RESTORATION OF SAGEBRUSH GRASSLAND FOR GREATER SAGE GROUSE HABITAT IN GRASSLANDS NATIONAL PARK, SASKATCHEWAN

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Land Reclamation and Remediation

Department of Renewable Resources

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ABSTRACT

Populations of Greater Sage-grouse (*Centrocercus urophasianus* Bonaparte [Phasianidae]; hereafter Sage-grouse) have been in decline in North America for the last 100 years; since 1988, the Canadian population has declined by 98 %. Initial declines of Sage-grouse populations were likely due to habitat loss, degradation, and fragmentation, which continue to be major contributors to ongoing declines. This research focused on developing methods to improve restoration of Sage-grouse habitat by increasing establishment, growth, and survival of Silver sagebrush (*Artemisia cana* Pursh), a critical component of Sage grouse habitat. Field research was conducted in Grasslands National Park (GNP), Saskatchewan, Canada.

Models that enable the calculation of seeding or planting densities to obtain desired sagebrush cover within specific time frames are essential for restoration. Cover and density of naturally occurring *Artemisia cana* stands were measured in 10 m x 10 m plots, with stem diameter, crown diameter, canopy cover, and age measured on individuals. Sagebrush mortality was estimated from stand age demographics, and seedling survival of other studies. Strong relationships between morphological characteristics and age were found. Age was significantly correlated with stem diameter (r^2 = 0.79) allowing non-destructive age estimations to be made for *Artemisia cana*. Age was also correlated to canopy cover (r^2 = 0.49 to 0.67) and allowed models of *Artemisia cana* landscape cover over time at different planting densities to be constructed. Largest cover increases can occur in areas that are grazed by cattle. Cover is maximized after 11 years in heavy cattle grazed areas, and after 21 years in light cattle grazed areas.

Artemisia cana emergence under field conditions has been extremely low. Seed dormancy and low germination were identified as possible factors reducing seedling emergence and were investigated. Seeds were cleaned and after ripened in cold storage for 4 to 18 months. Before germination in light or dark, a physical scarification treatment was applied. Pericarp removal and

after-ripening for 16 to 18 months marginally increased germination (approximately 10 %) of *Artemisia cana* under laboratory conditions. Even without treatment, *Artemisia cana* germination in a laboratory was very high. Results suggest that low success of *Artemisia cana* seeding in the field is not due to seed dormancy or poor germination but from limiting environmental factors.

Survival of outplanted *Artemisia* sp. seedlings has been low, with studies reporting 30 to 36 % survival after two years. Increasing nutrient availability during greenhouse growth via nutrient loading was investigated. Extending growth time in the greenhouse to 26 weeks and applying 175 and 245 mg nitrogen $plant^{-1}$ on exponential or modified exponential dosing schedules facilitated nutrient loading *Artemisia cana* seedlings. Seedlings were outplanted into a field plot and monitored for two growing seasons. Nutrient loaded seedlings had greater survival (80 %) than unloaded seedlings (57 %) and increased second season canopy development (1,040 cm^2 vs 680 cm^2). Elimination of herbaceous competition likely contributed to greater survival and is recommended for the first two years after outplanting. Use of nutrient loaded seedlings in restoration planting increased outputs for sagebrush landscape cover in light cattle grazed areas to 24 % and to 12 % in bison grazed areas.

The intense anthropogenic disturbance and alteration of potential Sage-grouse habitat necessitate that effects of land management be considered in its restoration. Research plots investigating revegetation (fall seeding, spring seeding, outplanting, control) and herbicide use to control non-native species were established in cattle grazed, bison grazed, watered, and ungrazed areas of GNP. Land management significantly altered soil properties and vegetation and invertebrate communities. Outplanting seedlings resulted in greater *Artemisia cana* cover than seeding. Very heavy grazing by cattle prevented adequate litter build up. Excess litter cover in bison grazed and ungrazed areas aided outplanted seedling survival but prevented broadcast seed from reaching the soil surface. Herbicide decreased non-native cover the first year after application but increased non-native cover thereafter. Herbicide did not negatively affect pre-

existing *Artemisia cana*. All sites had key components of Sage-grouse habitat and showed high potential for restoration success given land management modification. To maximize sagebrush landscape cover, nutrient loaded seedlings should be planted into appropriate microsites within areas where land management has achieved litter cover of 15 to 30 %.

PREFACE

Chapter 2 of this thesis has been accepted for publication as “Watkinson, AD, MA Naeth, and S Pruss. 2020. Storage time, physical scarification and light exposure effects on *Artemisia cana* seed germination. Native Plants Journal 21:4-13”. Several photographs have been removed from the thesis that were included in the journal publication.

Rules for nomenclature follow USDA NRCS (2020).

ACKNOWLEDGEMENTS

I acknowledge those individuals who made this project possible through their assistance and continued support. My most sincere thanks to the following.

My supervisors, Dr M Anne Naeth and Dr Shelley Pruss, and thesis committee member, Dr Cameron Carlyle.

Support staff of the Naeth Land Reclamation Research Group, Sarah Wilkinson and Stacy Campbell-Court.

My fellow graduate students in the Naeth Lab, the Land Reclamation International Graduate School, and the Department of Renewable Resources.

Grasslands National Park and staff, especially Dr Stefano Liccioli, Dr Maggi Sliwinski, Laura Gardiner, Samantha Fischer, Heather Facette, Matthew Johnson, and Nathan Young.

For field assistance, Adam Iverson, Ashley Kocsis, Abigayle Blackmore, Jordyn Renaud, Keana Boere, Rosheen Tetzlaff, Valish Ulrich, Jason Eerkes, Kiah Leicht, Hayley Webster, Lydia Kim, Kelsey Marchand, Valerie Miller, Alison Murata, Stephanie Ibsen, and Donna Watkinson.

For guidance in dendrochronology methods, Catherine McNalty, Northern Forestry Center.

For plant tissue analysis of total non-structural carbohydrates and nitrogen, Dr Kelvin Lein, Department of Agricultural, Food and Nutritional Science, University of Alberta.

For mentorship in the early stages of my doctoral program, Dr Brad Pinno, Department of Renewable Resources, University of Alberta.

This work was made possible through funding provided by Grasslands National Park (Parks Canada), Government of Saskatchewan through the Saskatchewan Fish and Wildlife Development Fund, and the Land Reclamation International Graduate School through the NSERC CREATE program. In-kind support and research materials were provided by Grasslands National Park (Parks Canada). The following organizations are gratefully acknowledged for their provision of scholarships: American Pheasant and Waterfowl Society, Government of Alberta, Government of Saskatchewan, Natural Sciences and Engineering Research Council of Canada, Syngenta Crop Protection Canada Inc., University of Alberta, University of Alberta Faculty of Agricultural, Life, and Environmental Sciences, and University of Alberta Department of Renewable Resources.

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"The sage and the grouse seem made for each other ... the sage is all things to these birds of the plains." – Rachel Carson, *Silent Spring*, 1962

1. INTRODUCTION

Populations of Greater Sage-grouse (*Centrocercus urophasianus* Bonaparte [Phasianidae]; hereafter Sage-grouse) have been in decline in North America for the last 100 years. Since the late 1980s there has been further reduction in the remaining Sage-grouse range in Alberta and Saskatchewan (Aldridge and Brigham 2003). Consequently, Sage-grouse was designated as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1998 and listed by the Species at Risk Act (SARA) in 2003 (Environment Canada 2014). Currently, the last two remaining active leks in Saskatchewan reside in Grasslands National Park.

Initial population decline of the Sage-grouse was likely caused by habitat loss, degradation, and fragmentation. This bird is a sagebrush obligate and its distribution in North America is closely linked to distribution of sagebrush species (*Artemisia* L. [Asteraceae]) (hereafter sagebrush) (Connelly et al. 2004). Although initial Sage-grouse population declines can be linked to habitat loss, recent dramatic declines in populations are not well understood. They are likely due to a combination of factors including continued habitat loss and degradation, disruption by industrial development, drought, predation, water impoundments, and disease (Gregg et al. 1994, Braun 1998, Aldridge and Brigham 2003, Crawford et al. 2004, Aldridge et al. 2008, Coates and Delehanty 2010, Harju et al. 2010, Blickley et al. 2012, Blomberg et al. 2012). Sage-grouse life stages have specific habitat requirements (Aldridge and Brigham 2002, Crawford et al. 2004, Hagen et al. 2007, Carpenter et al. 2010, Taylor et al. 2012) and remaining sagebrush range may not provide the quality of habitat needed to support mating, nesting, brood rearing, and winter survival. Canada's recovery strategy for the Sage-grouse (Environment Canada 2014) suggests that low quality habitat could support positive population growth through restoration of degraded habitat. Grasslands National Park, in collaboration with the Saskatchewan Research Council, has identified high priority sites for restoration based on their potential for success.

The research presented in this thesis investigated methods to improve establishment, growth, and survival of Silver sagebrush (*Artemisia cana* Pursh) (hereafter Silver sagebrush), with the

primary objective of improving quality of sagebrush habitat to facilitate the recovery of Sage-grouse. Experiments address three specific areas: sagebrush population ecology, improving greenhouse container growth and seeding of sagebrush, and impacts of land management on sagebrush restoration.

2. LITERATURE REVIEW

2.1. Greater Sage-Grouse Populations, Conservation, And Habitat

The Sage-grouse is the largest of North America's indigenous grouse species. Sage-grouse are ground dwelling; although capable of short distance flights, most movement is typically by foot. These birds are sexually dimorphic; males have an arched yellow comb above the eye, a black throat, a large white patch on the breast, long feathers at the back of the neck, and two large air sacs concealed within breast feathers that are inflated and deflated during courtship. Females have more cryptic plumage consisting of brown, black and buff markings which allow them to blend into their environment (Environment Canada 2014).

Populations have been in decline in Canada and the United States for the last 100 years and populations in North America were in a 2 % $year^{-1}$ decline between 1965 and 2003 (Connelly et al. 2004). Alberta and Saskatchewan's Sage-grouse populations have decreased 98 % from 1968 (Alberta) and 1988 (Saskatchewan) with approximately 200 to 300 Sage-grouse remaining in Canada (Environment Canada 2014, Qureshi 2019). In Saskatchewan, active leks have decreased from 42 in 1988 to 2 at present. The remaining active leks are within the boundaries of Grasslands National Park. Initial population decline was likely caused by direct loss and degradation of Sage-grouse habitat. As a sagebrush obligate, its distribution in North America is closely linked to distribution of sagebrush species (Connelly et al. 2004). In Canada, Sage-grouse historical range was approximately 100,000 km^2 in southern Alberta and Saskatchewan. As of 2003, only 6 % of the historical range remained (Aldridge and Brigham 2003). Limited distribution of sagebrush caused by anthropogenic activity including agriculture (over grazing, cultivation), industrial development (oil and gas exploration and operations), and transportation corridors have led to habitat range reduction and fragmentation.

Sage-grouse were designated as endangered by the Committee on the Status of Endangered Wildlife in Canada in 1998, listed as endangered by the Species at Risk Act in 2003, listed as potentially threatened in 1984, threatened in 1987 and endangered in 1999 by the province of Saskatchewan, and listed as endangered in 2000 by the province of Alberta (Environment

Canada 2014). In 2013, an Emergency Protection Order was issued by the Government of Canada to address imminent threats to survival, recovery and protection of the Sage-grouse (Government of Canada 2013). The order places restrictions on practices which may disturb Sage-grouse and their habitats on 1,672 km^2 of federal and provincial crown land in southwestern Saskatchewan and southeastern Alberta. The order aims to protect Sage-grouse by preventing habitat loss and fragmentation and providing time for habitat restoration.

Sage-grouse habitat needs vary throughout the year and are classified into four distinct types. These types are lek areas (for courtship displays), nesting sites, brood rearing sites, and over wintering sites. Seasonal movements between sites can exceed 75 km, with a home range of 125 to 2,764 km^2 (Connelly et al. 1988, Leonard et al. 2000, Smith 2013).

Lek habitat is generally on flat, open areas that are treeless, sparsely vegetated, and adjacent to sagebrush dominated areas (Dalke et al. 1963, Aldridge 2000). From early March until late May, male Sage-grouse congregate on leks and perform courtship displays to attract a mate (Adams et al. 2004a). Lek persistence is strongly correlated to amount of suitable nesting habitat within 5 to 7 km (Walker et al. 2007). Nests are usually within 3.2 km of occupied leks in uniformly distributed habitat and within 5 km in non-uniformly distributed habitat (Connelly et al. 2000).

In Canada, nesting occurs from mid May to late July (Adams et al. 2004a). Sage-grouse nest on the ground, in a shallow bowl lined with vegetation and feathers (Environment Canada 2014). Studies report sagebrush cover of approximately 20 to 30 % for successful nesting, with tall grass cover (> 18 cm) and some herbaceous cover to provide olfactory and visual cover from predators (Coggins 1998, Aldridge and Brigham 2002, Conover et al. 2011).

Females disperse after mating and rear their brood alone from late May to mid September (Adams et al. 2004a). Brood rearing habitat is usually located within 3 km of the nest during the first 2 to 3 weeks post hatch (Connelly et al. 2000). At this time, chicks are dependent on forbs and invertebrates for food. Crawford et al. (2004) report that chicks can consume up to 41 families of invertebrates and 33 genera of native forbs and grasses during their first month of life. Higher availability of forbs and invertebrates is expected to increase chick survival (Drut et al. 1994). Brood rearing habitat has approximately 10 to 15 % sagebrush cover with approximately 15 % grass and forb cover (Aldridge and Brigham 2002, Hagen et al. 2007, Environment Canada 2014).

Over wintering sites are used from early November through to late February (Adams et al. 2004a). In winter, Sage-grouse rely on sagebrush for shelter and food, comprising 100 % of their diet, and will travel tens of kilometers from nest and brood sites to find dense sagebrush wintering grounds

(Carpenter et al. 2010, Tack et al. 2012). Successful overwintering requires exposed sagebrush that is tall enough to remain above the snow (25 to 80 cm) (Aldridge 2000) with cover of 20 to 50 % (Eng and Schladweiler 1972, Connelly et al. 2004).

Sage-grouse consume sagebrush all year round; it comprises up to 60 % of their diet in summer and 100 % in winter (Wallestad and Eng 1975, Connelly et al. 2004). Forbs provide high quality forage and are an important component of summer and chick Sage-grouse diets. A diversity of species seems to be required including Prickly lettuce (*Lactuca serriola* L.), Common salsify (*Tragopogon dubius* Scop.), Common dandelion (*Taraxacum officinale* F.H. Wigg.), and Curlcup gumweed (*Grindelia squarrosa* (Pursh) Dunal.) (Miller and Eddleman 2001, Thompson et al. 2006). Insects are also essential requirements in chick diets, especially during the first month of life (Drut et al. 1994, Connelly et al. 2004). Chicks less than 21 days old require 15 g of invertebrates day^{-1} for survival and development and have been observed consuming grasshoppers (Orthoptera), beetles (Coleoptera) and ants (Hymenoptera) (Wallestad and Eng 1975, Johnson and Boyce 1990).

2.2. Sagebrush Ecology And Physiology

Sage-grouse distribution is tightly linked to that of sagebrush dominated ecosystems (Connelly et al. 2004). In Saskatchewan Sage-grouse are found in the Mixed Grassland ecoregion of the Prairie ecozone (Environment Canada 2014a). This region encompasses approximately 13 % (8.47 million ha) of Saskatchewan, with approximately half of the region cultivated for crops and the remainder utilized as rangelands for livestock production (Acton et al. 1998). Of these three anthropogenic uses, only native rangelands are viable Sage-grouse habitat. Sagebrush habitats have been significantly altered since European settlement in the 1800s; few areas remain intact and many only contain islands of sagebrush habitat within larger altered areas (Miller et al. 2011). In North America, sagebrush currently occupies < 60 % of its historical range (McArthur and Stevens 2004).

Mixed Grasslands are semi-arid; mean annual precipitation is 250 to 350 mm with moisture deficits in late summer caused by low precipitation and high evapotranspiration (Shorthouse 2010). Mean annual temperatures of the ecoregion are 3.5 °C, with 16 °C in summer (41.1 °C extreme high) and -10 °C in winter (-49.4 °C extreme low) (Environment Canada 2019). Winter and spring precipitation provide most of the available soil water to sagebrush communities; by mid summer available water in the soil surface is depleted (Shorthouse 2010).

Mixed Grasslands soils are predominantly Dark Brown Chernozems with parent materials of glacial till (Adams et al. 2004b); areas of Solonetzic soils are dispersed throughout. Areas that support sagebrush habitat are riparian, including older alluvial terraces on flood plains and alluvial fans in valleys (Weerstra 2001) with deep, loamy alluvial soils where higher water availability creates mesic areas. Topography of the Mixed Grasslands is dominantly undulating with sagebrush habitat generally occurring in flat areas with low to medium elevation.

Mixed Grasslands are treeless plains dominated by grasses and forbs. Grasses and sedges contribute 85 to 95 % of the above ground plant biomass, with forbs and shrubs making up the remainder (Rowe and Coupland 1984, Coupland 1992). Plant cover varies with soil and water availability, with large areas of bare ground in dry sites to almost 100 % cover in wet sagebrush communities. Perennials of sagebrush ecosystems have a discontinuous spatial arrangement, interspersed with open patches of biological crusts. These ecosystems are characterized by four distinct layers: shrubs and tall grasses 0.3 to 1.0 m high, forbs 0.2 to 0.6 m high, low growing grasses and forbs less than 0.2 m high, and biological crusts which include moss and lichen.

Silver sagebrush is the primary sagebrush species in Canada. It has three subspecies: Plains silver sagebrush (*Artemisia cana* ssp. *cana*), Bolander's silver sagebrush (*Artemisia cana* ssp. *bolanderi* (A. Gray) H.M. Hall & Clem.), and Mountain silver sagebrush (*Artemisia cana* ssp. *viscidula* (Osterh.) Beetle). Subspecies ranges are relatively geographically distinct. In Saskatchewan only *Artemisia cana* ssp. *cana* (hereafter Silver sagebrush) has been reported on mesic sites with relatively fertile soils, on well watered, deep soils, along stream bottoms and drainage ways (Thorpe 2002, Jones et al. 2005). *Artemisia tridentata* Nuttall (hereafter Big sagebrush) currently only occurs in the United States; with climate change it is predicted to expand into large areas of southern Saskatchewan by 2050 (Still and Richardson 2015).

Silver sagebrush individuals naturally grow in bunches distributed at various densities over the landscape, which have been categorized and described (i.e. class 2: a few sporadically occurring individuals, class 8: a few patches plus several sporadically occurring plants) by Jones et al. (2005). In southeastern Alberta, naturally occurring cover of Silver sagebrush has been reported to reach up to 14 % (Jones et al. 2005) and stem densities up to 5 m^{-2} were reported in southern Saskatchewan (Romo and Grilz 2002). A study conducted in western North Dakota, of 100 Silver sagebrush plants reported an average canopy diameter of 52 cm (Hazlett and Hoffman 1975). Old cultivated sites supported sagebrush patches and a continuous occurrence of well spaced individuals (Jones et al. 2005), which supports the hypothesis that Silver sagebrush can naturally colonize following a period of disturbance.

Silver sagebrush has a deep tap root and rhizome system (Jones et al. 2005). In Saskatchewan, tap roots were found at 2 to 4 m depth (Coupland and Johnson 1965). Taproots characteristically have widely spreading laterals in the upper 60 cm of soil, with few fine branches near the soil surface. Taproots absorb water from depths below that dominated by grass roots and are responsible for most deep soil water recharge in sagebrush habitats (Miller et al. 2011). Depending on grazing regime, mature Silver sagebrush plant height can reach 2.0 m (Shultz 2012). Leaves are thin and narrow, sometimes with 1 or 2 irregular lobes, 2 to 9 cm long. It flowers between August and September and seeds ripen in October (McArthur and Taylor 2004). Inflorescences are narrow with 2 to 3 flowering heads per branch (Shultz 2012). Flowering heads are bell shaped, 4 to 5 mm wide, 3 to 4 mm high, with 8 to 20 florets per head. Flowers are wind pollinated and develop in small heads in spike like panicles that occur terminally on branches of current season. One small seed (approximately 1 mm in length) is produced from each flower, with each plant potentially producing thousands of seeds. Individuals can start producing seed at 4 years of age (Romo and Grilz 2002). A transparent gelatinous envelope can develop around the seed upon contact with water, which may be an adaptation to enhance germination in adverse conditions by protecting the delicate embryo from desiccation (Clor et al. 1974, Harvey 1981, Kreitschitz and Valles 2007, Kreitschitz 2012). However, various factors including seed burial deep in the soil, seed predation, specific germination requirements, soil water limitations, competition, reduced seed-soil contact from litter build up, and adverse environmental conditions may limit reproduction of sagebrush from seed (Beetle 1960; Walton 1984, Romo and Grilz 2002).

Most sagebrush germination studies were conducted on Big sagebrush; limited information on seed germination patterns of a few sagebrush species may be broadly applicable to other species (Meyer et al. 1990, Meyer and Monsen 1991, 1992). Big sagebrush seeds require light (unknown exposure time or intensity) and are slow to germinate (Meyer 2008). Germination can increase when exposed to light and physical scarification of seed (Shepherd 1937, Goodwin 1956). Most shrubs, including Big sagebrush, require stratification or chilling for maximum germination (Stidham et al. 1980). Vegetative reproduction may be the primary means of Silver sagebrush establishment; approximately 63 % of plants arise from rhizomes and 37 % arise from seed (Wambolt et al. 1990). Rhizomes form a shallow complex network, with one parent plant being the source and connecting a series of sprouts (Wambolt et al. 1990). Most sprouts are found 50 to 100 cm from the parent, with few found within 50 cm (Wambolt et al. 1990).

Plants can grow up to 50 cm year^{-1} under moist conditions (McArthur and Taylor 2004). Silver sagebrush forms annual growth rings when secondary xylem forms concentric rings around the

stem during the growing season (Ferguson 1964). These rings are easily distinguished by a distinct cork layer. Often the decadent form of older stems results in an open pith. Subspecies of have reached 81 (*Artemisia tridentata* ssp. *vaseyana* (Rydb.) Beetle; Mountain big sagebrush), 75 (*Artemisia tridentata* ssp. *wyomingensis* Beetle & Young; Wyoming big sagebrush) and 55 (*Artemisia tridentata* ssp. *tridentata;* Basin big sagebrush) years of age (Perryman et al. 2001).

Silver sagebrush is commonly associated with Wheatgrass (*Agropyron* Gaertn.) – June grass (*Koeleria macrantha* (Ledeb.) J.A. Schultes) communities (Adams et al. 2004b). Silver sagebrush / Western wheatgrass (*Agropyron smithii* (Rydb.) Á. Löve) / June grass and Silver sagebrush / Northern wheatgrass (*Agropyron dasystachyum* (Hook.) Scribn. & J.G Sm.) / June grass are the two primary *Artemisia cana* communities in Saskatchewan Mixed Grasslands. These communities have 8 to 27 % bare soil, 35 to 45 % moss and lichen cover, and 50 to 69 % vegetation cover. Grass cover includes Western wheatgrass, June grass, Northern wheatgrass, Sandberg blue grass (*Poa sandbergii* J. Presl), Porcupine grass (*Hesperostipa spartea* (Trin.) Barkworth), Blue grass (*Poa* species), Blue grama grass (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths), and Plains reed grass (*Calamagrostis montanensis* Scribn. ex Vasey). Forb cover includes Common yarrow (*Achillea millefolium* L.), Everlasting species (*Antennaria* species), Pasture sage (*Artemisia frigida* Willd.), Prairie sage (*Artemisia ludoviciana* Nutt.), Golden bean (*Thermopsis rhombifolia* (Nutt. ex Pursh) Nutt. ex Richardson), Golden aster (*Heterotheca villosa* Pursh. Shinners), and Silky perennial lupine (*Lupinus sericeus* Pursh).

2.3. Disturbances In Sagebrush Grasslands

Anthropogenic activity is almost exclusively responsible for loss, fragmentation, and degradation of sagebrush habitat. Disturbance type can significantly influence cover, density, and height of sagebrush in the Mixed Grassland (Jones et al. 2005). Common anthropogenic disturbances in Saskatchewan include industrial activities, agriculture, wildfire, and construction of transportation corridors (Environment Canada 2014). Disturbance and degradation of sagebrush habitat caused by invasive species and climate change can be linked to anthropogenic activities. Saskatchewan and Alberta are rich in natural gas and oil resources and considerable industrial activities occur in the southern portion of these two provinces in arid grassland ecosystems. While oil and gas disturbances are relatively small, their impact on soil and plant communities, and subsequently wildlife, is potentially great. These disturbances reduce habitat connectivity on the landscape and can lead to introduction and spread of aggressive non-native plant species, which further degrade surrounding habitat (Braun et al. 2002, Gelbard and Belnap 2003).

Agricultural practices include cultivation, haying, grazing, and herbicide or pesticide application. Cultivation of sagebrush grasslands has led to abandonment of leks in Alberta and Saskatchewan (Dube 1993; Aldridge 1998, McAdam 2003). Reduction of habitat due to plowing can affect Sage-grouse populations as it exploits the flat terrain favoured by Sage-grouse for over winter sites. In a 202 km^2 study area in Montana, Sage-grouse populations decreased 73 % in 30 years, after plowing reduced Sage-grouse habitat by 16 % (Swenson et al. 1987). Cultivation has likely caused the geographical separation of Sage-grouse populations, resulting in genetically distinct populations (Bush et al. 2011). Silver sagebrush will decrease in abundance when subjected to heavy grazing (Adams et al. 2004a) and because it is relatively short in stature, there is also a high risk of livestock trampling. Heavy grazing can alter the plant-soil environment by increasing soil surface temperature and reducing soil water through reduction of litter (Adams et al. 2004b). When managed successfully, grazing can stimulate productivity of forbs that are important components of Sage-grouse diet. Thorpe and Goodwin (2003) found increased richness of forb species in grazed areas relative to ungrazed areas in Grasslands National Park.

Fire was historically common on the prairie landscape; today prescribed burns are often used to aid establishment of natural trajectories for plant community development and to manage non-native plant species. Maintaining fire intervals shorter than 50 years is critical to prevent woodland encroachment into prairie communities (Miller et al. 2011). Silver sagebrush is moderately resistant to fire and can resprout vigorously after a fire due to its complex rhizome system (Aldridge and Brigham 2002). White and Currie (1983) found this when burning occurred under favourable spring conditions, which reduced the Silver sagebrush kill rate. Wildfire frequency and size have increased significantly in many areas of the North American sagebrush shrubland since the 1980s, likely due to synergistic interactions with invading non-native grasses such as Cheatgrass (*Bromus tectorum* L.) (Balch et al. 2013). The positive feedback loop is known as the cheatgrass fire cycle and has been recognized as a primary mechanism altering sagebrush systems in the Great Basin (Chambers et al. 2014).

Coates et al. (2016) recommend strategically identifying areas for fire prevention versus restoration to improve Sage-grouse habitat. They suggest targeted management to accelerate sagebrush recovery in areas with moderate to high resilience to fire and resistance to Cheatgrass. When used as a restoration tool, burning can be conducted in spring or fall (Fischer et al. 1996, Pyle and Crawford 1996, Nelle et al. 2000). In general, fall burning will maintain plant composition and help remove litter, whereas spring burning can be used to alter plant community composition. Burning later in spring, when cool season grasses have established, can reduce the presence of

cool season invasive grasses, such as Cheatgrass, Smooth brome (*Bromus inermis* Leyss.), and Crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.). Fires that result in unburned islands of seed producing individuals can help colonize burned areas with rapid growth from seed and resprouting (Pausas et al. 2004).

Invasive and non-native species alter structure and dynamics of plant communities which can have significant impacts on habitat. In sagebrush grasslands, non-native species that aggressively invade native rangelands create monocultures of little use to native wildlife (Miller and Eddleman 2001, Rowland et al. 2006, Aldridge et al. 2008). These invasive, non-native grasses fill in bare patches of sagebrush habitat and provide more continuous cover than native perennial grasses associated with sagebrush, which can lead to increased occurrences of wildfire (Miller et al. 2011). In particular, cheatgrass is very competitive, making it difficult for new perennial grass and shrub seedlings to establish and native plant species to be restored.

Predictions for future climate of sagebrush grasslands include more variable and severe weather events, including drought and storms, higher temperatures and drier summer soils (Intergovernmental Panel on Climate Change 2014). In areas where three or more droughts occur per decade, Sage-grouse are more likely to be extirpated (Aldridge et al. 2008). In drought conditions, herbaceous cover at nests and availability of forbs and insects are reduced (Environment Canada 2014). Precipitation supports forb growth and boosts invertebrate abundance in upland mesic sites that are critical brood rearing habitat for Sage-grouse (Casazza et al. 2011). Subsequently, periods of increased precipitation increase Sage-grouse survival. However, heavy rainfall during egg laying or unseasonably cold temperatures with precipitation during the hatch period may result in nest failure (Wallestad 1975). McNeil et al. (2007) suggest the population decrease in Saskatchewan between 1999 and 2004 was in part due to increased frequency of cold and wet spring conditions.

The predictions for more variable and severe weather events which accompany climate change may increase the risk of extirpation of Sage-grouse, as the recovery time between severe weather events is reduced (Environment Canada 2014). Changes in climate will undoubtedly shift competitive advantage among plant species, with approximately 12 % of the current distribution of sagebrush predicted to be replaced by expansion of other woody vegetation for each 1 °C increase in temperature (Miller et al. 2011). Current climate models for southern SK predict a mean increase in annual temperature from 3.5 to 5.9 °C, with the number of days above 30 °C increasing from 22 to 38 (Prairie Climate Center 2019). Climate change could further complicate the ability to restore sagebrush habitats as sagebrush seedlings may be particularly susceptible

to climate influences on seedling recruitment. Perryman et al. (2001) found recruitment of Big sagebrush in semi-arid regions occurs in pulses consistent with favourable climate conditions. Gillespie and Loik (2004) found that an experimental pulse representing a 25 % increase in summer precipitation doubled transpiration of Big sagebrush seedlings. Although Maier et al. (2001) found Big sagebrush recruitment was greatest in years with above average winter precipitation following the first growing season, response of each Big sagebrush subspecies to precipitation patterns was variable.

2.4. Restoration Of Greater Sage-Grouse Habitat

Investigations of factors affecting sagebrush restoration are ongoing. Studies range from freezing tolerance of Silver sagebrush seedlings to large scale ecosystem experiments. At a large scale, many practices traditionally viewed as having a negative impact on sagebrush could be utilized in restoring these habitats if applied appropriately. Among the most important considerations for successful restoration of Sage-grouse habitat are establishing native grasses for visual cover from predators, establishing native forbs (which also facilitate insect abundance) to provide food source for nesting hens and newly hatched chicks, and establishing sagebrush in the appropriate amounts for lekking, nesting, brood rearing, or overwintering habitat. Limited resources can be maximized by identifying areas where restoration is likely to be most successful (seeded or planted vegetation established in desired amounts) and effective (will be used by Sage-grouse),

In North Dakota, recommendations for restoration of Sage-grouse habitat include use of at least one sagebrush species, and five species each of forbs and grasses; Wyoming recommends two shrub, four forb, two bunch grass, and one rhizomatous grass species (Dumroese et al. 2015). Arkle et al. (2014) concluded that re-establishing sagebrush cover to a level adequate for Sage-grouse residency will require more than 20 years with standard restoration methods. Avoiding Sage-grouse extirpation from Canada requires developing new and effective restoration methods that improve and accelerate sagebrush establishment, growth, and survival. It is unlikely that Sagebrush ecosystems can be restored to pre-settlement conditions, although enhancement and expansion of remaining sagebrush islands are possible. These remnants provide critical seed sources and habitat for endangered species and are thus valuable for restoration of adjacent areas. Where large areas of sagebrush are removed, natural re-establishment can take up to 50 years due to the absence of a long-lived seed bank, short dispersal distances, and ability to re-sprout from stumps or roots following disturbance (Jacobs et al. 2011). By seeding, outplanting, or using other introduction methods, vegetation establishment can be accelerated.

Acquiring the quantity and variety of seed desired for habitat restoration is often difficult, especially if seed must be wild collected or locally sourced. Non-locally sourced seeds are often avoided because they are assumed to produce inferior results to locally sourced seeds. Seed sourced from the site in which it will be sown is considered adapted to specific environmental conditions. However, obtaining locally sourced forb and grass seeds native to sagebrush habitats can be particularly difficult. Even when local seed sources are available, the quality and amount of seed available is often severely limited. Ecologically adapted varieties of seed (those bred without selection to improve characteristics such as drought resistance, height, etc.) are often favoured for use in restoration but have limited availability. Cultivars, seed from selectively breeding for a favourable genetic trait (e.g. drought resistance), are often avoided because their impacts on long term plant community development and on native species are unknown, and the perception is that they will outcompete native vegetation and be visually distinguishable from native varieties of vegetation. However, Jasper, Elk Island and Waterton Lakes National Parks have successfully used cultivars to restore vegetation without outcompeting existing or seeded native species (Pitchford 2000, Arychuck 2001, Naeth 2003, Stover et al. 2017).

In areas where there is existing sub-optimal habitat, many seeding techniques could be employed, with the exception of large, drill seeding equipment. Jacobs et al. (2011) recommend sagebrush be broadcast seeded followed by pressing, dragging or rolling, to improve seed-to-soil contact. Drill seeding can be employed in areas where sagebrush stands are not established, as soil disturbance is required for good seed-soil contact; this method may be most appropriate when establishing new communities on an already highly disturbed area (Dettweiler-Robinson et al. 2013). With drill seeding, seeds are buried at various depths; however, Silver sagebrush emergence is greatest from 2 to 5 mm depth, with no emergence below 25 mm (Romo and Grillz 2002). For Big sagebrush, seeding 0.11 to 0.22 kg ha^{-1} of pure live seed (PLS) is recommended to achieve adequate population establishment (Meyer 2008). Romo and Grilz (2002) found that Silver sagebrush establishment in southern Saskatchewan in research plots seeded at 20 g PLS m^{-2} had only 5.3 % of seedlings emerge even though germination in a laboratory was approximately 82 %. Over winter survival of those seedlings was 74 to 84 % and establishment was 96 to 98 %, resulting in 3.6 to 4.9 % of seeds establishing from broadcast application. Whether broadcast or drill seeded, establishment remains low. When Wyoming Big sagebrush was drill seeded at 11.2 g PLS ha^{-1} 2 % establishment resulted; when ground broadcast seeded at 22.4 g PLS ha^{-1} 6 % establishment resulted (Jacobs et al. 2011). When seeding rate was increased to 560.4 g PLS ha^{-1} and 1.12 kg PLS ha^{-1}, establishment was only 1 %.

Low establishment, from low germination and emergence can be overcome partially by seeding at the appropriate time, using seed with high purity that has been recently collected or stored appropriately for a short time. Sagebrush seed viability decreased after 2 to 3 years of storage under ambient conditions in a warehouse (Stevens et al. 1981, Jacobs et al. 2011). Storing seed in low humidity (6 to 8 %) and cool temperatures (less than 10 °C) can prolong seed viability (Meyer 2008; Jacobs et al. 2011). To maintain seed viability up to 5 years, Karrfalt and Shaw (2013) recommend cleaning to at least 80 % purity as soon as possible after collection, drying to 30 % relative humidity and storing at -8 °C or lower. Field germination is often much less than laboratory, and variation in climate of semiarid ecosystems can more strongly affect germination and establishment in some years than others (Dettweiler-Robinson et al. 2013). If conditions are not conducive for germination, seeds will lay dormant in the soil.

Seeding has generally resulted in poor establishment of sagebrush (Meyer 1990, Chambers et al. 1994, Romo and Grillz 2002); however, Shaw et al. (2015) suggest populations of sagebrush can be reliably established with seeding techniques if natural processes are mimicked. Locally sourced seeds are conditioned to local climate conditions and will germinate at the optimum time for the area, improving seedling survival. Seeding should occur in late fall or early winter when seeds naturally disperse and dormancy is regulated by appropriate light, moisture and temperature. Jacobs et al. (2011) recommend using recently collected, locally adapted seed, stored in cool, dry conditions for no more than 3 years; broadcast seeding in late fall at 0.22 to 0.55 kg PLS ha^{-1} onto a firm (but not compacted) seedbed with grazing deferred 2 to 5 years. Where creation of wildlife habitat is the primary objective, seeding sagebrush in a mixture of native forbs and bunch grasses is recommended. Huber-Sannwald and Pyke (2005) broadcast native seeds in dense Big sagebrush stands in Utah and found that neither shading nor root exclusion of adjacent vegetation negatively affected seedling survival of seeded native grass species.

Revegetation with outplanted seedlings will decrease risk of desiccation, wind and water erosion, and consumption by animals (Monsen and Stevens 2004). Outplanted seedlings can decrease erosion by reducing wind speed at the soil surface, diverting and slowing water flow, and quickly establishing roots to hold soil in place. Decreased risk of plant desiccation, and protection of seeds from wind and temperature fluctuations can be facilitated by outplanting. Growing seedlings to outplant can overcome low seed purity and germination, increasing vegetation establishment. Planting nursery stock may be more successful where soil disturbance is undesirable or direct seeding is not feasible or unlikely to succeed. Plants established in containers may produce less seed, have smaller above ground biomass and shallower root systems than those established

from seed (Jacobs et al. 2011). Although increasing container size increases the size of planting stock, it may not confer an advantage upon outplanting. Generally, stock is grown in conical 163.9 cm^3 containers that promote growth of deep-rooted seedlings. However, each greenhouse has its own specifications for growth conditions and seedling measures before outplanting. For example, at the Lucky Peak Forest Service Nursery (Boise, Idaho) Wyoming big sagebrush are grown in 103.2 cm^3 tubes and must be of 15.2 cm height, have 20.3 cm root length, and 0.2 cm stem caliper before planting (Shaw et al. 2015). Container stock can be grown in 1 year or less, and relative to broadcast or drill seeding, little seed is required to produce numerous plants.

Whether seeding or outplanting, establishment is greater when competition from grasses is reduced or removed. Planting technique is important for establishment, growth, and survival of outplanted seedlings. If they have been cold hardened into dormancy, they should be planted in early spring before native plants of the same species at the site break dormancy. If they are non-dormant, they must be planted after danger of frost. Exposed, dark, bare soil can cause increased soil surface temperatures which can damage the plant; temperatures above 54 °C are lethal to the phloem and cambial cells of plants (Shaw 2004). Therefore, soil should be compacted around the roots to avoid air pockets, then covered with a thin layer of light-coloured litter to reduce desiccation. Plants should be spaced based on restoration outcomes and tailored to habitat requirements. Wirth and Pyke (2011) recommend Big sagebrush be planted at 77 to 1,093 individuals ha^{-1}. Plants can be placed in appropriate microsites if outplanting by hand, or microsites can be produced by creating a shallow depression around the seedling to collect water. Outplanted, cold-hardened Wyoming big sagebrush had a 3-year post planting survival of 30 % (container stock) and 17 % (bare root stock); survival was enhanced by a hydrogel dip before planting but not by mycorrhizal amendments (Dettweiler-Robinson et al. 2013). On a Wyoming site, late spring planted seedling survival was 23 % over 5 years (Meikle 1999). Sagebrush seedlings will begin to flower at about 4 years of age, and grazing should be avoided until seeded or planted sagebrush is reproductive and can regenerate from grazing pressure.

Techniques for seeding, growing and planting sagebrush species continue to be developed and improved (Dumroese et al. 2015). An advantage to growing sagebrush in container stock is that growth media can be manipulated to influence seedling characteristics. Development of superior seedlings for planting during restoration could improve seedling establishment and survival. Use of plant parts, such as cuttings, could be investigated to develop revegetation techniques for sagebrush. Harvey (1981) successfully propagated plants through hardwood cuttings; after 17 weeks in culture, 87 % of cuttings had developed roots.

In North and South Dakota, Big sagebrush and Silver sagebrush density around active leks were 0.41 and 0.62 m^{-2} with 2.99 and 3.02 % canopy covers, respectively (Smith 2003). Sagebrush height, forb cover, and bare ground were significantly greater around active than inactive leks, suggesting balancing sagebrush density, height, forb cover, and bare ground are critical to successful restoration of Sage-grouse habitat. McAdam (2003) found mean density of mature Silver sagebrush in southwestern Saskatchewan was 0.16 m^{-2} at occupied and 0.12 m^{-2} at abandoned lek sites, with 1 to 189 Silver sagebrush plants in 5 m x 20 m quadrats. Although not linked to Sage-grouse habitat requirements, Romo and Grilz (2002) found stem densities reached 5 m^{-2} in natural Silver sagebrush in Saskatchewan. Optimal sagebrush density for Sage-grouse habitat restoration is influenced by factors such as land management, past disturbance, geographical location, and life stage of Sage-grouse. Therefore, when selecting optimal sagebrush density to restore sagebrush habitat, consideration must be given to a range of factors, including how to mitigate with the most impact.

3. SUMMARY OF KNOWLEDGE GAPS AND RESEARCH OBJECTIVES

The objective of this thesis is to develop best management practices and economical and ecologically effective methods to restore Silver sagebrush and the associated suite of native forbs and grasses in disturbed Sage-grouse habitat in southern Saskatchewan. Thesis chapters address the following knowledge gaps. Specific objectives for each chapter are detailed below.

Chapter II.

Sagebrush density and cover vary across the landscape. To restore sagebrush habitat, land reclamation practitioners must understand how individual size, age, and cover of Silver sagebrush relates to stand density, cover, and age in areas of specific grazing regimes. The objective of Chapter II was to develop a model to be used by restoration practitioners to calculate seeding or planting densities required to attain desired sagebrush cover within specific timeframes so habitat targets for Sage-grouse recovery can be achieved most effectively.

Chapter III.

Natural regeneration and broadcast seeding have been relatively unsuccessful methods to increase sagebrush cover over the landscape. Seed preparation and storage techniques such as cold storage and physical scarification could improve field germination with little effort. The objective of Chapter III was to assess seed preparation methods, including after-ripening and physical scarification, to increase *Artemisia cana* germination and decrease germination time.

Chapter IV.

Outplanting seedlings is often used in reclamation to increase plant cover and site biodiversity. Although outplanting may enhance plant survival and establishment compared to seeding, survival of outplanted *Artemisia* sp. seedlings is quite low. The objective of Chapter IV was to investigate whether nutrient loading *Artemisia cana* seedlings could increase internal nutrient content and improve performance once outplanted.

Chapter V.

Conventional revegetation techniques including broadcast seeding and outplanting, have varying levels of success depending on the ecosystem, intensity of degradation and current and past land management in the area. The objective of Chapter V was to investigate effects of land management on the efficacy of standard revegetation methods, including seeding and outplanting, and non-native vegetation control in areas identified as potential Sage-grouse habitat.

II. MODELLING *ARTEMISIA CANA* COVER AS A FUNCTION OF PLANT CANOPY COVER, DENSITY, AND AGE UNDER DIFFERENT GRAZING REGIMES

1. INTRODUCTION

Sagebrush (*Artemisia* L. [Asteraceae]) is one of the most common shrubs in North America and is a vital habitat component for many endangered grassland species (Meyer 2008). Sagebrush has been in decline since European settlement (c. 1850) and currently occupies less than 60 % of its historical range (McArthur and Stevens 2004). Loss, fragmentation, and degradation of sagebrush ecosystems due to anthropogenic activity have resulted in declining populations of sagebrush obligate species, many of which are at risk of extirpation from Canada. The Greater Sage-grouse (*Centrocercus urophasianus* Bonaparte [*Phasianidae*]) (hereafter Sage-grouse) is an iconic symbol of sagebrush habitat and is currently of prominent conservation concern. Its North American range has been reduced to 56 % of its pre-settlement state (Connelly et al. 2004) while the historic Canadian range has been reduced by 94 % and is currently less than 7,000 km^2 (Aldridge and Brigham 2003). Population declines of approximately 92 % over the last two decades (Environment Canada 2014) resulted in listing and protection of Sage-grouse under the Species at Risk Act (Environment Canada 2014). A recovery strategy for the species was developed; and among the strategies outlined, restoring sagebrush habitat was considered urgent and critical for population growth to occur (Lungle and Pruss 2008).

Research on sagebrush restoration is diverse, and ranges from small scale seed preparations to large scale ecosystem experiments. However, essential models that relate sagebrush density to cover are lacking. Vegetation targets are typically expressed in percent cover, while planting or seeding densities are used for restoration targets. For example, Sage-grouse has specific sagebrush requirements based on life stage, with as little as 5 % cover in leks, 15 % in brood rearing habitat, 30 % in nesting areas, and up to 50 % in winter habitat (Aldridge and Brigham 2002). These narrow ranges of sagebrush cover have been difficult to attain via restoration, thus a model that can accurately predict sagebrush cover based on planting and survival densities over time is critical for effective and successful restoration outcomes.

Silver sagebrush (*Artemisia cana* Pursh) is the second most widely distributed sagebrush species in North America (Connelly et al. 2004). Plains silver sagebrush (*Artemisia cana* ssp. *cana*; hereafter, *Artemisia cana*) is the only sagebrush species to occupy Canadian Sage-grouse range. Landscape distribution of *Artemisia cana* is patchy (Jones et al. 2005) with densities of 0.07 to

5.00 individuals m^{-2} reported in southwestern Saskatchewan and North Dakota (Hirsch 1985, Romo and Grilz 2002, McAdam 2003). Density and cover are rarely reported together. In south Dakota, *Artemisia cana* cover of 3.02 % corresponded with stem density of 0.62 m^{-2}, although age or mean size of individuals was not quantified (Smith 2003).

Age is expected to strongly influence density and cover of a planted (restored) sagebrush stand, with density of planted individuals decreasing due to mortality, while individual canopy cover is expected to increase with age. Although no studies currently relate sagebrush canopy cover and age, a strong relationship between *Artemisia tridentata* Nuttall (Big sagebrush) age and stem diameter was found (Perryman and Olson 2000, Landeen et al. 2019). Age-stem diameter correlations were found in many woody species (Brotherson et al. 1980, Brotherson et al. 1983, Hinchman and Birkeland 1995); other morphological traits, including canopy height, diameter (Crisp and Lange 1976), and bark thickness (Molina et al. 2016) have been used as age proxies.

The objective of this study was to develop a model to be used by restoration practitioners to predict post-restoration, sagebrush landscape cover to attain habitat targets within specific timeframes. The specific aims of this study were to: 1) model sagebrush morphological traits with plant age; 2) determine mortality rate and persistence of naturally occurring sagebrush; and 3) incorporate individual morphologic measurements, mortality rates, and density to model sagebrush landscape cover.

2. MATERIALS AND METHODS

2.1. Research Area

Our study was conducted in the Grasslands National Park, Saskatchewan, Canada. The regional ecosystem is mixed-grass prairie, dominated by *Artemisia cana* – Wheatgrass (*Agropyron* Gaertn.) – June grass (*Koeleria macrantha* (Ledeb.) J.A. Schultes) communities (Adams et al. 2004b). Soils are dominantly chernozemic, with patches of solonetz throughout, and have low soil water content during the growing season (Adams et al. 2004b). The climate is arid with an annual mean precipitation of 33 cm, received mostly as rainfall in May, June, and July (Environment Canada 2019). Daily June to August mean temperatures are 15 to 18 °C with extreme daily temperatures up to 41 °C (Environment Canada 2019).

The Park was established in 1981, with a total area of 900 km^2 divided into two blocks (West Block, East Block). In West Block, bison and cattle grazing occur in two distinct areas.

Approximately 17,800 ha have been grazed by 400 bison (± 100) since 2006 at a stocking density of 0.74 AUM ha^{-1}. Reported AUM (animal unit month) values were obtained from Grasslands National Park and calculated with herd age demographics, and one bison bull equivalent to 1.8 AUM, one bison cow equivalent to 1.5 AUM, and one bison yearling equivalent to 0.75 AUM. Before bison introduction, the area was grazed only by wild herbivores (mule deer, pronghorn antelope, etc.). Cattle grazing in the West Block is conducted through grazing leases and has occurred at stocking densities of 0.2 to 0.95 AUM ha^{-1} since the Park acquired the land in the 1980s. Park land was purchased without coercion from landowners when they were ready to sell; thus, each area has an unknown grazing history prior to the Park's acquisition. Cattle are generally on the land between April and October and are rotated through different areas throughout the year. In East Block, land was ungrazed since the 1980s, until cattle grazing was re-started in 2015 at stocking densities of approximately 0.45 AUM ha^{-1}.

Study sites were distributed throughout the 3 grazing areas (bison grazed, cattle grazed west block, cattle grazed east block) (Figure 2.1; Table S2.1). Within each grazing area, at least 3 sagebrush stands were identified for evaluation within each density range (0 to 0.25, 0.25 to 0.5, 0.5 to 0.75, 0.75 to 1.0, greater than 1.0 individuals m^{-1}). In no area did sagebrush density exceed 2.5 individuals m^{-1} and few areas had densities greater than 1.5 individuals m^{-1}. Assessments were made in June and July 2017 and 2018. In total, 48 stands were assessed (bison grazed n = 17, cattle grazed west n = 16, cattle grazed east n = 15) and 3,502 individuals were measured. Sites were located in lowland areas (Frenchman Valley in West Block and Horse Creek valley in East Block), with little or no slope, good range health, bare ground under 5 %, and no known prior disturbances (burning, mowing, plowing, etc.).

2.2. Sagebrush Measurement And Sampling

At each sampling site, a 10 m x 10 m plot was delineated around a representative portion of the sagebrush stand where cover and density were being assessed. Only individuals with stems originating inside plot boundaries were included in any measurements. Density was determined as equivalent to the number of individuals in the plot divided by 100 m^2. Cover was determined using the line intercept method, along 11 line transects, spaced at 1 m intervals, each 10 m long and running parallel through the plot (Figure 2.2). Total live sagebrush material that intersected each transect was divided by the length of the transect line, with spaces between foliage ≥ 3 cm excluded. The mean cover value from the 11 line transects was then used as a representative plot cover estimate.

For each individual sagebrush plant, largest stem diameter, crown diameter, and height were measured. Stem diameter was measured to the nearest mm with a caliper 5 cm from the ground surface. Crown diameter was measured to the nearest centimeter across the widest part of the crown. Height was measured to the nearest centimeter from the ground surface to tallest live plant part without elongating branches.

Within each plot, approximately 5 % of sagebrush plants were photographed from above to determine crown area, then destructively sampled for age determination. ImageJ image processing software (Laboratory for Optical and Computational Instrumentation, University of Wisconsin) was used to determine sagebrush canopy area. All sagebrush plants within 3 plots were photographed, so measurements of cover obtained from line intercepts could be compared to the sum of the canopy area of all plants in the plot. For age determination, stem cross sections were obtained by digging approximately 30 cm into the soil surrounding the stem(s). The root was cut as far below ground level as possible. In the laboratory, the stem was cut sequentially to obtain a cross section containing the pith and most annual growth rings (Ferguson 1964). The cross section was sanded using a palm sander with sequentially finer grit sandpaper (100 to 1200 grit) for each sanding. A high resolution scan of the cross section was made and used with Coorecorder (Cybis Elektronik, 2013) to determine age via ring count.

2.3. Statistics And Model Generation

Calculations and statistical analyses were conducted using the program R 3.4.1 (The R Foundation for Statistical Computing 2017) and CurveExpert Professional 2.6.5 (Daniel G Hyams, Hyams Development 2019). Over 90 linear and non-linear models were assessed to determine the best fit models for the relationships between stem dimeter and age, canopy cover and age, crown diameter and canopy cover, and stem diameter and canopy cover. Residuals, 95 % confidence interval, and Akaike information criterion (AIC) were used to select models with best fit using the fewest parameters. Analysis of covariance, at alpha 0.05, was used to elucidate differences in sagebrush height and cover between grazing areas, with age as the covariate.

Canopy cover for individual plants within each research plot was determined from photographs and/or crown diameter measurements. Canopy cover values for each plot were summed, then they were divided by plot area to determine plot cover. This value was compared to measured plot cover for each plot to determine if methods of determining cover (line-intercept or aerial photography) result in similar values or if discrepancies exist.

Equations that modelled plant morphological characteristics were integrated so that canopy cover was predicted by age or a morphological proxy for age (stem diameter). Predicted landscape cover (LC) was calculated as the product of mean canopy cover (CC) determined from morphological models, and from measured sagebrush density (D):

$LC = CC_M * D / 100$ [Eq. 1]

Predicted sagebrush cover values were compared to measured cover values and models were deemed acceptable if predicted values were within 10 % of the measured values, good if within 5 % of the measured values, and excellent if within 3 % of the measured values. Remaining sagebrush density (D_t, individuals m^{-2}) at time (t) was calculated as the product of remaining density of sagebrush (PD_t), using frequency data from each plot, and initial planting density (D_0, individuals m^{-2}):

$D_t = D_0 * PD_t$ [Eq. 2]

Integrating Eq. 1 and Eq 2. allowed predictions of sagebrush landscape cover (LC_t) contributed via outplanted individuals to be made:

$LC_t = CC_t * D_t / 100$ [Eq. 3]

3. RESULTS

3.1. Sagebrush Morphological Characteristics And Relationships

A significant linear relationship between sagebrush age and stem diameter was found ($F_{1,74} = 395.4$, $r^2 = 0.84$, $p < 0.001$) (Figure 2.3, Table 1). This enabled age predictions for individual plants not destructively sampled to be made from stem diameter measurements. Destructive sampling and age determination via ring count yielded sagebrush ages between 5 and 44 years. Ages predicted using the linear relationship between stem diameter and age were between 1 and 94 years. There were 17 individuals whose predicted ages were extrapolated beyond 44 years; 7 individuals were between 45 and 50 years, and only 5 individuals were predicted to be above 60 years. Mean sagebrush stand age was 2.5 to 13.1 years, with none of the grazing areas having more long-lived sagebrush than another.

Maximum sagebrush height recorded in the field was 110 cm in the bison grazed area, 91 cm in cattle grazed areas of East Block, and 111 cm in cattle grazed areas of West Block. Analysis of covariance revealed no significant differences in sagebrush height due to grazing, although height did significantly increase with age ($F_{1,69} = 16.97$, $p < 0.001$) (Figure 2.4). A significant linear

relationship between height and stem diameter was found ($p < 0.001$, Bison $F_{1,1218} = 1989$, Cattle East $F_{1,990} = 2242$, Cattle West $F_{1,927} = 1842$) (Table 1).

The largest canopy cover measured was 11,474 cm^2 (37 years) in the bison grazed area, 7,836 cm^2 (27 years) in the cattle grazed areas of East Block, and 26,542 cm^2 (stem diameter 30 mm, estimated age 24 years) in the cattle grazed area of West Block. Canopy cover was strongly correlated to canopy diameter with the relationship best described by a rational model (Table 1). Canopy diameter was moderately linearly correlated to stem diameter (Table 1) which did not allow accurate canopy cover estimates through incorporation of equations describing stem diameter - canopy diameter - canopy cover relationships. Canopy cover was moderately correlated to stem diameter and age in the bison and cattle east areas; in the cattle west area, canopy cover was only weakly correlated to stem diameter and age (Table 1). The reciprocal models relating age to canopy cover yielded maximum canopy cover of 3,800 cm^2 (at 38 years) in bison grazed areas, 2,700 cm^2 (at 21 years) in cattle grazed areas of East Block, and 1,150 cm^2 (at 12 years) in cattle grazed areas of West Block. Rational models describing canopy cover – stem diameter, and canopy cover – age (estimated from stem diameter), yielded greater maximum canopy cover than the reciprocal quadratic model described previously (Figure 2.5). Although not statistically tested, sagebrush in cattle grazed areas of West Block had more irregularly shaped canopies and developed open canopies at a younger age than in bison or cattle grazed areas of East Block (Figure 2.6).

3.2. Predicting Landscape Cover From Morphological Characteristics

In bison grazed and cattle grazed West Block areas, the most accurate predicted sagebrush plot cover values calculated by Eq. 1 were obtained by calculating mean canopy cover using the reciprocal quadratic relationship between canopy cover and age (with age determined from mean stem diameter of the sagebrush stand). Using this method of determining canopy cover, 100 % of predicted sagebrush cover values were within 10 % of the measured value, 89 % within 5 % of the measured value, and 71 % within 3 % of the measured value in bison grazed areas; in cattle grazed areas of West Block 100 % of predicted sagebrush cover values were within 3 % of the measured value. In cattle grazed areas of East Block, using the rational model for canopy cover and age (determined by stem diameter) best predicted measured plot cover; 100 % of predicted sagebrush cover values were within 3 % of the measured value.

The age demographic of the average sagebrush stand was left-skewed, with 40 % of individuals aged 1 to 3 years, 52 % 4 to 20 years, and 8 % over 20 years (Figure 2.7). Using age frequencies

from measured sagebrush stands and a 1-year post-outplanting seedling survival rate of 70 % (Naeth et al. 2019), percent remaining sagebrush density (PD) was expressed as a logistic model (Figure 2.8). Sagebrush density (D_t) at time (t) can therefore be expressed as:

$$D_t = - 2.89 * D_0 / (1 – 1.30 * \exp^{0.0528t}) \quad \text{[Eq 2.1]}$$

Integrating Eq. 2.1 with the canopy cover equations that best predicted landscape cover as per Eq. 1, and assuming initial seedling canopy cover of 100 cm^2 (Naeth et al. 2019), landscape sagebrush cover (LC) resulting from outplanted seedlings can be modelled as a function of time (t, years since planting) and planting density (D_0, individuals m^{-2}) (Figure 2.9):

Bison Grazed

$$LC = D_t * (150 + 4.71 * t / 1 – 0.0477 * t + 0.000679 * t^2) / 100 \quad \text{[Eq 3.1a]}$$

Cattle East

$$LC = D_t * (98.7 + 9.98 * t / 1 – 0.0638 * t + 0.00112 * t^2) / 100 \quad \text{[Eq 3.1b]}$$

Cattle Grazed West Block

$$LC = D_t * (1090 * \exp^{–(t – 14.1)\text{^}2 / 156}) / 100 \quad \text{[Eq 3.1c]}$$

Peak landscape cover is obtained 11 years after planting in cattle grazed areas of West Block and 27 years after planting in cattle grazed areas of East Block. In bison grazed areas individual canopy cover peaks after 37 years; paired with very high mortality rates and few individuals surviving up to 40 years, landscape cover in bison areas is greatest immediately after planting.

3.3. Discrepancies In Cover Based On Measurement Method

Sagebrush cover of research plots measured by line intercept (C_{LI}) were 0.11 to 17.1 %. In three plots, all individuals were photographed to determine canopy cover; plot cover obtained from canopy covers determined from photographs were not significantly different from plot cover obtained from canopy covers determined from the rational and reciprocal quadratic models (C_{CC}) ($F_{1,5} = 0.18$, $p = 0.70$). Plot C_{CC} was significantly greater than C_{LI} ($p < 0.001$). Plot C_{CC} can be modelled as a linear function of C_{LI}, with significant differences in slopes of these lines ($F_{3,44} = 138$, $r^2 = 0.90$, $p < 0.001$; Figure 2.10).

4. DISCUSSION

Strong relationships between sagebrush morphological characteristics enabled accurate landscape cover to be modeled with stand density and age, therefore specific Sage-grouse

habitats could be targeted in restoration. Despite *Artemisia cana* having multiple stems, correlated age and largest stem diameter is consistent with studies by Perryman and Olson (2000) and Landeen et al. (2019) on *Artemisia tridentata*. Using largest stem diameter as a proxy for age allowed modelling of other morphological characteristics of sagebrush for all 3,502 measured individuals. Landeen et al. (2019) suggest using stem diameter to determine age of a single individual is not recommended when accurate age estimates are required, as stem diameter variability of equivalent aged plants is large. However, based on model prediction of landscape cover, using mean stem diameter for a sagebrush group or stand can be appropriate and accurate.

Prior to this study, maximum age of naturally occurring *Artemisia cana* plants in southwestern Montana was reported as 31 years (Harvey 1981). Wambolt et al. (1990) reported mean age of *Artemisia cana* as 6.9 years ± 3.1 and concluded its life span was significantly shorter than that of *Artemisia tridentata,* likely due to primarily asexual reproduction. Reported ages of subspecies of *Artemisia tridentata* in Wyoming were up to 81 (ssp. *vaseyana* (Rydb.) Beetle), 75 (ssp. w*yomingensis* Beetle & Young), and 55 years (ssp. *tridentata*) (Perryman et al. 2001). Our results suggest *Artemisia cana* is much longer lived (maximum ring count age of 44 years, greatest predicted age based on age to stem diameter correlation of 94 years). Longer-lived individuals imply that less management will be required over time to sustain optimal habitat cover, as sagebrush will remain on the landscape longer. Larger individuals may contribute more to reproduction through extensive and persistent rhizome systems (Wambolt et al. 1990). Rhizomatous reproduction of *Artemisia cana* accounts for approximately 63 % of individuals, with reproduction occurring as soon as 3 years of age, and one rhizome capable of sprouting 52 individuals (Wambolt et al. 1990).

Our model outputs for predicted sagebrush cover are likely conservative, as estimates are for planted individuals and do not account for subsequent increases and maintenance of cover through reproduction. With adequate environmental conditions, planted individual plant reproduction could offset cover loss from planted individual mortality. We were unable to sample a large number of individuals whose stem diameter was greater than 55 mm because few existed on the landscape, and we were wary about destructively harvesting individuals who may be contributing disproportionately to reproduction. Therefore, extrapolation of the linear relationship of stem diameter and age past 44 years should be applied with discretion. Because so few individuals of this stem diameter exist on the landscape, we remain confident that our models

were not heavily impacted by possible issues with extrapolating this linear relationship past measured field values.

Although we were able to verify predicted landscape cover values from those measured in natural systems, our models of landscape cover for planted individuals cannot be validated at this time. There are no known long term (10+ years) studies of outplanted seedlings, with only 1 to 5-year post-outplanting survival of sagebrush seedlings reported (Meikle 1999, Dettweiler-Robinson 2013). Such studies would help to estimate mortality and persistence of outplanted individuals, which is expected to be different from natural systems. As data become available from long term studies, our model can be modified to better reflect mortality for outplanted seedlings.

Model outputs may also vary with factors that affect sagebrush growth, including differences in climate (now and future, including prolonged drought or precipitation pulses) (Gillespie and Loik 2004), position on slopes, site topography (Harvey 1981), competition (Schuman et al. 1998, Boyd and Svejcar 2011, Newhall et al. 2011, McAdoo et al. 2013), and soil type. Although we sampled various plant sizes and ages in areas with a gradient of densities and cover, other parameters which may have strengthened our models' predictive capabilities were not included (soil type, soil water holding capacity, growing season precipitation and temperature, etc.). Despite this, we obtained relatively accurate estimates of sagebrush cover using morphological characteristics, age, and density. This indicates that models are robust for lowland areas with good range health and could potentially be tailored for use throughout *Artemisia cana* dominated habitats.

Previous studies demonstrated that cover from visual assessments (quadrats) and line intercept methods can vary considerably (Connelly et al. 2003, Wambolt et al. 2006), with discrepancies of sagebrush cover up to 21 % (13.0 and 34 % for one area) (Wambolt et al. 2006). Using quadrats, where canopy cover is considered the surface area over which a plant has influence, can often over-estimate cover values (Connelly et al. 2003). Although we assessed sagebrush foliar cover by photographs to be as consistent with line intercept evaluations as possible, our discrepancies were also very large (up to 15 % difference from photographs than line intercept). As aerial photography through drone use is increasingly utilized to map and quantify sagebrush habitat, additional variation in reported cover in the literature should be expected. This could be an issue for Sage-grouse as small differences in sagebrush cover have ecological significance and could be problematic if inconsistent cover values are used for management recommendations. For example, 15 % sagebrush cover would classify an area as brood rearing habitat, while 30 % would qualify as nesting habitat. Sagebrush distribution is naturally patchy, so photographic methods of determining sagebrush cover could give a better representation of sagebrush cover

on the landscape, and aid in determining where transect lines should be placed to obtain the most accurate, and consistent, cover values. Our results highlight the need for consistency in measurement method within a given area and supports the need for a standardized, consistent method to measure sagebrush cover over landscape scales.

Although grazing is often cited as beneficial to range health, high and concentrated stocking densities can reduce sagebrush cover and density, while low grazing intensities can cause litter accumulation (Adams et al. 2004a), which hinders seedling establishment and growth (Walton et al. 1986, Wambolt et al. 1989). We found heavy grazing in West Block may be negatively affecting sagebrush cover through development of open canopies in relatively young individuals (13 years). The resulting smaller maximum canopy area per plant necessitates a greater number of individuals to achieve the same habitat cover targets, with more intensive management required (reseeding or replanting, rest from grazing, rotational grazing, exclusion). If seedlings are planted into areas of heavy grazing, the limited number of years that sagebrush can accumulate canopy size in combination with relatively small maximum canopy area, will limit what is achievable through restoration. In contrast, light cattle grazing interspersed with rest periods, allow sagebrush to develop large canopies and sustain landscape cover for several years longer. Although stocking densities of bison were within recommended ranges (0.37 to 0.61 AUM ha^{-1}) for *Artemisia cana – Agropyron* sp. communities (Adams et al. 2013), almost 20 years without grazing prior to bison re-introduction allowed significant litter accumulation, which likely contributed to the advanced age (approximately 38 years) at which sagebrush in this area developed maximum canopy cover. Considering approximately 8 % of individuals survive past 20 years, low projections for landscape cover in bison grazed areas are not unexpected. However, since excess litter can be removed during planting, we expect growth of outplanted seedlings not to be thus hindered, and for their growth to mirror that of sagebrush grown in lightly cattle grazed areas.

Landscape cover projections indicate planting density and site selection considerably affect expected sagebrush cover. Depending on timelines and resource availability, planting into the appropriate site could significantly improve restoration outcomes. Although planting into areas with light cattle grazing will result in greater landscape cover overall, by planting into more heavily grazed areas, sagebrush cover could substantially increase within a 10 year period, which may be imperative given current projections for Sage-grouse population in Canada.

Models of sagebrush distributions and growth are essential as the need for prairie habitat restoration grows. Prior to this study, maximum canopy cover of sagebrush, and age at which it was achieved, were unknown and estimated for restoration purposes. Sagebrush persistence and

long-term mortality rates were also unknown. This study provides essential information for successful restoration of *Artemisia cana*, that has been absent from the scientific literature. We obtained highly accurate estimates of sagebrush landscape cover using sagebrush morphological characteristics, age, and density that allowed projections of landscape cover post-outplanting to be made. Our models enable restoration practitioners to calculate seeding or planting densities required to obtain desired sagebrush cover values within a specific time frame, so habitat targets for Sage-grouse recovery can be attained most effectively.

5. CONCLUSIONS

Models of sagebrush distributions and growth are essential as the need for prairie habitat restoration grows. Prior to this study, maximum canopy cover of sagebrush, and age at which it was achieved, were unknown and estimated for restoration purposes. Sagebrush persistence and long-term mortality rates were also unknown. This study provides essential information for successful restoration of *Artemisia cana*, that has been absent from the scientific literature. We obtained highly accurate estimates of sagebrush landscape cover using sagebrush morphological characteristics, age, and density that allowed projections of landscape cover post-outplanting to be made. We found a strong relationship between stem diameter and age, which will allow age estimations of *Artemisia cana* to be made non-destructively. Our models enable restoration practitioners to calculate the seeding or planting densities required to obtain desired sagebrush cover values within a specific time frame, so habitat targets for Sage-grouse recovery can be attained most effectively.

Table 2.1. *Artemisia cana* morphology relationships; age, canopy cover, canopy diameter, height, and stem diameter.

Area	Relationship	Model	Equation	SE	df	r^2
All	A – SD	Linear	$A_{SD} = 0.786 * SD$	6.67	1, 112	0.79
Bison	H – SD	Linear	$H = 1.6 * SD + 16.5$	13.3	2,1218	0.62
	CD – SD	Linear	$CD = 2.14 * SD + 4.31$	19.7	2,1218	0.58
	CC – CD	Rational	$CC = 3.79E^{2} + 9.10E^{-1} * CD / 1 - 1.69E^{-2} * CD + 7.41E^{-5} * CD^{2}$	621.2	4, 27	0.94
	CC – SD	Rational	$CC = 2.06E^{2} + 1.19 * SD / 1 - 5.50E^{-2} * SD + 8.00E^{-4} * SD^{2}$	1082.3	4, 47	0.55
	CC – A	Reciprocal Quadratic	$CC = 1 / 2.68E^{-3} - 1.23E^{-4} * A + 1.57E^{-6} * A^{2}$	1023.8	3, 29	0.42
	CC – A_{SD}	Rational	$CC = 2.10E^{2} + 1.27 * A / 1 - 6.99E^{-2} * A + 1.29E^{-3} * A^{2}$	1093.5	4, 46	0.55
Cattle East	H – SD	Linear	$H = 1.3 * SD + 15.7$	8.4	2, 990	0.69
	CD – SD	Linear	$CD = 2.06 * SD + 0.56$	12.8	2,990	0.72
	CC – CD	Rational	$CC = -5.06E^{-2} + 7.82 * CD / 1 - 2.34E^{-2} * CD + 1.56E^{-4} * CD^{2}$	223.9	4, 24	0.97
	CC – SD	Rational	$CC = 97.4 + 7.86 * SD / 1 - 5.02E^{-2} * SD + 6.9E^{-4} * SD^{2}$	745.7	4, 29	0.67
	CC – A	Reciprocal Quadratic	$CC = 1 / (4.44E^{-3} - 3.95E^{-4} * A + 9.59E^{-6} * A^{2})$	1111.8	3, 24	0.33
	CC – A_{SD}	Rational	$CC = 97.4 + 10.0 * A / 1 - 6.38E^{-2} * A + 1.12E^{-3} * A^{2}$	745.7	4, 29	0.67
Cattle West	H – SD	Linear	$H = 1.9 * SD + 11.8$	13.1	2, 927	0.66
	CD – SD	Linear	$CD = 2.56 * SD + 6.33$	21.1	2, 927	0.58
	CC – CD	Reciprocal Quadratic	$CC = CD / (6.16E^{-2} - 6.40E^{-4} * CD + 1.86E^{-6} * CD^{2})$	420.0	3, 16	0.96
	CC – SD	Rational	$CC = 3.59E^{2} + 9.06 * SD / 1 - 6.85E^{-2} * SD + 1.27E^{-3} * SD^{2}$	3184.7	4, 71	0.37
	CC – A	Reciprocal Quadratic	$CC = 1 / (2.82E^{-3} - 2.78E^{-4} * A + 9.92E^{-6} * A^{2})$	784.0	3, 13	0.11
	CC – A_{SD}	Rational	$CC = 4.25E^{2} - 7.42 * A / 1 - 9.72E^{-2} * A + 2.45E^{-3} * A^{2}$	2008.9	4, 70	0.49

A = age, CC = canopy cover, CD = canopy diameter, H = height, SD = stem diameter.

Models presented were best fit based on Akaike information criterion, with SE, df and r^2 indicating standard error, degrees of freedom, and coefficient of determination, respectively.

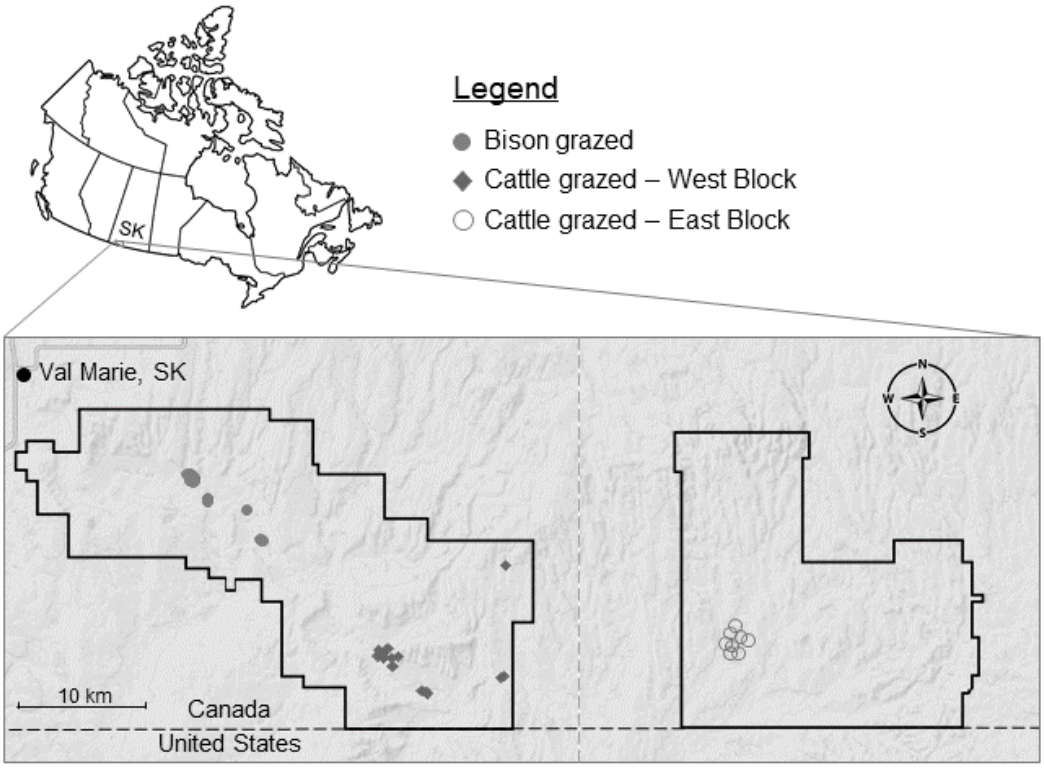

Figure 2.1. Locations of research plots distributed through cattle grazed and bison grazed areas of East and West Blocks of Grasslands National Park, Saskatchewan, Canada. East and West Block are separated by approximately 25 km, although the distance is reduced in this image.

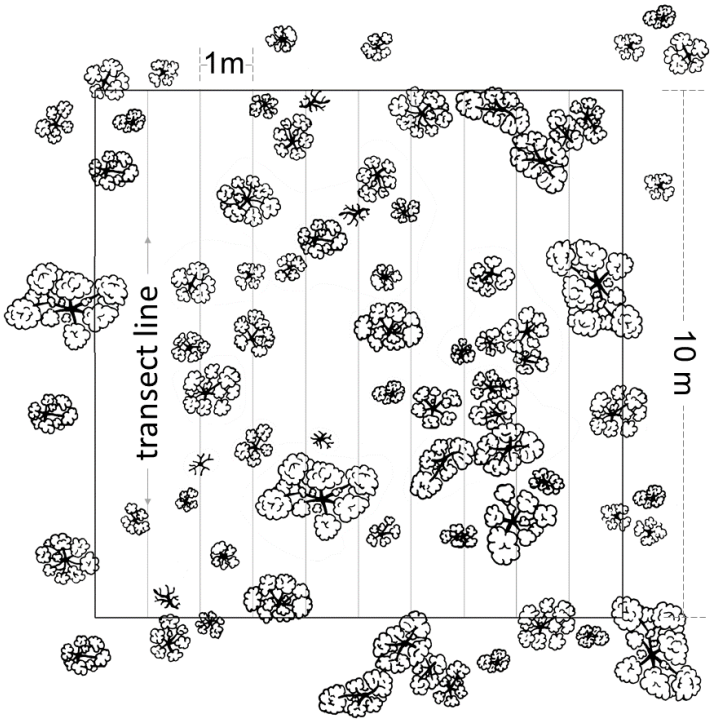

Figure 2.2. Schematic of a 10 m x 10 m plot, showing the 11 transect lines of 10 m length used to determine sagebrush cover over the entire plot, running parallel and at 1 m intervals.

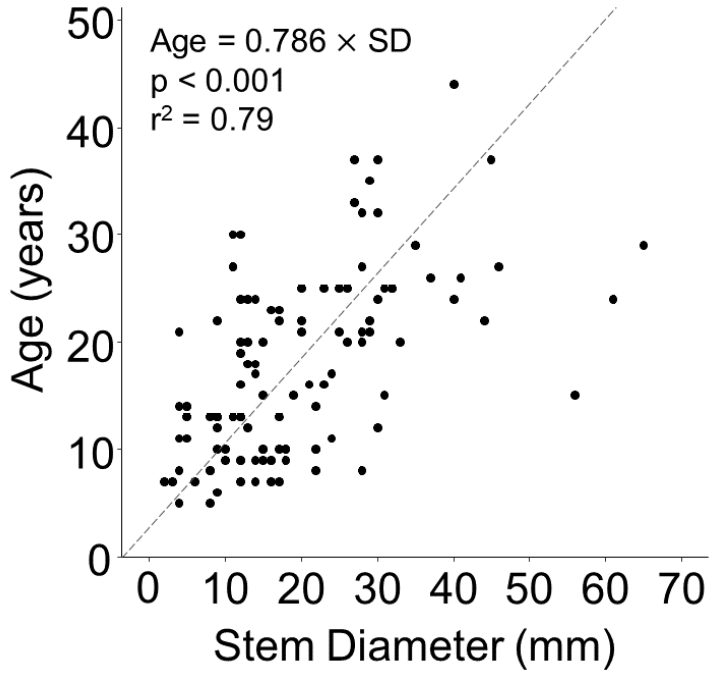

Figure 2.3. Linear regression model of age as a function of stem diameter (SD) across all grazing areas, n = 113.

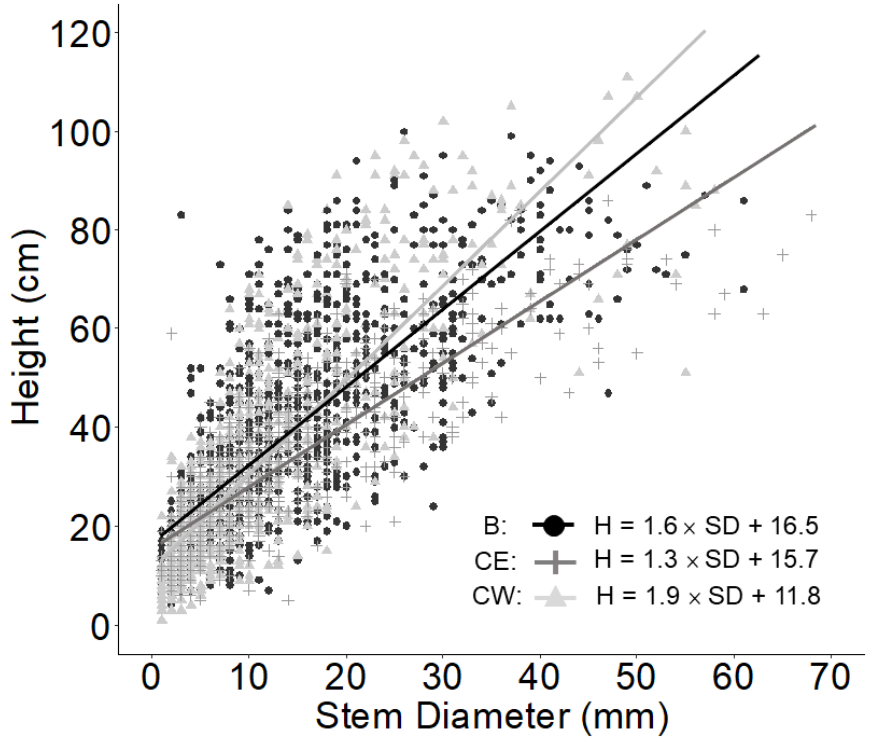

Figure 2.4. Sagebrush height as a function of stem diameter (proxy for age) in bison grazed (B), cattle grazed areas of East Block (CE), and cattle grazed areas of West Block (CW). n = 3136.

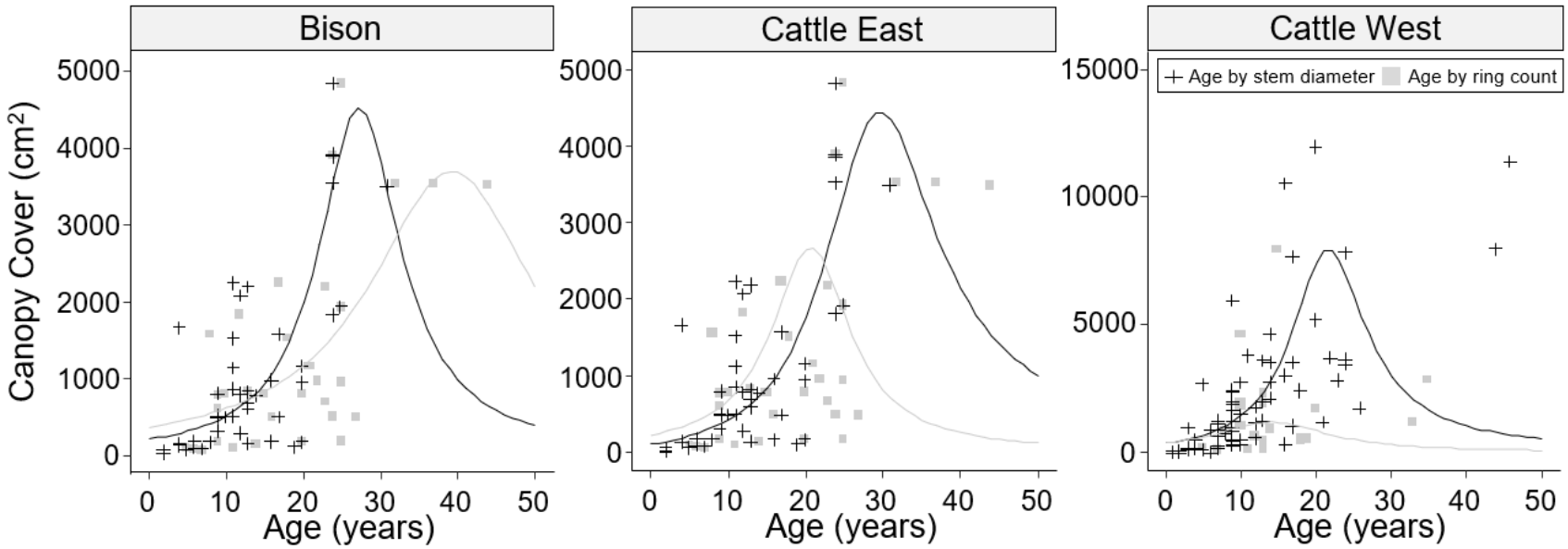

Figure 2.5. Models of canopy cover as a function of age. Canopy cover estimates differ significantly based on method to age sagebrush (predicted by stem diameter, or via ring count). (A) bison grazed area, n = 85; (B) cattle grazed area in East Block, n = 62; (C) cattle grazed area in West Block, n = 99.

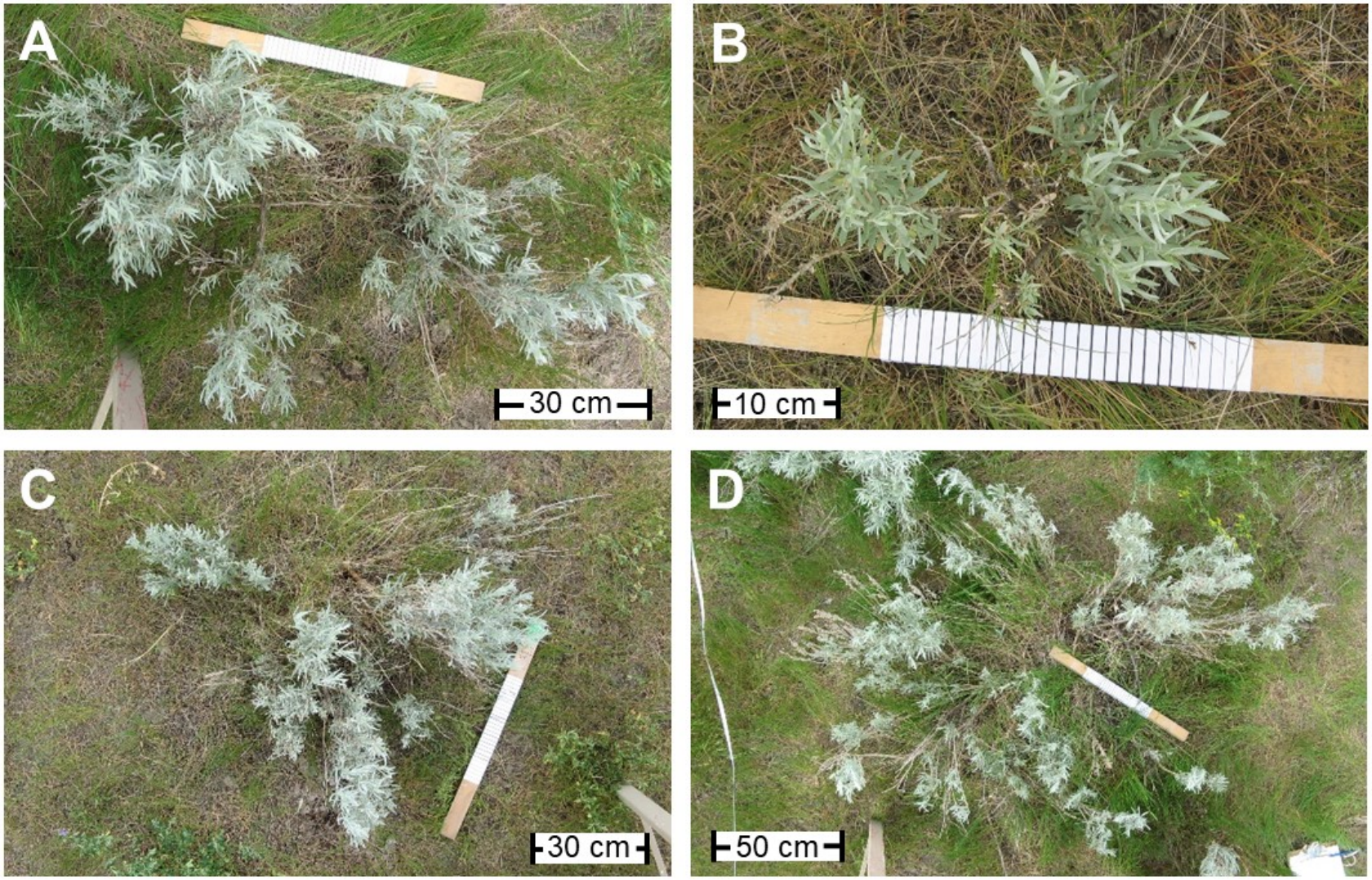

Figure 2.6. Examples of sagebrush with open canopies, measured within cattle grazed areas of West Block. Ages were predicted from stem diameter measurements as (A) 11 years, (B) 9 years, (C) 10 years, and (D) 46 years.

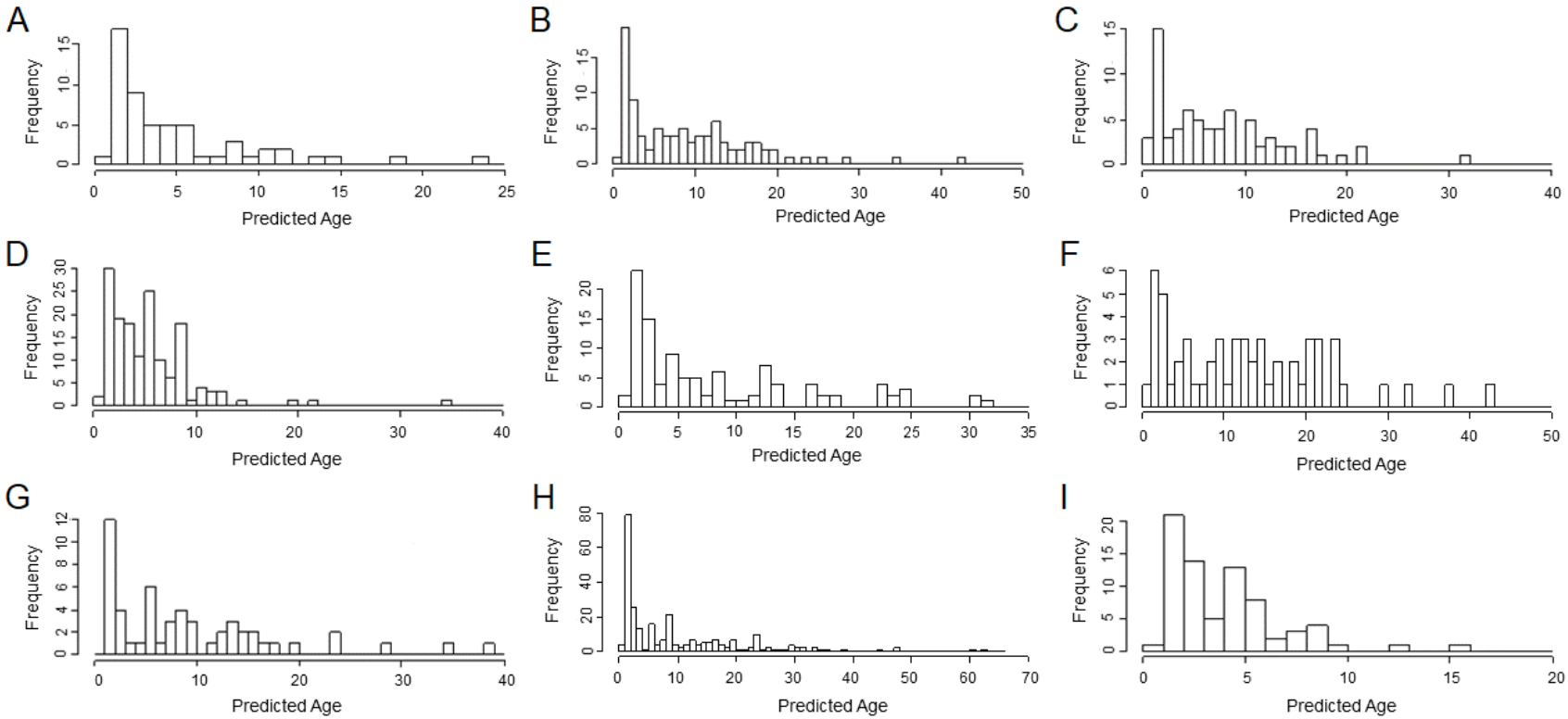

Figure 2.7. Histograms of typical sagebrush age distribution in plots in bison grazed (A, C, E, F, G, H) and cattle grazed (B, D, I) areas of West and East Blocks of Grasslands National Park.

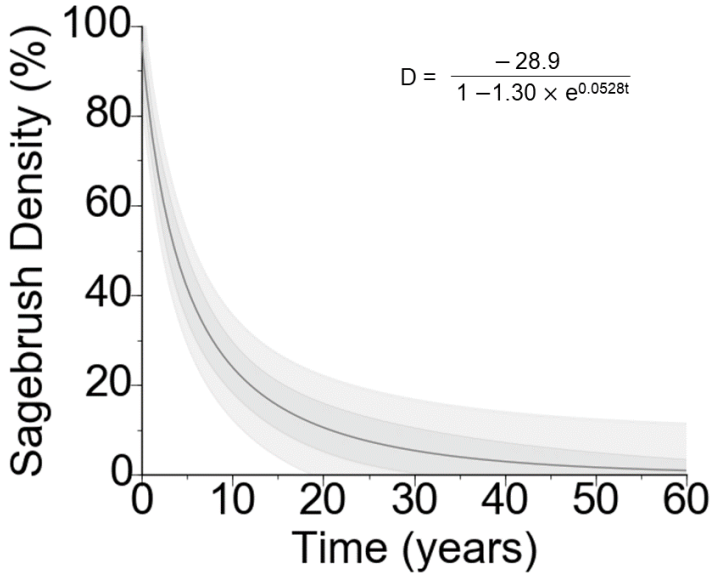

Figure 2.8. Logistic model showing percent density of sagebrush over time, given an initial stand density of 100 % at t = 0 (reproduction not occurring in this scenario). 95 % confidence interval shown as the darker gray shaded area, n = 62.

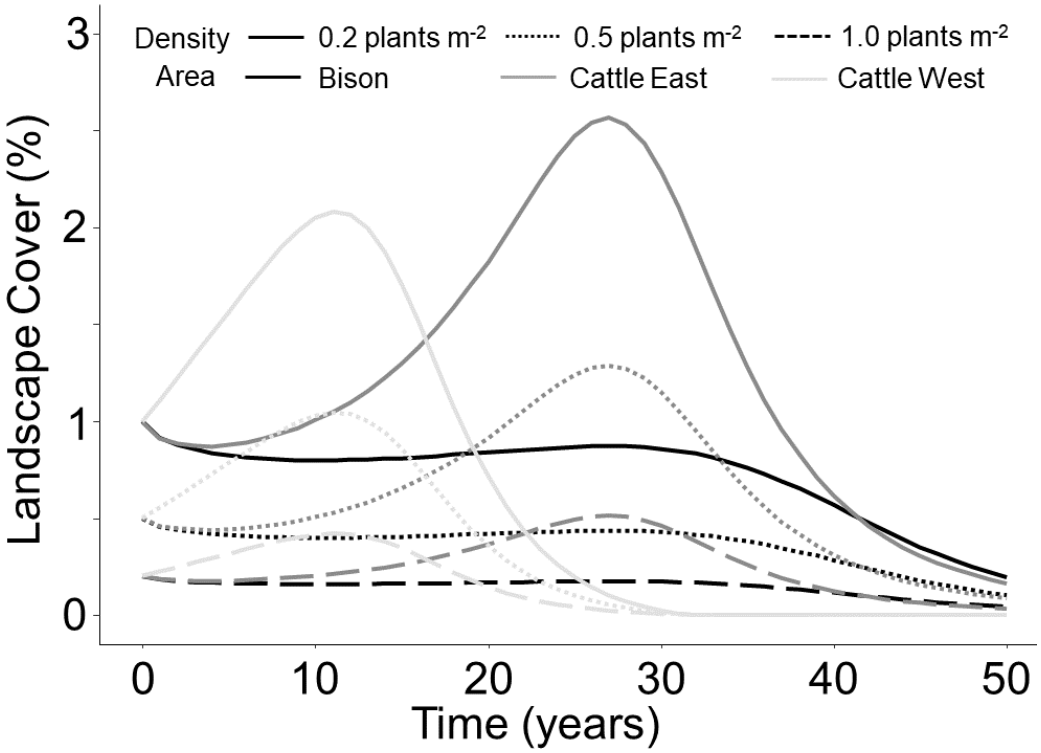

Figure 2.9. Projections for sagebrush cover contributed by planted individuals, modelled over time, at three planting densities, for bison and cattle grazed areas of Grasslands National Park. Maximum cover is obtained after 38 years in bison areas, 21 years in cattle east areas, and 13 years in cattle west areas. The model incorporates mortality of planted sagebrush but does not account for reproduction.

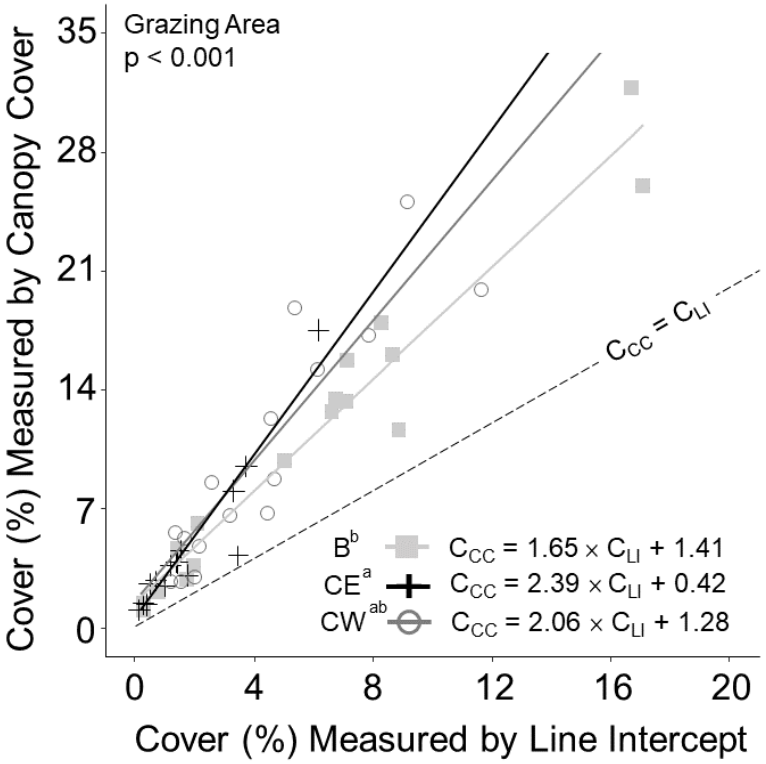

Figure 2.10. Sagebrush cover determined by summing canopy covers (C_{CC}) as a function of cover measured by line intercept method (C_{LI}) in bison grazed (B), cattle grazed areas of East Block (CE), and cattle grazed areas of West Block (CW). Linear equations are presented, with different letters denoting significant differences in slope.

SUPPLEMENTAL INFORMATION

Table S2.1. Location (UTM, 13U zone) of sagebrush stands measured within each grazing area and density category.

Density (individuals m^{-1})	Bison Grazed	Cattle Grazed - West	Cattle Grazed - East
0 - 0.25	313915 E 5449813 N 313823 E 5449798 N 317976 E 5447469 N	328352 E 5436035 N 328341 E 5436041 N 328276 E 5435901 N 328282 E 5435886 N	370048 E 5434259 N 369885 E 5435205 N 369766 E 5436101 N
0.25 - 0.5	313956 E 5449788 N 313942 E 5449854 N 313930 E 5449831 N 315074 E 5448221 N	328334 E 5436031 N 328310 E 5435993 N 337696 E 5442574 N	369741 E 5436066 N 369726 E 5436085 N 369845 E 5435614 N
0.5 - 0.75	313794 E 5450056 N 313954 E 5449773 N 315062 E 5448191 N	328333 E 5436019 N 337223 E 5434103 N 337224 E 5434112 N	370069 E 5434260 N 369881 E 5435260 N 369773 E 5435573 N
0.75 - 1.0	313949 E 5449792 N 315056 E 5448209 N 313808 E 5450054 N	331304 E 5433135 N 328557 E 5435768 N 328602 E 5435774 N	369873 E 5435237 N 370133 E 5434282 N 369854 E 5435628 N
> 1.0	313780 E 5450065 N 313841 E 5450074 N 319158 E 5444958 N 319133 E 5444937 N	331274 E 5433156 N 331258 E 5433158 N 328566 E 5435768 N	369992 E 5434238 N 369852 E 5435240 N 369778 E 5435546 N

n = 17 for bison grazed areas, n = 16 for cattle grazed areas of West Block, n = 15 for cattle grazed areas of East Block

III. STORAGE TIME, LIGHT EXPOSURE, AND PHYSICAL SCARIFICATION EFFECTS ON *ARTEMISIA CANA* SEED GERMINATION

1. INTRODUCTION

Sagebrush (*Artemisia* L. [Asteraceae]) is one of the most common shrubs in North America, with approximately 20 shrub species distributed throughout the prairie grassland and steppe ecoregions of the Great Basin (Meyer 2008). Big sagebrush (*Artemisia tridentata* Nutt.) is the most abundant sagebrush species, occupying approximately 600,000 km^2 of variable habitat (Meyer 2008). Silver sagebrush (*Artemisia cana* Pursh) is the second most widely distributed sagebrush species in North America, the dominant species in the Canadian range, and occupies approximately 140,000 km^2 (Connelly et al. 2004).

Over the last 100 years, much sagebrush habitat within temperate grasslands has been lost, fragmented, or degraded, resulting in declining populations of sagebrush obligate species. The clearest example of this is the Greater Sage-grouse (*Centrocercus urophasianus* Bonaparte [Phasianidae]), a species of prominent conservation concern. Its North American range has been reduced to 56 % of its pre-settlement state (Connelly et al. 2004); in Canada the historic range has been reduced by 94 % and is currently less than 7,000 km^2 (Aldridge and Brigham 2003). These range declines have led to extreme population declines. In Canada Greater Sage-grouse populations declined by 88 % between 1988 and 2006 (Environment Canada 2014). It was subsequently designated endangered by the Committee on the Status of Endangered Wildlife in Canada (1998) and listed as endangered by the Species at Risk Act (2003). The Recovery Strategy for the Greater Sage-grouse in Canada suggests that low quality sagebrush habitat could support population growth through restoration to optimal conditions for the species (Environment Canada 2014). Greater Sage-grouse has been identified as an umbrella species, thus habitat restoration for grouse will simultaneously benefit other at risk species. In Canada, there are more than 50 at risk prairie species with sagebrush grasslands habitat requirements that could benefit from Greater Sage-grouse habitat restoration (Environment Canada 2019). Consequently, the need for sagebrush seed is great and will likely increase as restoration efforts expand.

Artemisia cana flowers between August and September. Achenes are approximately 2 mm long and ripen in October and November (McArthur and Taylor 2004). To collect even a small amount of seed requires considerable effort and cost and seed supply are limited. Compounding these difficulties, *Artemisia cana* has reportedly low seeding success with seedling emergence of 5 to

6 % under field conditions (Romo and Grillz 2002). Low germination and seed dormancy may be limiting to seeding outcomes and restoration success. Most germination studies of the *Artemisia* genus have been conducted on *Artemisia tridentata*, but information on seed germination patterns of a few *Artemisia* species may be broadly applicable to other species based on previous studies (Meyer et al. 1990, Meyer and Monsen 1991, 1992 and Meyer 2008). Generally, sagebrush seeds have relatively low dormancy at dispersal, but may have a strong light requirement and be slow to germinate (Meyer 2008). Exposure to light may increase germination of *Artemisia tridentata* seed, and physical scarification may immediately increase germination (Shepherd 1937 and Goodwin 1956). The pericarp may prevent or delay germination by mechanically restricting embryo expansion, by reducing rate of imbibition or by releasing chemical inhibitors of germination (Mekenian and Willemsen 1975, Cousens et al. 2010, Sperber et al. 2017). Dormancy and light requirement may be removed by a period of after ripening (dry storage) or moist chilling (stratification) (Meyer 2008, Baskin and Baskin 2014). *Artemisia cana* was immediately germinable but has greater germination if seed first experience a period of cold temperature (Eddleman 1977, Walton 1984). Environmental conditions at seed collection sites can influence germination characteristics; seed collected in areas having long, snowy winters can be more dormant, light requiring, and slower to germinate than seeds collected in areas with short, mild winters and hot dry springs (Meyer 2008).

The objective of this study was to assess seed preparation methods to increase *Artemisia cana* germination and decrease germination time. Seed preparation methods that improved germination of other *Artemisia* species were investigated, including after ripening and physical scarification. We hypothesized that *Artemisia cana* seed would exhibit a strong light requirement for germination and that after ripening and physical scarification would increase seed germination in dark conditions. To test this hypothesis we conducted a study to determine: (1) if after ripening and/or dry storage time affects germination of *Artemisia cana* in light and/or dark; (2) if physical scarification affects germination of *Artemisia cana* in light and/or dark; and (3) if seeds originating from different collection sites and macrohabitats within the same ecosystem and latitude have different responses to germination conditions and seed preparation.

2. MATERIALS AND METHODS

Artemisia cana seed was collected from Grasslands National Park, Saskatchewan (13U 313766 E 5450204 N) in November 2015 and 2016. Seed was collected at various locations throughout the

Park in November 2015 and from two locations in November 2016 (Table 3.1). A portion of seed collected in 2016 was separated by collection site so differences in germination could be analysed. Another portion of 2016 seed was combined with seed from other collection years so collection year differences in germination could be analysed. After collection, seeds were hand cleaned to approximately 15 % seed (by weight) and dry stored in paper bags for 4, 5, 6, 16, 17, and 18 months in a cold room or refrigerator (1 to 5 °C). Storage duration represented likely restoration scenarios where seed collected in late November would be used as early as late March (4 months storage), April (5 months storage), or May (6 months storage), or stored for multiple years. Scarification treatments were applied after dry storage (scarified, non-scarified) by lightly rubbing seeds for approximately 15 sec with 120 grit sandpaper.

Seeds were germinated with light exposure via indirect natural light (approximately 12 hr photoperiod; peak PPFD = 82 μmol m^{-2} s^{-1}), and in dark via blacked-out cupboards. Sagebrush seed was germinated for a minimum of 21 days at an ambient temperature of 18 to 20 °C, on a moist paper towel in a sealed, transparent petri dish (Meyer 2008). Paper towels were kept moist throughout the experiment with deionized water. For each combination of treatments (seed collection location and seed collection year x after ripening x physical scarification x light exposure) five replicates (petri dishes) of 10 seeds were germinated. Seeds were monitored daily; seeds that germinated were removed and noted, and germination was recorded when root and epicotyl had emerged from the seed. Non-germinated seed was not tested for viability; therefore results represent germinability x viability of sagebrush seed.

Calculations and statistical analyses were conducted using R 3.4.1. (The R Foundation for Statistical Computing 2017). Generalized linear models with four fixed factors were performed on untransformed data to determine effect of seed collection site or seed collection year (two levels: 2015, 2016 or 2016-A, 2016-B) x after ripening and dry storage (3 levels for 2015 seed; 6 levels for 2016 seed) x physical scarification (two levels) x light exposure (two levels) on germination (maximum germination and time to maximum germination). Maximum germination was considered the number of seeds that germinated within the 21 days of monitoring and was modeled with a binomial distribution (germinated vs non-germinated). Time to maximum germination was the days required for maximum germination to occur (e.g. 9 of 10 seeds germinated in the monitoring period and all 9 seeds germinated within 4 days of the start of the experiment) and was modeled with a Gaussian distribution. Least square means test with Tukey pairwise comparisons and an alpha of 0.05 was run post hoc to the general linear models running if a significant difference was detected at $p \leq 0.05$.

3. RESULTS

Over all treatments, time to maximum germination was 1 to 14 days, and maximum germination was 30 to 100 %. Of the seeds that germinated, 54 % germinated by day 3 of observation, 81 % by day 4, and 92 % by day 5. No seeds germinated after 14 days.

3.1. Seed Collected In 2015

The second order interactions of scarification with storage time and scarification with light had a significant effect on maximum germination of seed collected in 2015 (Figure 3.1). The third order interaction was not significant. In general, scarified seed had greater maximum germination than non-scarified seed ($p < 0.01$). This result was especially distinct in seed stored for 17 months, where scarification resulted in maximum germination, not significantly different from seed stored for 16 or 18 months. When scarified seed germinated in dark conditions, maximum germination was equivalent to seed (scarified and non-scarified) germinated in light, and significantly greater than non-scarified seed germinated in dark ($p < 0.03$). Second order interactions of scarification with storage time and storage time with light had a significant effect on time to maximum germination (Figure 3.2).

The third order interaction was not significant. Scarified seed had significantly shorter time to achieve maximum germination than non-scarified seed ($p < 0.001$). This result is especially distinct in seed stored for 18 months, where scarified seed had significantly shorter time to maximum germination than all other treatment combinations ($p < 0.05$). Even in the absence of light, scarified seed stored for 18 months had significantly shorter time to maximum germination than seed stored for fewer months and germinated in light ($p < 0.01$).

3.2. Seed Collected In 2016 (Composite Sample)

Maximum germination of seed collected in 2016 was not significantly affected by the main effects of the experiment, or by interactions of storage time, scarification, or light (Figure 3.1). Storage time and scarification both significantly affected time to maximum germination of seed collected in 2016 (Figure 3.2). Light, second order interactions, and third order interaction were not significant. Scarifying seed resulted in significantly shorter time to maximum germination ($p < 0.01$). Increasing storage time decreased time to maximum germination ($p < 0.05$). Time to maximum germination was significantly less when seed was stored for 16, 17, and 18 months (2.6 ± 0.2, 2.5 ± 0.2, 2.8 ± 0.3 days on average to reach maximum germination) than when seed

was stored for only 4, 5, and 6 months (3.9 ± 0.4, 4.6 ± 0.4, 3.8 ± 0.4 days on average to reach maximum germination).

3.3. Differences In Germination Due To Collection Year

The second order interaction of storage time with scarification was significant in predicting maximum germination when year was introduced into the general linear model (Figure 3.1). The main effects of year and light, and fourth order, third order, and other second order interactions were not significant. For seed collected in 2015 and 2016 and germinated in either light or dark conditions, scarification significantly increased maximum germination ($p < 0.05$), except for seed stored 16 months where maximum germination was greatest, and scarification had no effect. The fourth order interaction between year of collection, storage time, scarification, and light significantly affected time to maximum germination for seed collected in 2015 and 2016 ($p < 0.01$) (Figure 3.2). Storage time and scarification generally decreased time to maximum germination, while seed collected in 2015 had longer time to maximum germination. Depending on year, scarification and storage time, light had mixed results on time to maximum germination. Without scarification, light during germination generally decreased time to maximum germination (e.g. seed collected in 2015 or 2016, stored 17 months, non-scarified: light resulted in significantly shorter time to maximum germination). When seed was non-scarified, light during germination had no effect on time to maximum germination (e.g. for scarified seed stored 16 months and collected in 2016, light resulted in greater time to maximum germination; for scarified seed stored for 16 months and collected in 2015, light resulted in lower time to maximum germination).

3.4. Differences In Germination Due To Collection Site

The third order interaction of storage time with scarification with light was the only significant factor in predicting maximum germination when seed collection site was added to the general linear model (Figure 3.3). The main effect of collection site (or seed population), fourth order, and other third order interactions were not significant. Increasing storage time generally decreased maximum germination ($p < 0.01$), while scarification increased maximum germination ($p < 0.001$). Light significantly increased maximum germination for scarified seed but not for non-scarified seed. When seed was stored for 4 months, regardless of scarification or light conditions during germination, maximum germination was high. The fourth order interaction between seed collection site with storage time with scarification with light was significant in predicting maximum germination times for seed collected in 2016 (Figure 3.4). Storing seed for 5 months (rather than

4 or 6 months) significantly increased time to maximum germination of seed in both populations ($p < 0.05$) unless seed was scarified and germinated in light. Time to maximum germination was low regardless of scarification or light treatment for seed stored for 6 months; for seed stored for 4 months, scarification decreased time to maximum germination regardless of light condition. The two seed populations had similar response to storage time and scarification; however, germination in light always decreased time to maximum germination in population A, while light only decreased time to maximum germination for non-scarified seed in population B.

4. DISCUSSION

The rapid and relatively uniform germination of *Artemisia cana* seed we found was unexpected, as studies on *Artemisia tridentata* reported low germination and seed that germinated up to 56 days after exposure to moisture (Goodwin 1956, Meyer 1990, Meyer and Monsen 1992). High germination in our laboratory suggests low success of *Artemisia cana* seeding in the field is not due to germination limitations, but to limiting environmental factors, early seedling death (Eddleman 1979), seasonal climate conditions (Nosova 1973), soil water relationships, litter (Beetle 1960), soil crusting, and seed desiccation. To improve *Artemisia cana*, seeding outcomes future research on increasing establishment and growth of sagebrush would be beneficial.

That physical scarification negated light requirements for germination may be applied to seedling development in a greenhouse where rapid and uniform germination is desired, but seed desiccation is unlikely. Scarification is unlikely to improve sagebrush seeding outcomes. In the field, pericarp removal may disrupt mechanisms for germination timing and could increase seed desiccation risk. If fall seeding, retaining mechanisms to inhibit germination in the dark could be critical so seeds do not germinate until snow has melted and moisture and light are available. If spring seeding, removing the seed coat and pericarp could reduce the seed's ability to cope with limiting environmental factors, resulting in water loss and desiccation. Many *Artemisia* species, including *Artemisia cana*, produce a transparent, gelatinous coating (mucilage envelope) after hydration. Mucilage is hypothesized to improve seed dispersal, colonization, and seedling emergence; reduce seedling mortality by improving seed attachment to soil particles, and enhance germination conditions by protecting the embryo from desiccation and mechanical injury (Clor et al. 1974, Harvey 1981, Kreitschitz and Valles 2007, Kreitschitz 2012, Yang et al. 2012).

Our results suggest that if seed is stored for an over winter period, increased storage time is not necessary for seed to germinate. We did not test germination of newly harvested *Artemisia cana*

seed, which would have allowed us to conclude whether after ripening is necessary for seed to break dormancy. Increasing storage time did help seed germinate more quickly. Decreasing germination time can be beneficial in restoration applications as smaller windows of optimal environmental conditions for seed germination can be exploited. When restoration occurs on large scales, common in sagebrush ecosystems, even small increases in germination can have a large impact. This is especially pertinent to the Greater Sage-grouse, where habitat cover targets can be reached by increasing sagebrush cover by only 5 to 10 % (Environment Canada 2014).

Similar germination responses to storage time, scarification, and light of seed collected in different years, from the same general area, may be due to average precipitation and temperature in both years. Drought years usually result in sagebrush with limited seed production and variable quality (Young et al. 1989). Other studies on *Artemisia tridentata* germination and dormancy suggest sub-populations adapted to localized conditions (winter length and temperature) affect seed dormancy and germination (Meyer and Monsen 1991, 1992). The two *Artemisia cana* populations in this study were similarly affected by scarification and storage time; populations were from the same latitude and within a 25 km radius, so no localized adaptations were expected.

Only marginal increases in maximum germination with scarification and after ripening indicate that *Artemisia cana* could be non-dormant, although it's likely that dormancy may be primarily inhibited by temperature. Meyer et al. (1990) found that at 15 °C, 100 % of *Artemisia tridentata* seeds germinated in light conditions and 81.5 % germinated in the dark after 14 days; at 1 °C, 50 % of seeds germinated after 56 days. *Artemisia cana* is adapted to germinate under snow for early spring emergence and dormancy may be primarily inhibited by temperature rather than seed having a strong light requirement, as our results indicate that seed had high germination even in the dark. We did not investigate the effects of temperature on *Artemisia cana* seed germination.

We did not test seed viability of ungerminated seed; therefore, our results represent germination x viability. When stored in ambient warehouse conditions, sagebrush seed viability starts to decrease after 2 to 3 years of storage (Stevens et al. 1981); seed viability of *Artemisia tridentata* drops rapidly over the first 12 months after collection, from 81 to 92 % to 22 to 49 % (Wijayratne and Pyke 2012). Our results did not indicate any difference in germination (and purity) of seed stored for 4 to 6 months versus 16 to 18 months. However, cold storage prolongs seed viability (Karrfalt and Shaw 2013) and storage of seed at 5 °C may prevented discernable drops in viability over the 18 months of storage.

Based on the results of our study, germination patterns from some *Artemisia tridentata* are applicable to *Artemisia cana*, but ranges for germination were not useful in predicting germination

of our seed. Differences in germination are likely a result of plant adaptations or responses to environmental conditions. Much existing research on *Artemisia* species has been conducted on *Artemisia tridentata* because of its wide range and its dominance in North American prairies, while fewer studies focused on *Artemisia cana*. Thus, many restoration practices for *Artemisia cana* have been based on *Artemisia tridentata* ecology and physiology. Although some similarities exist in germination patterns of *Artemisia cana* and *Artemisia tridentata,* their ecological and physiological differences are documented. Therefore, we recommend restoration methods for *Artemisia cana* based on studies of *Artemisia tridentata* ecology should be tested on a small scale before application on a landscape level.

To improve seeding outcomes and restoration success, future research should focus on developing methods to overcome limiting field conditions preventing seedling emergence (such as seed desiccation, water availability and erosion). These methods could be paired with after ripening and scarification of seed to further improve seedling emergence in the field. Cold storage could help preserve the longevity of the seed, increasing restoration success while maximizing labour, time and cost.

5. CONCLUSIONS

Germination of *Artemisia cana* seed under laboratory conditions was very high and occurred rapidly and uniformly. Seed collected in two different years, and from two distinct populations, exhibited the same response to physical scarification, after ripening and light conditions. Pericarp removal via physical scarification and after ripening for 16 to 18 months marginally increased germination by approximately 10 %. *Artemisia cana* could be non-dormant, or dormancy may be controlled by factors unexamined in this study. Low success of *Artemisia cana* seeding in the field is likely not due to poor germination, but from limiting environmental factors.

Table 3.1. *Artemisia cana* seed groupings for analysis, location, and collection date in Grasslands National Park in 2015 and 2016.

Seed Group	Date Collected	Collection Coordinates (UTM, Zone 13U)
2015	November 2015	Composite
2016	November 2016	Composite
2016 (A)	November 4-9, 2016	313054 E 5453183 N, 313766 E 5450204 N
2016 (B)	November 21-24, 2016	318323 E 5446209 N, 314996 E 5448074 N

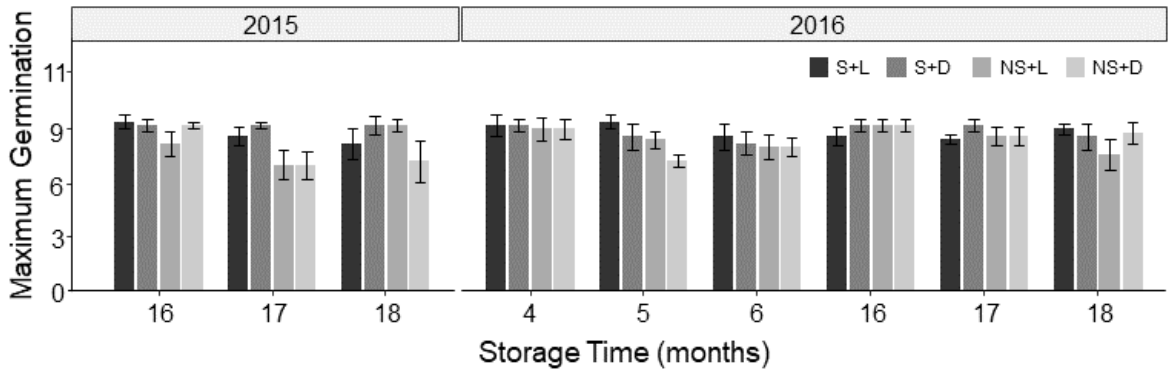

Figure 3.1. Maximum germination (seeds out of 10) for composite seed samples collected in Grasslands National Park in 2015 and 2016. Treatments include storage time (4, 5, 6, 16, 17, 18 months), scarification (S) or no scarification (NS), and light (L) or dark (D) conditions during germination. Standard error bars are shown. n = 60 for 2015, n = 120 for 2016.

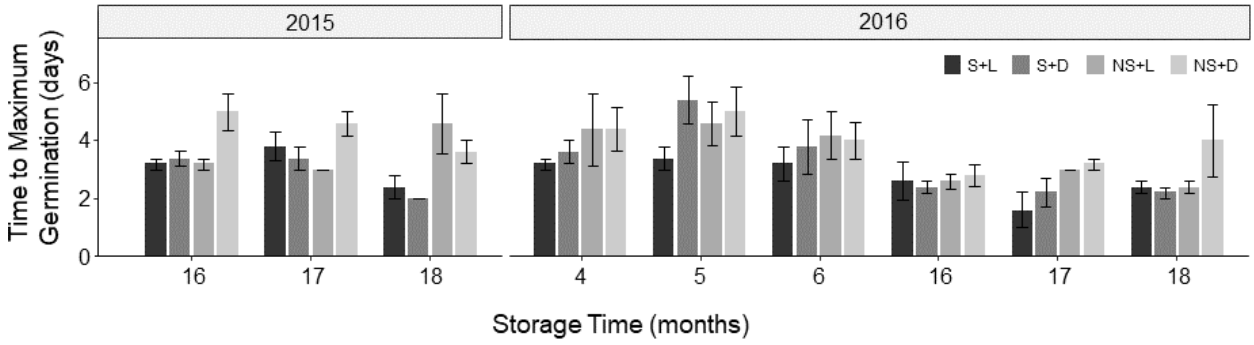

Figure 3.2. Time to maximum germination (days) for composite seed samples collected in Grasslands National Park in 2015 and 2016. Treatments include storage time (4, 5, 6, 16, 17, 18 months), scarification (S) or no scarification (NS), and light (L) or dark (D) conditions during germination. Standard error bars are shown. n = 60 for 2015, n = 120 for 2016.

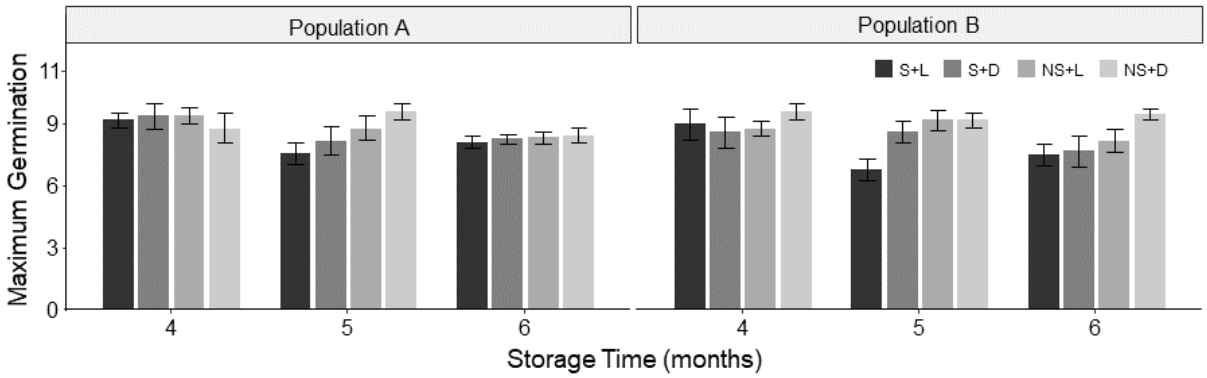

Figure 3.3. Maximum germination (seeds out of 10) for seed samples (populations) collected from two different areas in Grasslands National Park in 2016. Treatments include storage time (4, 5, 6 months), scarification (S) or no scarification (NS), and light (L) or dark (D) conditions during germination. Standard error bars are shown. n = 60 for each population.

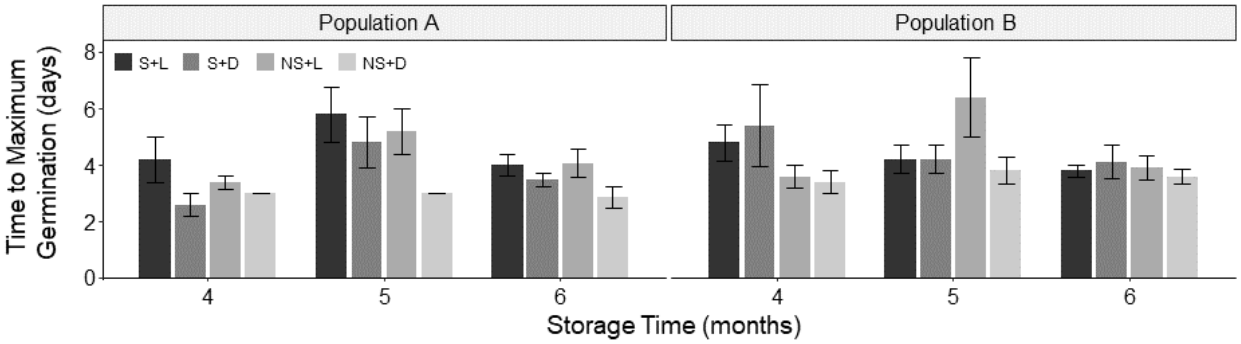

Figure 3.4. Time to maximum germination (days) for seed samples (populations) collected from two different areas within Grasslands National Park in 2016. Treatments include storage time (4, 5, 6 months), scarification (S) or no scarification (NS), and light (L) or dark (D) conditions during germination. Standard error bars are shown. n = 60 for each population.

IV. NUTRIENT LOADING TO PROMOTE MORPHOLOGICAL QUALITY AND SURVIVAL OF *ARTEMISIA CANA* SEEDLINGS POST-OUTPLANTING

1. INTRODUCTION

Sagebrush (*Artemisia* L. [Asteraceae]) is a vital habitat component of many endangered prairie faunal species (Meyer 2008). Sagebrush habitat has been reduced to less than 60 % of its historic range by anthropogenic activities (McArthur and Stevens 2004); remaining habitat is often severely fragmented and at sub-optimal condition for sagebrush obligate species use, resulting in their population declines. In Canada, more than 50 at risk prairie species are associated with sagebrush grasslands habitat (Environment Canada 2019). The most poignant example is the Greater sage-grouse (*Centrocercus urophasianus* Bonaparte [Phasianidae]) (hereafter Sage-grouse) whose current population in North America is approximately 3 % of historic estimates and whose range has been reduced to 56 % of its pre-settlement state (Connelly et al. 2004). In Canada, populations declined by 88 to 92 % in the last two decades (Environment Canada 2014), and range is approximately 6 % of its historic estimate (Aldridge and Brigham 2003). Only a few hundred individuals remain in two small populations in Alberta and Saskatchewan (Heinrichs et al. 2019). The recovery strategy for Sage-Grouse in Canada recommends restoration of low-quality sagebrush habitat to optimal conditions for supporting its population growth (Environment Canada 2014) which could positively affect habitat and populations of other at risk prairie species.

Silver sagebrush (*Artemisia cana* Pursh) is the dominant sagebrush species in Canada and the second most widely distributed in North America, occupying approximately 140,000 km^2 (Connelly et al. 2004, Meyer 2008). Plains silver sagebrush (*Artemisia cana* Pursh ssp. *cana* (hereafter *Artemisia cana*) is the only sagebrush species to occupy the range of Sage-grouse in Canada (Figure 4.1). Conventional revegetation methods for *Artemisia cana* have had limited success. Romo and Grilz (2002) studied its establishment in southern Saskatchewan and found that although laboratory germination was 82 %, only 5.3 % of seeds produced seedlings in the field; two year survival of seedlings was 84 %. Outplanting container stock seedlings was generally more successful than seeding, as limitations of germination were overcome, and plants were of adequate vigour. However, outplanting success is still low. Dettweiler-Robinson et al. (2013) found outplanted Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis* Beetle & Young) had 30 % survival after three years with container stock and 17 % with bare root stock. On a Wyoming site, late spring planted seedling survival was 23 % after five years (Meikle 1999).

Seedling traits, such as nutrient content in tissue (reserves) and competitive ability (related to size), can improve survival of planted individuals and confer resistance to summer drought or water limited conditions (Villar-Salvador et al. 2012). Seedling quality may be improved through manipulation of nutrient availability during nursery production and multiple studies suggest that well fertilized seedlings perform better after outplanting (Malik and Timmer 1996, Timmer 1997, Imo and Timmer 1999, McAllister and Timmer 1998, Salifu and Timmer 2003b, Oliet et al. 2009, Galvez et al. 2011, Hu 2012, Landhäusser et al. 2012, Schott et al. 2016). Improved performance is attributed to depletion of nutrient reserves (accumulated during greenhouse growth) which are used for new growth (Malik and Timmer 1996, Salifu and Timmer 2003a). Nutrient loading is a fertilizer regime applied to nursery stock that increases plant nutrient reserves through storage of nutrients (Imo and Timmer 2002). Nutrient loaded seedlings exhibit the capacity to re-translocate nutrients for current growth and show increased root growth and resistance to nutrient and water stress after outplanting (Timmer and Munson 1991, Malik and Timmer 1996, Timmer 1997).

The objective of this study was to investigate whether exponential and modified exponential nutrient loading of *Artemisia cana* seedlings could increase internal nutrient content and improve performance once outplanted. We hypothesized that exponential and modified exponential fertilizer loading would result in nutrient accumulation in seedlings and in greater growth and survival of outplanted seedlings. Two related studies were conducted: 1) to determine time required for exponential growth to cease, allowing storage of nutrients; and 2) to determine optimal fertilizer loading and whether increased nutrient status of seedlings resulted in increased growth and survival post-outplanting.

2. MATERIALS AND METHODS

2.1. Seed Collection, Greenhouse Conditions, And Nutrient Additions

Seed was collected from healthy, naturally occurring populations of *Artemisia cana* in Grasslands National Park in fall 2016. Seed was dried, cleaned to approximately 15 % purity (by weight), stored in brown paper bags, and held in cold storage (1 °C) for approximately 10 months before use. Seeds were germinated on moist paper towels, at approximately 22 °C in direct and indirect sunlight. Seeds were left to germinate for two days.

Pots of approximately 7.5 cm depth (6.3 cm diameter, 75 mL cavity) were filled with a peat moss and perlite mix growth medium (Sun Gro Horticulture, Sunshine Loosefill Aggregate 4 (LA4) 60 to 70 % Canadian sphagnum peat moss and 30 to 40 % coarse perlite, Agawam, MA). Once

filled, pots were saturated with deionized water until freely draining from the bottom. After 24 hrs of drainage, saturation and drainage were repeated twice to achieve full saturation of the growth medium prior to planting.

Two germinated sagebrush seeds were placed into each pot. After one week, pots were thinned to one seedling each. Pots were distributed randomly in treatment blocks and placed into growth chambers with conditions set to 16 hrs light, 22 °C (± 2) temperature ramping down from 22 °C at 21:00 h to 18 °C at 24:00 h, and up from 18 °C at 06:00 h to 22 °C at 11:00 h. Humidity fluctuated from 50 to 85 %. Pots were watered three times weekly with 10 mL deionized water.

Nutrient (fertilizer) treatments were prepared by adding a commercial water soluble fertilizer (20-8-20 N:P:K) weekly to administered water. Quantity of fertilizer was calculated to achieve nitrogen (N) additions of 70, 105, 175, or 245 mg per growth period. Constant dose treatments were calculated based on total N to be added over the growth period, divided by number of growth weeks. Weekly additions for exponential treatments were calculated based on the steady state model (Timmer 1997; Salifu and Timmer 2003b) using the equation:

$$N_T = N_S (\exp^{rt} - 1) \quad \text{[Eq. 1]}$$

where r is the relative addition rate required to increase N_S (N content in seed or initial N content) to a final N content ($N_T + N_S$), and N_T is the desired amount to be added in a number (t) of fertilizer applications (number of growth weeks).

Weekly N additions for modified exponential treatments were applied to raise N addition slightly at the start of fertilizer application and calculated with the equation:

$$N_C = N_0 (\exp^{-rt} - 1) \quad \text{[Eq. 2]}$$

where N_0 is the final amount (approaching 0) of nutrient added over the compensation period; and N_C the compensating quantity of nutrient, corresponding to the difference between last and penultimate fertilizer applications. This quantity was subtracted from the final N application; thus the modified exponential regime ensured steady state nutrient culture reflected by stable internal nutrient concentrations during the fertilizing period.

2.2. 11 Week Greenhouse Experiment

Artemisia cana seedlings were grown for 11 weeks, which is the approximate production time for sagebrush seedlings in Shand Greenhouse (Estevan, SK) (Pyra 2016). Four fertilizer treatments were delivered weekly: single dose (SD), constant dose (CD), exponential dose (EX), modified exponential dose (ME) delivering 70 mg N per plant over the growth period, and a water only

control (CW). Good transplanting performance was expected with at least 70 mg of N plant^{-1} (Van den Driessche 1988), aligning with standard nursery practice for developing *Artemisia cana* seedlings for restoration. Shand Greenhouse employs a constant dose fertilizer regime where approximately 70 mg of N is added per plant over the growing period (Pyra 2016). Weekly N additions to meet growing season requirements are detailed in Supplemental Information (Table S4.1). Each treatment was applied to 35 (± 5) sagebrush seedlings at 3, 5, 7, 9, and 11 weeks. Five seedlings from each treatment were randomly selected for height, health, and leaf area measurement and sampled for biomass measurement and nutrient content analysis.

2.3. 26 Week Greenhouse And 2 Year Field Experiment

Artemisia cana seedlings were grown 25 weeks. Four fertilizer treatments and 4 total N additions (SD70, CD70, CD175, EX70, EX105, EX175, EX245, ME70, ME105, ME175, ME245), and a water only control (CW) were applied weekly. Each treatment was applied to 35 (± 5) seedlings. Weekly N additions were to meet growing season requirements (Table S4.2). After 25 weeks, 3 seedlings from each treatment were randomly chosen for leaf area, height, and health measurement, then removed for biomass and nutrient content determination.

Remaining seedlings were transported to Grasslands National Park and maintained outdoors in full sun (water via precipitation) for one week before planting into a 16 m x 16 m plot (13U 335780 E 5449707 N) on 7 May 2018. Only seedlings with a health score of at least 4 out of 5 (see section 2.4) were outplanted. Seedlings were planted 1 m apart in 16 rows of 16 individuals (256 total individuals) in a hole approximately twice as deep and wide as the root ball. Seedlings were removed from containers, submerged in distilled water to loosen bound roots, placed in the hole, then soil lightly filled around the root ball. From each treatment, 20 to 23 replicates were planted in randomly determined positions in the plot.

Before outplanting, the plot was a garden cleared of all vegetation via plowing and hand weeding, so sagebrush growth and survival could be analyzed in absence of competition. On 2 July 2018 the plot was thoroughly hand weeded, and landscaping fabric installed to prevent weed growth. Holes of approximately 30 cm diameter were cut in the landscaping fabric around seedlings so as not to impede growth and straw was lightly packed on top of exposed soil surrounding the seedling to prevent soil water loss. Seedling health, height, and canopy area were measured 3 times during the first growing season (3, 31 July and 2 October 2018) and twice during the second growing season (3 June and 2 July 2019). On 2 October 2018, 5 seedlings of each nutrient loading treatment were randomly removed for biomass measurement and nutrient content analysis.

Growing season temperature and precipitation were monitored using three meteorological stations in Grasslands National Park. Annual and growing season means were calculated from meteorological data collected by Grasslands National Park, between 1995 and 2018.

2.4. Plant Measurements And Sample Analysis

Seedling height was measured from growth media or soil surface to the tallest living part of the plant without elongating branches or stems. Health was determined on a 1 to 5 scale: 1 for plants with 20 % or less living (green) material, 2 for plants with 21 to 40 % living material, 3 for plants with 41 to 60 % living material, 4 for plants with 61 to 80 % living material, and 5 for plants with greater than 80 % living material. To determine above and below ground biomass, plant samples were washed with deionized water, dried at 60 °C for 48 hrs, then weighed.

To determine total leaf area (photosynthetic area), all leaves were removed from stems, pressed flat under glass, and photographed from above (scale bar in frame). ImageJ software was used to isolate leaves from the background and to measure total leaf area (Laboratory for Optical and Computational Instrumentation, University of Wisconsin). To determine canopy area, a photograph of the sagebrush seedling was taken from above. ImageJ software was used to isolate sagebrush from the background and measure total canopy area.

Total N content of roots and leaves was assessed via combustion using a Flash 2000 elemental analyzer on approximately 5 mg of dried and finely ground sample (Dumas 1831). Non-structural carbohydrate analysis was performed by gas chromatography after sequential digestion of approximately 1 g of dried and finely ground sample using a modified version of the Englyst method (Englyst et al. 1992). Modification was to add 1 mL of sodium borohydride in dimethyl sulfoxide to reduce heat generation during reduction and acetylation (detailed methods in Supplemental Information, Method S4.1). Starch extraction, via hydrolysis in 0.2 M sulfuric acid and subsequent analysis using gas chromatography, was completed separately to confirm results of sequential digestion (detailed methods in Supplemental Information, Method S4.2)

2.5. Statistical Analysis And Calculations

Calculations and statistical analyses were conducted using R 3.4.1 (The R Foundation for Statistical Computing 2019). Morphological and nutritional data were analyzed by analysis of co-variance (ANCOVA) for 11 and the 26 week experiments to determine how plant traits differed over time and between fertilization treatments (time as covariate). Morphological and nutritional

data that met normality and variance assumptions were analyzed by analysis of variance (ANOVA) to determine if fertilization treatment and seedling age significantly affected seedling health, height or canopy cover. ANOVA was followed by Tukey's pairwise comparisons test if means were significantly different. If data did not meet normality assumptions, data was log transformed. To determine if plant position in the plot affected health, height, and canopy cover of seedlings a linear mixed effects model was used with fertilization treatment and seedling age as fixed factors and plant position as the random effect. Significance of the random effect was tested by comparing Akaike information criteria (AIC) of the two models (one with the random effect and one without). Survival was analyzed with generalized linear models with binomial distribution, to determine effect of fertilization treatment and seedling age. Relative growth curves were constructed using exponential models of dry mass over time and assessed with coefficient of determination (r^2) values. Significance was assessed at an alpha of 0.05.

3. RESULTS

3.1. 11 Week Greenhouse Experiment

Nutrient loading was not achieved in 11 weeks. Biomass did not plateau (Figures 4.2, 4.3.c, 4.3.d), which did not allow N accumulation in roots or leaves (Figures 4.3.e, 4.3.f). As expected, seedling height, leaf area, root and leaf mass increased over time (Figure 4.3.). Seedling height and leaf area were not significantly different between 9 and 11 weeks of growth; height was greater in seedlings receiving modified exponential fertilization than those that received water only ($F_{4,45} = 4.73$, $p < 0.01$), while leaf area was greater in seedlings that received large doses of fertilization at the outset of the experiment (SD, CD, ME) than those that did not (CW, EX) ($F_{4,45} = 4.73$, $p < 0.01$). Lower growth of seedlings receiving only water or exponential dose fertilizer implied seedlings were nutrient deficient at some point in the growing period (Figure 4.2).

After 11 weeks of growth, root mass and leaf mass were significantly greater than after 9 weeks of growth ($F_{1,48} = 10.56$, $p < 0.01$; $F_{1,48} = 8.24$, $p < 0.01$), although there were no differences in seedling root or leaf mass between fertilization treatments. Root and leaf nitrogen decreased over time, but was not significantly different after 9 and 11 weeks of growth ($F_{1,48} = 0.02$, $p > 0.05$; $F_{1,48} = 2.32$, $p > 0.05$) or between different fertilization treatments ($F_{4,45} = 1.38$, $p > 0.05$; $F_{4,45} = 0.06$, $p > 0.05$). Total non-structural carbohydrate (NSC) content increased over time in roots and leaves, starch increased over time in leaves but decreased in roots while fructans increased in both leaves and roots (Figure 4.4). NSC, starch and fructans were not significantly

different after 9 or 11 weeks of growth ($p > 0.10$) or between fertilization treatments after 11 weeks of growth ($p > 0.10$). Seedling health decreased over time and was significantly lower after 11 weeks (4.3 / 5) than after 5 (4.9 / 5) or 9 (4.8 / 5) weeks ($F_{2,68} = 11.19$, $p < 0.001$).

3.2. 26 Week Greenhouse And 2 Year Field Experiment

3.2.1. Greenhouse

During the last three weeks in the greenhouse, seedling height, health, and root mass were stable, and not significantly different with fertilizer treatments ($p > 0.05$). Leaf mass did not differ with treatments but was significantly greater in seedlings grown for 26 weeks (0.62 g ± 0.04) than for 23 weeks (0.50 g ± 0.03) ($F_{1,70} = 5.51$, $p < 0.05$).

After 26 weeks, height, root mass, leaf mass, leaf area, and content of NSC, starch and fructans in leaves did not differ significantly with fertilizer treatments. Health was significantly greater in exponentially fertilized seedlings than in those receiving only water or single dose fertilizer at the beginning of the growth period ($F_{11,24} = 3.06$, $p < 0.05$). Some seedlings were nutrient loaded by 26 weeks; seedlings receiving 175 or 245 mg of N (constant, exponential, or modified exponential doses), had significantly greater root and leaf N content ($F_{11,24} = 5.44$, $p < 0.001$; $F_{11,24} = 8.28$, $p < 0.001$). Storage of N in roots and leaves was at 1:1 ratio. Root total NSC, starch, and fructans content was significantly different with treatment ($F_{11,23} = 4.48$, $p < 0.01$; $F_{11,23} = 3.34$, $p < 0.01$; $F_{11,23} = 4.43$, $p < 0.01$). In general, root NSC, starch and fructans were significantly greater in seedlings receiving lower amounts of N throughout the growing period (70 mg).

3.2.2. First field season

Between May and October of the first field season (2018), 101.7 mm of precipitation fell; 50 % of normal. Summer temperatures were 10 to 30 °C and not below 0 °C until October. Seedling health was significantly greater at the end of the first growing season than when outplanted ($F_{1,95} = 57.8$, $p < 0.001$). Seedlings receiving water only (CW) or one large dose of fertilizer at growth initiation (S70) showed significant health increases from health scores when outplanted (Figure 4.5.a).

Seedling height was significantly greater at the end of the first growing season than at outplanting ($F_{1,95} = 39.2$, $p < 0.001$), with mean increases of 50 to 290 % (Figure 4.5.b). N content increased in roots ($F_{1,95} = 37.6$, $p < 0.001$) (Figure 4.5.c) and leaves ($F_{1,95} = 94.3$, $p < 0.001$) (Figure 4.5.d). Significant N increases occurred in seedlings that accumulated the smallest amount of N during greenhouse growth (seedlings receiving water only or 70 mg N fertilizer). Total NSC content of roots ($F_{1,94} = 51.1$, $p < 0.001$) (Figure 4.5.e) and leaves ($F_{1,95} = 37.8$, $p < 0.001$) (Figure 4.5.f), and

fructans content of roots ($F_{1,94} = 97.6$, $p < 0.001$) (Figure 4.8.g) decreased since outplanting, with significant NSC decreases generally in seedlings that accumulated greatest NSC before outplanting (seedlings receiving water only or 70 mg N fertilizer). Root starch increased since outplanting ($F_{1,94} = 59.7$, $p < 0.001$) (Figure 4.5.i) and leaf starch decreased ($F_{1,95} = 68.2$, $p < 0.001$) (Figure 4.5.j). Significant decreases in leaf starch were in seedlings that accumulated the most starch prior to outplanting (seedlings receiving water only, 70 mg N fertilizer, or modified exponential fertilizer). Leaf fructans content was not significantly different since outplanting or between treatments.

3.2.3. Overwintering and second field season

During the first overwintering, temperatures stayed below 0 °C until April 2019. Between May and August in the second growing season (2019), 223.2 mm of precipitation fell, just above mean normal. Summer temperatures were cooler than in the first growing season, staying between 7 and 28 °C. Over the two field seasons, seedling survival differed significantly with treatment (X^2 (11, n = 256) = 36.7, $p < 0.001$) and age (X^2 (4, n = 256) = 83.23, $p < 0.001$). Survival of seedlings receiving 245 mg N in exponential doses (E245) was significantly greater than seedlings receiving water only (CW) and 70 mg N in single (S70) or constant (C70) doses (Figure 4.6). Mortality decreased with age, was significantly greater in the first field season than the second ($F_{1,787} = 7.66$, $p < 0.01$), and significantly greater at the beginning of the first field season than the end ($F_{1,476} = 6.13$, $p < 0.05$) (Figure 4.6).

Unexpectedly, within 52 days of planting, 5 new seedlings sprouted from the original outplants and by the end of the first growing season 2 additional seedlings sprouted. It is unclear which seedlings resprouted as they were not excavated, but 100 % of sprouts were adjacent to a nutrient loaded seedling (M175, M245, E175, E245). Height, canopy cover, and health of new seedlings were comparable to the original seedlings (Figure 4.7). Survival of new seedlings over winter was 85.7 %, with the loss of 1 new seedling, which had a significantly lower health score (2 / 5) than the other new seedlings (4.2 / 5). In all seedlings, health score interacted with fertilizer treatment to significantly affect survival ($F_{47,741} = 2.29$, $p < 0.0001$). Seedlings receiving only water or 70 mg of N in the greenhouse, with a health score of 3 or lower, had significantly lower survival than those with health scores of 4 or 5, regardless of fertilizer treatment.

Over time (both field seasons), seedling health ($F_{4,56} = 89.1$, $p < 0.0001$), height ($F_{4,56} = 499.5$, $p < 0.0001$), and canopy cover ($F_{4,56} = 139.1$, $p < 0.0001$) significantly increased (Figure 4.7). The largest increases occurred in the second field season, after remaining relatively consistent in the

first. At the end of the second field season, health of all individuals was high, with 90 % of individuals considered 5 of 5, 9 % 4 of 5 and only 1 % 3 of 5. Height and canopy cover were significantly different between seedlings that received different fertilizer treatments during the 26 week greenhouse phase ($F_{11,260} = 2.42$, $p < 0.01$; $F_{11,260} = 2.66$, $p < 0.01$) (Figure 4.7). Seedlings that had received greater amounts of nitrogen during greenhouse growth (245 mg) had significantly greater heights than those that received less, except for those seedlings that received 70 mg of nitrogen in one large, single dose (S70). Canopy cover was significantly greater in those seedlings having received 175 of 245 mg nitrogen at a modified exponential schedule. Individual plants, or their positions in the plot, significantly affected seedling height ($p < 0.0001$, AIC with covariate = 139, AIC without covariate = 394), canopy cover ($p < 0.0001$, AIC with covariate = 112, AIC without covariate = 590), but did not significantly affect seedling health ($p < 0.05$, AIC with covariate = 2467, AIC without covariate = 2472). Seedlings positioned in the north-east quadrant had smaller heights and canopy covers than seedlings in the other quadrants (especially south-west quadrant) (Figure 4.8).

4. DISCUSSION

The results of our study highlight the importance of N reserves in survival and growth of outplanted *Artemisia cana*. To our knowledge, this is the first study investigating the potential of nutrient loading a shrub species, although the method has been successful in many tree species (Malik and Timmer 1996, McAllister and Timmer 1998, Xu and Timmer 1999, Salifu and Timmer 2003a, 2003b, Birge et al. 2006, Oliet et al. 2009, Galvez et al. 2011, Schott et al. 2013, 2016, Pokharel and Chang 2016). We now know *Artemisia cana* seedlings can be nutrient loaded if time in the greenhouse is sufficient for exponential growth of seedlings to cease. The standard practice of growing *Artemisia cana* for 12 weeks is insufficient for nutrient loading. Seedlings grown for 12 weeks by Shand Greenhouse, using approximately 70 mg N $plant^{-1}$ (Pyra 2016) were used to restore areas of Grasslands National Park between 2016 and 2018. Mean survival was 30 % after one growing season and overwintering and height increases were small (approximately 15 cm) (Naeth et al. 2019).

Artemisia cana growth was exponential, and thus exponential and modified exponential fertilizer treatments were most beneficial to plant development. When nutrients are provided in excess of that required for growth, relative growth rate is maximized, and nutrients can accumulate in tissues. In our study, 175 and 245 mg N $plant^{-1}$ enabled development of nutrient loaded *Artemisia*

cana seedlings, evidenced by the lack of difference in seedling mass or height at the end of greenhouse growth but greater accumulation of N in seedlings receiving higher fertilizer treatments. Lack of toxicity in any seedlings receiving 245 mg N plant^{-1} indicates nitrogen additions could be further increased to optimize nitrogen storage in tissues, although the amount of N accumulated in leaves of our seedlings was comparable to, or greater than, that of other woody species which reported maximum N concentrations of 3.56 % (Perry and Hickman 2000).

Stored N can be used to maintain growth while supply is limited (Chapin et al. 1990), as it is a major component of amino acids and integral for leaf growth and expansion as a building block of chlorophyll. In spring, plant growth often starts before N uptake has started from the soil; therefore, stored N may be important for seedling growth immediately after outplanting. Seedlings in our study that had not accumulated N during greenhouse growth, were able to accumulate N after outplanting, in both leaf and roots. Seedlings that accumulated N during greenhouse growth did not accumulate more in the field, implying that N was not limiting in the field and stored N was not required to continue rapid growth once outplanted. As we did not label N supply, we cannot conclude if stored N was remobilized for growth after outplanting.

Several studies found nutrient loading increased biomass after outplanting (Malik and Timmer 1995, McAllister and Timmer 1998, Salifu and Timmer 2003a, Salifu and Timmer 2003b, Salifu et al. 2005, Oliet et al. 2009, Pokharel and Chang 2016, Schott et al. 2016) and suggest large nutrient reserves in seedlings before outplanting increase biomass production through remobilization of internal N (McAllister and Timmer 1998, Millard and Gellet 2010). Greater canopy cover and height at end of the second growing season in N loaded seedlings may have been due to factors other than N leaf content prior to outplanting. We think this is likely the case, as at the end of the first growing season, N content was equivalent in all seedlings regardless of N content at outplanting. We were unable to measure root growth after outplanting, but it is possible that seedlings with greater N content were able to increase root production, and therefore increase nutrient acquisition for growth in the second field season. This idea is supported by nutrient loaded seedlings demonstrating significantly greater, second season height and canopy covers than those seedlings that were not nutrient loaded. Previous studies reported increased root production in nutrient loaded seedlings and hypothesized this could confer resistance to nutrient and water stress (Malik and Timmer 1996, Timmer and Munson 1991).

Resprouting of seedlings in our study was unexpected, as previous research on *Artemisia cana* stem and rhizome age indicates that 3 to 5 years is required before rhizomatous reproduction occurs (Wambolt et al. 1990). The same study reports that 37 % of *Artemisia*

cana seedlings arise from seed, while 63 % of individuals arise from rhizomes, making rhizomatous reproduction important for maintaining landscape cover of sagebrush. Resprouting of seedlings within the fist year of planting could help offset mortality rates that reduce overall sagebrush cover. Large contributions to landscape cover from greater individual canopy cover could help reach habitat cover targets more quickly and without increasing planting densities.

The role of NSC in plants is not well understood. Some suggest NSC can be remobilized for use in rapid growth, or when carbon input is reduced by shade, drought, or disturbances such as herbivory (Chapin et al. 1990, Millard and Grelet 2010), and excess NSC promotes resprouting of some species (Kabeya and Sakai 2005). Others suggest specific carbohydrates like fructans may confer some cold stress tolerance (Van de Ende 2013, Tarkowski and Van de Ende 2015). Our results suggest *Artemisia cana* seedlings use fructans as their main storage carbohydrate, and that it accumulates mostly in roots. This is consistent with work reporting Asteraceae plants use inulin, a type of fructan, as their primary storage carbohydrate (Pollock 1986, Hendry 1993). It is unclear if seedlings in our study remobilized NSC for growth; although NSC in seedlings dropped significantly over the growing season, this did not correlate with increases in seedling height or canopy area. High concentrations of fructans in seedling roots at the end of the first growing season suggest either NSC is purposefully being retained at a threshold level, possibly for cold stress tolerance; or is not reusable by the plant, meaning carbon is sequestered instead of stored (Hoch et al. 2003, Körner 2003, Würth et al. 2005, Spann et al. 2008).

The C/N balance theory suggests that when nutrient availability limits plant growth, they tend to accumulate excess carbon as NSC (Chapin et al. 1990, Wiley and Helliker 2012). Our results support this theory, as evidenced by increased NSC but decreased N storage in seedlings receiving low N fertilizer treatments. Kabeya and Sakai (2005) reported increased NSC in roots of plants receiving low N treatments, and low NSC in plants receiving high N treatments. If reserves of both N and NSC are not attainable in *Artemisia cana* seedlings simultaneously, several factors should be considered prior to seedling development to ensure they have attributes to most improve growth and survival once outplanted. Our results indicate N was important for increasing seedling survival through the first growing season and over wintering, even if changes in seedling morphology cannot be directly attributed to N storage. Since seedling mortality is greatest during the first 120 days after outplanting (Harvey 1981, Dettweiler-Robinson et al. 2013), increasing N storage in seedlings prior to outplanting is likely most effective to improve restoration.

Our seedling survival post-outplanting is atypically high relative to other studies, which report average survival of outplanted *Artemisia* sp. seedlings of 30 to 36 % after two years (Evans and

Lih 2005, Newhall et al. 2011, Wirth and Pyke 2011, Dettweiler-Robinson et al. 2013, McAdoo et al. 2013). Increased survival in our study could be attributed to use of nutrient loaded seedlings, exclusion of competition in the field, or favourable environmental conditions. Due to resource limitations we were unable to conduct a split plot design to investigate effects of competition. Many studies reported greater seedling survival and vigour when herbaceous competition was reduced or eliminated (Schuman et al. 1998, Boyd and Svejcar 2011, Newhall et al. 2011, McAdoo et al. 2013). Without controlling competition, seedling survival, height, and canopy cover would likely decrease due to competition for below and above ground resources. Precipitation can significantly affect seedling establishment, and likely contributed to less growth in the first field season than the second.

Our results support the idea that microsite selection is an important factor in determining seedling survival and growth. Given adequate environmental conditions, reproduction of planted individuals could significantly offset loss of cover from mortality of planted individuals. In a restoration setting, expectations for seedling growth and survival will need to be adjusted for soil type (sandy soils retain less nutrients and water than loam soils), site topography (south facing slopes generally have lower seedling survival) (Harvey 1981), presence of invasive species, and land management.

5. CONCLUSIONS

Our study highlights the need to extend the length of time *Artemisia cana* are grown in greenhouses to maximize seedling growth and nutrient reserves before outplanting. Current, standard practices for growing *Artemisia cana* are insufficient to develop nutrient loaded seedlings. Although it is unclear if nutrient status influenced *Artemisia cana* seedling morphology, survival and second season growth of nutrient loaded seedlings was greater than that of conventionally fertilized seedlings. Increasing growing time of *Artemisia cana* in greenhouses to allow nutrient loading may increase restoration costs initially but is likely to save costs over the long term by improving restoration outcomes through increased plant growth, survival and reproduction. Use of nutrient loaded seedlings that have higher survival and contribute more canopy cover per plant to habitat cover targets will decrease the need for replanting and management. To increase seedling survival and growth, competition should be controlled for the first two growing seasons after outplanting to allow seedlings to establish and acquire competitive ability (increase height, root growth root, leaf area).

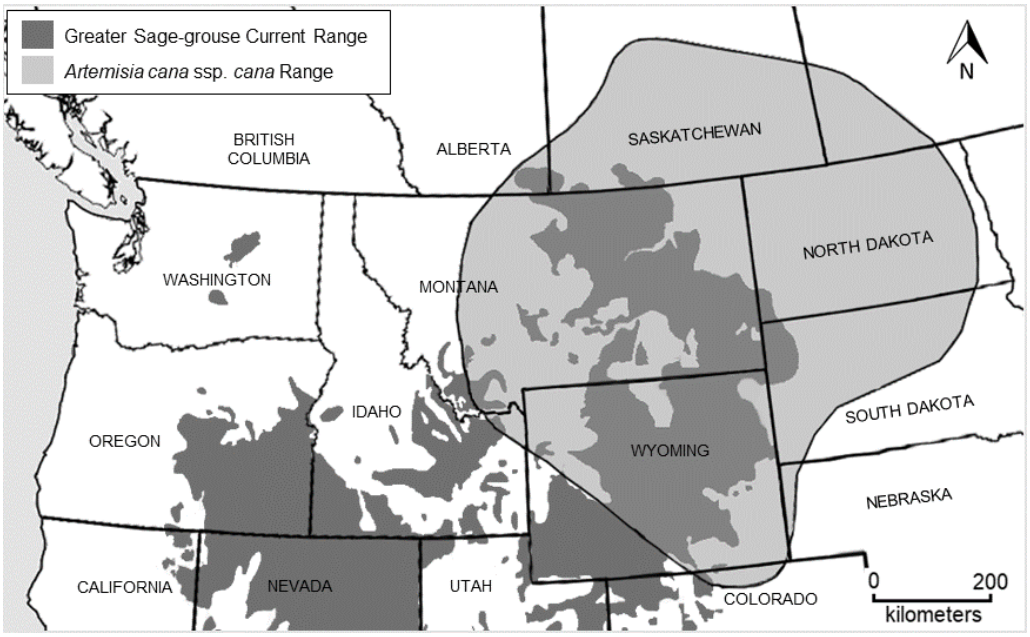

Figure 4.1. Current distribution of Sage-grouse in North America, showing Canadian and northern portions of the American range, with distribution of *Artemisia cana* ssp. *cana* overlaid.

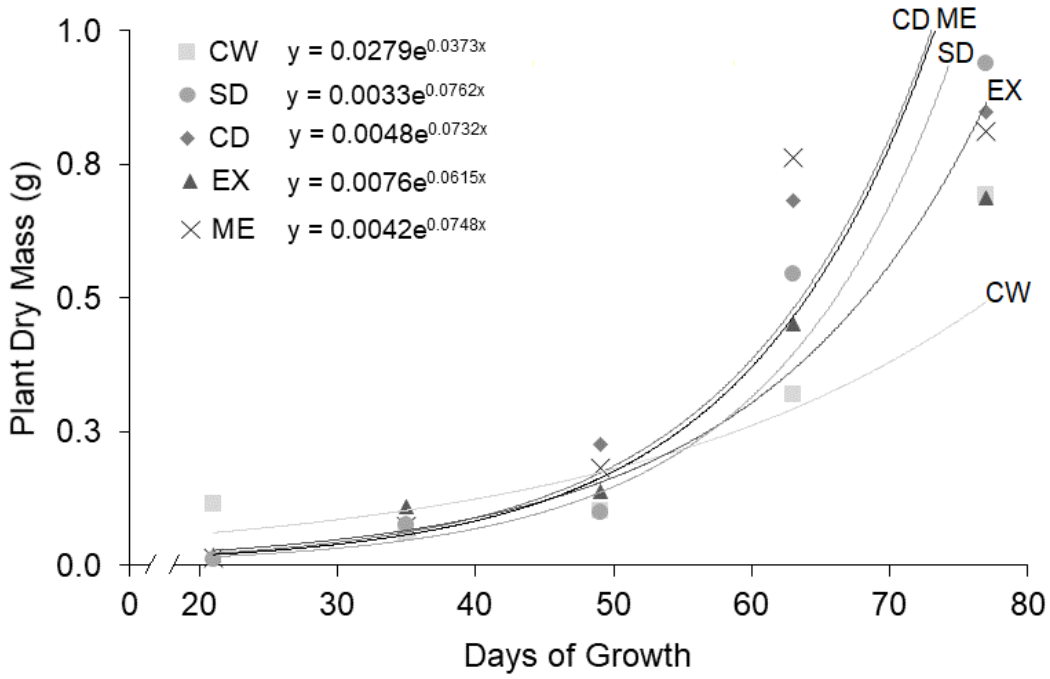

Figure 4.2. Mean dry mass (g) of seedlings grown for 77 days under fertilizer treatments (CW: control with water only, SD: single dose, CD: constant dose, EX: exponential fertilization, ME: modified exponential fertilizer). Exponential model equations representing relative growth displayed. n = 5 for each treatment per harvest (at 21, 35, 49, 63, 77 days).

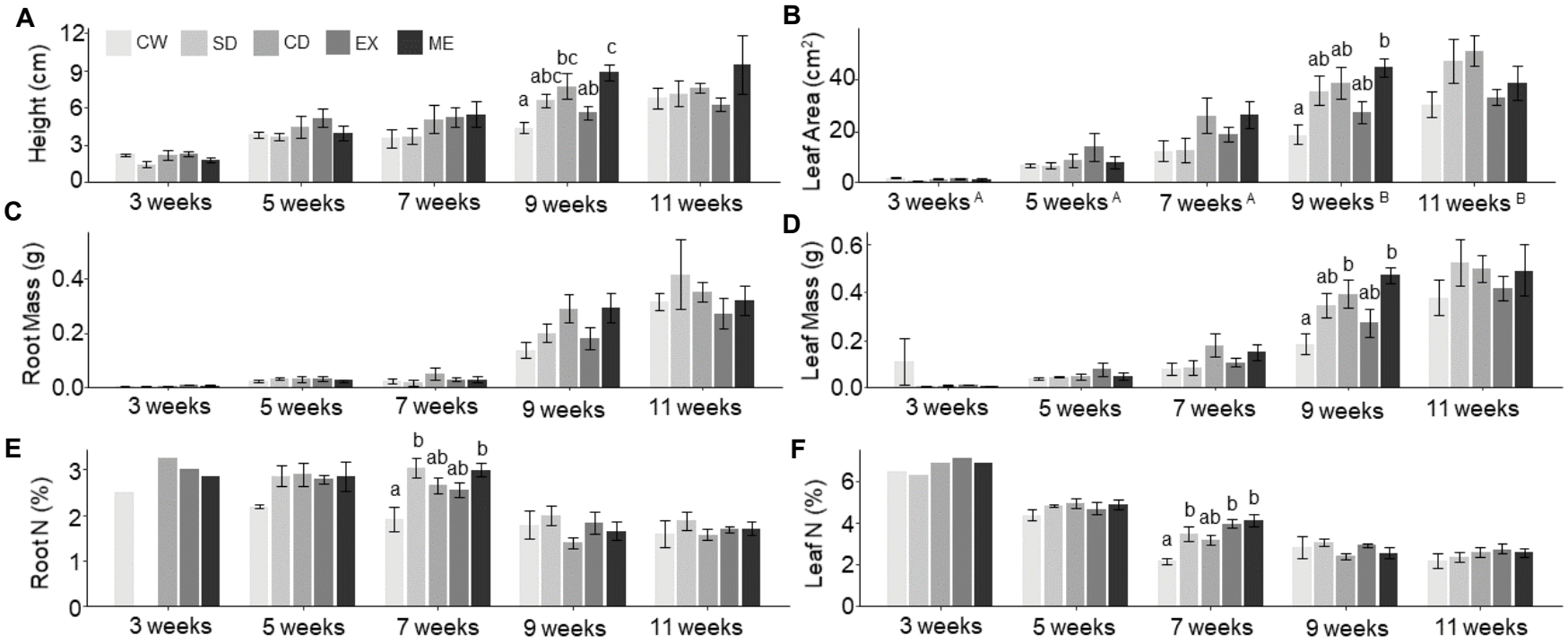

Figure 4.3. Height, leaf area, root and leaf mass, and root and leaf nitrogen content of *Artemisia cana* seedlings grown for 11 weeks under fertilizer treatments (CW: control with water only, SD: single dose, CD: constant dose, EX: exponential fertilizer, ME: modified exponential fertilizer). Over 11 weeks seedlings received 70 mg N. Bars represent standard error. Letters indicate significant differences in the indicated morphological trait between treatments (within a week). n = 5 for each treatment (per week), except for root and leaf nitrogen at 3 weeks which were composite samples due to small amounts of plant material.

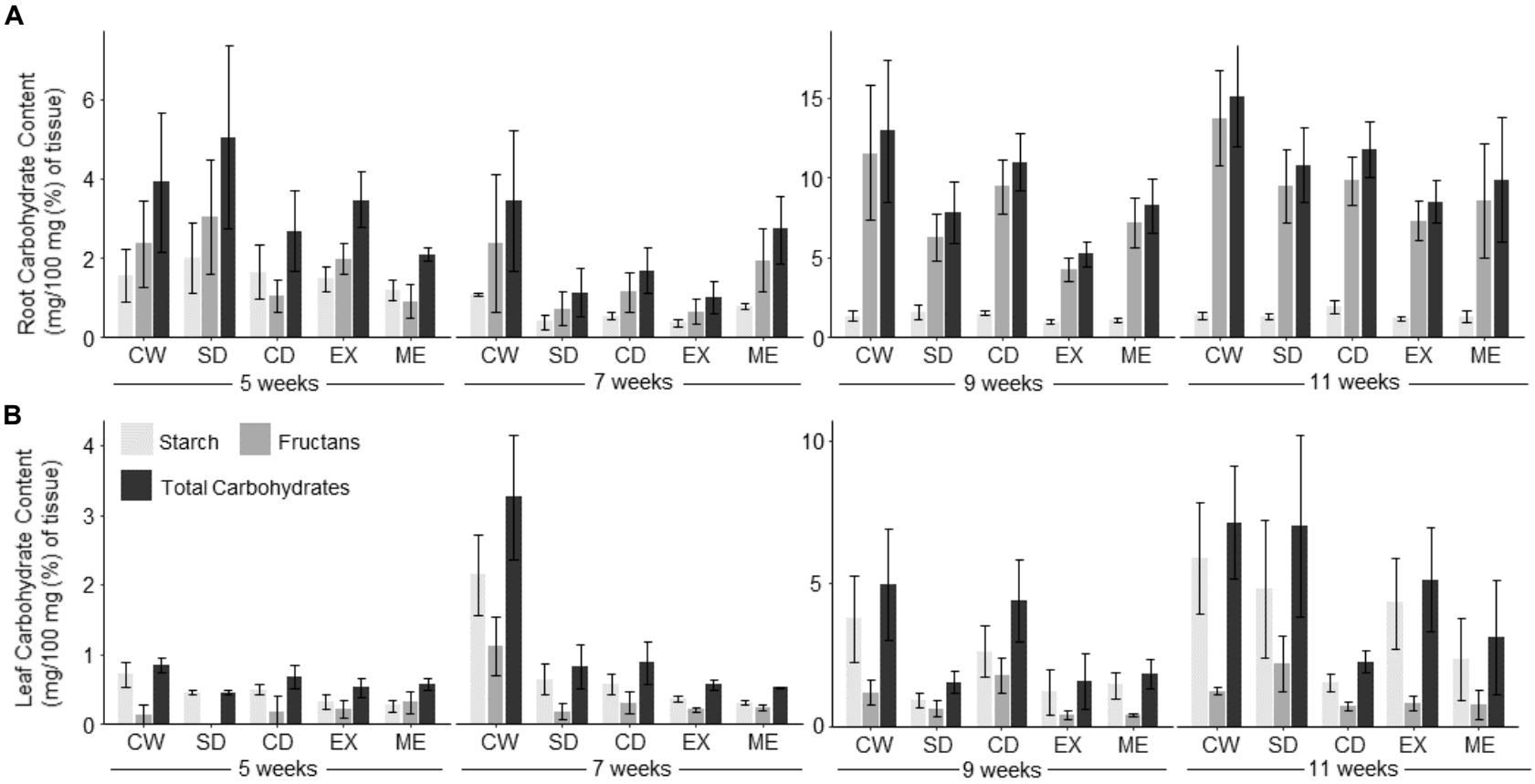

Figure 4.4. Carbohydrate content of roots (A) and leaves (B) of sagebrush seedlings grown under fertilizer treatments (CW: control with water only, SD: single dose, CD: constant dose, EX: exponential fertilizer, ME: modified exponential fertilizer) during the 11 week growth experiment. Bars represent standard error. n = 78 for roots, n = 93 for leaves.

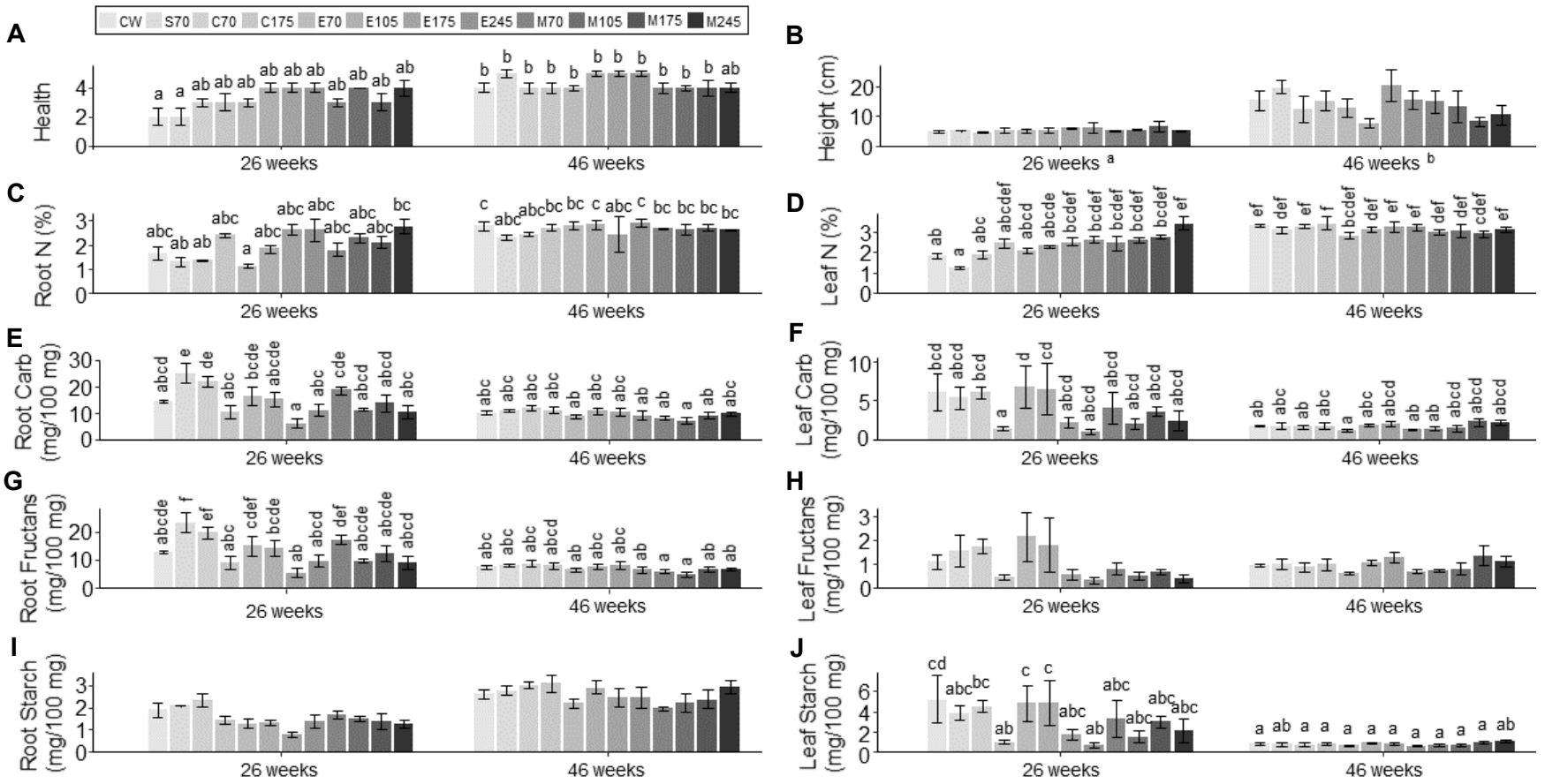

Figure 4.5. Health, height, and root and leaf nitrogen, carbohydrates, fructans, and starch of *Artemisia cana* seedlings grown for 26 weeks in a nursery and 20 weeks in a field (46 week total) under fertilizer treatments (CW: control with water only, S: single dose, C: constant dose, E: exponential fertilizer, M: modified exponential fertilizer with 70, 105, 175, and 245 mg N over the 26 week nursery period). Bars represent standard error. Letters indicate significant differences in the indicated seedling characteristic. n = 3 for each treatment grown for 26 weeks and n = 5 for each treatment grown for 46 weeks.

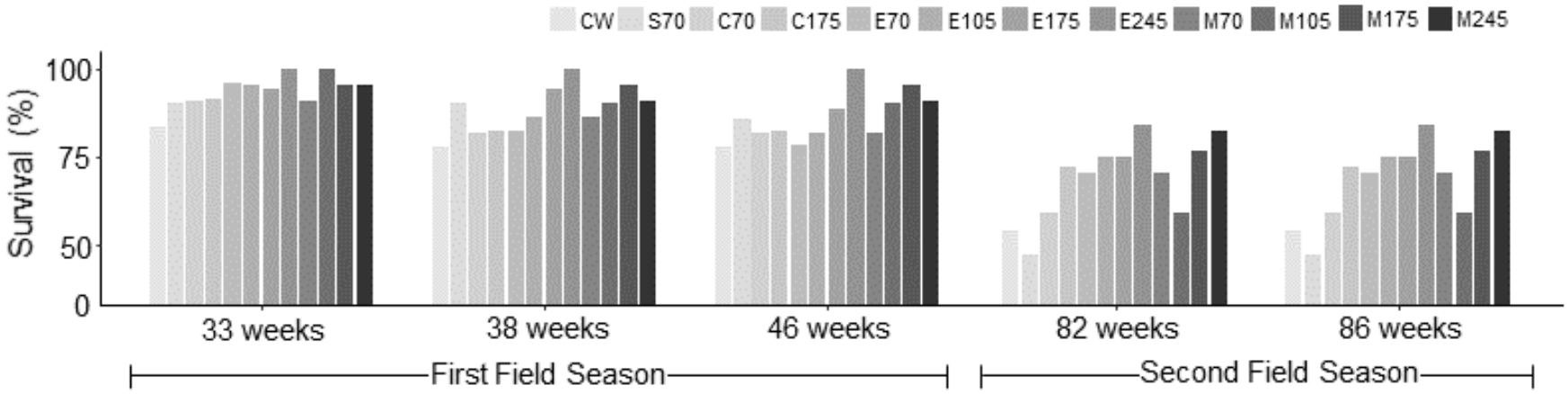

Figure 4.6. Seedling survival after outplanting assessed at seedling ages 33, 38, 46, 82, and 86 weeks. Seedlings were grown for 26 weeks before outplanting under fertilizer treatments (CW: control with water only, S: single dose, C: constant dose, E: exponential fertilizer, M: modified exponential fertilizer with 70, 105, 175 and 245 mg N over the 26 week nursery period). n = 256 for 33, 38 and 46 weeks each, n = 197 for 82 and 86 weeks each.

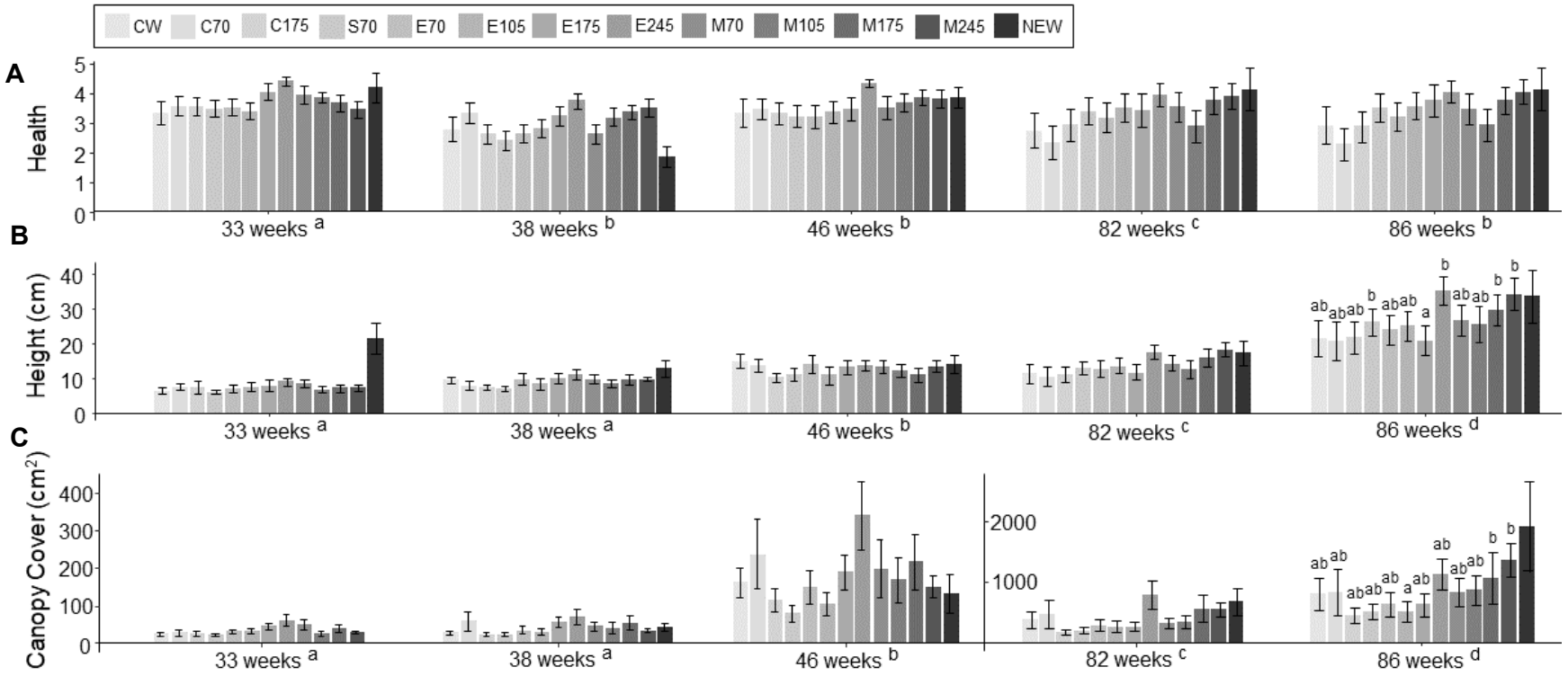

Figure 4.7. Height (A), canopy cover (B), and health (C) of seedlings grown for 26 weeks in a nursery and 7, 12, 20, 60, and 64 weeks in a field (33, 36, 46, 82, and 86 week total) under fertilizer treatments (CW: control with water only, S: single dose, C: constant dose, E: exponential fertilizer, M: modified exponential fertilizer with 70, 105, 175, and 245 mg N over the 26 week nursery period). Bars represent standard error. Letters indicate significant differences in the indicated seedling characteristic between seedling ages. n = 256 for 33, 38, and 46 weeks and n = 197 for 82 and 86 weeks.

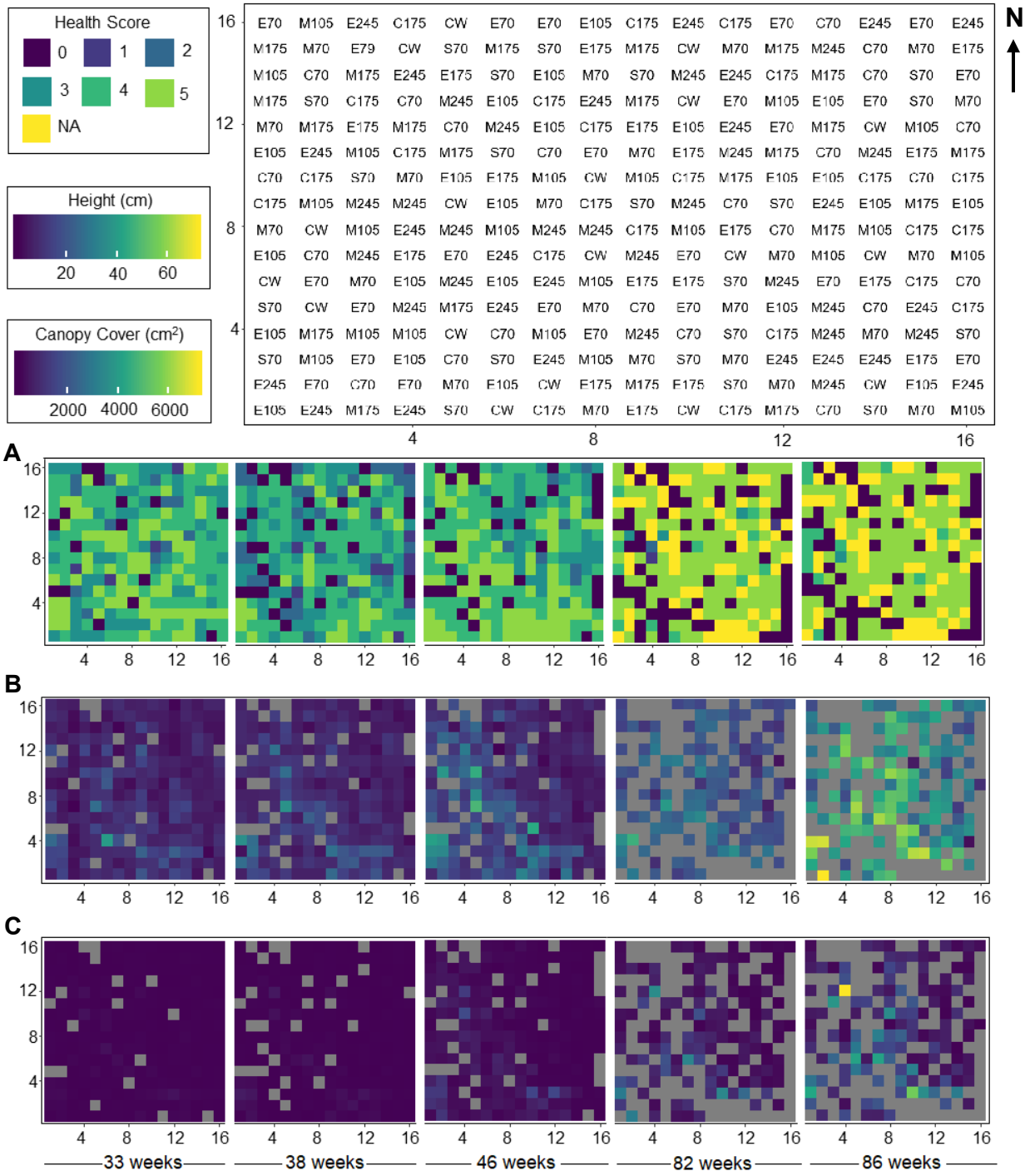

Figure 4.8. Heat map of the field plot showing seedling health (A), height (B), and canopy cover (C) at ages 33, 36, 46, 82, and 86 weeks. The legend in the top right indicates fertilizer treatments for each position in the 16 x 16 plot, with quadrants indicated by cardinal directions in each corner. For 33, 38, and 46 weeks n = 256, for 82 and 86 weeks n = 197. Gray boxes in height and canopy cover maps indicate dead or sampled seedlings.

SUPPLEMENTAL INFORMATION

Table S4.1. Weekly nitrogen additions for seedlings grown over 11 weeks in greenhouse, with 70 mg N delivered over the growing period.

Week	Single	Constant	Exponential	Modified Exponential
1	70.00	7.00	0.27	5.35
2	0.00	7.00	0.45	3.34
3	0.00	7.00	0.76	2.40
4	0.00	7.00	1.27	2.21
5	0.00	7.00	2.14	2.67
6	0.00	7.00	3.58	3.88
7	0.00	7.00	6.00	6.00
8	0.00	7.00	10.05	10.05
9	0.00	7.00	16.85	11.15
10	0.00	7.00	28.24	22.54

Table S4.2. Weekly nitrogen additions for seedlings grown over 26 weeks in greenhouse.

Week	Single	Constant		Exponential				Modified			
	70	70	175	70	105	175	245	70	105	175	245
1	70.00	2.80	7.00	0.19	0.20	0.23	0.25	0.44	0.73	1.49	2.46
2	0.00	2.80	7.00	0.22	0.25	0.28	0.31	0.45	0.70	1.32	2.06
3	0.00	2.80	7.00	0.26	0.30	0.35	0.38	0.47	0.69	1.20	1.77
4	0.00	2.80	7.00	0.31	0.36	0.43	0.48	0.50	0.70	1.13	1.57
5	0.00	2.80	7.00	0.37	0.43	0.52	0.59	0.54	0.73	1.10	1.46
6	0.00	2.80	7.00	0.43	0.52	0.64	0.74	0.59	0.78	1.12	1.42
7	0.00	2.80	7.00	0.51	0.63	0.79	0.92	0.65	0.85	1.18	1.46
8	0.00	2.80	7.00	0.61	0.75	0.97	1.15	0.74	0.95	1.29	1.57
9	0.00	2.80	7.00	0.72	0.91	1.20	1.43	0.84	1.08	1.46	1.77
10	0.00	2.80	7.00	0.85	1.09	1.47	1.78	0.96	1.24	1.69	2.05
11	0.00	2.80	7.00	1.01	1.32	1.81	2.22	1.01	1.32	1.81	2.22
12	0.00	2.80	7.00	1.20	1.59	2.23	2.77	1.20	1.59	2.23	2.77
13	0.00	2.80	7.00	1.42	1.91	2.74	3.45	1.42	1.91	2.74	3.45
14	0.00	2.80	7.00	1.69	2.30	3.37	4.30	1.69	2.30	3.37	4.30
15	0.00	2.80	7.00	2.00	2.77	4.14	5.36	2.00	2.77	4.14	5.36
16	0.00	2.80	7.00	2.37	3.34	5.09	6.68	2.37	3.34	5.09	6.68
17	0.00	2.80	7.00	2.81	4.02	6.26	8.32	2.81	4.02	6.26	8.32
18	0.00	2.80	7.00	3.33	4.85	7.69	10.37	3.33	4.85	7.69	10.37
19	0.00	2.80	7.00	3.95	5.84	9.46	12.92	3.95	5.84	9.46	12.92
20	0.00	2.80	7.00	4.68	7.03	11.63	16.10	4.68	7.03	11.63	16.10
21	0.00	2.80	7.00	5.54	8.47	14.29	20.07	5.54	8.47	14.29	20.07
22	0.00	2.80	7.00	6.57	10.20	17.57	25.01	6.57	10.20	17.57	25.01
23	0.00	2.80	7.00	7.79	12.29	21.61	31.16	7.79	12.29	21.61	31.16
24	0.00	2.80	7.00	9.23	14.81	26.57	38.83	8.38	13.29	23.52	34.05
25	0.00	2.80	7.00	10.94	17.83	32.66	48.39	10.09	16.32	29.61	43.61

Method S4.1. Rationale and detailed procedure for sugar derivatization (alditol acetates) for gas chromatography analysis.

The alditol acetate procedure used in this study was a modified version of that by Englyst et al. (1992). With the Englyst procedure, 1 mL of hydrolysate is made basic with addition of concentrated ammonium hydroxide (NH_4OH) and reduction is accomplished by adding 100 µl of sodium borohydride in 3 M NH_4OH (100 mg mL^{-1}). The reduction has an initial rapid increase in breakdown of sodium borohydride which creates excess heat. Excess heat is also created when the acetylation reagents are initially added. The modification made was to add 1 mL of sodium borohydride in dimethyl sulfoxide at the reduction step where 0.1 mL of the hydrolysate (made basic with concentrated NH_4OH) is added to 1 mL of sodium borohydride in dimethyl sulfoxide (10 mg mL^{-1}). This modification creates a milder digestion than that of the Englyst procedure as evidenced by little, if any, heat generated during reduction and acetylation steps. This modification should yield a direct measure of fructose. The alditol acetate of fructose is the same as that of mannose; thus fructose is observed as a mannose peak in gas chromatography analysis. However, depending on conditions, a portion of fructose will derivatize to the alditol acetate of glucose. With the Englyst method approximately 66 % of the fructose is expressed as glucose; with the modification approximately 25 % of the fructose is expressed as glucose, possibly allowing a clearer distinction between glucose derived from starch and glucose derived from the acetylation fructose. The modification allows more ease in analysis of large numbers of samples.

1. Sample Preparation
   a. Transfer 1 mL of starch extract into a 16 x 125 mm test tube (see starch extraction/digestion procedure).
   b. Add 50 µL of concentrated NH_4OH and mix. Check pH to ensure it is basic.
2. Reduction
   a. Transfer 100 µL of sample (from 1) to a 16 x 100 mm test tube containing 1 mL of sodium borohydride (10 mg mL^{-1}) in dimethyl sulfoxide (DMSO).
   b. Incubate in a water bath at 45 ºC for 1 hour mixing frequently by vortexing
   c. Remove samples from water bath and add 200 µL glacial acetic acid to destroy remaining sodium borohydride. Mix well.
   d. Let sit to cool (approximately 5 minutes).
3. Acetylation
   a. Add 200 µL 1-methylimazole and mix well using a vortex mixer.
   b. Add 2 mL acetic anhydride and mix well.
   c. Steps a and b, including mixing, should proceed as quickly as possible.
   d. Allow acetylation to proceed for 10 minutes, mixing frequently with a vortex mixer.
   e. Add 5 mL of water to stop acetylation by destroying the remainder of the acetic anhydride. Mix well.
   f. Allow samples to cool completely.
4. Extraction of alditol acetates
   a. Add 4 mL of dichloromethane, capo tubes, and mix vigorously by shaking.
   b. Centrifuge 10 minutes at 1500 rpm to separate layers.
   c. Remove top aqueous layer using a water aspirator.
   d. Add 5 mL water, mix vigorously, centrifuge 10 minutes at 1500 rpm and draw of the top aqueous layer using a water aspirator.
   e. Add 5 mL water, mix vigorously, centrifuge 10 minutes at 1500 rpm and draw of the top aqueous layer using a water aspirator.
   f. Dry off the dichloromethane at 40 ºC under a stream of nitrogen gas.
   g. Add 1 mL of dichloromethane to re-dissolve alditol acetates, transfer to a gas chromatography vial.
5. Analysis using gas chromatography

Method S4.2. Rationale and detailed procedure for digestion and extraction of starch.

Starch has limited solubility in aqueous solutions. To extract it from plant based materials, it is best to digest with starch digesting enzymes leaving free glucose which is readily soluble in aqueous solutions. Fructans, the other major source of non-structural polysaccharides, are also readily soluble in aqueous mixtures and will be extracted with the recently released glucose. Starch could be measured as the free glucose in this solution. Fructans require hydrolysis with a mild acid (0.1 M or 0.2 M sulfuric acid) to be broken down. These sugars (fructose, glucose) can be measured by gas chromatography. Thus, completing a starch digestion followed by hydrolysis with mild acid will allow total non-structural carbohydrates (total available carbohydrates) to be determined in the sample. This procedure describes the first part of that procedure, digestion of starch using the Megazyme®© Total Starch Assay Procedure. Because samples were not well ground due to small sample volume, it was necessary to adapt the procedure described in the kit instructions (using procedure d, excluding ethanol, followed by procedure a), especially with time of incubation in dimethyl sulfoxide and enzymes, to obtain accurate results. Optimum times were assessed by sequential incubation prior to starting the assay procedure. Dimethyl sulfoxide incubation was extended to 20 minutes from 5 minutes and incubation in thermostable α-amylase was increased to 40 minutes from 12 minutes. Given the longer incubation in dimethyl sulfoxide, it was not necessary to wet samples with ethanol. Ethanol appeared to be an impediment to extraction of starch from the poorly ground leaf samples but had less effect in better ground samples. The procedure was monitored using the starch provided in the Megazyme®© Total Starch Assay kit.

1. Sample Preparation
   a. Weigh 100 mg of sample in 16 x 125 mm test tube.
2. Part (d) Megazyme procedure
   a. Add 2 mL dimethyl sulfoxide and stir on a vortex mixer.
   b. Place in a vigorously boiling water bath for 20 minutes mixing repeatedly with a vortex mixer.
3. Part (a) Megazyme procedure
   a. Add 3 mL of α-amylase (bottle 1 diluted 1:30 in reagent 1; 100 mM sodium acetate buffer, pH 5.0).
   b. Transfer to a boiling water bath, incubate 40 minutes, mix every 5 to 10 minutes on a vortex mixer.
   c. Transfer tubes to a 50 °C water bath.
   d. Add 0.1 mL of amyloglucosidase (bottle 2), stir on a vortex mixer and incubate for 30 minutes.
4. Remove tubes from water bath and add 5 mL water (approximate 10 mL).
5. Mix well and centrifuge at 3000 rpm for 10 minutes.
6. Transfer portion of aqueous layer to 16 x 125 mm culture tube (separate from undigested residue).
   a. Transfer 1 mL of aqueous to a separate 16 x 125 mm culture tube for carbohydrate analysis by gas chromatography.
7. Seal all culture tubes and freeze.

V. LAND MANAGEMENT EFFECTS ON SAGEBRUSH HABITAT RESTORATION

1. INTRODUCTION

Native grasslands are some of the most altered habitats globally, and those of North America are among the most at risk ecosystems (Noss et al. 1995, Samson and Knopf 1996, Miller et al. 2011). Sagebrush (*Artemisia* L. [Asteraceae]) is one of the most common shrubs in North America, distributed throughout the prairie grassland and steppe ecoregions of the Great Basin (Meyer 2008). Distribution of sagebrush obligate species is closely linked to sagebrush as these shrubs are used for nesting, predator protection, and to fulfill dietary requirements. Loss, fragmentation, and degradation of sagebrush ecosystems caused by anthropogenic activity have resulted in declining populations of sagebrush obligate species (Crawford et al. 2004), most prominently, the Greater Sage-grouse (*Centrocercus urophasianus* Bonaparte [Phasianidae]; hereafter Sage-grouse). Its North American range has been reduced to 56 % of its pre-settlement state (Connelly et al. 2004); in Canada the historic range has been reduced by 94 % and is currently less than 7,000 km^2 (Aldridge and Brigham 2003). Extreme population declines led to listing and protection under the Species at Risk Act (SARA) in 2003 (Environment Canada 2014). The 2014 SARA recovery strategy suggests that low quality sagebrush habitat could support population growth through restoration to conditions that are optimal for the species (Environment Canada 2014).

Silver sagebrush (*Artemisia cana* Pursh) occurs in the Mixedgrass subregion of Canadian grasslands. It is the second most widely distributed sagebrush species in North America, occupying approximately 140,000 km^2 (Connelly et al. 2004). Plains silver sagebrush (*Artemisia cana* ssp. *cana*; hereafter, *Artemisia cana*) is the only sagebrush species to occupy Canadian Sage-grouse range. Sage-grouse have varying sagebrush requirements dependent on life stage, with as little as 5 % cover in leks, up to 50 % in winter habitat, 15 % in brood rearing habitat, and 30 % in nesting areas (Aldridge and Brigham 2002). Grasses provide cover from predators (Hagen et al. 2007), and forbs provide high quality forage for Sage-grouse prior to nesting and through chick rearing (Coggins 1998). Invertebrates are essential in chick diets, especially in the first month of life when 15 g day^{-1} are required for survival (Drut et al. 1994, Connelly et al. 2004).

Despite its distribution and importance in endangered species habitat, there has been relatively little research on methods to restore or enhance *Artemisia cana* habitat. With high anthropogenic disturbance in potential Sage-grouse habitat, studies that focus on land management and its effects on restoration outcomes will be critical for successful landscape restoration. Cattle grazing

is a common land management practice in sagebrush grasslands. Grazing intensity, rotation, and pasture size contribute to how grazing affects vegetation and range health (Knick 1999, Beck and Mitchell 2000, Anderson and Inouye 2001, Adams et al. 2004a). Reported effects of cattle grazing have been mixed, with some of the studies demonstrating that grazing will aid species establishment and increased plant cover and diversity (Gibson et al. 1987, Smith et al. 2000, Bullock et al. 2001, Wilsey and Martin 2015). Others report decreases in cover of native species with cattle grazing (Gornish and dos Santos 2015), especially in resource poor environments (Bakker at al. 2006, Beck et al. 2015). With recent and continuing bison re-introductions in the Northern Great Plans, effects of bison grazing also need to be considered. Bison cause less trampling and erosion damage than cattle and can help maintain forbs (Vinton et al. 1993, Steuter and Hidinger 1999), and in relation to ungrazed areas, bison grazed areas have greater vegetation richness and diversity (Hartnett et al. 1996).

Altering type and intensity of herbivore grazing can alter conditions at the soil surface by altering abundance of litter and bare ground (Willms and Quinton 1995). Excess bare ground and reduced litter cover may increase loss of seed from desiccation (Fowler 1986, Boeken and Orenstein 2001), erosion from wind or rain, and predation (Willms and Quinton 1995) and also lead to increased establishment of weeds (Bergelson et al. 1993). Excess litter may hinder revegetation by preventing seed from reaching the soil surface (Williams 1984), but adequate litter cover could aid in seedling survival by reducing soil surface temperatures and increasing soil water content (Naeth et al. 1991a, 1991b, Naeth and Chanasyk 1995). Herbicides commonly used to maintain a desirable native vegetation composition and control competition from non-native species, could increase seedling survival of outplanted sagebrush (Schuman et al. 1998, Boyd and Svejcar 2011, Newhall et al. 2011, McAdoo et al. 2013) but may be less effective with greater litter depth and cover which intercepts application to soil or plants.

The objective of this study was to investigate effects of land management in Grasslands National Park on potential sites for Sage-grouse habitat restoration and determine how the efficacy of standard revegetation methods (fall or spring seeding, outplanting seedlings), and non-native vegetation control (herbicide) may differ due to site qualities. We hypothesized that: 1) initial site conditions, assessed by baseline soil, invertebrate, and vegetation measurements would differ between each land management area, and 2) revegetation and non-native vegetation control would be negatively affected in areas where site condition was diminished (increased bare ground, decreased soil nutrients and litter cover, etc.).

2. MATERIALS AND METHODS

2.1. Research Sites And Establishment Of Research Plots

2.1.1. Research sites

Research was conducted in Grasslands National Park, Saskatchewan, from 2016 to 2019. The Park is 900 km^2, divided in two blocks (West Block, East Block) and is situated in the Mixed Grassland ecoregion. Soils are dominantly chernozemic with patches of solonetz and have low water content during the growing season (Adams et al. 2004b).

Four research sites were established, each with a distinct land management history: cattle grazed, bison grazed, watered, and ungrazed. Each of the 4 research sites was established independently and was unreplicated across the Park. This prohibits generalization of our results to areas outside the Park with similar land management histories, or assigning causation directly to land management, but allowed us to make recommendations for Sage-grouse habitat restoration at potential sites within the Park.

The bison grazed research site was located in the West Block bison range (13U 308725 E 5445201 N). Approximately 17,800 ha had been grazed by 400 bison (± 100) since 2006 at a stocking density of 0.74 AUM ha^{-1}. Reported AUMs (animal unit month) were obtained from Grasslands National Park and calculated with herd age demographics with one bison bull equivalent to 1.8 AUM, one bison cow equivalent to 1.5 AUM, and one bison yearling equivalent to 0.75 AUM. GPS collar data indicate that bison do not frequent this area often (Liccioli 2020). Prior to bison introduction the area was grazed only by wild herbivores (mule deer, pronghorn antelope, etc.) since Grasslands National Park acquired it in the late 1980s. The cattle grazed research site was located in West Block (13U 324026 E 5441119 N) and was grazed regularly, through grazing leases at stocking densities of 0.2 to 0.95 AUM ha^{-1} since the property was obtained by Grasslands National Park in the 1980s. Park land was purchased without coercion from landowners when they were ready to sell; thus, each area has an unknown grazing history prior to the Park's acquisition. Cattle are generally on the land between April and October and are rotated through different areas throughout the year. The watered research site has ephemeral spring flooding and was located in the bison grazing range (13U 308830 E 5445436 N) in an area bison under-utilize relative to the rest of their range (Liccioli 2020). On each of 21 July, 25 July, and 1 August 2017, approximately 500 L of water was applied by Parks Canada staff to the site (0.25 ha) using a water trailer with a fire hose to increase soil water content. The ungrazed

research site located in East Block (13U 369389 E 5434337 N) was not actively managed from 1986 to 2008, but prior to this was lightly grazed by cattle owned by private ranchers. This research area was surrounded with an electric fence to prevent stray cattle from foraging.

2.1.2. Establishment of research plots

At each site, 72 plots, 2 m x 2 m in size, were established in May 2016, each separated by at least 2 m. A full-factorial, completely randomized combination of 4 revegetation methods (fall seeding, spring seeding, spring planting, no seeding or planting) and 3 non-native plant species management methods (non-natives present with herbicide, non-natives present no herbicide, no non-natives, no herbicide). Each revegetation x herbicide treatment was replicated 6 times.

2.2. Revegetation And Non-Native Species Control Treatments

Fall and spring seeding treatments consisted of 8 native grasses, 7 native forbs, and *Artemisia cana* to achieve 10, 20, and 15 % plant cover, respectively (Table 5.1). Seed was wild collected in Grasslands National Park or sourced by collectors within 300 km of the Park (Table S1). For seeding calculations, germination and viability estimates for each seed lot were made by testing 5 replicates of 10 seeds for germination on moist paper towels in indirect sunlight. Emergence, establishment, and over-winter survival were conservatively estimated at 50 % (Calculation S1, Table S2). On 4 and 5 October 2016 and 19 to 31 May 2017 seed was hand broadcast in revegetation plots at 17 kg ha^{-1} (0.5 kg *Artemisia cana*, 5.9 kg forbs, 10.6 kg grasses).

Some *Artemisia cana* seed was used to grow seedlings in root trainers (12 cm deep) at the University of Alberta in January 2017 until outplanted to research plots in May 2017. Five, 3 to 5 cm tall *Artemisia cana* seedlings were outplanted in plots, 5 days after herbicide application. A hole approximately twice as deep and wide as the root ball was dug. Above ground vegetation was removed by hand from 10 cm around the seedling perimeter to reduce competition for light, water, and nutrients. Soil clods were broken by hand before being backfilled around the seedling and lightly packed. Litter was used to cover the seedling base to reduce soil water evaporation.

Glyphosate herbicide (Crush'R 540, active ingredient 540 g L^{-1}, AgriStar, Calgary, Alberta) in a 2 % solution with water was applied 15 to 19 May 2017 with a backpack sprayer by Parks Canada staff under University of Alberta direction. Herbicide was applied to foliage of all non-native plants in herbicide treatment plots 5 days after pitfall traps removal and before seeding and transplanting. Glyphosate is a broad-spectrum herbicide which is commonly utilized on annual broadleaf weeds and competitive grasses.

2.3. Data Collection And Sample Analysis

2.3.1. Temperature and precipitation

Growing season precipitation was monitored using three meteorological stations located in central West Block, southwestern East Block and eastern East Block, Grasslands National Park. Monthly temperature means were obtained from the Environment Canada's historical climate data for Val Marie, Saskatchewan (2019). Growing season temperature and precipitation means were calculated from meteorological data collected between 1995 and 2019.

2.3.2. Soil sampling and analyses

In June 2016, before revegetation and non-native species control treatments were applied, soil samples from 0 to 15 cm depth were collected across each research area. Three composite samples per site were submitted for analysis. Samples were analyzed at a commercial laboratory according to standard methods from Carter and Gregorich (2008), unless otherwise noted, for particle size by hydrometer method, pH and electrical conductivity by saturated paste, available nitrogen by ammonium fluoride and sulfuric acid extraction (Laverty and Bollo-Kamara 1988), available phosphorus and potassium by modified Kelowna extraction (Qian et al. 1994), and total nitrogen and total carbon by LECO furnace method.

2.3.3. Invertebrate sampling and analyses

From 8 to 10 May 2017, before revegetation and non-native species control treatments were applied, 24 pitfall traps were installed with even distribution across each 0.25 ha research site. Pitfall traps were deployed in early May to correspond with peak foraging time of Sage-grouse hens and chicks. Trap design was based on methods of Lowe et al. (2010). Approximately 20 mL of dilute ethylene glycol filled the trap bottoms to kill entering invertebrates. Traps were collected after 5 nights. Contents were preserved in 70 % isopropyl alcohol after collection and stored for further assessment. In April 2018, invertebrates from each trap were weighed, identified, and enumerated to taxonomic order.

2.3.4. Vegetation assessment

Between late June and early July 2016, 2017, 2018, and 2019 vegetation was assessed in 3 randomly located 0.1 m^2 quadrats in each plot. In each quadrat, plant foliar cover, ground cover, *Artemisia cana* cover, litter depth, and dominant grass heights were measured. Assessments in 2016 were made prior to application of revegetation and non-native species control treatments.

Assessments in 2017, 2018 and 2019 were made 2, 14, and 16 months post-treatment application. Plant foliar cover was visually estimated by species and ground cover (2.5 cm above surface) including bare ground, litter, lichen, moss, and other vegetation. *Artemisia cana* cover was determined in 0.1 m^2 quadrats in plots with transplants, by counting the live transplants and assigning each a cover value of 2 %. Litter depth was measured at 3 random locations in each plot. Height of dominant grass species was measured for 3 representative plants per quadrat and means for each of the species were calculated.

Artemisia cana transplant height and health were assessed in June 2017, 2018, and 2019 and percent survival calculated for each plot. Height was measured from the ground to tallest, living plant part. Plant heath was on a 0 to 5 scale; 0 for plants that could not be located, 1 for dead (0 % live), 2 for necrotic (< 25 % live), 3 for severely chlorotic or wilting (25 to 50 % live), 4 for chlorotic or wilting (51 to 75 % live), and 5 for healthy (> 75 % live).

2.4. Statistical Analyses And Calculations

Calculations and statistical analyses were conducted in R 3.4.1. (The R Foundation for Statistical Computing 2019). The condition of each land management area before revegetation and non-native species control treatment application were described with soil properties; invertebrate community composition, abundance and weight; vegetation community composition; and total forb and grass cover.

Analysis of variance (ANOVA), with alpha 0.05, was used to assess land management (cattle grazed, bison grazed, watered, ungrazed) effects on soil properties (sand, silt, clay; pH; electrical conductivity; available nitrogen, phosphorus, potassium; total, inorganic, organic carbon; total nitrogen; bare ground; litter cover and depth). Least square means test with Tukey pairwise comparisons and alpha 0.05 was run post hoc if a significant difference was detected. Non-metric multidimensional scaling with permutational multivariate analysis of variance (using distance matrices) was used to determine dissimilarity of land management areas based on abundance of invertebrates within each taxonomic order, and plant species richness and abundance data collected in 2016.

Treatments were assessed by comparing data prior to treatment application (2016) with data after treatment application in 2017, 2018, and 2019. Differences in total grass cover, total forb cover, and cover of seeded grasses, due to revegetation treatment were assessed using a Kruskal-Wallis multiple comparisons test, with significance at alpha 0.05. Dunn test, with Bonferroni

adjusted p values and alpha 0.05, was run post hoc if significant differences were detected with Kruskal-Wallis. Pairwise differences in total grass cover, total forb cover, and cover of seeded grasses, due to herbicide treatments were assessed with a Mann-Whitney test with alpha 0.05.

3. RESULTS

3.1. Temperature And Precipitation

Summer (May to September) precipitation in Grasslands National Park (ranges indicate multiple weather stations) was 267 to 300 mm in 2016, 61 mm in 2017, 101 mm in 2018, and 258 to 265 mm in 2019 (Table 5.2). Although precipitation is patchy within the Park (Val Marie measured 3.9 mm precipitation in June 2017, Gergovia station approximately 10 km away measured 20.2 mm), 2017 was reportedly the driest spring in 100 years (Johnson 2017). 2018 also had little precipitation, especially in May when vegetation demand for soil water is greatest. Mean summer temperatures were 15.1 °C in 2016, 15.8 °C in 2017, 15.6 °C in 2018, and 14.4 °C in 2019 (Table 5.2). Monthly temperatures were extremely high in 2017 and 2018; July 2017 was 6 °C warmer than average and a new record for extreme daily maximum temperature (40.9 °C) was set in August 2018.

3.2. Land Management

3.2.1. Soils

Soils at each land management area were of loam, sandy loam and silt loam textures (Table 5.3). Each area had significantly different available and total nitrogen, available potassium, total and organic carbon (Table 5.3). Soils had low electrical conductivity. The pH of the cattle grazed area was slightly alkaline, which decreases availability of some plant micronutrients (copper, iron, manganese, zinc). All areas had sufficient soil phosphorous, potassium, and nitrogen, good carbon to nitrogen ratios (approximately 10:1), and low organic matter (1.9 to 3.1 %). Proportion of bare ground was significantly higher and litter cover and depth significantly lower in the cattle grazed area than the other land management areas. The proportion of bare ground in each area was acceptable for healthy range sites (Adams et al. 2016).

3.2.2. Invertebrates

Hymenoptera (wasps, bees, ants) were the most abundant invertebrates at all sites, followed by Diptera (true flies). Sites had similar invertebrate richness, with differences in proportions of each order. The cattle grazed area had lowest invertebrate diversity, yet greatest invertebrate total weight within the sampling area; the ungrazed area had lowest invertebrate total weight and highest diversity. There was a high degree of similarity between each land management area, especially bison grazed and watered areas (Figure 5.1), although the invertebrate community was significantly affected by land management ($F_{3,92} = 10.9$, $p < 0.001$). The ungrazed area was distinguished by an abundance of Phalangida (daddy longlegs) and Gastropoda (snails, slugs), while the cattle grazed area was distinguished by an abundance of Hymenoptera, Coleoptera (beetles), and Orthoptera (grasshoppers, locusts, crickets).

3.2.3. Vegetation

Vegetation cover and richness were similar in bison grazed, ungrazed, and watered land management areas (Figure 5.2). The cattle grazed area lacked litter, and had more lichen and moss layers, which were covered by litter in other areas. The cattle grazed area was dominated by grasses and forbs of dry or subxeric habitats, such as Needle and thread grass (*Stipa comata* Trin. & Rupr.), and many grazing increasers, such as Pasture sage (*Artemisia frigida* Willd.) (Table 5.5). Bison grazed and watered areas were dominated by species of depressions or wet habitats, such as Western wheatgrass (*Agropyron smithii* (Rydb.) Á. Löve), with generalist species such as Plains reed grass (*Calamagrostis montanensis* Scribn. ex Vasey). The ungrazed area had a mix of generalist species and those that prefer wet and dry areas. Cattle and bison grazed areas had significantly greater native grass cover than watered or ungrazed areas ($Chi^2 = 70.3$, $p < 0.0001$) and were above habitat requirements for Sage-grouse (Figure 5.3). Forb cover was significantly greater in cattle and bison grazed areas than in the ungrazed area ($Chi^2 = 11.8$, $p < 0.01$). Sage-grouse habitat requirements for total forb cover were met consistently in cattle and bison grazed areas, and at some points throughout the study for watered and ungrazed areas (Figure 5.4).

3.3. Non-Native Species Control

In 2016, Crested wheatgrass (*Agropyron pectiniforme* Roem. & Schult.) was the only non-native grass species and was only found in the cattle grazed area. Its abundance and cover varied from year to year, with cover decreasing both with and without herbicide (Figure 5.3). Herbicide

reduced cover of non-native grasses in 2017 ($W = 216$, $p < 0.01$), and it remained low through 2018 and 2019. Herbicide reduced native grass species abundance in all land management areas, greatest in cattle grazed (Figure 5.3). Throughout the study, the cattle grazed area had greatest native grass cover (in plots not receiving herbicide) ($Chi^2 = 50.6$, $p < 0.0001$), but lowest native grass cover in plots receiving herbicide ($Chi^2 = 28.1$, $p < 0.0001$). By 2019, in all areas except cattle grazed, native grass cover in herbicide plots increased to near equivalent cover of that in untreated plots.

In total, 7 non-native forb species were observed in one or more plots: common dandelion (*Taraxacum offiniale* Weber), Goat's beard (*Tragopogon dubius* Scop.), Branched pepper grass (*Lepidum ramosissium* A. Nels.), Lamb's-quarters (*Chenopodium album* L.), Yellow sweet clover (*Melilotus officinalis* (L.) Lamb), Tansy mustard (*Descurainia sophia* (L.) Webb), and Alfalfa (*Medicago staiva* L.). Common dandelion and Goat's beard were present in all land management areas. In the cattle grazed area, Branched pepper grass and Lamb's-quarters were observed only after herbicide application, Yellow sweet clover increased from 1 to 4 % cover to 2 to 20 % cover after herbicide application, and Tansy mustard was only observed after herbicide application but was present in herbicide sprayed and unsprayed plots. In the bison grazed and watered areas, Tansy mustard was found at less than 1 % cover (on fewer than 5 plots) in newly exposed areas in herbicide sprayed plots. Alfalfa was found once (2 % cover) in 2017, in one plot in the ungrazed area. Herbicide decreased cover of non-native forbs immediately after application but not cover of non-native forbs long term (Figure 5.4). Herbicide increased cover of native forbs (Figure 5.4), generally by increasing richness and abundance of species present.

In the cattle grazed area, native forb cover significantly decreased in herbicide sprayed plots in 2017 ($W = 264$, $p < 0.001$), but increased in 2018 ($W = 889$, $p < 0.01$) and 2019 ($W = 17.5$, $p < 0.0001$). Herbicide decreased non-native forb abundance in the cattle grazed area in 2017 ($W = 294$, $p < 0.0001$) and 2018 ($W = 67.5$, $p < 0.01$); in 2019 non-native cover was significantly greater with herbicide ($W = 69.5$, $p < 0.05$). In bison grazed areas native forb cover was not significantly affected by herbicide ($W = 161$, $p = 0.99$), but in 2019, herbicide significantly lowered non-native cover ($W = 196.5$, $p < 0.05$). In watered areas, herbicide significantly increased native forb cover in 2018 only ($W = 889$, $p < 0.01$) and did not affect non-native forb cover. In the ungrazed area, herbicide increased native forb cover in 2018 ($W = 889$, $p < 0.01$) and 2019 ($W = 51.5$, $p < 0.01$), and did not significantly affect non-native forb cover.

3.4. Revegetation

Cover of seeded grass species in 2019 did not significantly differ between controls and seeding treatments or between herbicide and revegetation treatments (Figure 5.5). Western porcupine grass (*Stipa curtiseta* (Hitchc.) Barkworth), and Green needlegrass (*Stipa viridula* Trin.) were not present in 2019, and only a few of the other seeded species potentially established. In the bison grazed area, *Agropyron smithii* and Blue grama grass (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths) cover was greater (not statistically significant) in seeded treatments than in controls, but only with herbicide.

There was no evidence of establishment of any seeded forb species. *Dalea purpurea*, *Ratibida columnifera*, *Liatris punctata,* and *Linum lewisii* were not observed in any of the revegetation or control plots in 2019. Three flowered avens (*Geum triflorum* Pursh) and Blanket flower (*Gaillardia aristata* Pursh) were found in only one plot each, both in the cattle grazed area. Shining arnica (*Arnica fulgens* Pursh) was the most abundant seeded forb, and was present in 29 plots (of 360) in cattle grazed, bison grazed, and watered areas. When present, the mean cover of *Arnica fulgens* was 1.8 ± 0.3 %.

Establishment of *Artemisia cana* from seed was low and could not be statistically analyzed. In the watered area, 1 new individual established in a no herbicide, fall seeded plot. In the bison grazed area, 3 new individuals established in plots without herbicide, 1 individual each in 2 fall seeded plots and a spring seeded plot. In the cattle grazed area, herbicide killed, or reduced cover of, pre-existing *Artemisia cana*, but may have aided establishment from seed. Of plots with pre-existing *Artemisia cana*, 57 % decreased in density and cover, all with herbicide. Those that maintained or increased cover and density were without herbicide and were a relatively even mix of fall or spring seeded, and a control. 6 new individuals were observed in 3 plots that had no pre-existing *Artemisia cana*: 1 in a herbicide spring seeded plot, 4 in a herbicide fall seeded plot, and 1 in a no herbicide fall seeded plot. In the ungrazed area, herbicide was not detrimental to pre-existing *Artemisia cana*, but may have prevented establishment of new individuals. Of plots with pre-existing *Artemisia cana*, only those without herbicide increased cover and density, while those that decreased or maintained cover and density of *Artemisia cana* had a relatively even mix of herbicide and non-herbicide and fall or spring seeded, or control revegetation. Without pre-existing *Artemisia cana*, 2 % of plots had new individuals; 71 % were without herbicide and either spring seeded or controls with a mean of 3.2 ± 1.0 new individuals. The remaining 29 % of plots had herbicide applied and were spring seeded with a mean 3 ± 2 new individuals.

Survival of outplanted *Artemisia cana* seedlings was low (Figure 5.6). In the cattle grazed area, there were no surviving seedlings one year after outplanting. Two years after outplanting, the ungrazed area had greatest seedling survival at 13 %. Two year survival of seedlings in the bison grazed area was 4.4 % and 6.7 % in the watered area. Mean height of surviving seedlings was 14.7 ± 2.7 cm in bison grazed, 15.8 ± 6.4 cm in watered, and 17.9 ± 2.5 cm in the ungrazed area, two years after outplanting.

4. DISCUSSION

Natural, annual variation of vegetation cover and richness was evident from changes in control plots over time and may have had a relatively large effect on cover and richness of seeded plots. Despite that, all aspects of Sage-grouse habitat requirements (grass and forb cover, invertebrate abundance) were highly dissimilar between cattle grazed and the other land management areas. Many studies have shown benefits of cattle grazing in grasslands restoration (Gibson et al. 1987, Smith et al. 2000, Bullock et al. 2001, Wilsey and Martin 2015), by aiding species establishment and increasing plant cover and diversity. Others have reported decreases in cover of native species with cattle grazing (Gornish and dos Santos 2015), especially in resource poor environments (Bakker at al. 2006, Beck et al. 2015). Although impacts of livestock grazing specifically on Sage-grouse habitat are contextual (Guthery 1996), well managed grazing is thought to stimulate growth, and/or increase availability of forbs eaten by grouse (Neel 1980, Evans 1986, Thorpe and Goodwin 2003).

The cattle grazed area of our study was subjected to various grazing intensities and rotations since its acquisition by Grasslands National Park; although its prior grazing history is unknown. Over-grazing reduces revegetation success through low litter cover and high bare ground (Henderson et al. 2004). This led to erosion at our sites, manifesting as larger proportions of sand, increased soil pH (Dormaar and Willms 1998), low organic matter (Steffens et al. 2008), more non-native plant species which exploit bare ground, and more plant species that increase with grazing pressure. Reduced litter through heavy grazing can increase soil surface temperatures and reduce soil water content (Naeth et al. 1991a, 1991b, Naeth and Chanasyk 1995), which alters the plant-soil environment (Adams et al. 2004b, Deutsch et al. 2010).

Effects of over-grazing on restoration of Sage-grouse habitat and grassland health are numerous. Increases in bare ground increase visibility of Sage-grouse and their chicks, increasing their vulnerability to predation. Soil surface erosion with lack of litter could reduce effectiveness of

broadcast seeding, as seed blows away before emergence; any remaining seed has an increased risk of desiccation from exposure and greater soil surface temperatures. Vegetation establishment will be reduced by lower soil water content, and soil nutrients via erosion could hinder growth of new seedlings. However, if management is adapted to include effective rest periods and reduced stocking densities, litter will accumulate and soil organic matter will increase (Naeth et al. 1991c, Adams et al. 2004b), resulting in cattle grazed areas with good potential for restoration success. Cattle grazing can promote seed-soil contact, grass growth, and openings in vegetation for seedlings to establish. This was evidenced in our cattle grazed areas, with greatest grass cover, vegetation diversity and richness, and total invertebrate weight from all insect families that chicks consume. Total invertebrate weight may be more important to Sage-grouse than total number as small invertebrates are less likely to be seen and consumed. Chicks less than 21 days old require 15 g of invertebrates day^{-1} for survival and development and consume grasshoppers (Orthoptera), beetles (Coleoptera), and ants (Hymenoptera) (Patterson 1952, Klebenow and Gray 1968, Wallestad and Eng 1975, Johnson and Boyce 1990).

The ungrazed land management area, and bison and watered areas to some extent, were affected by increased litter accumulation. Increased litter can reduce productivity of a site (Adams et al. 2016) and could have contributed to reduced grass and forb cover at the ungrazed, bison and watered areas. Research on willow grouse indicates that chicks will avoid areas with dense vegetation or litter to avoid increasing their energy demands which could put them at higher risk of mortality (Erikstad and Spidsø 1982). Excess litter at the ungrazed, bison and waters areas likely hindered seeding success due to reduced seed-soil contact after broadcast seeding.

Reduced light at the soil surface may have hindered germination when seed did reach the soil surface. Despite low success of seeding treatments, these sites enabled outplanted seedlings to establish and survive. Drought conditions in 2017 and 2018 likely favoured sites which facilitated seedlings in obtaining water and nutrients. Litter and soil texture most likely influenced seedling survival in these conditions. Loam soils at the bison grazed and ungrazed areas provided good root-soil contact, and ideal pore space, drainage, and water retention. Silt loams at the watered site had greater potential for plant nutrient retention, and although they have low infiltration rates, water holding capacity is good. Increased litter cover and depth likely moderated soil-surface temperatures and reduced soil water evaporation from soil surfaces (Naeth et al. 1991a,b, 1990).

If the restoration timeline restricts site preparation prior to revegetation, it would be most efficient to remove excess litter through increased stocking densities, extended grazing periods, or prescribed fire in ungrazed or bison grazed areas, rather than waiting to accumulate litter and soil

organic matter in over-grazed areas. Many studies emphasize maintaining protective litter cover for Sage-grouse, but few indicate the level at which litter becomes problematic. Adams et al. (2004a) state that deeper litter cover from light grazing by cattle results in areas which best meet habitat requirements for Sage-grouse; however, there are very few Sage-grouse habitat assessments that report litter cover. One study on Sage-grouse habitat in mixedgrass prairie of North Dakota reported greater nest success with litter cover of 13 % versus 7 % (Herman-Brunson et al. 2009). A study in sagebrush steppe in Wyoming reported hens selected areas with greater litter cover (17.8 % versus 14.5 %) for nesting (Holloran et al. 2005). Studies on range health indicate that ecologically sustainable stocking rates for the *Artemisia cana* – *Agropyron smithii* range plant communities on loam sites is 0.37 to 0.61 AUM ha^{-1}, with lower rates for blowouts, saline or sandy sites (Adams et al. 2013). Stocking rate recommendations were not met by any management area in this study. Although the bison grazed area exceeded stocking density recommendations, excess litter likely accumulated because bison grazing is heterogeneous large ranges and the research sites were located in under-utilized areas (Licciolo 2020). The ungrazed, bison grazed, and watered sites have high potential for successful restoration via seedling outplanting. However, litter reduction through increased grazing or fire will need to occur for broadcast seeding to be an effective revegetation method in these areas. Reduction of the litter cover and volume may also enable natural establishment of forbs whose reproduction is mainly by seed.

Although *Artemisia* seedling survival in our study was lower than the mean two year survival rate of 30 to 36 % of outplanted *Artemisia tridentata* and *Artemisia cana* seedlings reported in other studies (Evans and Lih 2005, Newhall et al. 2011, Wirth and Pyke 2011, Dettweiler-Robinson et al. 2013, McAdoo et al. 2013), reducing or eliminating herbaceous competition could result in greater survival and vigour of *Artemisia* seedlings (Schuman et al. 1998, Boyd and Svejcar 2011, Newhall et al. 2011, McAdoo et al. 2013). Herbicide may be a viable option to improve seedling survival by reducing competition, as well as opening areas for good soil-seed contact after broadcast seeding. Finally, increasing seedling internal nutrient reserves and vigour prior to outplanting through nutrient loading may increase seedling survival (Malik and Timmer 1996, Timmer 1997, Imo and Timmer 1999, McAllister and Timmer 1998, Salifu and Timmer 2003, Oliet et al. 2009, Galvez et al. 2011, Hu 2012, Landhäusser et al. 2012, Schott et al. 2016).

Differences in forage selection by bison and cattle may have contributed to greater numbers of native forbs in bison grazed areas. Bison select grasses preferentially, while cattle will consume both forbs and grasses (Steuter and Hidinger 1999), although results are confounded by differing

grazing systems employed for each herbivore (Helzer and Steuter 2005). Bison generally have large ranges and are left to graze year long, while cattle are rotated through smaller pastures throughout the year or for part of the year (Steuter and Hidinger 1999, Helzer and Steuter 2005). In smaller pastures, forbs are quickly depleted as they are preferential forage for cattle. Although herbicide increased native forb cover in all land management areas, it also increased cover of non-native forbs where they were already problematic. This indicates that long-term management to reduce non-native species is required, but that short-term increases in native forb cover where non-natives are not an issue could be obtained through herbicide application. Although not achieved in our study due to extreme drought and sites that were not adequately prepared for revegetation, increasing plant diversity through seeding could confer resistance to invasion by non-native vegetation (Dickson and Busby 2009, Nemec et al. 2013). A seed mix with a high diversity of forbs, including those adapted to different environmental conditions could provide resilience to drought as community composition will shift accordingly, while overall cover may be maintained. To increase the success of establishment from seed, Dickson and Busby (2009) suggest forbs be seeded at higher densities, and that forb and grass seed be spatially separated to reduce competition. To reduce seed costs, forbs could be seeded into managed areas to reduce competition from grasses. If seed costs are highly prohibitive to restoration, a variety of seed mixes may be used for specific site conditions, so species that are best adapted and most likely to dominate a given area are seeded into those areas.

Although this study was conducted during severe drought, which may limit generalization of our results to other restoration efforts, climate change is predicted to increase drought in many areas, including Grasslands National Park. Current climate models for southern Saskatchewan predict a mean increase in annual temperature from 3.5 to 5.9 °C, with the number of days above 30 °C increasing from 22 to 38 (Prairie Climate Center 2019). Climate change could further complicate sagebrush habitat restoration as sagebrush seedlings may be particularly susceptible to climate influences on seedling recruitment. Therefore, our study may represent likely restoration outcomes for future climate scenarios, rather than during an exceptional weather event, highlighting the importance of appropriate land management to facilitate successful restoration.

5. CONCLUSIONS

Our study highlights the need for appropriate land management to achieve successful revegetation for Sage-grouse habitat restoration. Land management significantly affected soil

properties, and invertebrate and vegetation communities. Broadcast seeding was the least effective revegetation method and although outplanting seedlings resulted in greater establishment of *Artemisia cana*, survival was very low. One-time herbicide application was not successful in reducing non-native vegetation cover over the long term and negatively impacted native plant cover. All sites in this study have potential for successful restoration to Sage-grouse habitat given modification to their management practices and selection of appropriate revegetation methods.

Table 5.1. Native species and seed weights sown on land management research plots (each 4 m^2) in October 2016 (fall seeding) and May 2017 (spring seeding).

Scientific Name	Common Name	Seed Per Plot (g / 4 m^2)	Seeding Rate (g m^{-2})
Artemisia cana	Silver sagebrush	0.219	0.055
Agropyron smithii	Western wheatgrass	0.878	0.220
Agropyron dasystachyum	Northern wheatgrass	0.598	0.150
Poa sandbergii	Sandberg blue grass	0.223	0.056
Stipa viridula	Green needlegrass	0.409	0.102
Stipa comata	Needle and thread grass	0.666	0.167
Stipa curtiseta	Western porcupine grass	1.728	0.432
Koeleria macrantha	Prairie june grass	0.026	0.007
Bouteloua gracilis	Blue grama grass	0.060	0.015
Geum triflorum	Three flowered avens	0.252	0.063
Ratibida columnifera	Upright prairie coneflower	0.303	0.076
Gaillardia aristata	Blanket flower	0.797	0.199
Dalea purpurea	Purple prairie clover	0.589	0.147
Arnica fulgens	Shining arnica	0.346	0.087
Liatris punctata	Dotted blazing star	0.105	0.026
Linum lewisii	Wild blue flax	0.139	0.035
Total Weight of Seed		7.338	1.835

Table 5.2. Temperature and precipitation for Grasslands National Park 2016 to 2019.

	Normals	2016	2017	2018	2019
Precipitation (mm)					
May	51.7	79.7	13.4	9.0	19.9
June	65.3	34.5	15.1	17.4	104.8
July	54.0	129.6	11.4	9.9	9.8
Aug	33.8	7.0	13.0	24.2	63.6
Sept	27.6	50.1	8.9	41.2	67.5
Daily Average Temperature (°C)					
May	11.0	11.1	11.2	14.0	8.8
June	15.8	16.6	15.5	16.5	15.4
July	18.3	18.2	21.7	19.2	18.0
Aug	17.7	17.3	17.8	18.0	17.6
Sept	11.6	12.2	12.6	10.1	12.1
Daily Maximum Temperature (°C)					
May	18.2	19.6	20.6	23.2	17.4
June	23.3	25.3	25.0	25.8	23.7
July	26.2	25.9	32.1	29.4	27.3
Aug	26.2	26.3	28.0	28.3	26.1
Sept	19.9	20.5	21.0	17.0	18.9
Daily Minimum Temperature (°C)					
May	3.7	2.5	1.8	4.7	0.3
June	8.4	7.7	6.0	7.2	7.2
July	10.4	10.4	11.1	8.9	8.8
Aug	9.2	8.3	7.6	7.6	9.2
Sept	3.2	3.9	3.2	3.2	5.4
Extreme Maximum Temperature (°C)					
May	36.0 *	30.4	29.9	31.5	29.8
June	38.5 *	32.6	32.8	32.6	32.3
July	41.1 **	31.2	38.5	39.3	35.7
Aug	40.9 ***	33.6	35.0	40.9	35.8
Sept	36.1 ****	31.3	32.6	30.2	31.0

* 1988, ** 1937, *** 2018, **** 1967

Monthly normals are calculated from meteorological data collected 1995 to 2019.

Table 5.3. Soil parameters for land management areas June 2016, prior to revegetation or non-native vegetation control treatments.

Soil Parameter	Land Management Area				ANOVA Output
	Cattle Grazed	Bison Grazed	Watered	Ungrazed	
Sand (%)	63.0^{a} ± 1.41	38.2^{b} ± 1.89	32.0^{b} ± 1.20	38.4^{b} ± 1.15	F = 32.1, p < 0.001
Silt (%)	22.1^{b} ± 1.25	42.8^{a} ± 0.93	45.5^{a} ± 3.89	40.6^{a} ± 1.00	F = 16.5, p < 0.001
Clay (%)	14.9^{b} ± 0.29	19.0^{ab} ± 1.30	22.5^{a} ± 1.96	21.0^{ab} ± 0.58	F = 4.98, p < 0.05
Bare ground (%)	6.0^{a} ± 0.48	1.7^{b} ± 0.23	0.59^{b} ± 0.17	0.60^{b} ± 0.45	F = 50.8, p < 0.0001
Litter cover (%)	6.7^{d} ± 0.32	59.2^{c} ± 3.17	72.7^{b} ± 2.05	83.8^{a} ± 1.40	F = 286.0, p < 0.0001
Litter depth (cm)	1.3^{a} ± 0.08	4.9^{b} ± 0.27	6.7^{c} ± 0.30	5.8^{b} ± 0.21	F = 106.5, p < 0.0001
Conductivity (dS m^{-1})	0.89 ± 0.015	0.85 ± 0.126	1.08 ± 0.079	1.02 ± 0.097	p > 0.05
Soil pH	7.2 ± 0.02	6.5 ± 0.33	6.5 ± 0.32	6.5 ± 0.16	p > 0.05
Available N (mg kg^{-1})	13.9^{c} ± 0.70	16.1^{bc} ± 0.45	37.6^{a} ± 5.68	32.1^{ab} ± 1.49	F = 10.7, p < 0.01
Available P (mg kg^{-1})	7.9 ± 0.94	4.8 ± 0.32	8.5 ± 2.05	5.5 ± 0.57	p > 0.05
Available K (mg kg^{-1})	242^{b} ± 8.30	280^{ab} ± 7.36	392^{a} ± 48.29	297^{ab} ± 4.98	F = 5.84, p < 0.05
Total C (%)	1.35^{b} ± 0.05	1.71^{ab} ± 0.13	2.13^{a} ± 0.09	1.58^{b} ± 0.07	F = 9.35, p < 0.01
Total Inorganic C (%)	0.125 ± 0.015	0.094 ± 0.024	0.181 ± 0.072	0.055 ± 0.003	p > 0.05
Total Organic C (%)	1.23^{c} ± 0.04	1.63^{ab} ± 0.09	1.95^{a} ± 0.03	1.53^{bc} ± 0.07	F = 16.9, p < 0.001
Total N (%)	0.124^{c} ± 0.003	0.158^{b} ± 0.008	0.193^{a} ± 0.003	0.156^{bc} ± 0.009	F = 14.9, p < 0.01

Means ± standard error are presented. Different letters indicate significant differences in the specific soil parameter between the land management areas. n = 72 for bare ground, litter cover, and litter depth for each land management area, otherwise n = 3 for each land management area.

Table 5.4. Invertebrate diversity (Simpson's diversity), total weight, and abundance of individuals within each order, May 2017, prior to applying non-native vegetation controls.

Invertebrate Parameter	Land Management Area			
	Cattle Grazed	Bison Grazed	Watered	Ungrazed
Diversity	0.49	0.64	0.65	0.79
Total weight (g)	12.4	4.94	8.48	3.64
Abundance				
Arachnida	10.17 ± 1.55	6.46 ± 0.63	7.83 ± 0.89	7.67 ± 0.69
Chilipoda	0.08 ± 0.06	0.38 ± 0.12	0.04 ± 0.04	0 ± 0
Coleoptera	10.38 ± 1.69	3.67 ± 0.33	7.21 ± 0.69	2.92 ± 0.42
Collembola	0.67 ± 0.20	1.58 ± 0.32	1.29 ± 0.36	0.92 ± 0.28
Diptera	11.38 ± 1.40	30.83 ± 8.47	48.04 ± 8.74	10.13 ± 2.17
Gastropoda	0 ± 0	0 ± 0	0 ± 0	0.04 ± 0.04
Hemiptera	2.29 ± 0.50	3.71 ± 1.01	2.58 ± 0.50	2.29 ± 0.44
Hymenoptera	84.67 ± 30.79	46.54 ± 10.53	27.13 ± 3.80	12.58 ± 1.60
Lepidoptera	0.71 ± 0.19	0.92 ± 0.29	0.79 ± 0.28	0.21 ± 0.12
Orthoptera	1.38 ± 0.29	0.04 ± 0.04	0.08 ± 0.06	0.08 ± 0.06
Phalangida	0 ± 0	0.25 ± 0.25	0 ± 0	3.38 ± 0.68

n = 24 pitfall traps for each 0.25 ha land management area.

Table 5.5. Vegetation richness, diversity, and dominance in non-herbicide sprayed plots for land management areas June 2019.

Land Area	Diversity	Richness	Dominant Species
Cattle grazed	0.91	39	*Bouteloua gracilis* 3.6 %, *Stipa comata* 2.9 %, *Koeleria macrantha* 2.2 %, *Agropyron pectiniforme* 1.4 %, *Artemisia frigida* 2.7 %, *Plantago patagonica* 2 %, *Taraxacum officinale* 1.4 %, *Linum rigidum* 1 %
Bison grazed	0.88	25	*Agropyron smithii* 1.3 %, *Agropyron dasystachyum* 1 %, *Calamagrostis montanesis* 1 %, *Antennaria aprica* 3.4 %, *Achillea millefolium* 0.5 %, *Sphaeralcea coccinea* 0.5 %
Watered	0.89	29	*Agropyron smithii* 1.7 %, *Festuca hallii* 0.9 %, *Agropyron dasystachyum* 0.6 %, *Taraxacum officinale* 2.8 %, *Antennaria aprica* 2.1 %, *Sphaeralcea coccinea* 1 %, *Achillea millefolium* 0.6 %, *Cerastium arvense* 0.6 %
Ungrazed	0.90	24	*Agropyron smithii* 2.4 %, *Agropyron dasystachyum* 1.8 %, *Koeleria macrantha* 0.9 %, *Taraxacum officinale* 1.1 %, *Achillea millefolium* 1 %

*Simpson's diversity index calculated as 1 - Σ (n / N)
Mean dominant species cover is reported. n = 48 for each land management area.

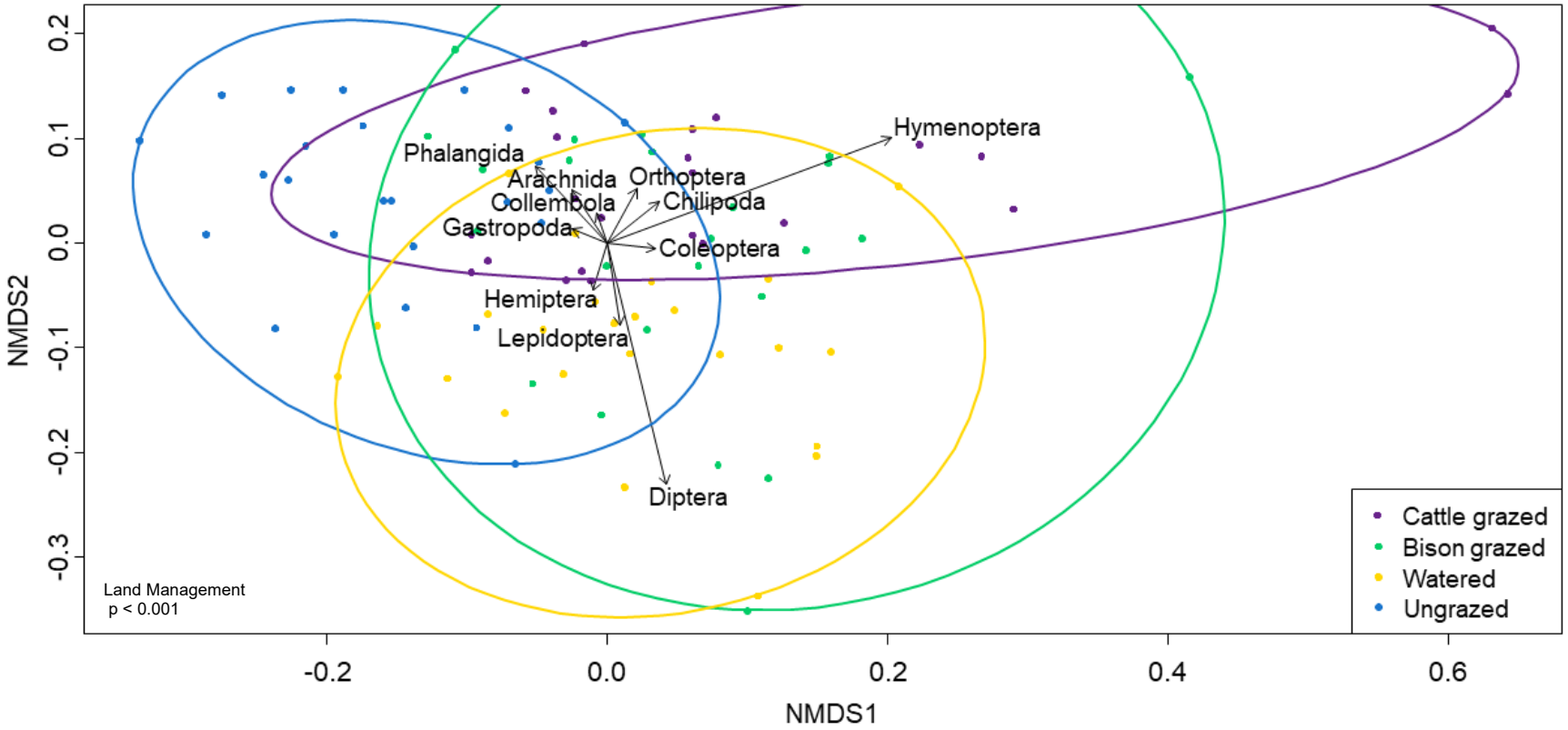

Figure 5.1. Non-metric multidimensional scaling analysis for invertebrates captured via pitfall trap within land management areas, prior to herbicide application. Plots positioned closer together are more similar than plots positioned farther apart. Plots located at the tip of the vectors are more strongly associated with that invertebrate order than plots located farther from the tips of the vectors. The length of the vector is correlated to the strength of the relationship of that variable with the ordination. n = 96.

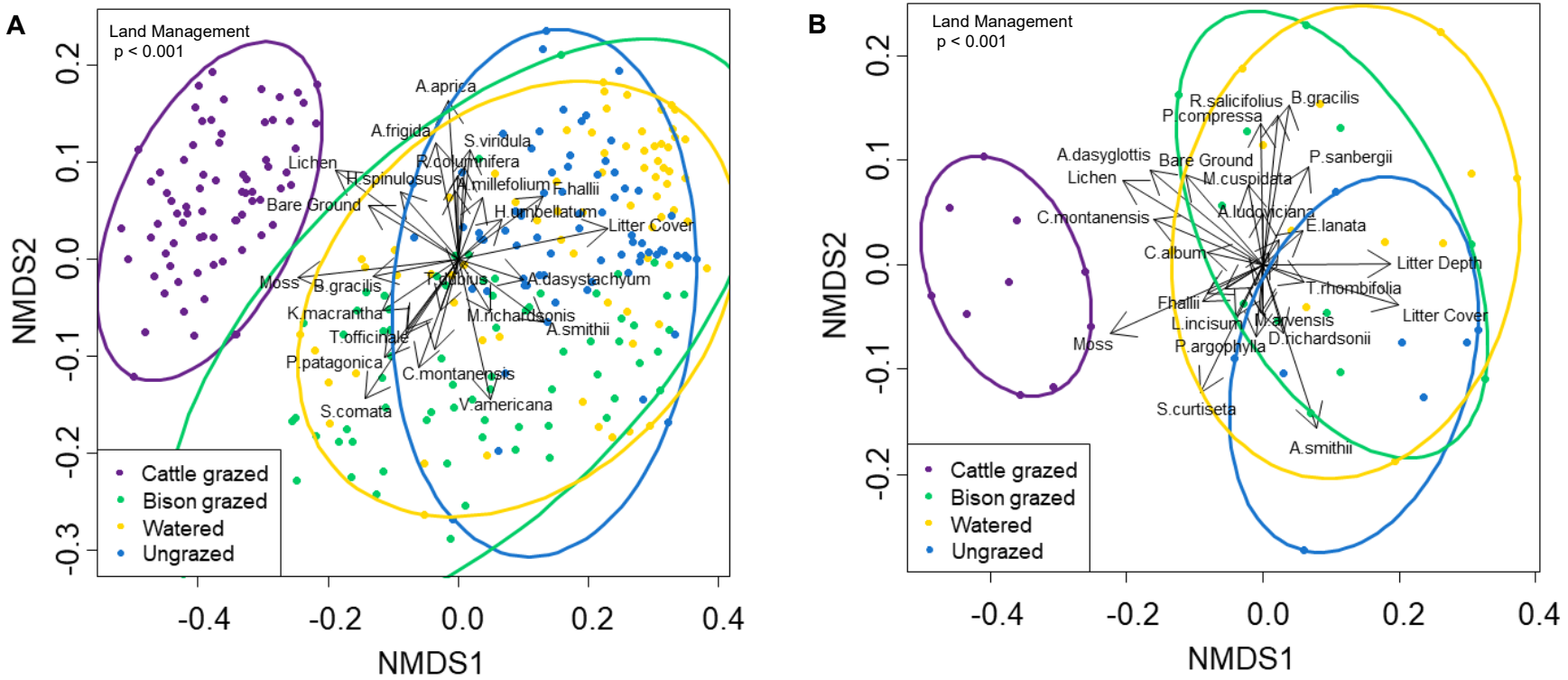

Figure 5.2. Non-metric multidimensional scaling analysis for vegetation cover within land management areas, (A) in 2016 prior to any non-native vegetation management or revegetation treatments and (B) in 2019 for control plots. Plots positioned closer together are more similar than plots positioned farther apart. Plots located at the tip of the vectors are more strongly associated with that vegetation parameter than plots located farther from the tips of the vectors. The length of the vector is correlated to the strength of the relationship of that variable with the ordination. (A) n = 360, (B) n = 42.

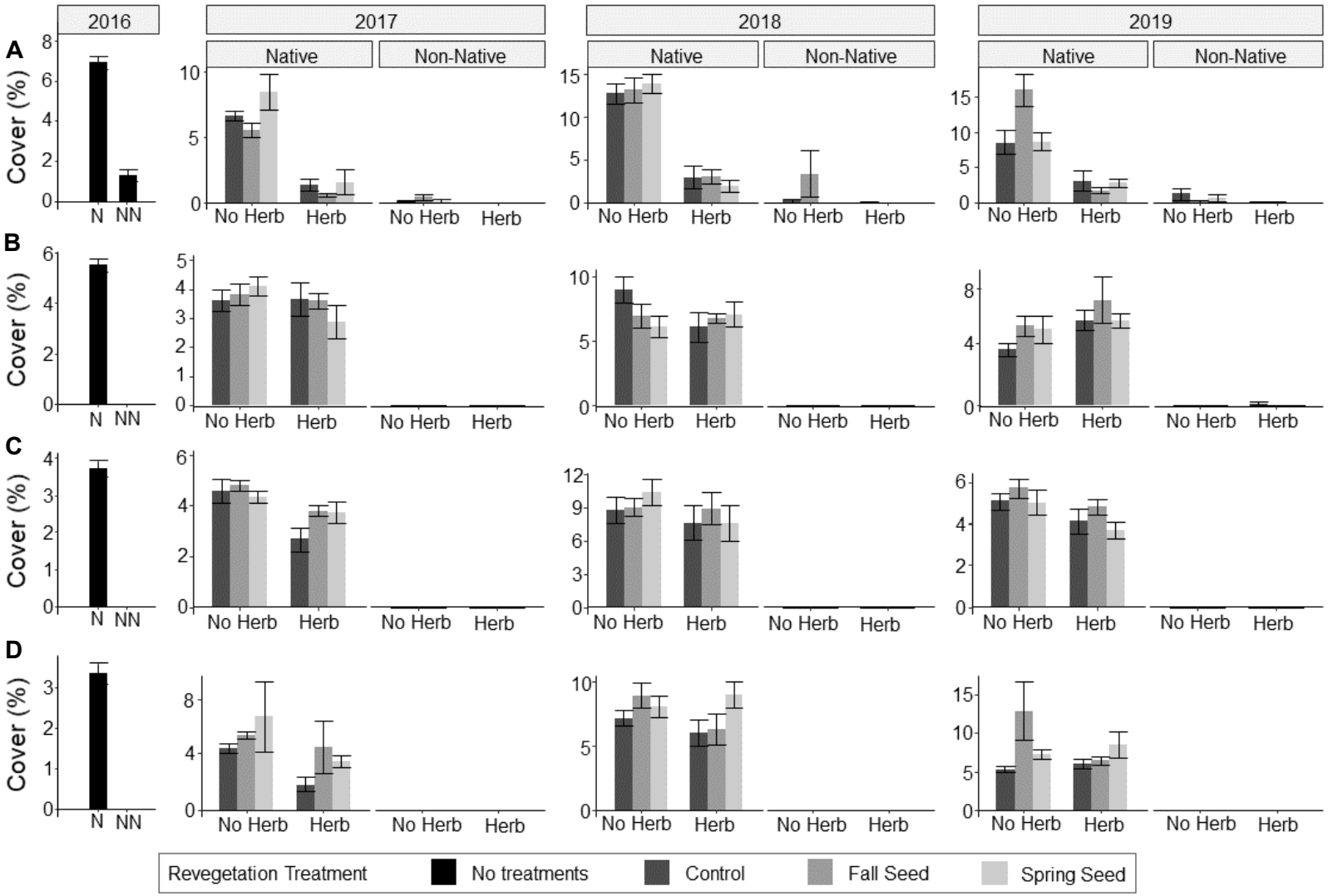

Figure 5.3. Total grass cover of native and non-native species in treated (revegetation x non-native vegetation control) plots in cattle grazed (A), bison grazed (B), watered (C), and ungrazed (D) land management areas from 2016 to 2019. Bars represent standard error. No treatments were applied in 2016, data presented only for native (N) and non-native (N) grasses. Herbicide (herb) was applied May 2017, fall seed in October 2016 and spring seed in May 2017. n = 72 for each land management area per year.

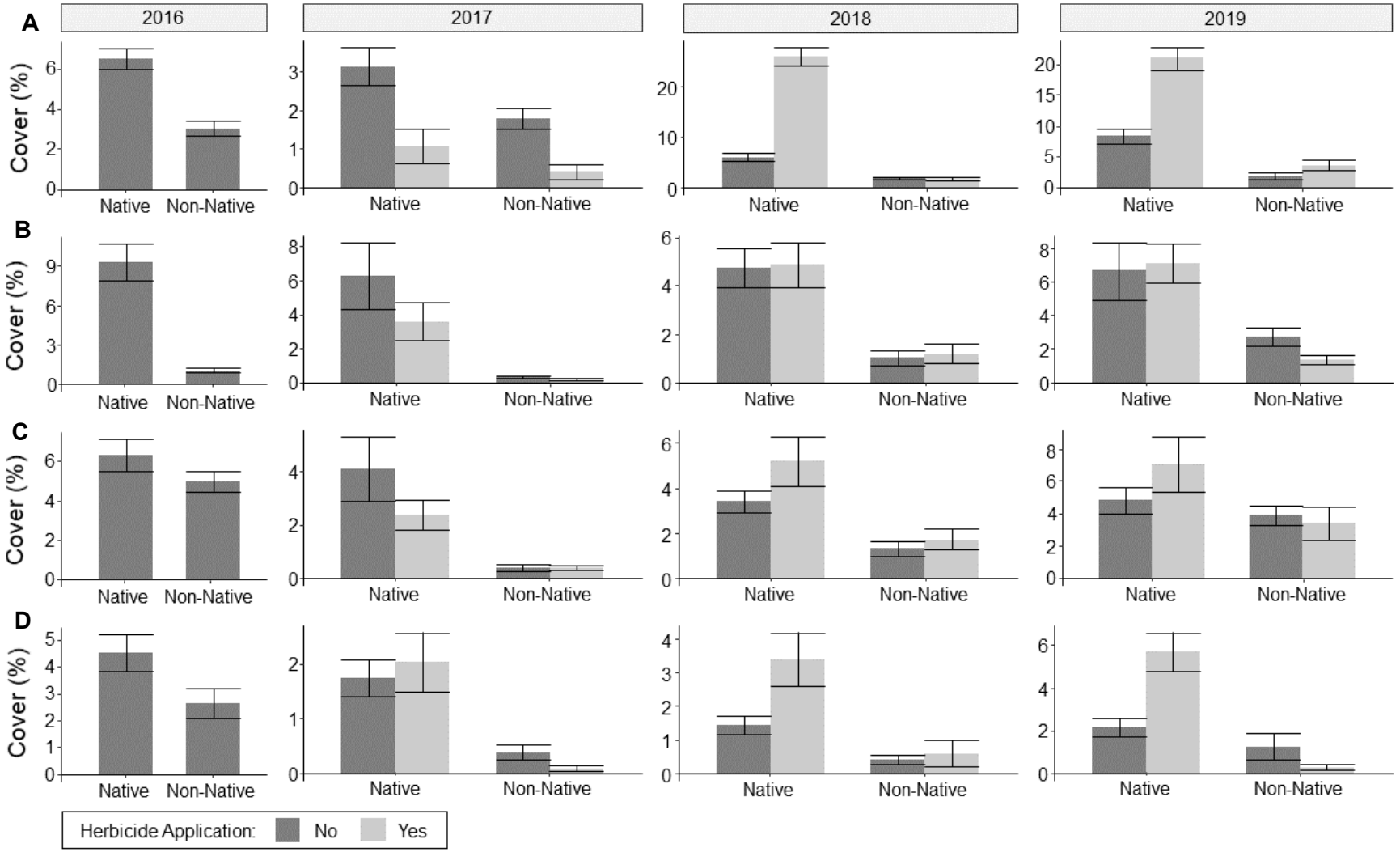

Figure 5.4. Total forb cover of native and non-native species in cattle grazed (A), bison grazed (B), watered (C), and ungrazed (D) land management areas from 2016 to 2019. Bars represent standard error. No treatments were applied in 2016, data presented only for native (N) and non-native (N) forbs. Herbicide (herb) was applied May 2017, fall seed in October 2016 and spring seed in May 2017. n = 72 for each land management area per year.

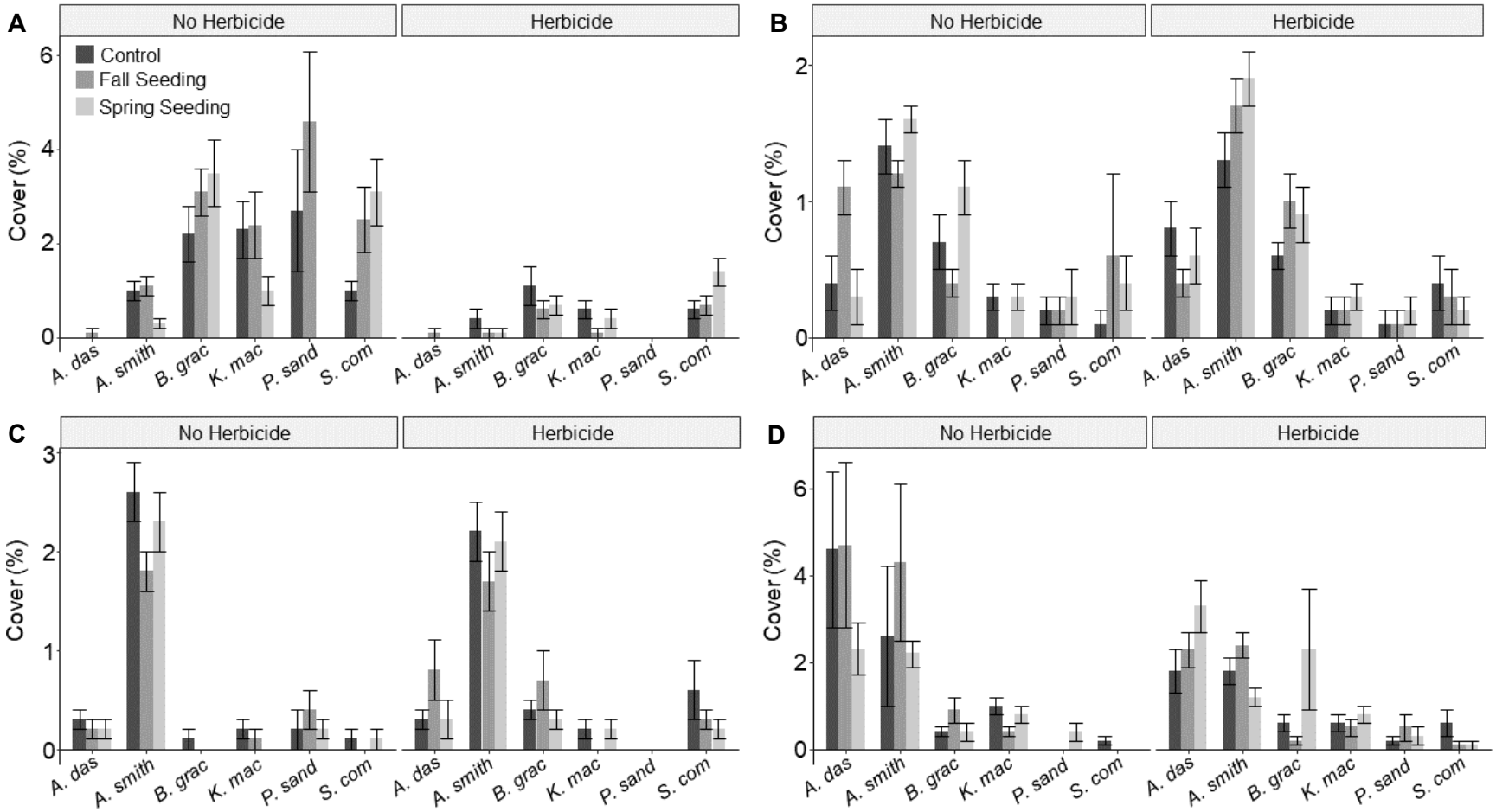

Figure 5.5. Cover of seeded grass species in 2019. Bars represent standard error. n = 8 for revegetation and herbicide treatment combinations (A) cattle grazed, (B) bison grazed, (C) watered, (D) ungrazed.

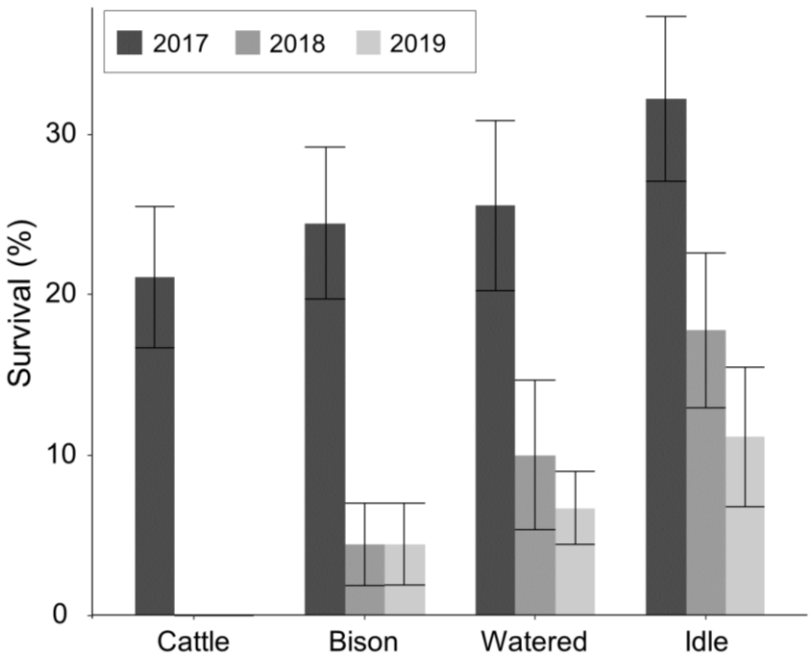

Figure 5.6. Survival of outplanted *Artemisia cana* seedlings in land management areas. Seedlings were planted in early May 2017, and survival was assessed each June. Bars represent standard error. n = 5 seedlings per plot, n = 18 plots per land management area.

SUPPLEMENTAL INFORMATION

Table S5.1. Sources and collection year of seed used in revegetation treatments in the land management experiment in October 2016 and May 2017.

Scientific Name	Common Name	Source	Year
Artemisia cana	Silver sagebrush	Grasslands National Park	2015
Agropyron smithii	Western wheatgrass	Grasslands National Park	2015
Agropyron dasystachyum	Northern wheatgrass	Grasslands National Park	2015
Poa sandbergii	Sandberg blue grass	Grasslands National Park	2016
Stipa viridula	Green needlegrass	GNP, Skinner*	2015
Stipa comata	Needle and thread grass	GNP, Skinner*	2015
Stipa curtiseta	Western porcupine grass	GNP, Skinner*	2013
Koeleria macrantha	Prairie june grass	Grasslands National Park	2016
Bouteloua gracilis	Blue grama grass	Grasslands National Park	2016
Geum triflorum	Three flowered avens	Alberta Porcupine Hills	2015
Ratibida columnifera	Upright prairie coneflower	Grasslands National Park	2016
Gaillardia aristata	Blanket flower	Grasslands National Park	2016
Dalea purpurea	Purple prairie clover	Alberta Porcupine Hills	2015
Arnica fulgens	Shining arnica	Grasslands National Park	2016
Liatris punctata	Dotted blazing star	Grasslands National Park	2016

* seed collected in Grasslands National Park then grown out for one generation near Roblin, Manitoba by John Skinner

Calculation S5.1. Steps And Calculations To Determine Seeding Rate

(1) Obtain the following values necessary for calculating seeding rate:
- Seed per kilogram of bulk seed (bulk seed includes chaff).
- Germination and viability of seed. If germination test completed without testing for viability of non-germinated seed, then value obtained corresponds to germination x viability (G x V).
- Estimate rate of emergence, establishment and over-winter survival of seed.
- Determine the target cover of vegetation (%) and the number of plants required per m^2 to achieve target cover. Convert cover to density by estimating the cover 1 plant provides and multiplying that to meet target cover value.

(2) Calculate the percentage of seeds that will result in a plant (SRP) using germination (G), viability (V), emergence (EM), establishment (EST) and over-winter survival estimates (OWS).
SRP = (G x V) * EM * EST * OWS
SRP = 0.85 * 0.5 * 0.5 * 0.5
SRP = 10.6 % of all seed turns into a plant (or 0.106 plants $seed^{-1}$)

(3) Calculate number of seeds required (NSR) to obtain target density (TD) of plants per m^2 by dividing target density by the percentage of seeds that result in a plant (SRP).
NSR = TD / SRP
NSR = 3 plants m^{-2} / 0.106 plants $seed^{-1}$
NSR = 28.3 seed m^{-2}

(4) Calculate seeding rate, as the weight of seed required (WSR) to achieve target density (TD) of plants per m^2 by dividing number of seeds required to obtain target density by the number of seed in 1 kg of bulk seed sample.
WSR = NSR / seed in 1 kg bulk seed
WSR = 28.3 seed m^{-2} / 225,000 seed kg^{-1}
WSR = 0.000126 kg m^{-2} (1.26 g seed m^{-2})
Double seeding rate if broadcast seeding: SR = 2.52 g seed m^{-2}

(5) Calculate weight of seed (WS) needed for a given area (e.g. research plot, all research plots). In this example, a research plot is 4 m^2 and there are 288 research plots total.
WS per research plot = WSR * plot size
WS per plot = 2.52 g seed m^{-2} * 4 m^2
WS per plot = 10.08 g seed for one research plot
WS for all plots = WS per plot * number of plots
WS all plots = 10.08 g seed per plot * 288 plots
WS all plots = 2,903 g seed for all research plots (2.903 kg seed)

Table S5.2. Seeding rate calculations and parameters for seed used in revegetation treatments in the land management experiment in October 2016 and May 2017.

Species	Seed per kg of Bulk Seed	GxV	EMG	EST	OWS	Number of Seeds that Results in Plant	Target Cover (%)	Density Plants For Cover Target (plants m^{-2})	No. Seed Required For Target Density (seed m^{-2})	Weight of Seed Required to Meet Target Density (g m^{-2})	Broadcast Seeding Rate (g m^{-2})	Seed (g) Required for 4 m^2 Plot
Artemisia cana	224,400	0.85	0.5	0.5	0.5	0.106	15	0.7	6.59	0.029	0.059	0.235
Agropyron smithii	210,000	0.75	0.5	0.5	0.5	0.094	1	4	42.67	0.203	0.406	1.625
Agropyron dasystachyum	308,000	0.75	0.5	0.5	0.5	0.094	1	4	42.67	0.139	0.277	1.108
Poa sandbergii	1,240,000	0.75	0.5	0.5	0.5	0.094	1.5	6	64.00	0.052	0.103	0.413
Stipa viridula	338,300	0.75	0.5	0.5	0.5	0.094	3	3	32.00	0.095	0.189	0.757
Stipa curtiseta	160,000	0.50	0.5	0.5	0.5	0.063	1	4	64.00	0.400	0.800	3.200
Stipa comata	207,500	0.50	0.5	0.5	0.5	0.063	0.5	2	32.00	0.154	0.308	1.234
Koeleria macrantha	3,612,500	0.75	0.5	0.5	0.5	0.094	0.5	2	21.33	0.006	0.012	0.047
Boutelou gracilis	1,535,500	0.75	0.5	0.5	0.5	0.094	0.5	2	21.33	0.014	0.028	0.111
Dalea purpurea	391,176	0.60	0.5	0.5	0.5	0.075	4	4	53.33	0.136	0.273	1.091
Liatris punctata	151,137	0.60	0.5	0.5	0.5	0.075	0.6	0.3	4.00	0.026	0.053	0.212
Geum triflorum	604,546	0.68	0.5	0.5	0.5	0.085	3	3	35.29	0.058	0.117	0.467
Ratibida columnifera	949,999	0.60	0.5	0.5	0.5	0.075	5	5	66.67	0.070	0.140	0.561
Gaillardia aristata	289,128	0.60	0.5	0.5	0.5	0.075	4	4	53.33	0.184	0.369	1.476
Arnica fulgens	332,500	0.60	0.5	0.5	0.5	0.075	2	2	26.67	0.080	0.160	0.642
Linum lewisii	415,625	0.60	0.5	0.5	0.5	0.075	1	0.5	13.33	0.032	0.064	0.257

* GxV = Germination x Viability, EMG = Emergence, EST = Establishment, OWS = Over-winter Survival

VI. SYNTHESIS OF RESEARCH

1. INTRODUCTION

Populations of Greater Sage-grouse (*Centrocercus urophasianus* Bonaparte [Phasianidae]; hereafter Sage-grouse) have been in decline in North America for the last 100 years; since 1988, the Canadian population has declined by 98 %. Initial population declines were likely caused by habitat loss, degradation, and fragmentation. Recent dramatic declines are likely due to a combination of factors including continued habitat loss and degradation, disruption by industrial development, drought, predation, water impoundments, and disease. The Canadian population is predicted to approach zero between 2020 and 2030 if no suitable habitat management or conservation strategies are applied. Federal protection by the Species at Risk Act and an Emergency Protection Order, numerous habitat enhancement and restoration projects, formation of working groups, and captive breeding and reintroduction programs have adverted this prediction and Canada is currently estimated to be home to 300 to 350 individuals. Details and references for these points can be found in Chapter 1.

2. RESEARCH SUMMARY

Our research focused on developing methods to improve restoration of Sage-grouse habitat by increasing establishment, growth, and survival of Silver sagebrush (*Artemisia cana* Pursh), the shrub from which Sage-grouse obtained their name and one that is integral to the bird's continued existence. Research was conducted at University of Alberta and in Grasslands National Park, home to the last two Sage-grouse populations in Saskatchewan.

2.1. Modelling Sagebrush Cover

Habitat restoration targets for vegetation are expressed in percent cover, while planting and seeding densities are required to inform restoration. Our research revealed strong relationships between sagebrush morphological characteristics and age. These data enabled me to construct a model of sagebrush cover over time at different planting densities. Model predictions were sufficiently accurate for specific ranges of sagebrush habitat cover to be targeted in restoration. The models indicate that the largest increases in sagebrush cover can occur in areas that are, or have recently been, grazed by cattle, at 0.2 to 0.95 AUM ha^{-1}. Even with relatively high planting

densities (1.0 plants m^{-2}), high mortality of young plants limits the amount of landscape cover that can be obtained. Considering only cover added by planted individuals and not their reproduction, cover is maximized at 2.1 % after 11 years in heavy cattle grazed areas and 2.6 % after 27 years in light cattle grazed areas.

2.2. Germination Of Sagebrush Seed

Artemisia cana emergence under field conditions has been extremely low and is often attributed to seed dormancy and low germination. Removal of the pericarp and after ripening marginally increased germination percentages (approximately 10 % increase) of *Artemisia cana* under laboratory conditions. Even without treatment, *Artemisia cana* germination in a laboratory was very high, and dormancy is likely not the limiting factor to poor emergence of seed in the field. Our research results suggest that low success of *Artemisia cana* seeding in the field is not due to poor germination but to limiting environmental factors.

2.3. Nutrient Loading Sagebrush Seedlings

Outplanted *Artemisia* sp. seedling survival has been low, with 30 to 36 % survival reported after two years. Increasing nutrient availability during greenhouse growth increased nutrient reserves and competitive ability for several other woody species and was considered theoretically possible for *Artemisia cana*. Extending growth time in the greenhouse and applying larger amounts of nitrogen on exponential or modified exponential dosing schedules led to *Artemisia cana* seedlings being nutrient loaded. Nutrient loaded seedlings likely had increased root growth after outplanting, which conferred greater survival and increased second season canopy development. Elimination of herbaceous competition likely contributed to greater survival and is recommended for the first two years after outplanting.

2.4. Effects Of Land Management On Revegetation

Anthropogenic disturbance and alteration of historical Sage-grouse habitat necessitate that restoration efforts account for effects of land management which has significantly altered soil properties, vegetation, and invertebrate communities. Outplanting seedlings resulted in greater *Artemisia cana* cover than seeding. Litter cover likely played a key role in determining degree of revegetation success. Over-grazing by cattle prevented adequate litter build up, leaving broadcast seed and outplanted seedlings exposed and susceptible to increased soil surface temperature

and wind speed and reduced soil available water. Excess litter cover in bison grazed and ungrazed areas may have aided outplanted seedling survival but prevented broadcast seed from reaching the soil surface. Herbicide decreased non-native species in the short term and did not negatively affect pre-existing *Artemisia cana*. All sites had key components of Sage-grouse habitat and show high potential for restoration success if land management is modified to appropriate stocking densities to reduce litter in some areas and accumulate it in others, with rest periods in severely overgrazed areas.

3. SYNTHESIS

3.1. Adapting Sagebrush Cover Models For Nutrient Loaded Seedlings

Nutrient loading increased outplanted seedling survival to 80 % (versus 57 %) two years after outplanting. Removal of localized herbaceous competition likely contributed to increased seedling survival in our study versus others. Nutrient loaded seedlings had significantly greater canopy cover; after two years their cover was approximately 1,200 cm^2, relative to 500 cm^2 for non loaded. In natural systems, it took *Artemisia cana* 5 years to reach the cover obtained by non-nutrient loaded, outplanted seedlings, and 10 to 16 years to that obtained by nutrient loaded seedlings.

Using increased seedling survival from the nutrient loading study of 90 % (year 1) and 80 % (year 2) to determine sagebrush density over time post-outplanting, we obtain the logistic power equation: $D_t = D_0 / 1 + (t / 5.38)^{1.35}$. This results in small increases in sagebrush cover outputs in all grazing areas. At a planting density of 1 plant m^{-2}, peak sagebrush cover increases from 0.9 % (at planting) to 1.5 % (in 32 years) in bison grazed areas, from 2.6 to 4.1 % after 28 years in light cattle grazed areas, and from 2.1 to 2.7 % after 11 years in heavy cattle grazed areas.

Sagebrush cover projections increase even more substantially, when enhanced canopy cover of nutrient loaded seedlings is factored into the sagebrush cover model. Changing year two canopy covers to those of nutrient loaded sagebrush, while keeping annual canopy cover increases the same as those in natural systems (conservative since long-term effects of nutrient loading on sagebrush growth are unknown), results in sagebrush landscape cover increasing to 43.1 % in bison grazed areas, 139 % in light cattle grazed areas, and 40.2 % in heavy cattle grazed areas after 34, 21, and 12 years, respectively (Figure 6.1).

For the same time periods maximum individual plant canopy cover is approximately 3,000 cm^2 in bison grazed areas, 5,200 cm^2 in light cattle grazed areas, and 2,200 cm^2 in heavy cattle grazed

areas. Based on our field measurements of sagebrush, predicted canopy covers are realistic. For example, in the light cattle grazed area, one sagebrush plant, determined by ring count to be 20 years old, had a canopy cover of 5,100 cm^2. Maximum canopy covers predicted by the model are smaller than a number of measured canopy covers. Several exceeded 10,000 cm^2 for plants estimated from stem diameters to be 16 to 46 years. The largest individuals measured in our study had canopy covers of 24,895 and 26,542 cm^2 for plants estimated to be 39 and 24 years, respectively. This indicates that the model may still be a conservative estimate of sagebrush cover and highlights the need for long-term monitoring plots of planted or seeded *Artemisia cana* to better understand its growth over time and how cover might be sustained through reproduction.

3.2. Climate Variation

Results from the land management study highlight how a variable climate, and one particularly dry and/or hot year, can greatly reduce success of revegetation efforts. Without increasing the number of seedlings planted, staggering planting over multiple years could reduce risk associated with climate variation. This approach may be more feasible than conducting a larger, one-time planting event, especially for smaller operations that are limited by equipment, time, and/or volunteers and workers. In 2018, restoration of 2.9 ha of Greater Sage-grouse habitat in Grasslands National Park required approximately 18 individuals, 5 days (630 person hours) to plant 6,000 *Artemisia cana* seedlings at 0.2 plant m^{-2}. An area of similar size could not likely be planted at a higher density with the same resources, given how many more person hours would be required to do so.

Adapting the sagebrush cover model for staggered planting decreases peak landscape cover by only 2.0 % in bison grazed areas, 19.7 % in light cattle grazed areas, and 3.4 % in heavy cattle grazed areas (Figure 6.2). Given predictions for increased drought occurrence and length in southern Saskatchewan, staggered planting ensures that a particularly dry year does not remove all planted sagebrush cover, as seedlings are most susceptible to dry conditions in the first year of growth. Staggered planting would allow for adaptations to be made based on the previous years' outcomes, including enhanced site selection, planting techniques, or improved engagement with volunteers.

Climate change is predicted to increase drought conditions in many areas of the Canadian prairies, including Grasslands National Park (see Chapter 1). Current climate models for southern Saskatchewan predict a mean increase in annual temperature from 3.5 to 5.9 °C, with the number of days above 30 °C increasing from 22 to 38. Climate change will likely further complicate the

ability to restore sagebrush habitats as sagebrush seedlings may be particularly susceptible to drier and hotter weather. Based on our work and that of others (see Chapter 1), land management adaptations to reduce effects of climate change on newly outplanted seedlings will be critical. For example, litter depth and cover can reduce erosion, soil surface temperatures and evaporation of soil water, leading to maintenance of soil nutrients through improved nutrient cycling. All are critically important to seedlings in their first year of outplanting.

3.3. Land Management, Planting Site Selection, And Preparation

This and other research shows that seed bed preparation is critical for successful revegetation, especially when broadcast seeding. Nutrient loading seedlings, scarifying seed to make nutrient additions more effective, and modelling sagebrush cover to ensure planting densities meet habitat requirements will only be effective in reaching habitat goals if land management in planting sites are changed. For example, non-nutrient loaded seedlings planted into clay loam soil and devoid of herbaceous vegetation had two year survivals of 47 to 75 %; seedlings planted directly into native prairie of varying disturbance levels had 0 to 11 % two year survival. These results could arise from an absence of competition, preferable site conditions, and climate variations between the two planting years (although both years were hot and dry, there was 40 mm more precipitation in 2018 than 2017).

Where overgrazing has not altered soil conditions, reduction or elimination of herbaceous competition in an area of 30 cm radius of the planted seedling could substantially increase seedling survival. This was not done in the land management study, as it would have removed a significant amount of other vegetation in the 2 m x 2 m plots, in which 5 seedlings were planted. Many other studies (Chapter 1) show greater seedling survival and vigour when herbaceous competition was reduced or eliminated by herbicides and mowing. Use of glyphosate herbicide in the land management study showed this could be a feasible way to promote establishment of *Artemisia cana* as it's use did not negatively affect existing *Artemisia cana* plants but opened spaces in dense vegetation. Hand pulling weeds is likely not a feasible option for large-scale restoration work but was effective in reducing competition in the nutrient loading study because the plot was relatively small (16 m x 16 m). Root exclusion apparatus, currently used to isolate below ground plant parts to examine respiration, could reduce below ground competition to seedlings if the apparatus could be modified to biodegrade after one year.

Based on the land management study, we know survival of outplanted *Artemisia cana* is greatly influenced by site characteristics. The nutrient loading study indicates microsite selection being

important for survival and growth. Within the 0.0256 ha nutrient loading plot, placement within the plot had a significant effect on individual survival and growth. Greater seedling survival and growth was observed when seedlings were planted into areas that accumulate and retain more water, such as loam to clay loam with absence of coarse materials like gravel and pebbles. Results from the land management experiment support this, although greater seedling survival could not be attributed solely to soil texture because it was not an isolated factor in the experiment. In Grasslands National Park, soil texture is highly variable within small areas where patches of soil containing large amounts of coarse debris are often adjacent to clay loam or loam soils.

Based on our and other research showing low establishment of *Artemisia cana* from seed, care should be taken to select sites with soils that promote reproduction via rhizomes. The nutrient study demonstrated that sprouting could occur within one month after planting. Thus, planting into appropriate microsites to ensure the ability of plants to reproduce rhizomatously could greatly increase sagebrush landscape cover. Therefore, large-scale management combined with microsite selection and verification will be important for successful restoration.

4. FUTURE RESEARCH DIRECTIONS

4.1. Use Of *Artemisia Cana* Cuttings

Outplanting seedlings is more effective in developing landscape sagebrush cover than seeding but requires more resources than broadcast seeding (greenhouse costs, planting equipment, time to plant an area). Cuttings may be a viable option to increase sagebrush cover, while reducing costs and time associated with seed collection, cleaning, and preparation, and greenhouse growth of seedlings. The literature on use of cuttings is almost non-existent. Development of cuttings and their survival and growth post-field deployment could expedite restoration of Sage-grouse habitat in its northern range. It could be especially useful for small or remote operations, as a relatively small area is required to develop many cuttings and can be done on site. Cutting stems from healthy *Artemisia cana* plants is not likely to cause significant or irreversible damage to the individual as *Artemisia cana* can resprout from the crown after disturbance.

4.2. Studies To Improve Establishment From Seed

Although *Artemisia cana* seed production is high, natural propagation is achieved primarily through rhizomatous reproduction, indicating seeds are not ideal for restoring sagebrush habitat.

Our results are consistent with others and suggest difficulty in establishing *Artemisia cana* from seed is due to environmental factors. If these factors could be overcome, revegetation of *Artemisia cana* from seed could be an efficient way to restore large areas of land in degraded native prairie. Use of conglomerated and pelletized seed shows promise in enhanced deployment and improved emergence. Including additives tailored to site requirements, such as fungicides, plant growth hormones, or fertilizers could greatly improve establishment and growth.

4.3. Use Of *Artemisia Tridentata Wyomingensis*

Currently, *Artemisia cana* is the only *Artemisia* species occupying the Canadian range of the Greater Sage-grouse, but *Artemisia tridentata* Nutt. subsp. *wyomingensis* Beetle & Young (Wyoming big sagebrush) is predicted to expand into large areas, including southern Saskatchewan (see Chapter 1). *Artemisia tridentata wyomingensis* is utilized by Sage-grouse, but occupies drier areas than *Artemisia cana*, and is dominant in soils with low water holding capacity. Use of *Artemisia tridentata wyomingensis* could substantially increase landscape cover of sagebrush, especially in areas *Artemisia cana* is unlikely to occupy at a significant density. Future research could investigate the potential use of *Artemisia tridentata wyomingensis* in concurrence with *Artemisia cana* for restoration of Sage-grouse habitat in southern Saskatchewan. Because the two species occupy different niche environments, it is unlikely they would be in direct competition with each other. Occupation of drier sites by *Artemisia tridentata wyomingensis* could potentially prevent encroachment of problematic species, such as *Sarcobatus vermiculatus* (Hook.) Torr. (Greasewood), *Symphoricarpos occidentalis* Hook. (Buckbrush), and *Symphoricarpos albus* (L.) Blake (Snowberry).

4.4. Establishment Of Long-Term Monitoring Plots

Literature regarding long-term studies on the restoration of *Artemisia cana* are lacking. Understanding how *Artemisia cana* growth and canopy development responds to changes in climate, particularly soil water availability, will be essential to develop sustainable sagebrush cover at a landscape level. Investigating sagebrush cover in natural systems is a good first step. However, the sagebrush cover models developed in this thesis cannot be validated for restoration use without long term research plots dedicated to studying survival and growth of planted *Artemisia cana*. Ideally, long-term monitoring plots could be established in areas with different site conditions (soil texture, upland vs. lowland, prior management, presence of non-native species, etc.) with adjacent climate stations installed. This type of study, or establishment of a few long-

term monitoring plots of this design, could substantially increase our understanding of sagebrush growth, and factors that influence it, allowing restoration practitioners to make more informed decisions and improve restoration outcomes. With climate change and future predictions, this could be important. Grasslands National Park is an ideal location to establish these plots, as the Park is likely to persist well into the future and the land within its boundaries to remain protected.

5. RECOMMENDATIONS AND CONCLUSIONS

Although many threats to Sage-grouse are being addressed, there is still significant work to be done in habitat restoration. The results of our research are a promising step towards achieving the sagebrush cover required by Sage-grouse. Results will help overcome the largest impediments in restoring sagebrush by: 1) providing important information about sagebrush growth and persistence overtime to inform seeding and planting rates; 2) overcoming exceedingly high first year mortality rates of outplanted seedlings; and 3) understanding how to better manage and select areas for restoration of Sage-grouse habitat. Use of all recommended methods could significantly expedite the restoration process, improving habitat for Sage-grouse and other prairie species which depend on sagebrush to survive.

- Without changes in land management, or use of methods to improve seeding outcomes, broadcast seeding into native prairie is likely to result in little to no plant establishment and is not recommended.
- Outplanting *Artemisia cana* seedlings is recommended as an effective way to increase sagebrush cover on the landscape.
- *Artemisia cana* seedlings should be nutrient loaded in the greenhouse for at least 25 weeks to increase survival and canopy cover development after outplanting.
- Seed used to develop nutrient loaded seedlings should be left to after ripen for 6 to 18 months, then scarified to achieve uniform germination, and allow weekly nutrient additions to be most effective in matching changing growth requirements.
- To maximize increased sagebrush landscape cover with limited resources, seedlings should be planted into appropriate microsites within cattle grazed areas of East Block or areas with similar environmental conditions. Seedlings should not be planted into clay soils or soils that contain a large fraction of coarse debris.
- Sites with adequate litter cover (greater than 15 %) should be selected for seedling planting to reduce the detrimental effects of climate change.

- Seedlings should be planted over multiple years to help reduce risk of seedling loss due to unfavourable climate variations, as staggered planting is projected to minimally reduce sagebrush landscape cover.
- Competition should be excluded from an area of at least 30 cm radius surrounding outplanted seedlings to promote survival and growth. Potential mechanisms include spot herbicide application, hand pulling at planting, and biodegradable root exclusion tubes.
- Management modifications should be made in areas where revegetation is not planned to help meet Sage-grouse habitat requirements. For example, litter reduction in areas currently ungrazed, or subjected to light grazing, could help naturally increase grass and forb cover.

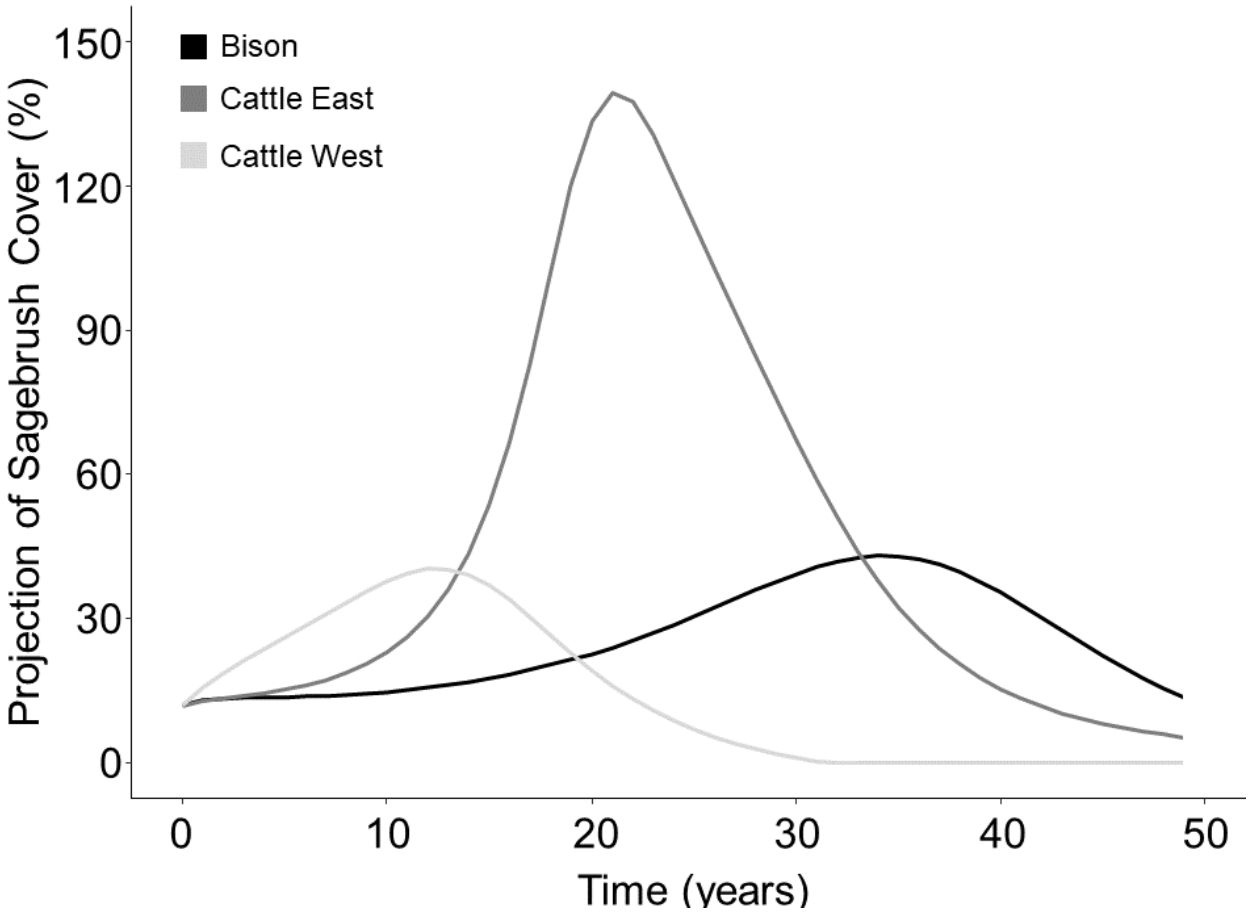

Figure 6.1. Projections for sagebrush cover contributed by nutrient loaded seedlings planted into three grazing areas (bison, light cattle, heavy cattle) of Grasslands National Park. Maximum cover is obtained after 36 years in bison areas, 21 years in cattle east areas, and 13 years in cattle west areas. The model incorporates mortality of the nutrient loaded sagebrush, as 17 % after 2 years, and increased initial canopy cover of nutrient loaded individuals as approximately 1,200 cm^2. Reproduction of planted individuals is not considered in the model.

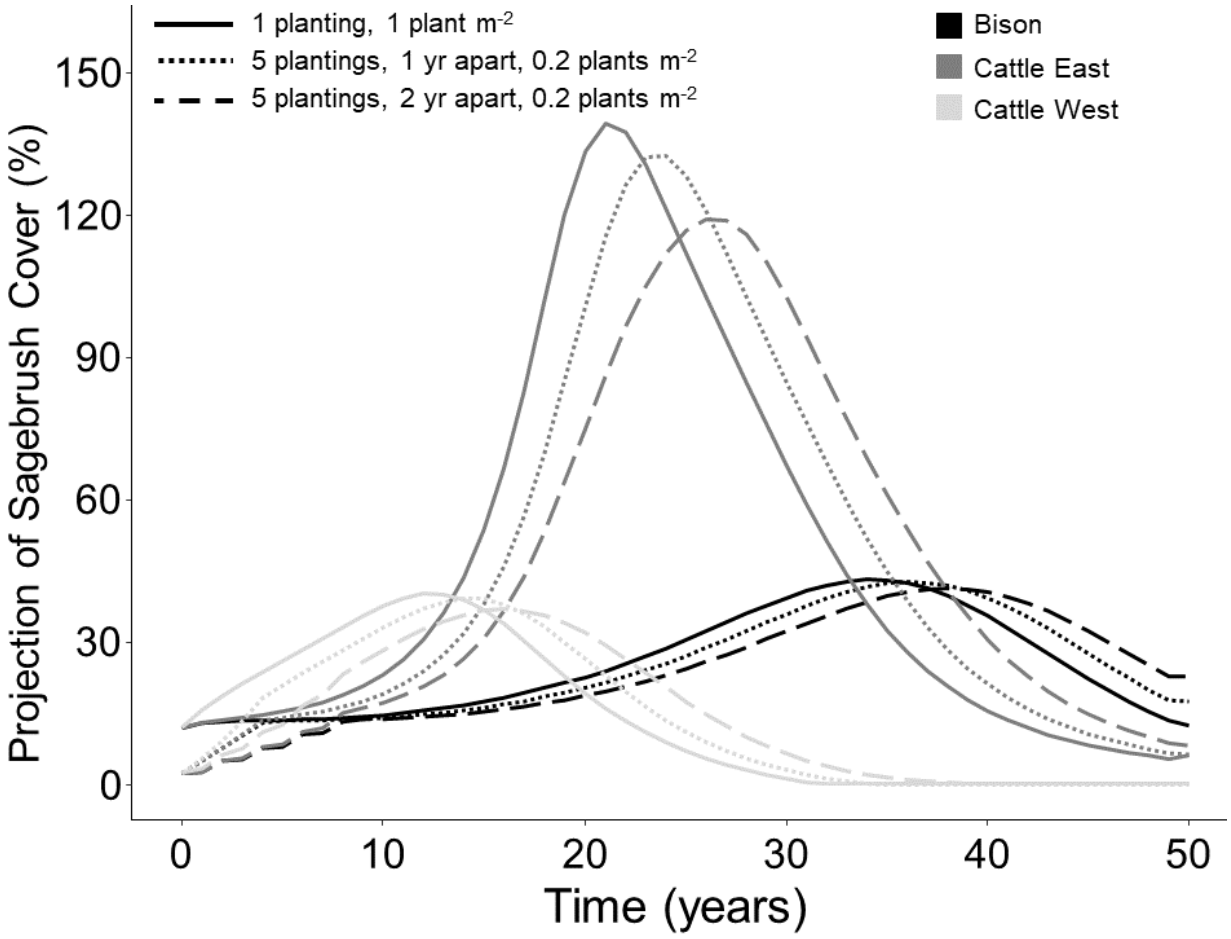

Figure 6.2. Projections for sagebrush cover contributed by nutrient loaded seedlings planted into three grazing areas (bison, light cattle, heavy cattle) of Grasslands National Park, with staggered planting events. In the three scenarios, number of individuals planted is the same, but is done over 1 year, 5 years or 10 years. The model incorporates mortality of the nutrient loaded sagebrush, as 80 % after 2 years, and increased initial canopy cover of nutrient loaded individuals as approximately 1,200 cm^2. Reproduction of planted individuals is not considered in the model.

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