Time 4), 286 days (Time 5), and 365 days (Time 6).

N concentration (mg N g ⁻¹)						
Amendment	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
Control	2.19	3.57	3.61	4.37	4.19	5.33
Bison	1.63	3.37	4.53	4.30	4.22	4.60
Urea	3.40	3.91	4.81	5.60	4.97	4.99
Ungulate	2.51	3.55	4.16	5.34	4.89	4.89
P concentration (mg P g ⁻¹)						
Amendment	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
Control	1.62	1.98	1.89	2.01	1.78	1.62
Bison	1.69	1.84	1.89	2.03	1.84	1.84
Urea	1.67	1.98	1.92	1.92	2.08	1.90
Ungulate	1.87	2.01	1.97	2.06	1.98	1.71

Table 3-5. Content of N and P (mg bag⁻¹) for litter over time for the Control and for all amendments Bison, Urea, and Ungulate. The six collection times were: 1 day (Time 1), 176 days (Time 2), 223 days (Time 3), 258 days (Time 4), 286 days (Time 5), and 365 days (Time 6).

N content (mg N bag ⁻¹)						
Amend	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
1	5.90	7.61	7.43	8.43	8.21	9.48
2	4.71	8.09	9.71	8.72	9.16	9.66
3	9.33	8.71	10.15	11.13	10.01	8.42
4	7.31	8.32	9.52	11.32	10.30	9.16
P content (mg P bag ⁻¹)						
Amend	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
1	4.38	4.21	3.89	3.86	3.52	2.90
2	4.88	4.44	4.12	4.18	4.05	3.87
3	4.62	4.42	4.05	3.86	4.23	3.25
4	5.48	4.72	4.52	4.37	4.19	3.24

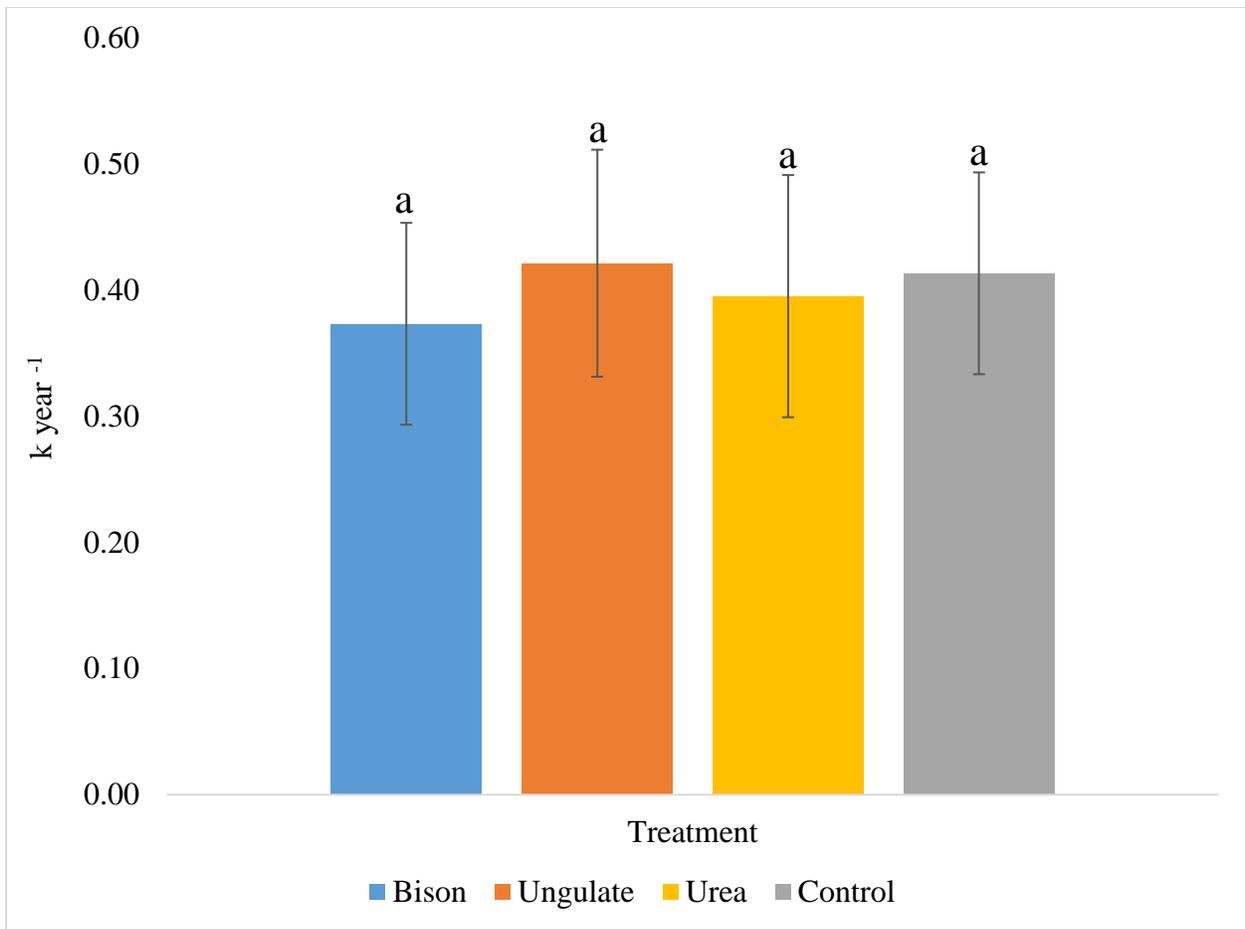


Figure 3-7. Average k values for bison amendment (Bison), wild ungulate blend amendment (Ungulate), urea amendment (Urea), and leaf litter alone (Control). Means that are statistically identical are marked above error bars with the same letter (a).

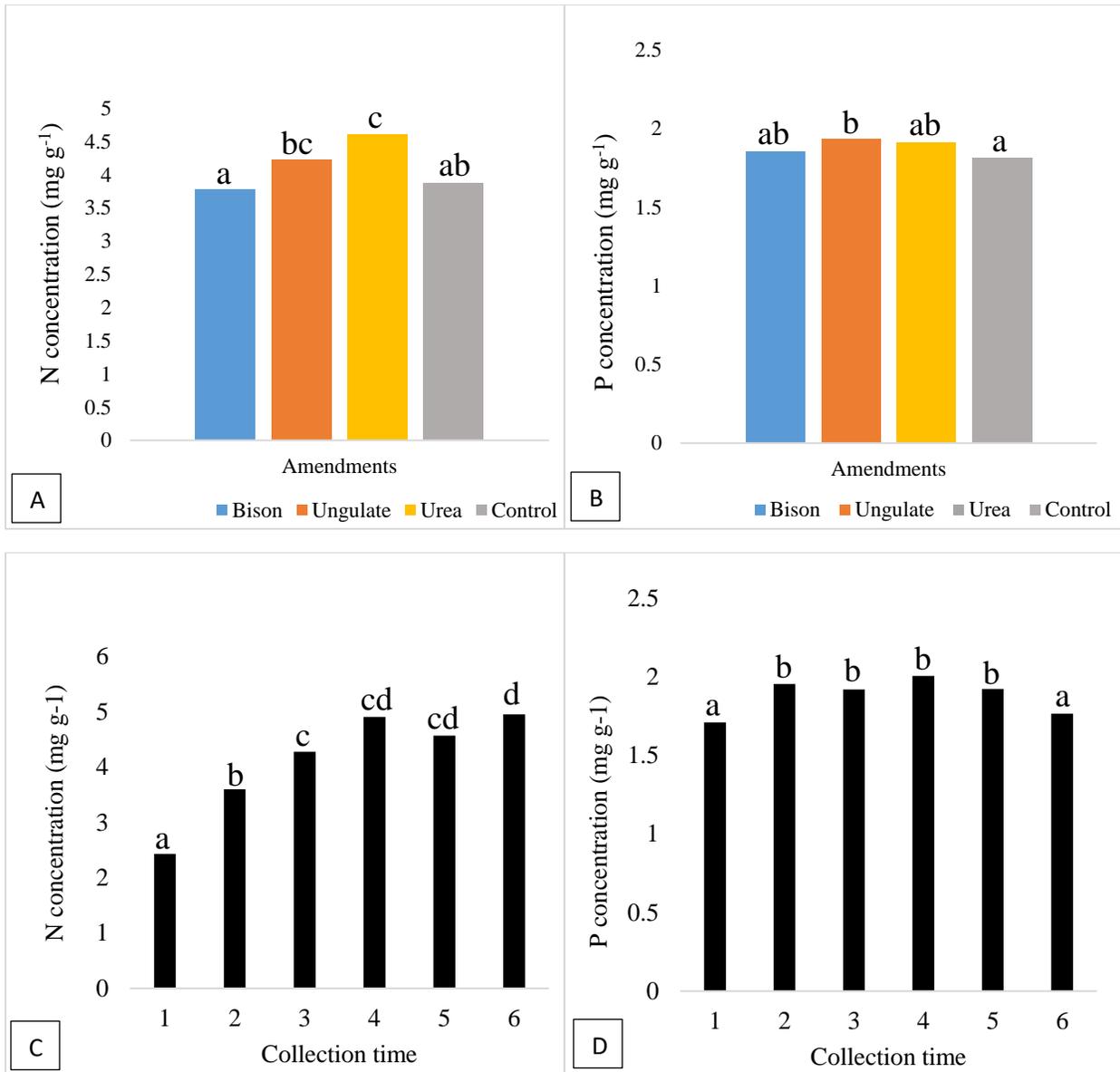


Figure 3-2. A. Concentration of N (mg N g⁻¹) in the leaf litter relative to the Control in response to amendment treatments Bison, Ungulate, and Urea. B. Concentration of P (mg N g⁻¹) in the leaf litter relative to the Control in response to amendment treatments Bison, Ungulate, and Urea. C. Concentration of N (mg N g⁻¹) at the six Collection Times. D. Concentration of P (mg P g⁻¹) at the six Collection Times. The times were: 1 day (1), 176 days (2), 223 days (3), 258 days (4), 286 days (5), and 365 days (6). Means that are statistically identical are marked above bars with the same letter (a, b, c, d, or a combination of the above).

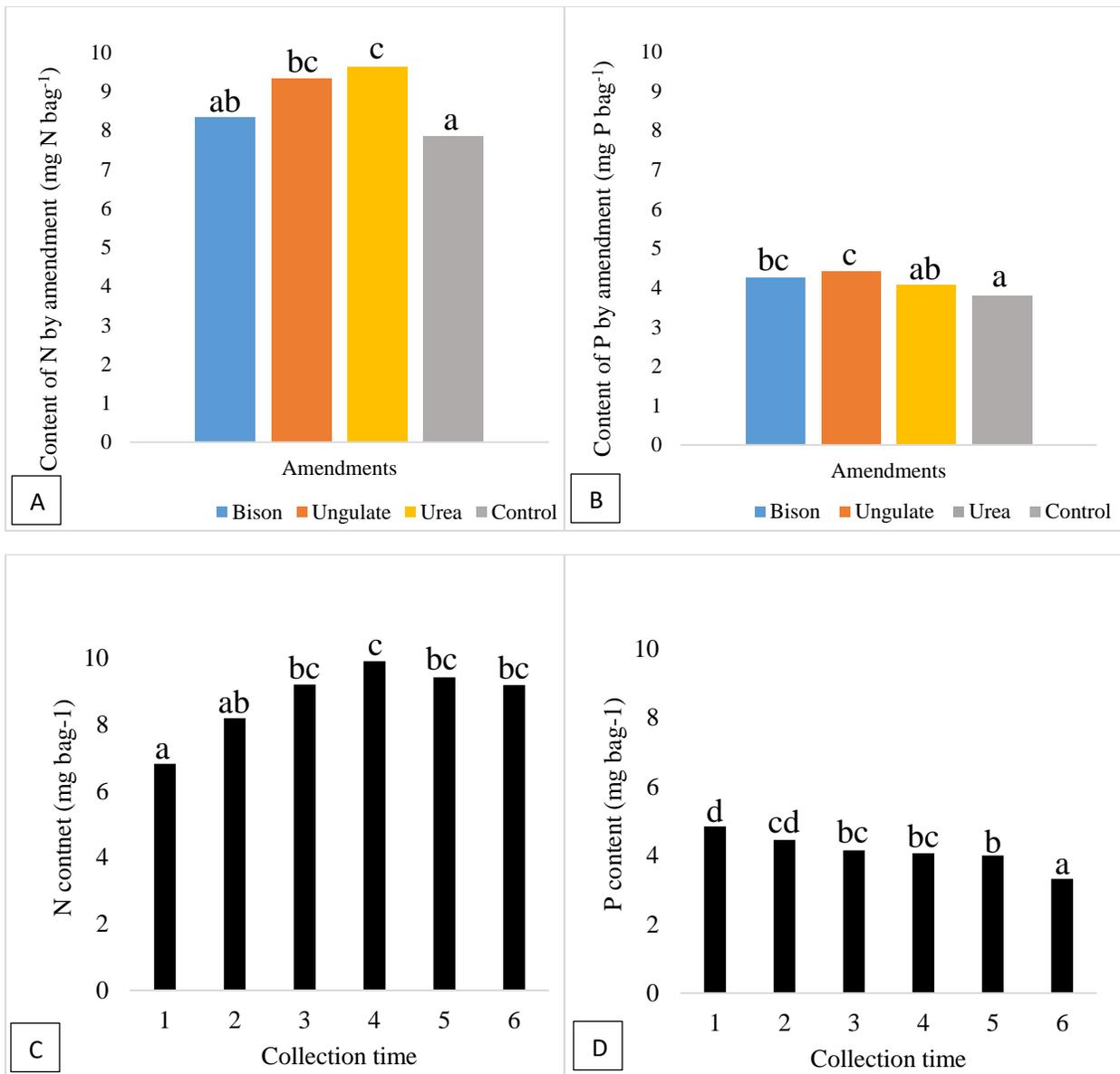


Figure 3-3. A. Content of N (mg N bag⁻¹) in the leaf litter relative to the Control in response to amendment treatments Bison, Ungulate, and Urea. B. Content of P (mg N bag⁻¹) in the leaf litter relative to the Control in response to amendment treatments Bison, Ungulate, and Urea. C. Content of N (mg N bag⁻¹) at the six Collection Times. D. Content of P (mg P bag⁻¹) at the six Collection Times. The times were: 1 day (1), 176 days (2), 223 days (3), 258 days (4), 286 days (5), and 365 days (6). Means that are statistically identical are marked above bars with the same letter (a, b, c, d, or a combination of the above).

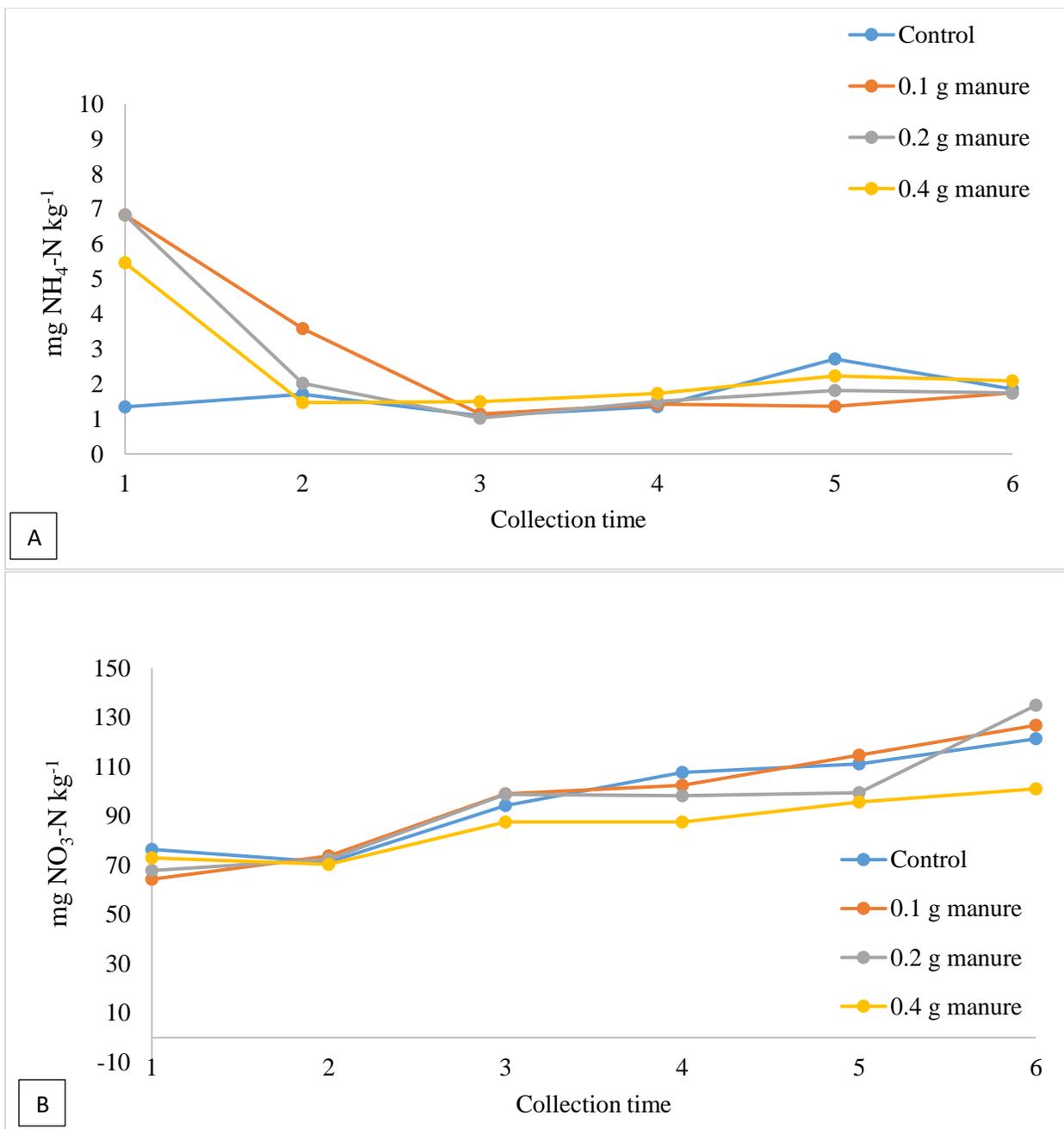


Figure 3-4. A. Concentration of $\text{NH}_4\text{-N}$ ($\text{mg N kg}^{-1}\text{soil}$) in soil incubations given bison manure. B. Concentration of $\text{NO}_3\text{-N}$ ($\text{mg N kg}^{-1}\text{soil}$) in soil incubations given bison manure. Determinations were made at six collection times: immediately after placement (1), one day (2), one week (3), two weeks (4), four weeks (5), eight weeks (6).

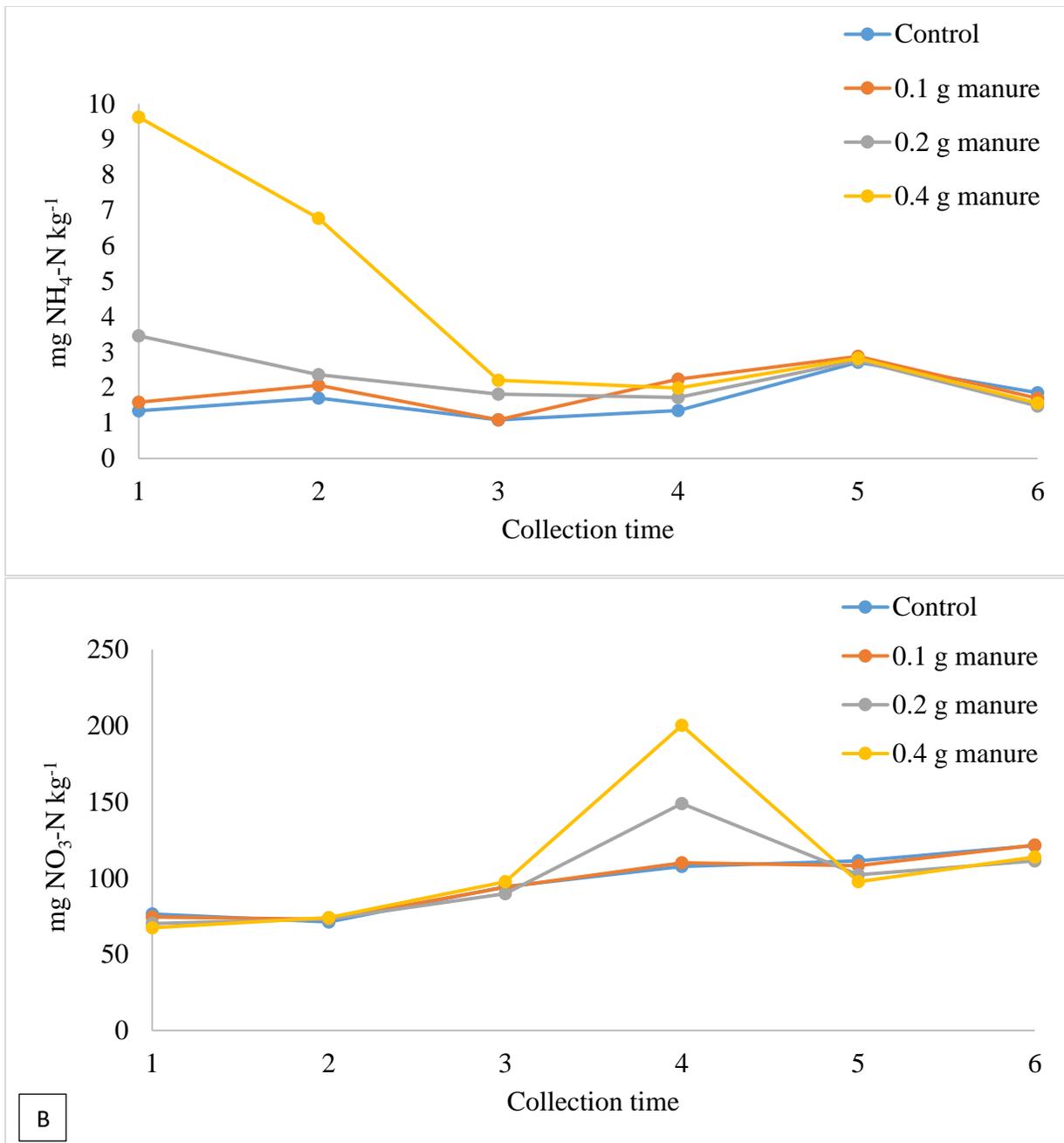


Figure 3-5. A. Concentration of NH₄-N (mg N kg⁻¹soil) in soil incubations given ungulate blend manure. B. Concentration of NO₃-N (mg N kg⁻¹soil) in soil incubations given ungulate manure. Determinations were made at six collection times: immediately after placement (1), one day (2), one week (3), two weeks (4), four weeks (5), eight weeks (6).

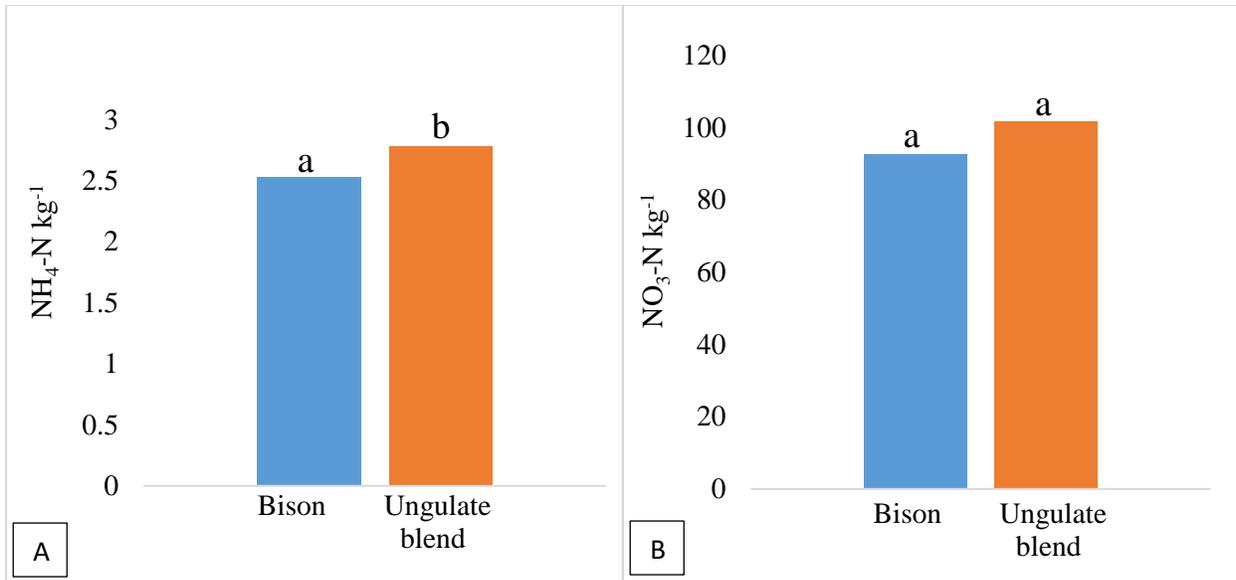


Figure 3-6. A. Concentration of $\text{NH}_4\text{-N}$ in soil given either bison or ungulate manure. B. Concentration $\text{NO}_3\text{-N}$ in soil given either bison or ungulate manure. Means that are statistically identical are marked above bars with the same letter (a, b).

4 Chapter Four: DETERMINATION OF HISTORIC EXTENT OF GRASSLANDS IN
RIDING MOUNTAIN NATIONAL PARK WITH SOIL PITS TO DETERMINE
ORGANIC CARBON WITH DEPTH

Kathryn Barr and Terence McGonigle

Chapter summary

Woody plant encroachment into grasslands is a well documented phenomenon. Riding Mountain National Park (RMNP) has been noted as a site of concern, because its grasslands have been greatly reduced due to forest encroachment. Soil heterogeneity is associated with forest and areas with encroachment of woody species into prairie. In grasslands, a large portion of the organic matter input is from deep fibrous root systems. In forest, most of the organic input is from leaves falling to the forest floor. Root litter at depth causes grasslands to have most of the organic matter distributed through the A horizon of the mineral soil, while forests store much organic matter within the O horizon above the mineral soil. The use of soil organic carbon (SOC) at depth was tested as a method to determine historic grassland locations in RMNP. Soil pits were dug and soil SOC was analyzed with depth. Stoniness along with soil texture was also included to better characterize the sites. Soil pits were dug at long-term forested sites, long-term prairie sites, and recently forested sites. Site selection was based on historical aerial photographs. Samples were taken from the pits at depth intervals of 10 cm and analyzed in the laboratory. Overall, the sites presented great variability. Long-term forest sites showed higher percentages of silt and clay. Prairie or recently forested sites had higher percentages of sand. Both recently forested and prairie sites had higher stoniness. The majority of the long-term forested sites had high SOC close to the surface, which then abruptly decreased after 30 cm of depth. In contrast, prairies sites showed pockets of SOC at depth, consistent with deposition of root material. Overall, the variability among sites precludes the use of SOC at depth to reliably indicate historic grassland extent. However, association of stoniness at depth with current or recent grassland, association of fine-texture with long-term forest sites, as well as deeper SOC in recently forested compared to long-term forest sites, indicates that some stony sites with coarse soil textures are predisposed to cycle periodically between grassland and forest cover.

4.1 INTRODUCTION

Woody plant encroachment into grasslands and savannas is a well documented phenomenon (Archer, 1994; McPherson, 1997; Grover and Musick, 1990) that has been increasing worldwide (Brown and Archer, 1999). The prairie-forest transition zones spans across the eastern edges of the mixed-grass and tall-grass prairies, ranging from Alberta, Saskatchewan, Manitoba, and southwards into Texas. Within these transition zones, the boundaries between forest and prairies can either be abrupt or gradual. Local environmental conditions, such as topography and soil, dictate the form of this transition. One of the most apparent limiting factors within transition zones is the ability of the soil to retain water. In general, fine-textured soils hold more moisture than coarse-textured soils, although some of the water held in the small void spaces of clays is not available to plants. Well-drained soils are favorable for grass growth. However, well-drained soils can hinder the expansion of trees, due to the low moisture content at certain times of the year (Anderson, 1982).

In Manitoba, aspen (*Populus tremuloides*) encroaches on grassland. Aspen is known to be a pioneer species, is characterized by rapid establishment on disturbed sites (Myking et al., 2011), and eventually gives way to spruce-fir forest. Although commonly seen as an early succession tree, some aspen stands remain stable and free of coniferous trees for hundreds of years (Cryer and Murray, 1992). Aspen in stable stands are found mainly on mollisols, which are soils with a dark, organically enriched mineral soil layer. Mollisols normally contain base-rich minerals and are associated with grassland vegetation. A mollic horizon is a surface layer in the mineral soil that contains at least 6 g kg⁻¹ organic C. Actively encroaching aspen stands tend to move roots into soils with a thin mollic surface layer. Over time, as leaf fall increases, the soil surface becomes organically enriched by leaf litter. When aspen is replaced by spruce and fir,

and when sufficient precipitation occurs, the mollic horizon may evolve into an albic horizon when the soil pH drops to 6.0 or lower (Cryer and Murray, 1992). An albic horizon is below the surface organic horizon and at the upper surface of the mineral soil. The albic horizon becomes pale in colour, because it has lost sesquioxides and organic materials to depth.

Depending on the relative proportions of grassland and forest cover, ecosystem properties are affected differently (Breshears and Barnes, 1999). Soil heterogeneity is associated with forest and areas with encroachment of woody species into prairie. Woody plants increase nutrient heterogeneity by taking up nutrients over a large rooting area and depositing them under their canopy. Patchiness also arises from precipitation being intercepted by canopies, which then enters the soil as stemflow (Kleb and Wilson, 1997).

The abundance of organic C in the soil affects and is affected by plant production, as has been recognized for over a century (Dokuchaev, 1883). Different plant communities leave unique footprints within the soil. In grasslands, a large portion of the organic matter input is from deep fibrous root systems. In forest, most of the organic matter input is due to leaves falling to the forest floor. Root litter at depth causes grasslands to have most of the organic matter in the A horizon of the mineral soil, while forests store much organic matter within the O horizon above the mineral soil (Brady and Weil, 2002).

The patterns of SOC storage are controlled by the balance of C inputs from plant production and outputs from decomposition (Schlesinger, 1977). In addition to plant productivity, soil texture plays an important role in organic C storage. Increasing clay content lowers C outputs, because clay stabilizes soil organic matter (Paul, 1984).

Jackson et al. (1996) studied the allocations of above- and below-ground biomass in grass-, shrub-, and tree-dominated systems. Temperate forests have high above-ground allocation of biomass and shallower C profiles than temperate grasslands, whereas above-ground allocation of biomass is lower in grasslands.

An analysis on over 2700 soil profiles in three global databases showed that root distributions affect the vertical placement of C in the soil. Trees had the shallowest root profiles, grasses were intermediate, and shrubs had the deepest root deployment. Above-ground allocation of biomass also influences C input, because it affects the relative amount of C that eventually falls to the soil surface (Omonode and Vyn, 2006).

The allocation of SOC is influenced by different factors at different depths. Precipitation and climate are the best predictors of total SOC in the top 20 cm of soil, whereas clay content is the better predictor in deeper layers due to slower recycling of SOC (Jobbagy and Jackson, 2000).

The objective of this study was to determine of historic extent of grasslands in RMNP with soil pits by analyzing SOC with depth. Landscape management at RMNP seeks to maintain and expand grassland cover from present levels in order to provide habitat diversity for elk populations, which are regarded presently as suboptimal. More detailed knowledge of the historic extent of grasslands would allow for a more focussed deployment of resources in the effort to enhance grassland cover. The hypothesis was that a surface layer concentration of SOC would be associated with permanent forest cover, whereas historic grasslands would be revealed by greater representation of SOC with depth in the profile.

4.1.1 STUDY SITE

The study was conducted at RMNP, Manitoba. Most of RMNP lies in the Mixedwood Section of the Boreal Forest Region (B.18a) (Rowe, 1972). This region is characterized by well-drained soils, which are dominated by species like aspen and white spruce (*Picea glauca*). The study was conducted at the Deep Lake in forest-grassland transition zones, long-term forested areas, and long-term prairie.

Environmental conditions were taken from Wasagaming, a town located within RMNP at 50 39.300N 99 56.517W. Historical weather data of 29 years was obtained between the years 1981 and 2010. The historical daily temperature averages were 17°C for July and 15.9°C for August. Total averaged annual rainfall was 372.1 mm, with the most rainfall in June of 80.1 mm, followed by July with 66.7 mm and then by August with 59.2 mm. Total precipitation averaged 488 mm per year. In 2016, the mean average temperature for July was 17.1°C, and total precipitation was 82.9 mm. For August, average daily temperature was 15.7°C, with total precipitation of 79.9 mm (Environment Canada, 2017). A soil survey by D. F. Acton (1975) presents maps that include the current study sites, categorizing them by soil texture modifier, compositional category, surface expression, slope, parental material, and soil series (Appendix 1).

4.2 METHODS AND MATERIALS

To verify historic extent of grasslands at RMNP, a series of soil pits were dug in known long-term forest, areas that are believed to have been grasslands and that are now recently forested, and long-term grasslands. The locations were determined by overlaying air photographs from 1959 and 2013 at Deep Lake. Polygons were established around each of the three vegetation-history zones, and four random points were placed in each polygon using random

numbers to generate GPS coordinates (Figure 1). The soil pits were 1-m deep by 1-m long, and a soil probe was used to take cores horizontally at 10 cm depth intervals. Four cores were taken at each depth in order to average across variability at a single depth. Two sets of cores were collected from each site, one to be analyzed in the laboratory, and one to archive. After the samples were collected, the pits were re-filled, and the sod was placed back to its original position. Samples were taken up to 100 cm in depth at most sites, but they were limited at two sites to 60 cm and 80 cm by abundant stones or clay. Only the upper 60 cm are reported here in order to provide an equal comparison among sites.

4.2.1 STONINESS

In the laboratory, the soil samples were air dried for 24 hours and passed through a 2 mm sieve to separate coarse material from fine material. Subsamples from both the coarse and fine material were oven dried at 105°C to find dry weight. Percent coarse material was calculated as a measure of stoniness.

4.2.2 SOIL CARBON AT DEPTH

Soil organic C was determined by loss on ignition (LOI) for all samples (Nelson and Sommers, 1996). Subsamples from the fine material were passed through a 500 µm sieve prior to use for LOI. Before adding the soil samples, crucibles were placed in combustion furnace at 400°C for two hours to burn off organic matter residue. Crucibles were placed in desiccator until cool enough to weigh. The mass of each crucible was recorded, and then 1 g of soil was added to each crucible. Each run included one or two soil standards. In the sets that included two soil standards, those standards were a marine sediment with 3.27% organic C and the in-house soil standard with 4.23% organic C. For the sets that only included one soil standard, the in-house soil standard was used. Both standards were used initially in order to confirm the organic C level

in the in-house soil standard, which was then used on its own. Once the 1 g soil was added, the crucible was placed in the oven at 105°C for 24 hours. Subsequently, the mass of the crucible and the oven-dry soil was recorded. The crucible and the oven dry soil were then placed in the combustion furnace at 400°C for 16 hours. The samples were left to cool in the desiccator before the weight of soil and crucible was recorded. Loss of mass from 105°C to 400°C corresponds to organic matter, and results were reported as g organic C kg⁻¹ dry soil.

4.2.3 SOIL TEXTURE

Air-dried soil passed through a 2 mm sieve was used to determine soil texture. A subsample of 30 g was mixed with 100 ml of sodium metaphosphate in a 1000 ml cylinder and left to sit overnight to allow the particles of clay to separate. Reverse osmosis water was then added to the 1000 ml mark, and the column was mixed vigorously with a plunger. A first reading was taken at 40 seconds with a hydrometer, at which point the sand has settled and the silt and clay particles remained suspended in the water column. A second measurement was taken after seven hours, at which point the silt particles had settled and only clay particles remained suspended.

4.3 RESULTS

Stoniness varied widely among sites. In forested sites (Figure 2), stoniness at the surface ranged between 2% and 26%. Surface stoniness at prairie sites ranged between 8% and 13%. Stoniness at recently forested sites ranged between 9% and 47%. In both recently forested and prairie sites, a stony layer was visible around 30 cm in depth, which is mostly absent from the forest sites. High stoniness persisted through depth to 60 cm in recently forested sites, ranging between 35% and 60%. Long-term prairie sites show a similar pattern, with the exception of

prairie site No. 1, who showed a more gradual increase in stoniness with depth. In contrast, three out of the four long-term forest sites had less than 30% stoniness through the profile.

Forested sites at the surface ranged between 17.6 and 81.4 g C kg⁻¹ (Figure 4-3). Three out of the four forest sites showed high SOC in the top 10 cm, but one site appeared to be an outlier. At 20 cm, there was a substantial drop in SOC under forest, which then remained relatively consistent throughout the rest of the profile. Long-term prairie sites had a surface SOC between 42.5 and 66.7 g C kg⁻¹ through the profile. Under prairie, SOC resurges at different depths, with pockets of SOC appearing. Overall, SOC is maintained at higher percentages at depth under prairie than under long-term forest. In recently forested sites, surface SOC ranged between 39.4 and 103.4 g C kg⁻¹. Three out of the four recently forested sites acted like long-term forest sites, with SOC dropping off rapidly with depth. RF2 is the exception, where pockets of SOC reappeared at depth.

Soil textures showed a persistence of sand through the profile in long-term prairie sites (Figure 4-4), ranging from 55% to 95%. The profile starts off as a loamy sand at the surface and leads towards sand or loamy sand as depth increases for all four sites (Table 4-1). The percentage of silt and clay remain low throughout the profile for all four prairie sites, with silt ranging from 0% to 23%, and clay ranging from 2% to 22%.

Compared to the other land uses, long-term forest showed the highest variability in texture among sites (Figure 4-5). Overall, the percent of sand ranged from 13% to 93% through the profile, while silt ranged from 2% to 42%, and clay ranged from 5% to 58%. Long-term forest sites No. 2 and No. 4 varied greatly in contrast to long-term forest sites No.1 and No. 3. Sites No. 2 and No. 4 had high sand in the first 30 cm, but prominence of clay and silt at greater depth. In contrast, sites No. 1 and No. 4 showed a high percentage of sand and a low percentage

of silt and clay through the profile. Forest site No. 2 started at 10 cm with a sandy loam and ended with a silty clay loam at depth. Forest site No. 4 started at 10 cm with a sandy loam and ended with clay loam at depth. Forest site No. 1 started at 10 cm with loamy sand and ended with sandy loam at depth, which was similar to forest site No. 3 that started at 10 cm with sandy loam and ended with loamy sand at depth (Table 4-2).

Recently forested sites showed soil texture similar to long-term prairie, with a high percentage of sand throughout the profile and a low percentage of silt and clay. Overall, the percent of sand ranged from 70% to 95% though out the profile, silt ranged from 2% to 18%, and clay ranged from 2% to 13%. Recently forested site No.1 starts off with loamy sand at 10 cm and ends off with sand at depth (Table 4-3). A sandy loam at the surface and a sand at depth was seen at recently-forested sites No. 2, 3, and 4.

4.4 DISCUSSION AND CONCLUSION

Jobbagy and Jackson (2000) stated that clay content was the best predictor of amount of organic matter in deeper layers due to slower nutrient cycling, causing SOC pools at depth. Long-term forest sites No. 2 and No. 4 showed higher percentages of silt and clay throughout the profile, and they had more SOC than long-term forest sites No. 1 and No. 3.

In both recently-forested and prairie sites, a stony layer is visible around 30 cm in depth, which is mostly absent from the forest sites. The stony layer suggests that, historically, areas with this layer of stoniness originally only allowed for prairies to grow, whereas areas where this layer was absent allowed for growth of forests. The persistence of gravel at depth in all recently forested site suggests that stoniness may facilitate encroachment to occur.

Long-term forested sites had high SOC in the first 10 cm, with the exception of forest site No. 1, but that SOC then drops off suddenly with depth. The abrupt decrease in SOC is consistent with the literature for forests (Brady and Weil, 2002), whereas long-term prairie sites showed pockets of SOC at depth. Similar patterns with depth for SOC for both prairie and recently forested sites, both being reasonably distinct from long-term forested sites, suggest that a cyclic process of forest encroachment and retreat has occurred historically in areas that we see today as recent forest entry into former grassland. Although SOC at depth showed an overall trend specific for each of the different zones studied, the variability among pits within each zone was prominent. Thus, caution is needed for interpretation of the results. High variability can be attributed, in part, to the small sample size. Repeating this study with a larger sample size might show more definite trends for each of the different zones.

Variability among sites precludes the determination of SOC at depth as an indicator of historic grassland cover. Recently-forested sites exhibit trends similar to either grassland or forests in terms of soil texture and SOC, suggesting that some recently-forested sites converted from grasslands. The time frame over which recently-forested sites may have alternated repeatedly between forest cover and grassland cover is likely in centuries and presumably relates to historic occurrences of fire and patterns of moisture availability. Notwithstanding this historic variation in fire and moisture, lack of pronounced stoniness appears to confer a predisposition to permanent forest cover in certain areas. Based on the data collected in the present study, recommendation is made to focus efforts to restore grassland on areas containing at least 40% coarse fraction at some point within the top 60 cm of the profile.

Table 4-1. Soil texture from long term prairie sites up to 60 cm in depth.

Site	Depth (cm)	Soil Texture
Long Term Prairie 1	10	Loamy sand
	20	Sandy loam
	30	Loamy sand
	40	Loamy sand
	50	Sand
	60	Sand
Long Term Prairie 2	10	Loamy sand
	20	Loamy sand
	30	Loamy sand
	40	Sandy clay loam
	50	Loamy sand
	60	Loamy sand
Long Term Prairie 3	10	Loamy sand
	20	Sandy loam
	30	Sandy loam
	40	Loamy sand
	50	Sand
	60	Loamy sand
Long Term Prairie 4	10	Loamy sand
	20	Loamy sand
	30	Loamy sand
	40	Loamy sand
	50	Loamy sand
	60	Sand

Table 4-2. Soil texture from long term forest sites up to 60 cm in depth.

Site	Depth (cm)	Soil Texture
Long Term Forest 1	10	Loamy sand
	20	Sandy loam
	30	Sandy loam
	40	Sandy loam
	50	Loamy sand
	60	Sandy loam
Long Term Forest 2	10	Sandy loam
	20	Sandy clay loam
	30	Sandy loam
	40	Sandy clay loam
	50	Clay
	60	Silty clay loam
Long Term Forest 3	10	Sandy loam
	20	Sandy loam
	30	Loamy sand
	40	Loamy sand
	50	Loamy sand
	60	Loamy sand
Long Term Forest 4	10	Sandy loam
	20	Loam
	30	Loam
	40	Loam
	50	Clay loam
	60	Clay loam

Table 4-3. Soil texture from recently forested sites up to 60 cm in depth.

Site	Depth (cm)	Soil Texture
Recently Forested 1	10	Sandy loam
	20	Sandy loam
	30	Loamy sand
	40	Sand
	50	Sand
	60	Sand
Recently Forested 2	10	Loamy sand
	20	Sandy loam
	30	Sandy loam
	40	Loamy sand
	50	Loamy sand
	60	Sand
Recently Forested 3	10	Loamy sand
	20	Loamy sand
	30	Sand
	40	Sand
	50	Sand
	60	Sand
Recently Forested 3	10	Loamy sand
	20	Loamy sand
	30	Loamy sand
	40	Sand
	50	Sand
	60	Sand

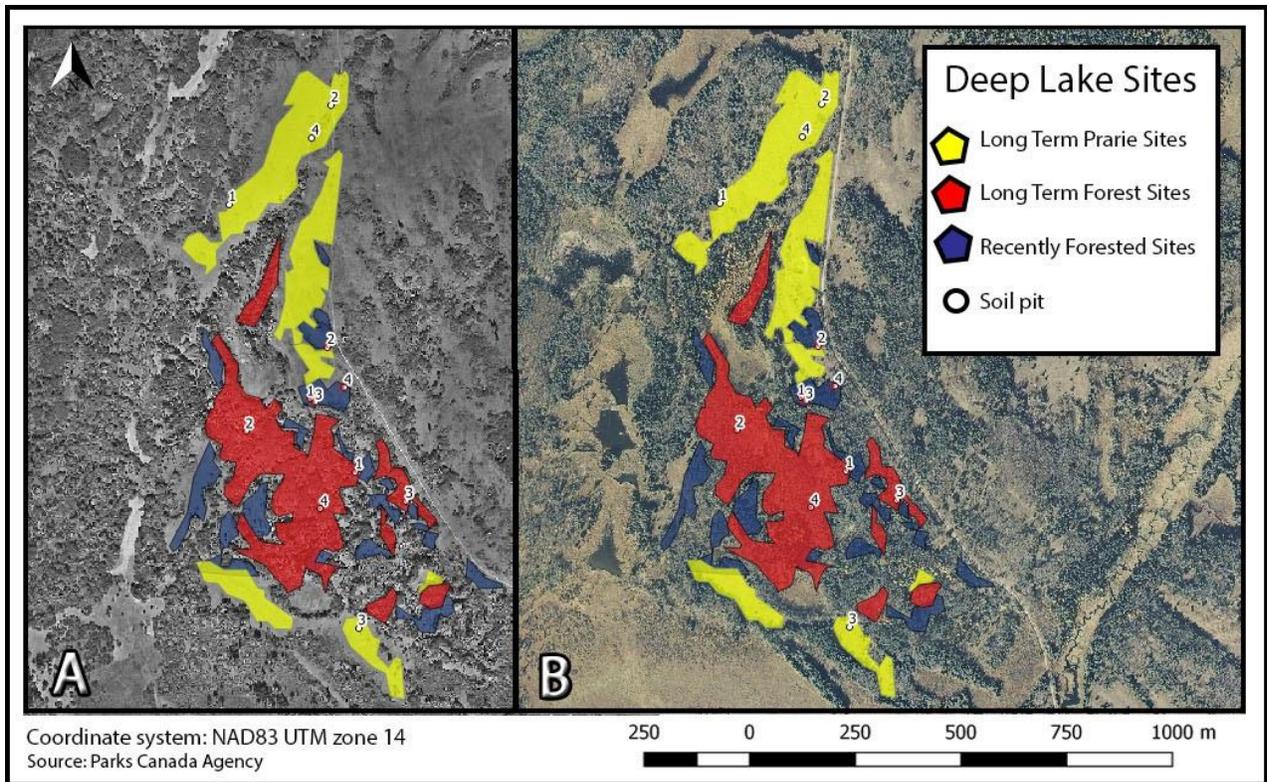


Figure 4-1. Deep Lake study site at RMNP. Section A is an air photo from 1959 with polygons overlaid. Section B is an air photo from 2013 with polygons overlaid. There are four sites within each section. Yellow indicates long-term prairie, red indicates long-term forest, and blue indicates recently-forested sites.

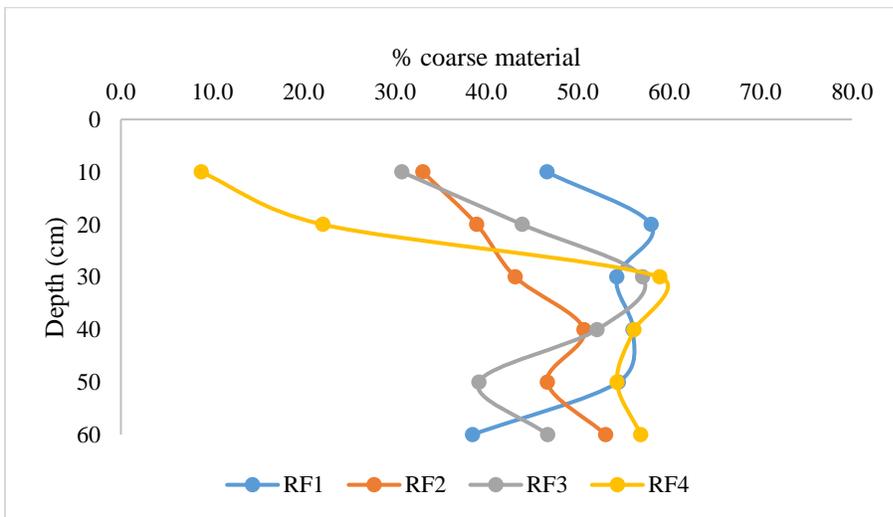
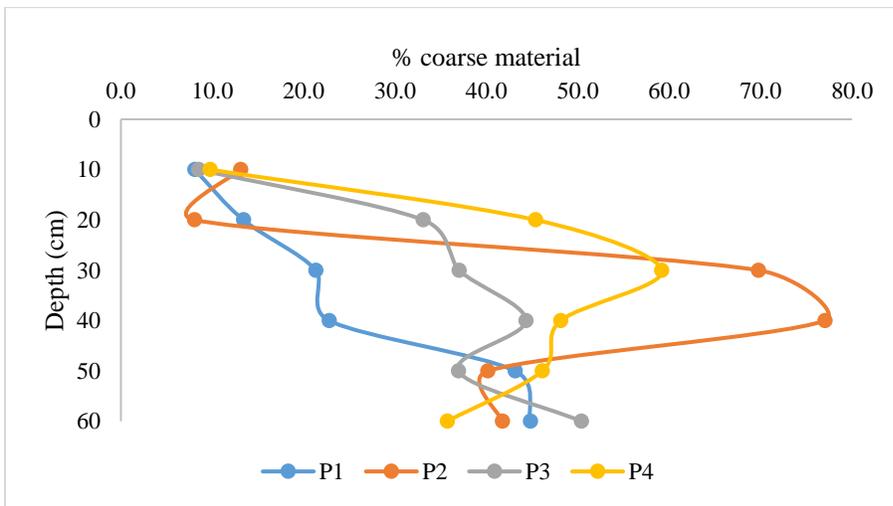
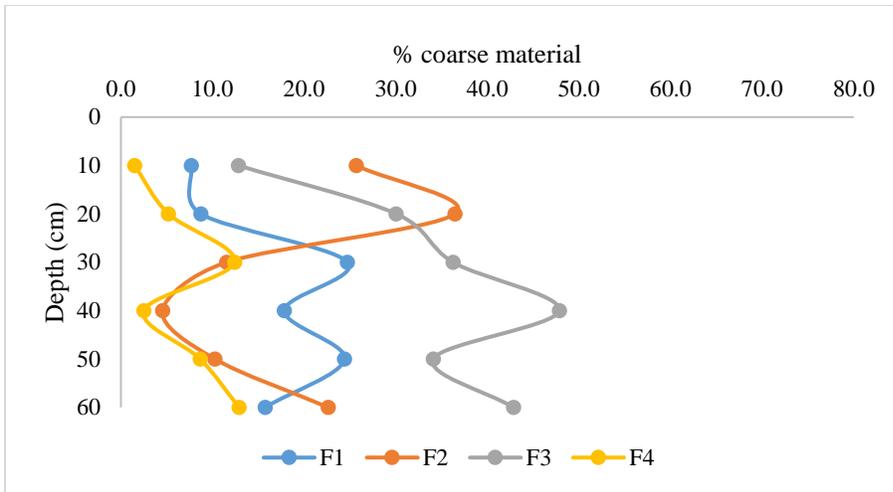


Figure 4-2. Stoniness, as percentage coarse material, from long-term prairie (P), long-term forest (F) and recently-forested sites (RF) through the profile up to 60 cm.

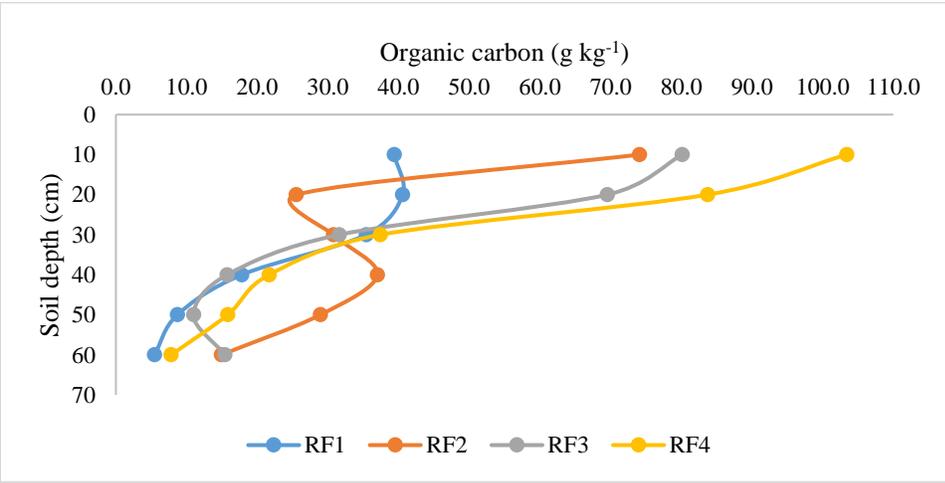
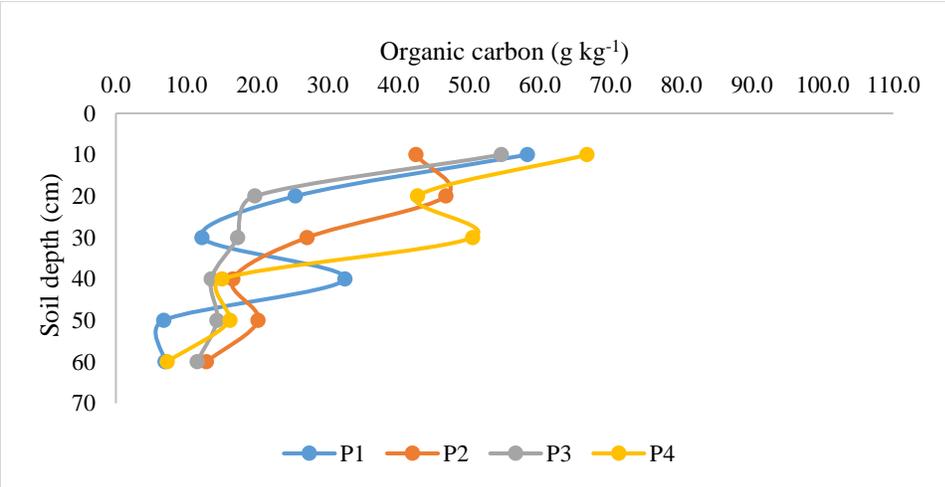
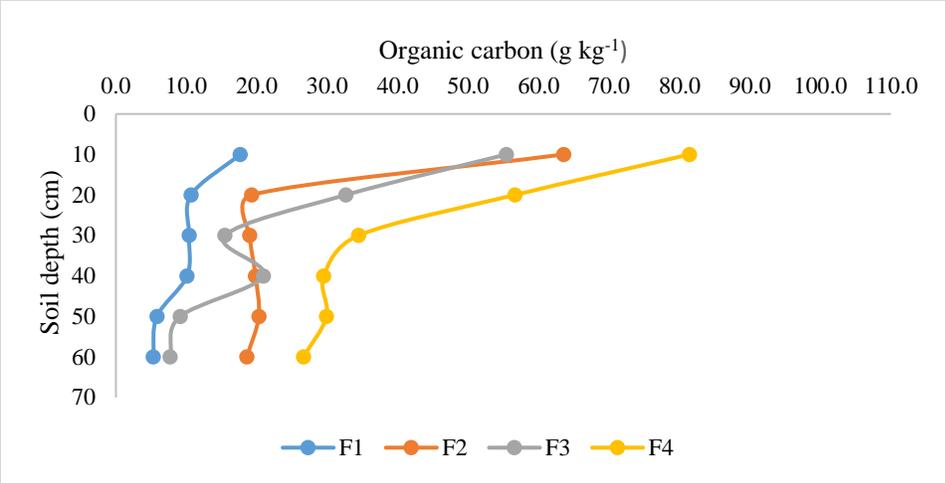


Figure 4-3. Soil organic carbon determined by loss on ignition for long-term prairie (P), long-term forest (F) and recently-forested sites (RF) through the profile up to 60 cm.

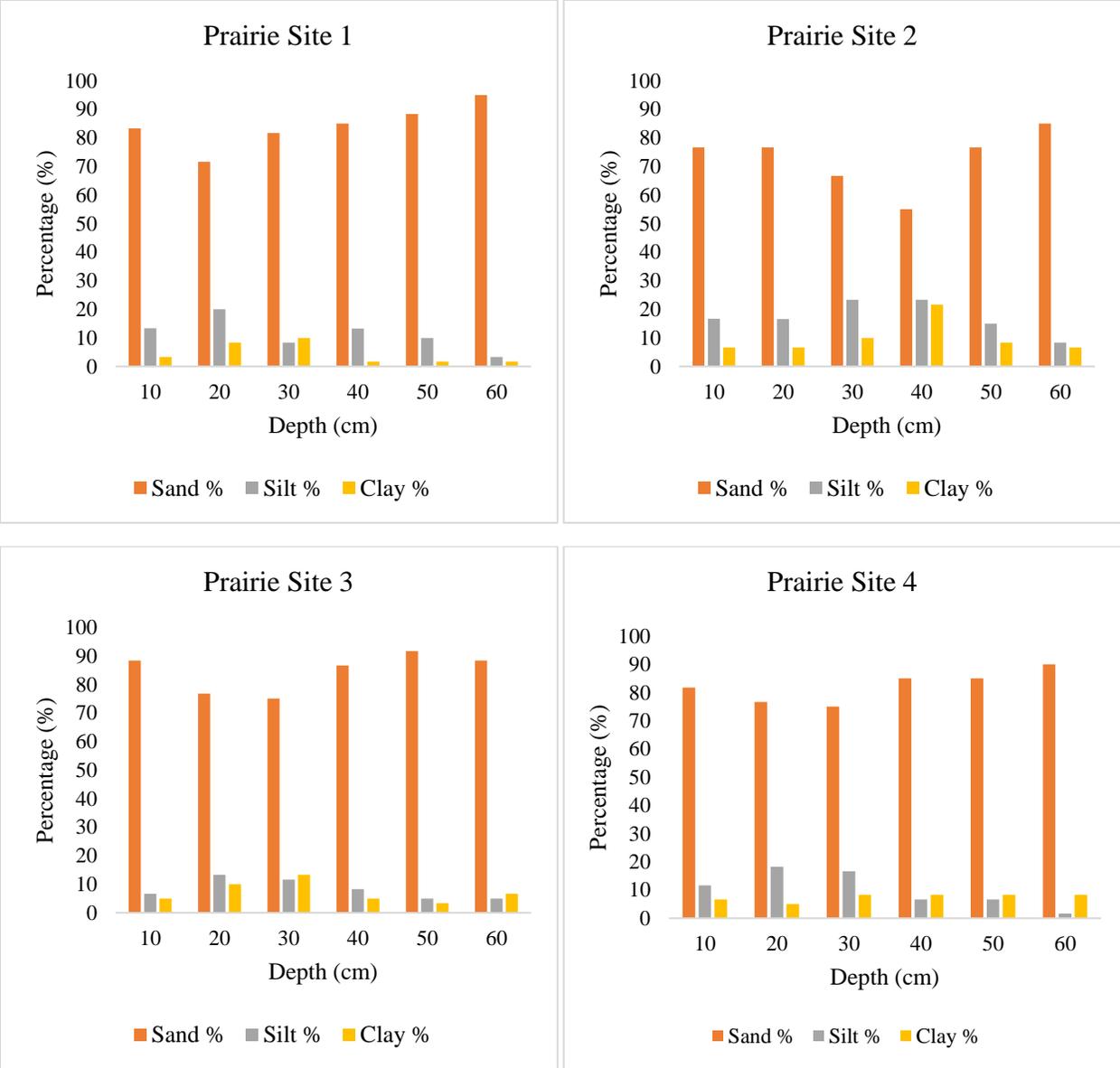


Figure 4-4. Soil texture for long term-prairie sites showing percent of sand, silt and clay at depths in intervals of 10 cm up to 60 cm.

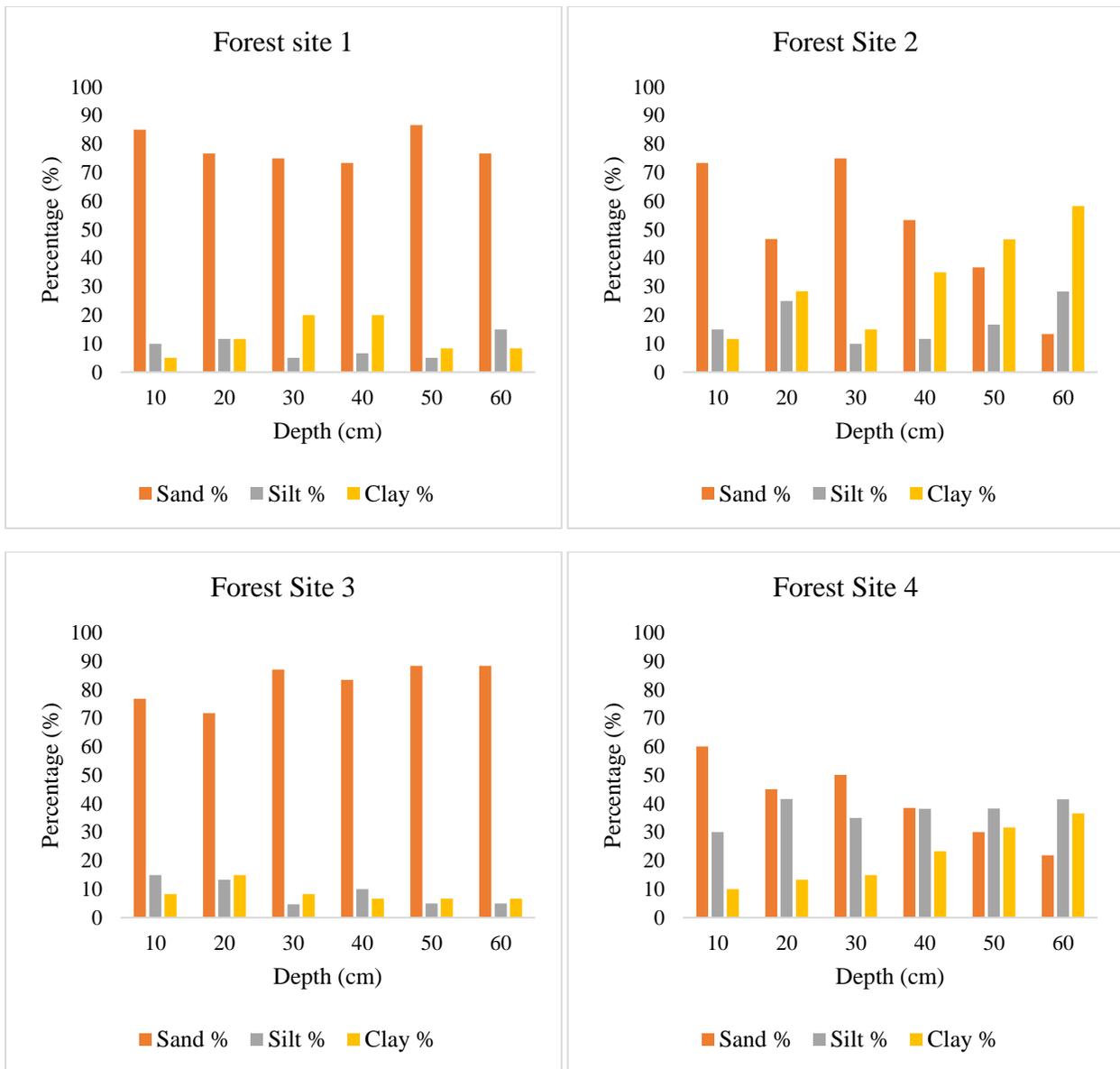


Figure 4-5. Soil textures for long-term forest sites showing percent of sand, silt and clay at depths in intervals of 10 cm up to 60 cm.

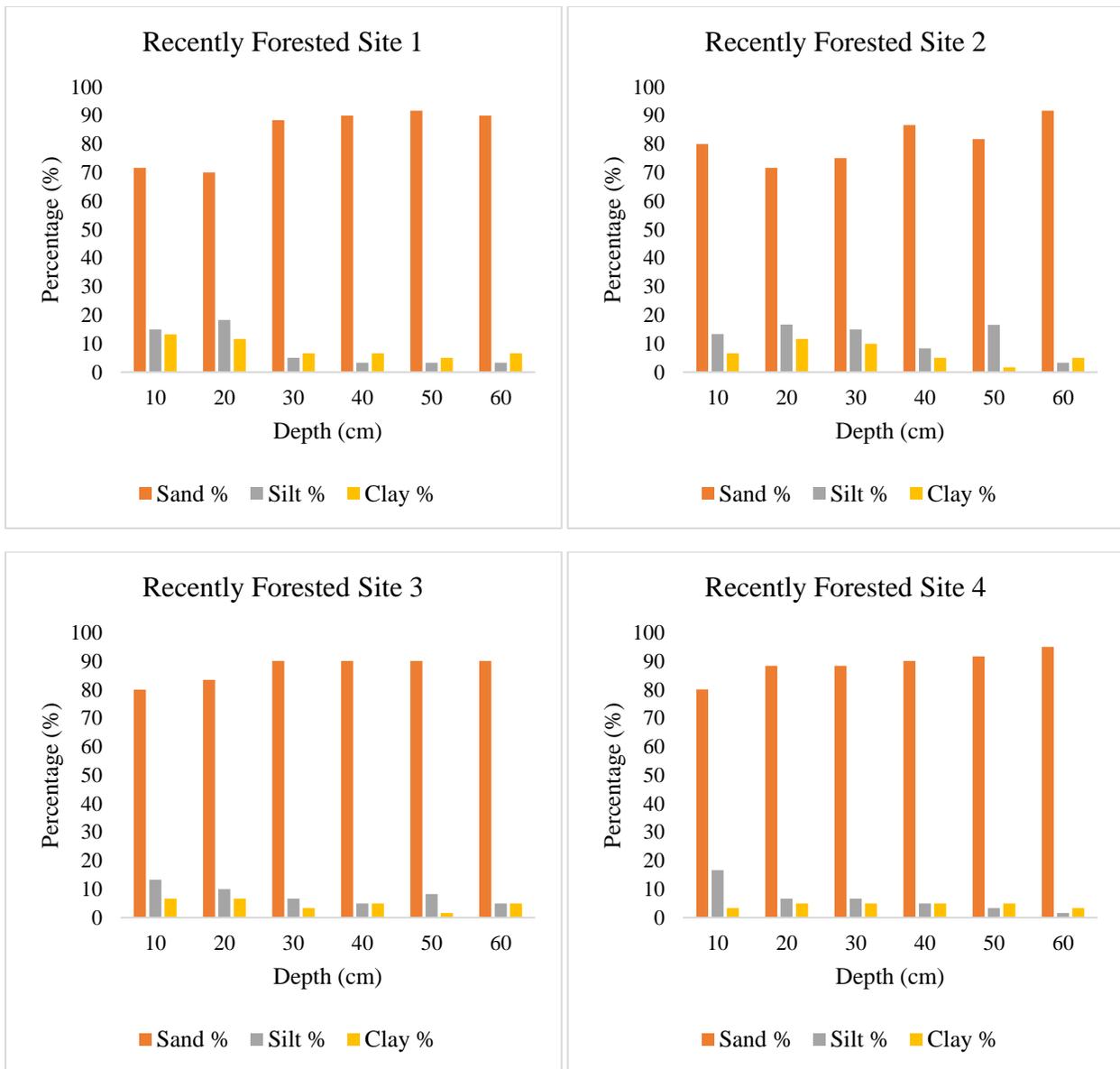


Figure 4-6. Soil textures for recently-forested sites showing percent of sand, silt and clay at depths in intervals of 10 cm up to 60 cm.

5 Chapter Five: SYNTHESIS AND CONCLUSION

A study on aspen encroachment was conducted to: 1) determine the relationship between browsing and burn history on aspen regrowth; 2) investigate the effect of ungulate manure on aspen litter breakdown; and 3) establish a method to determine location of historic grasslands with the use of SOC.

Chapter Two established that browsing on its own had an effect on aspen regrowth, but there was no interaction between burn history and browse. When applied at 100% leaf removal, independent of burn treatment, the percentage cover of aspen decreased over two years. However, when leaf removal was applied at 50%, there was no significant difference in regrowth when compared to the control. There was also a difference between the two burn treatments. Sites burned in spring 2016 had a higher increase in percentage cover over the two years when compared to the sites that had not been exposed to fire for decades. It was also established that the location of browse impacts aspen vigor. Suckers with the apical shoot removed showed significantly lower vertical growth than suckers with only lateral foliage removed. Burn treatments also affected the understory species composition. Recently burned sites showed greater species richness and evenness compared to the sites with no burn for the long-term. Sites with no recent burn history were dominated more strongly by Kentucky bluegrass.

Chapter Three established that neither one of the two manure amendments affected the decay rate of the aspen litter. The concentration of N, averaged over all treatments, increased over time. Litter amended with urea and ungulate manure had significantly higher concentrations of N, whereas litter amended with bison manure had a concentration of N that was statistically similar to the control litter. In terms of P concentration, there was an initial increase, but P was ultimately lost to the system as mineralization proceeded. The release of plant-available N to soil

from the manures in the form of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in soil was not greatly affected by either manure amendment. There was no dose-related effect of either type of manure on $\text{NO}_3\text{-N}$ release in soil. The lack of a dose response indicates that the release of $\text{NO}_3\text{-N}$ was from native organic matter already present in the soil. Both manure types stimulated an initial burst of $\text{NH}_4\text{-N}$, and there was a dose response for $\text{NH}_4\text{-N}$ release, which indicates that the $\text{NH}_4\text{-N}$ was derived from the manure amendment. Addition of wild ungulate manure to soil increased both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ compared to adding the bison manure, but in modest amounts. Although this difference was statistically significant, it is probably not biologically significant, because the amount of mineral N present in the soil was already substantial. Overall, manure seems to have no meaningful impact to provide available soil N for plants. This leads to the conclusion that bison manure and manure from wild ungulates are not expected to influence plant growth in the season of application, but that they simply contribute to longer-term replenishment of soil organic matter.

Chapter Four established that, on average, long-term forest sites had higher percentages of silt and clay throughout the profile than long-term prairie or recently-forested sites. A stony layer was present at depth in recently-forested and prairie sites, but this stoniness was mostly absent from the forest sites. The majority of the long-term forested sites had high SOC close to the surface, which then abruptly decreases at depth. Long-term prairie sites on the other hand showed pockets of SOC at depth, consistent with deposition of root material. These patterns are in keeping with a model that a cyclic process of forest encroachment and subsequent retreat has occurred historically in the recently forested sites. Overall, the variability among sites precludes the use of SOC at depth to indicate historic grassland extent.

Overall synthesis of this study is as follows. Similarities in soil properties between long-term prairie and recently-forested sites suggest that the encroachment of forest into prairies is a cyclic process where trees encroach and dieback. Historic photographs show that encroachment has been ongoing in the last century, and active management may be necessary to promote return to prairie. One tree species of particular concern is aspen, a fire adapted early colonizer. Prescribed burns are currently used to preserve the integrity of the remaining prairies, but a combination of browsing and burning had not previously been assessed at RMNP. The simulated browsing results show that browsing applied at 100% is effective to control aspen regrowth, but ineffective when applied at 50% leaf removal. Growth of aspen suckers over two years was significantly higher for suckers that grew after a burn than for suckers in sites that had not been burned for decades. This difference indicated that if aspen suckers are left untouched after a burn, they will grow back and recolonize quickly. Although there was no interaction between browsing and burning, aspen left alone after a burn will continue to encroach. Increasing the number of ungulates at RMNP may offer a solution to reduce encroachment, because high levels of browse thwart aspen sucker growth. With a higher density of ungulates, there will be more feces interacting with leaf litter and the soil. Mixing local wild ungulate manure with aspen leaf litter did not affect the decay rate of the litter. Fecal incubations in soil showed that although the manure amendments do increase the amount of plant-available N, mineral N release is not substantial enough to have an immediate biological effect on plant growth. Hence, increase fecal deposition should not have a significant effect on the system in the short-term, but simply contributes to long-term replenishment of organic matter.

Increasing the numbers of wild browsers within the park presents a series of difficulties. The permeable barrier of RMNP allows for animals to enter and leave RMNP without restriction.

Animals that leave RMNP can cause damage to neighbouring farmland, which in turn creates conflict. Animals that wander out of RMNP are also vulnerable to hunters and higher road density, which presents greater risk of increased mortality. Browsers might also permanently migrate out of RMNP, having no impact on the vegetation within the park boundary. Bringing cattle back into the park is not an option, because cattle degrade the natural prairies to an ecosystem dominated strongly by Kentucky blue-grass. This degradation of the ecosystem conflicts with the Parks Canada mandate to conserve ecological integrity within National Parks. An increase in the frequency of prescribed burns would likely help stop aspen encroachment, but weather conditions often prevent burns being undertaken in some years. Management suggestions are to maintain the browser intensity as high as possible and to do prescribe burns as frequently as possible. Physical removal of saplings is also an alternative to stop the advancement of woody species into prairies.

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APPENDICES

Appendix 2-1. Analysis of variance table for simulated browsing experiment. The table presents burn treatments (burn), clipping treatments (clip), and interaction between burn treatment and clipping treatment (burn*clip).

July 2016					
Source	DF	SS	MS	F	p
burn	1	0.00729	0.00729	0.07	0.7958
clip	2	0.01894	0.00947	0.09	0.9155
burn*clip	2	0.01410	0.00705	0.07	0.9364
Error	30	3.20987	0.10700		
Total	35	3.25021			
Grand Mean 2.1704 CV 15.07					
August 2016					
Source	DF	SS	MS	F	p
burn	1	0.16113	0.16113	1.59	0.2173
clip	2	2.52877	1.26439	12.46	0.0001
burn*clip	2	0.21773	0.10887	1.07	0.3547
Error	30	3.04352	0.10145		
Total	35	5.95116			
Grand Mean 1.9383 CV 16.43					
June 2017					
Source	DF	SS	MS	F	p
burn	1	0.28920	0.28920	5.52	0.0255
clip	2	0.98643	0.49322	9.42	0.0007
burn*clip	2	0.06909	0.03454	0.66	0.5243
Error	30	1.57061	0.05235		
Total	35	2.91533			
Grand Mean 2.0360 CV 11.24					
August 2017					
Source	DF	SS	MS	F	p
burn	1	1.2550	1.25504	10.67	0.0027
clip	2	9.9145	4.95724	42.15	0.0000
burn*clip	2	0.4721	0.23606	2.01	0.1520
Error	30	3.5283	0.11761		
Total	35	15.1700			
Grand Mean 1.6921 CV 20.27					

Appendix 2-2. Complete list of species identification of 2016 with average percent cover (%) over all sites.

Species	Common name	Percent cover (%)
<i>Poa pratensis</i>	Kentucky bluegrass	167.0
<i>Festuca hallii</i>	rough fescue	52.8
<i>Solidago canadensis</i>	Canada goldenrod	32.9
<i>Carex inops</i> ssp. <i>heliophila</i>	sun sedge	22.4
<i>Galium boreale</i>	northern bedstraw	21.3
<i>Lathyrus ochroleucus</i>	cream peavine	15.4
<i>Monarda fistulosa</i>	wild bergamot	14.9
<i>Fragaria virginiana</i>	wild strawberry	14.1
<i>Hesperostipa spartea</i>	porcupine grass	13.8
<i>Solidago rigida</i> ssp. <i>humilis</i>	stiff goldenrod	11.3
<i>Symphyotrichum laeve</i>	smooth aster	11.1
<i>Vicia americana</i>	American vetch	10.3
<i>Symphoricarpos occidentalis</i>	western snowberry	10.0
<i>Amelanchier alnifolia</i>	saskatoon	10.0
<i>Achillea millefolium</i>	common yarrow	7.8
<i>Lithospermum canescens</i>	hoary puccoon	7.8
<i>Elymus trachycaulus</i> ssp. <i>trachycaulus</i>	slender wheatgrass	7.4
<i>Artemisia ludoviciana</i>	prairie Sage	6.9
<i>Agastache foeniculum</i>	giant hyssop	5.8
<i>Leymus innovatus</i> ssp. <i>innovatus</i>	downy ryegrass	5.8
<i>Populus tremuloides</i>	aspen	5.8
<i>Oryzopsis asperifolia</i>	roughleaf ricegrass	5.8
<i>Muhlenbergia glomerata</i>	marsh muhly	5.8
<i>Rosa acicularis</i>	prickly rose	5.7
<i>Phalaris arundinacea</i>	reed canary grass	5.3
<i>Smilacina stellata</i>	starry false Solomon's seal	5.2
<i>Bromus inermis</i>	smooth brome	4.9
<i>Thalictrum venulosum</i>	veiny meadow rue	4.3
<i>Arctostaphylos uva-ursi</i>	bearberry	4.2
<i>Achnatherum richardsonii</i>	Richardson's needlegrass	4.0
<i>Anemone canadensis</i>	Canada anemone	4.0
<i>Elymus trachycaulus</i> ssp. <i>subsecundus</i>	awned wheatgrass	4.0
<i>Prunus virginiana</i>	chokecherry	3.8
<i>Bromus ciliatus</i>	fringed brome	3.3
<i>Rosa woodsii</i>	Woods' rose	2.9
<i>Equisetum sylvaticum</i>	wood horsetail	2.8
<i>Piptatheropsis canadensis</i>	Canada rice grass	2.8
<i>Potentilla arguta</i>	tall cinquefoil	2.8
<i>Elymus trachycaulus</i> ssp. <i>trachycaulus</i>	slender wheatgrass	2.7

<i>Sonchus arvensis</i>	field milk thistle	2.5
<i>Piptatheropsis pungens</i>	mountain ricegrass	2.4
<i>Artemisia dracunculus</i>	tarragon	2.1
<i>Schizachne purpurascens</i>	purple oat grass	2.1
<i>Corydalis aurea</i>	golden corydalis	2.0
<i>Agrostis scabra</i>	hair grass	1.7
<i>Avenula hookeri</i>	spikeoat	1.4
<i>Anthoxanthum hirtum</i>	sweet grass	1.3
<i>Lactuca pulchella</i>	blue lettuce	1.2
<i>Lysimachia ciliata</i>	fringed loosestrife	1.2
<i>Campanula rotundifolia</i>	harebell	1.1
<i>Solidago missouriensis</i>	Missouri goldenrod	1.1
<i>Hedysarum alpinum</i>	alpine sweetvetch	1.1
<i>Comandra umbellata</i>	bastard toadflax	1.0
<i>Rhamnus alnifolia</i>	alderleaf buckthorn	0.96
<i>Epilobium angustifolium</i>	fireweed	0.79
<i>Koeleria macrantha</i>	prairie junegrass	0.75
<i>Salix bebbiana</i>	Bebb's willow	0.75
<i>Spiraea alba</i>	meadowsweet	0.67
bryophyte	moss	0.63
<i>Salix petiolaris</i>	meadow willow	0.58
<i>Sisyrinchium montanum</i>	blue-eyed grass	0.54
<i>Geum triflorum</i>	prairie smoke	0.50
<i>Liatris ligulistylis</i>	blazing star	0.42
<i>Potentilla gracilis</i>	slender cinquefoil	0.42
<i>Solidago simplex</i>	Mt. Albert goldenrod	0.42
<i>Poa compressa</i>	Canada bluegrass	0.38
<i>Elymus lanceolatus</i> ssp. <i>lanceolatus</i>	thickspike wheatgrass	0.33
<i>Viola canadensis</i> var. <i>rugulosa</i>	Canadian white violet	0.33
<i>Lonicera dioica</i>	twining honeysuckle	0.33
<i>Stellaria longipes</i> ssp. <i>longipes</i>	long-stalked starwort	0.33
<i>Elymus trachycaulus</i> ssp. <i>trachycaulus</i>	slender wheatgrass	0.29
<i>Cirsium arvense</i>	Canada thistle	0.29
<i>Hieracium umbellatum</i>	Canadian hawkweed	0.25
<i>Taraxicum officinale</i>	common dandelion	0.25
<i>Viola canadensis</i> var. <i>rugulosa</i>	tall white violet	0.25
<i>Maianthemum canadense</i>	Canada mayflower	0.21
<i>Agoseris glauca</i>	prairie agoseris	0.17
<i>Stachys palustris</i>	hedge-nettle	0.17
<i>Hesperostipa curisetia</i>	needle grass	0.17
<i>Erigeron glabellus</i>	streamside fleabane	0.13
<i>Elymus canadensis</i>	Canada wild rye	0.13
<i>Ribes oxycanthoides</i>	Canadian gooseberry	0.13

<i>Rudbeckia serotina</i>	black-eyed Susan	0.13
<i>Salix bebbiana</i>	Bebb's willow	0.13
<i>Agrohordeum macounii</i>	Macoun's Barley	0.08
<i>Anemone cylindrica</i>	candle anemone	0.08
<i>Apocynum cannabinum</i>	Indian hemp	0.08
<i>Danthonia spicata</i>	poverty oatgrass	0.08
<i>Dracocephalum parviflorum</i>	dragonhead mint	0.08
<i>Petasites frigidus</i> ssp. <i>sagittatus</i>	arrowleaf sweet coltsfoot	0.08
<i>Mentha arvensis</i>	wild mint	0.08
<i>Muhlenbergia richardsonis</i>	mat muhly	0.08
<i>Viola renifolia</i> var. <i>brainerdii</i>	kidneyleaf violet	0.08
Forb 1	no specimen	0.04
<i>Arabis pycnocarpa</i> ssp. <i>pycnocarpa</i>	hairy rock cress	0.04
<i>Koeleria macrantha</i>	prairie Junegrass	0.04
<i>Muhlenbergia cuspidata</i>	plains muhly	0.04
<i>Poa glauca</i>	glaucous bluegrass	0.04
<i>Lysimachia ciliata</i>	fringed loosestrife	0.04
<i>Trifolium repens</i>	white clover	0.04
<i>Ulmus americana</i>	American elm	0.04
<i>Zizia aptera</i>	heart-leaf golden alexander	0.04
<i>Viola canadensis</i> var. <i>rugulosa</i>	tall white violet	0.04
<i>Calamagrostis stricta</i> ssp. <i>inexpansa</i>	northern reedgrass	0.04
	standing dead	101.8
	Total average percent cover	564.1

Appendix 2-3. Complete list of species identification of 2017 with average percent cover (%) over all sites.

Species	Common name	Percent cover (%)
<i>Poa pratensis</i>	Kentucky bluegrass	145.8
<i>Carex inops</i> ssp. <i>heliophila</i>	sun sedge	27.9
<i>Symphoricarpos occidentalis</i>	western snowberry	25.2
<i>Fragaria virginiana</i>	wild strawberry	22.0
<i>Festuca hallii</i>	rough fescue	21.7
<i>Solidago canadensis</i>	Canada goldenrod	20.9
<i>Galium boreale</i>	northern bedstraw	19.4
<i>Liatris ligulistylis</i>	blazing star	14.4
<i>Amelanchier angustifolia</i>	saskatoon	14.3
<i>Schizachne purpurascens</i>	purple oat grass	28.3
<i>Populus tremuloides</i>	aspen	13.6
<i>Lathyrus venosus</i>	veiny pea	11.01
<i>Rosa woodsia</i>	Woods' rose	10.2
<i>Polygala senega</i>	Seneca snakeroot	10.1
<i>Leymus innovatus</i> ssp. <i>innovatus</i>	downy rye-grass	10.1
<i>Monarda fistulosa</i>	wild bergamot	9.3
<i>Rosa acicularis</i>	prickly rose	9.0
<i>Symphyotrichum laeve</i>	smooth aster	7.9
<i>Calamagrostis canadensis</i>	Canada reed grass	7.8
<i>Vicia americana</i>	American vetch	5.9
<i>Thalictrum venulosum</i>	veiny meadow rue	5.0
<i>Prunus virginiana</i>	chokecherry	4.0
<i>Smilacina stellata</i>	starry false Solomon's seal	3.8
<i>Lathyrus ochroleucus</i>	cream peavine	3.6
<i>Arctostaphylos urva-ursi</i>	bearberry	3.4
<i>Achillea millefolium</i>	common yarrow	3.3
<i>Agastache foeniculum</i>	giant hyssop	3.3
<i>Solidago rigida</i> ssp. <i>humilis</i>	stiff goldenrod	2.8
<i>Hedysarum alpinum</i>	alpine sweetvetch	2.8
<i>Elymus repens</i>	couch grass	2.6
<i>Rubus idaeus</i>	raspberry	2.6
<i>Artemisia ludoviciana</i>	prairie sage	2.3
<i>Lonicera dioica</i>	twining honeysuckle	2.1
<i>Muhlenbergia glomerata</i>	marsh muhly	2.6
<i>Poa compressa</i>	Canada bluegrass	2.0
<i>Potentilla arguta</i>	tall cinquefoil	2.0
<i>Stachys palustris</i>	hedge nettle	2.0
<i>Artemisia dracunculus</i>	tarragon	1.8
<i>Rosa arkansana</i>	prairie rose	1.8

<i>Smilax lasioneura</i>	blue ridge carrionflower	1.8
<i>Bromus inermis</i>	Smooth brome	1.7
<i>Geum triflorum</i>	prairie smoke	1.6
<i>Hesperostipa spartea</i>	porcupine grass	1.6
<i>Symphotrichum ciliolatum</i>	Lindley's aster	1.5
<i>Corylus cornuta</i>	beaked hazelnut	1.4
<i>Apocynum cannabinum</i>	Indian hemp	1.3
<i>Campanula rotundifolia</i>	harebell	1.3
<i>Lithospermum canescens</i>	hoary puccoon	1.2
<i>Poa palustris</i>	fowl bluegrass	1.2
<i>Ribes oxyacanthoides</i>	Canadian gooseberry	1.1
<i>Salix bebbiana</i>	Bebb's willow	1.0
<i>Anthoxanthum hirtum</i>	sweet grass	1.4
<i>Bromus ciliatus</i>	fringed brome	1.5
<i>Rosa blanda</i>	smooth rose	0.83
<i>Hesperostipa curtisetia</i>	needle grass	0.67
<i>Viola canadensis</i> var. <i>rugulosa</i>	Canadian white violet	0.58
<i>Lysimachia ciliata</i>	fringed loosestrife	0.53
<i>Elymus trachycaulus</i> ssp. <i>subsecundus</i>	awned wheatgrass	0.47
<i>Solidago simplex</i>	Mt. Albert goldenrod	0.56
<i>Anemone canadensis</i>	Canada anemone	0.44
<i>Shepherdia canadensis</i>	buffaloberry	0.39
Grass 12	no specimen	0.33
<i>Cirsium arvense</i>	Canada thistle	0.31
<i>Poa interior</i>	inland bluegrass	0.31
<i>Agoseris glauca</i>	prairie agoseris	0.31
Grass 36	no specimen	0.31
<i>Aralia nudicaulis</i>	wild sarsaparilla	0.28
<i>Hieracium umbellatum</i>	northern hawkweed	0.25
<i>Sisyrinchium montanum</i>	blue-eyed grass	0.22
<i>Carex rossii</i>	Ross' sedge	0.19
<i>Schizachne purpurascens</i>	purple oat grass	0.19
<i>Lactuca pulchella</i>	blue lettuce	0.19
<i>Elymus trachycaulus</i> ssp. <i>trachycaulus</i>	slender wheatgrass	0.17
<i>Taraxicum officinale</i>	common dandelion	0.17
<i>Spiraea alba</i>	meadowsweet	0.14
<i>Viola adunca</i>	early blue violet	0.14
<i>Erigeron glabellus</i>	streamside fleabane	0.08
<i>Mertensia paniculata</i>	northern bluebells	0.08
<i>Elymus trachycaulus</i> ssp. <i>trachycaulus</i>	slender wheatgrass	0.08
<i>Hesperostipa curtisetia</i>	needle grass	0.08
<i>Zizia aptera</i>	heart-leaf golden alexander	0.08
Forb 18	no specimen	0.06

<i>Stellaria longipes</i> ssp. <i>longipes</i>	long-stalked starwort	0.03
<i>Allium stellatum</i>	prairie onion	0.03
	standing dead	168.3
	total average percent cover	517.1

Appendix 3-2. (A) ANOVA table for aspen leaf litter decay ($k \text{ year}^{-1}$). (B) ANOVA tables for concentration of N and P (mg N g^{-1} , mg P g^{-1}) in litter bags. (C) ANOVA tables for content of N and P (mg N bag^{-1} , mg P bag^{-1}). ANOVA Sources list Rep for replicate site in the field, Treatment for manure amendment, and Time for collection time. Interaction between Treatment and Time is abbreviated to Treat*Time.

(A) Randomized complete block analysis of variance for k (year^{-1})					
Source	DF	SS	MS	F	p
Site	5	0.12236	0.02447		
Treatment	3	0.00535	0.00178	0.94	0.45
Error	15	0.02843	0.00190		
Total	23	0.15613			
(B) Two-way analysis of variance for N concentration (mg N g^{-1})					
Source	DF	SS	MS	F	p
Rep	6	12.881	2.1469	5.24	<0.001
Time	5	108.925	21.7849	53.13	<0.001
Treatment	3	14.848	4.9493	12.07	<0.001
Time*Treat	15	12.080	0.8053	1.96	0.024
Error	110	45.107	0.4101		
Total	139				
Two-way analysis of variance for P concentration (mg P g^{-1})					
Source	DF	SS	MS	F	p
Rep	6	1.11283	0.18547	5.57	<0.001
Time	5	1.51512	0.30302	9.11	<0.001
Treatment	3	0.29485	0.09828	2.95	0.036
Time*Treat	15	0.68290	0.04553	1.37	0.18
Error	116	3.65978	0.03327		
Total	139				
(C) Two-way analysis of variance for N content (mg N bag^{-1})					
Source	DF	SS	MS	F	p
Rep	6	21.076	3.5127	1.21	0.31
Time	5	144.814	28.9628	9.99	<0.001
Treatment	3	72.620	24.2066	8.35	<0.001
Time*Treat	15	87.176	5.8117	2.00	0.021
Error	116	318.855	2.8987		
Total	139				
Two-way analysis of variance for P content (mg P bag^{-1})					
Source	DF	SS	MS	F	p
Rep	6	16.1838	2.69731	9.81	<0.001
Time	5	30.1603	6.03206	21.94	<0.001
Treatment	3	7.6308	2.54360	9.25	<0.001
Time*Treat	15	4.1028	0.27352	0.99	0.47
Error	116	30.2454	0.27496		
Total	139				

Appendix 3-2. Kruskal-Wallis non-parametric analyses of soil incubations. Statistical summary is given for both the Kruskal-Wallis statistic and the Conover and Iman (1981) analysis of variance of the ranked values.

For NO ₃ -N by amendment type					
Kruskal-Wallis statistic=4.5816; p=0.10					
Source	DF	SS	MS	F	p
Between	2	67189	33594.5	2.31	0.10
Within	416	6062780	14574.0		
Total	418	6129969			
Cases Included 419 Missing Cases 1					
For NO ₃ -N by amendment rate					
Kruskal-Wallis statistic=6.9804; p=0.07					
Source	DF	SS	MS	F	p
Between	3	102367	34122.3	2.35	0.07
Within	415	6027603	14524.3		
Total	418	6129970			
Cases Included 419 Missing Cases 1					
For NO ₃ -N by time					
Kruskal-Wallis statistic=300.056; p<0.001					
Source	DF	SS	MS	F	p
Between	5	4400319	880064	210	<0.001
Within	413	1729651	4188		
Total	418	6129969			
Cases Included 419 Missing Cases 1					
For NH ₄ -N by time					
Kruskal-Wallis statistic=129.121; p<0.001					
Source	DF	SS	MS	F	p
Between	5	1902599	380520	36.9	<0.001
Within	414	4271363	10317		
Total	419	6173962			
Cases Included 420 Missing Cases 0					
For NH ₄ -N by amendment type					
Kruskal-Wallis statistic=19.7284; p<0.001					
Source	DF	SS	MS	F	p
Between	2	290697	145349	10.3	<0.001
Within	417	5883265	14109		
Total	419	6173962			
Cases Included 420 Missing Cases 0					
For NH ₄ -N by amendment rate					
Kruskal-Wallis statistic=31.2356; p<0.001					
Source	DF	SS	MS	F	p
Between	3	460257	153419	11.2	<0.001
Within	416	5713705	13735		
Total	419	6173962			
Cases Included 420 Missing Cases 0					
Ungulate compared to Control for NO ₃ -N by time					

Kruskal-Wallis statistic=188.387; p<0.001					
Source	DF	SS	MS	F	p
Between	5	908028	181606	174	<0.001
Within	234	243952	1043		
Total	239	1151980			
Cases Included 240 Missing Cases 0					
Ungulate compared to Control for NO ₃ -N by amendment type					
Kruskal-Wallis statistic=0.3336; p=0.56					
Source	DF	SS	MS	F	p
Between	1	1608	1608.02	0.33	0.56
Within	238	1150372	4833.50		
Total	239	1151980			
Cases Included 240 Missing Cases 0					
Ungulate compared to Control for NO ₃ -N by amendment rate					
Kruskal-Wallis statistic=1.9847; p=0.58					
Source	DF	SS	MS	F	p
Between	3	9566	3188.69	0.66	0.58
Within	236	1142414	4840.74		
Total	239	1151980			
Cases Included 240 Missing Cases 0					
Ungulate compared to Control for NH ₄ -N by time					
Kruskal-Wallis statistic=69.0687; p<0.001					
Source	DF	SS	MS	F	p
Between	5	332911	66582.1	19.0	<0.001
Within	234	819068	3500.3		
Total	239	1151978			
Cases Included 420 Missing Cases 0					
Ungulate compared to Control for NH ₄ -N by amendment type					
Kruskal-Wallis statistic=18.8870; p<0.001					
Source	DF	SS	MS	F	p
Between	2	290697	145349	10.3	<0.001
Within	417	5883265	14109		
Total	419	6173962			
Cases Included 420 Missing Cases 0					
For NH ₄ -N by amendment rate					
Kruskal-Wallis statistic=42.0089; p<0.001					
Source	DF	SS	MS	F	p
Between	1	91035	91035.0	20.4	<0.001
Within	238	1060943	4457.7		
Total	239	1151979			
Cases Included 420 Missing Cases 0					
Bison compared to Control for NO ₃ -N by time					
Kruskal-Wallis statistic= 168.088; p<0.001					
Source	DF	SS	MS	F	p
Between	5	803461	160692	112	<0.001
Within	233	334179	1434		

Total	238	1137640			
Cases Included 239 Missing Cases 1					
Bison compared to Control for NO ₃ -N by amendment type					
Kruskal-Wallis statistic=3.2033; p=0.07					
Source	DF	SS	MS	F	p
Between	1	15312	15311.8	3.23	0.07
Within	237	1122328	4735.6		
Total	238	1137640			
Cases Included 239 Missing Cases 1					
Bison compared to Control for NO ₃ -N by amendment rate					
Kruskal-Wallis statistic=13.4704; p=0.004					
Source	DF	SS	MS	F	p
Between	3	64389	21462.9	4.70	0.003
Within	235	1073251	4567.0		
Total	238	1137639			
Cases Included 239 Missing Cases 1					
Bison compared to Control for NH ₄ -N by time					
Kruskal-Wallis statistic=79.0008; p<0.001					
Source	DF	SS	MS	F	p
Between	5	380784	76156.8	23.1	<0.001
Within	234	771196	3295.7		
Total	239	1151980			
Cases Included 420 Missing Cases 0					
Bison compared to Control for NH ₄ -N by amendment type					
Kruskal-Wallis statistic=7.7976; p=0.005					
Source	DF	SS	MS	F	p
Between	1	37584	37584.4	8.03	0.005
Within	238	1114395	4682.3		
Total	239	1151979			
Cases Included 420 Missing Cases 0					
Bison compared to Control for NH ₄ -N by amendment rate					
Kruskal-Wallis statistic=9.1367; p=0.03					
Source	DF	SS	MS	F	p
Between	3	44039	14679.6	3.13	0.03
Within	236	1107941	4694.7		
Total	239	1151980			
Cases Included 420 Missing Cases 0					
Bison compared to Ungulate for NO ₃ -N					
Kruskal-Wallis statistic=2.9863; p=0.08					
Source	DF	SS	MS	F	p
Between	1	32163	32162.9	3.00	0.08
Within	357	3823497	10710.1		
Total	358	3855660			
Cases Included 359 Missing Cases 1					
Bison compared to Ungulate for NH ₄ -N					
Kruskal-Wallis statistic=5.1939; p= 0.02					

Source	DF	SS	MS	F	p
Between	1	56250	56250.0	5.26	0.02
Within	358	3831718	10703.1		
Total	359	3887968			
Cases Included 360 Missing Cases 0					

Appendix 4-1. Soil pit sites classified based on “A landform mapping system for Canadian soil surveys” by D. F. Acton (1975), including soil texture modifier, compositional category, surface expression, slope, parental material and soil series

Site	Texture Modifier	Compositional Category	Surface Expression	Slope	Parental Material	Soil Series
Long Term Forest 1	Coarse loamy	Glaciofluvial	Horizontal	Steeply sloping (15%-30%)	Outwash coarse gravelly	Seech
Long Term Forest 2	Coarse loamy	Glaciofluvial	Hummocky	Moderately sloping (5%-9%)	Outwash coarse gravelly	Seech
Long Term Forest 3	Coarse loamy	Glaciofluvial	Horizontal	Steeply sloping (15%-30%)	Outwash coarse gravelly	Seech
Long Term Forest 4	Mesic	Bog	Flat	Very gently sloped (0.5%-2%)	Organic forest peat	Baynham
Long Term Prairie 1	Mesic	Glaciofluvial	Undulating	Very gently sloped (0.5%-2%)	Outwash coarse gravelly	Heron peaty phase
Long Term Prairie 2	Mesic	Glaciofluvial	Undulating	Very gently sloped (0.5%-2%)	Outwash coarse gravelly	Heron peaty phase
Long Term Prairie 3	Mesic	Fen compositional	Horizontal	Very gently sloped (0.5%-2%)	Organic fen peat	Kircro
Long Term Prairie 4	Mesic	Glaciofluvial	Undulating	Very gently sloped (0.5%-2%)	Outwash coarse gravelly	Heron peaty phase
Recently Forested 1	Coarse loamy	Glaciofluvial	Hummocky	Steeply sloping (15%-30%)	Outwash coarse gravelly	Seech
Recently Forested 2	Coarse loamy	Glaciofluvial	Hummocky	Moderately sloping (5%-9%)	Outwash coarse gravelly	Seech
Recently Forested 3	Coarse loamy	Glaciofluvial	Hummocky	Steeply sloping (15%-30%)	Outwash coarse gravelly	Seech
Recently Forested 4	Coarse loamy	Glaciofluvial	Hummocky	Steeply sloping (15%-30%)	Outwash coarse gravelly	Seech