REJUVENATION OF DEPLETED PASTURE USING BLOAT-FREE LEGUMES FOR HIGH PERFORMANCE CATTLE GRAZING

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A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfilment of the
Requirements for the Degree

MASTER OF SCIENCE

Department of Biological Sciences
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

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Date of Defence: November 28, 2017

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Dedication

I dedicate this thesis to my mother, Tara Khatiwada.

Abstract

Direct seeding into existing pasture is expected to reduce time for rejuvenation and loss of productivity. To accomplish this, experiments were conducted to determine appropriate method of pasture rejuvenation using bloat-free forage legumes. Four sainfoin (*Onobrychis viciifolia* Scop.) and three cicer milkvetch (CMV) (*Astragalus cicer* L.) populations were seeded into alfalfa and grass pastures using three seeding methods to compare their ability to establish and persist in the mixtures. At Lethbridge where the old pasture was predominantly alfalfa, significantly higher ($p<0.001$) proportion of newly established plants were observed when the pasture was completely plowed and reseeded with both alfalfa and test populations (cultivation method) than directly seeding sainfoin or CMV for rejuvenation. Between the two test populations sainfoin contributed higher ($p<0.05$) proportions of plants to the pasture mix compared to the CMV populations. Two of the three new sainfoin populations, AAC Mountainview and LRC3432 seeded in alternate rows with alfalfa contributed >20% ($p< 0.05$) in total dry matter (DM) yield at each harvest. In grass pastures at Ponoka and Red Deer maximum biomass contribution from test populations when drilled were 2.3% and 8.2% respectively. Grass-legume mixtures with >10% legume stands increased soil available nitrogen in 2 years. Although in two years sainfoin and CMV mixed pastures did not sequester organic carbon in soil, they increased microbial carbon biomass and enzyme associated with C-cycling. Seeding of two sainfoin populations AAC Mountainview and LRC3432 into established alfalfa pasture could improve productivity of existing pasture while preventing bloat in grazing cattle.

Acknowledgement

I acknowledge funding from Alberta Livestock and Meat Agency (ALMA) for this project and express my sincere appreciation and deep gratitude to my supervisory committee members, Drs. James Thomas, Surya Acharya, Rene Barendregt and Francis Larney for their guidance, support and continuous encouragement throughout and during the completion of this thesis. They also provided some insightful ideas in preparing this thesis. I am especially thankful to Dr. Acharya for his continuous supervision during my studies. I could not have asked for a better supervisor than him.

I feel so fortunate to work with forage breeding team of Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre. Special thanks to Doug Messenger, Doug Friebel, Brett Hill, Anthony Curtis, Bonnie Tovell, Derrick Kanashiro, Chelsea Jensen and Brittany Campbell for helping me during my research work. I am thankful Dr. Newton Lupwayi for his guidance and supervision in analysing soil samples. I also thank Kamala Sapkota and Udaya Subedi for their support in my field work. Appreciation also goes to the Department of Biological Sciences, University of Lethbridge for support in the form of a graduate scholarship.

Finally I thank my sister, Deepti and my wife Ambika for their encouragement and infinite love.

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List of Abbreviations

AB:	Alberta
ADF:	Acid Detergent Fibre
C:	Carbon
CMV:	Cicer MilkVetch
CP:	Crude Protein
CT:	Condensed Tannins
DDM:	Dry Matter Digestibility
DM:	Dry Matter
DMI:	Dry Matter Intake
LRDC:	Lethbridge Research and Development Centre
N:	Nitrogen
NDF:	Neutral Detergent Fibre
RFV:	Relative Feed Value
WSA:	Water Stable Aggregates

Chapter 1 : General Introduction

It is known that sainfoin (*Onobrychis viciifolia* Scop.), a condensed tannin-containing legume, when present in alfalfa (*Medicago sativa* L.) pasture prevents bloat in grazing cattle (Berg et al., 2000; McMahon et al., 1999). Sainfoin has many good agronomic qualities and is as palatable and nutritious as alfalfa. When consumed with alfalfa, tannins from sainfoin reduce the proteolysis of alfalfa proteins in the rumen and increase the protein absorption and utilization efficiency by ruminants (Wang et al., 2006). However, old sainfoin cultivars do not persist in alfalfa stands for long and do not regrow at the same rate as alfalfa after grazing making the mixed pasture vulnerable to bloat.

Lethbridge Research and Development Centre (LRDC) forage breeding and research program has developed a number of new sainfoin populations specifically for their improved ability to survive in mixed alfalfa stands and regrowth after cutting or grazing. A multi-year and multi-location study indicated that newly developed sainfoin in pure and mixed stands with alfalfa can perform better than old check cultivar Nova, in western Canada both in terms of their dry matter (DM) production and proportion in the mixed stands over years (Acharya et al., 2013). Due to their ability to survive and grow quickly after cutting new sainfoin populations in mixed stands reduced bloat incidence and severity in steers by 98% compared to Nova mixtures under conditions for maximizing bloat occurrence (Sottie et al., 2014), although in this study cattle preference for alfalfa (55%) was slightly higher than sainfoin (45%). Interestingly the average daily gains (ADG kg/d) and live weight gains (LWG kg/ha) in paddocks with high (>25%) or low (<10%) proportion of sainfoin were the same (Sottie et al., 2014).

Cicer milkvetch (*Astragalus cicer* L.) (CMV) is a non-bloating forage legume known for its long life; wide adaptation and ability to retain its forage quality into late fall in western Canada (Acharya et al., 2006). It was not used to its potential as the old cultivars took long to establish. In recent years LRDC forage breeding program has developed two new CMV cultivars (AC Oxley II and Veldt) with ability to establish quickly and produce more biomass than the old cultivar Oxley (Acharya, 2001 and 2009). LRDC forage research program also developed a protocol for rapid establishment of difficult to establish crops such as CMV (Acharya et al., 2006) which is being used for forage establishment in western Canada. There is anecdotal information that CMV grows and establishes in mixed forage stands and may even increase in proportion over time.

The new populations of sainfoin or CMV were never tested for their ability to establish in existing alfalfa or grass pastures. Due to lack of experimental evidence and an appropriate protocol producers cannot utilize the new populations for rejuvenating their pastures. It is estimated that the inability to graze high performance alfalfa pasture likely costs Canadian cattle producers $30 to $50 million per year in lost productivity, a figure that has undoubtedly grown with the increasing cost of grain-based diets (Acharya et al., 2013).

1.1. Hypotheses

- Sainfoin and CMV populations differ in their ability to establish in existing alfalfa and grass pastures.
- Direct seeding for rejuvenation differs from the traditional method of plow down and establishment.
- Soil parameters vary before and after rejuvenation.

1.2. Objectives

The main objectives of this applied research project will be to generate information on: 1) ability of four sainfoin and three CMV populations to establish in existing alfalfa and alfalfa/grass mixed stands; 2) differences among new and old populations for their ability to establish in existing or recently terminated stands; 3) type of equipment that can be used for rejuvenating existing pastures or recently taken out stands; and 4) changes in soil nutrient status, microbial population dynamics, and carbon sequestration before and after pasture rejuvenation.

Chapter 2 : Literature Review

2.1. Introduction

Nutrient deficiency in pasture land and soil compaction due to livestock related activities limit pasture productivity (Lardner et al., 2002; Malhi et al., 2000; Schmeer et al., 2014). Grazing intensity and climatic factors affect above ground primary productivity and nutritional value of forages (Ren et al., 2015). Grazing pressure can have detrimental effects on regrowth ability of grassland species (Fanselow et al., 2011). Poorly managed pastureland quickly loses its productivity and plant species diversity (Malhi et al., 2000). In other words nutrient deficiency, soil compaction, age of forage stand, high grazing intensity, climatic factors and poor management of pasture land are some of the causes for pasture depletion. In addition, shift to grain-based diet due to increasing incidence of pasture bloat from legume-based pastures leads to neglect or under-utilization of such pastures leaving them to deplete. Alfalfa is an example of under-utilized forage legume for grazing in Canada. There are two major problems with alfalfa: bloat in grazing cattle and autotoxicity during establishment. Bloat discourages cattle producers from using this high quality and productive forage, in high performance grazing systems and alfalfa has been found to exhibit autotoxicity (Hedge and Miller, 1990), which means seeding alfalfa for renovation of old alfalfa pastures is not recommended as the new plants suffer from a condition known as ‘alfalfa sickness’. These deterrents lead to reduced potential pasture productivity from alfalfa pastures which are estimated to cost producers $30 to $50 million per year in Canada (Acharya et al., 2013).

Rejuvenation can be a pasture management strategy for rapid improvement of depleted pastures (Acharya, 2015). The act of rejuvenation can help turn depleted pastures into productive, nutritious and bloat-safe pastures in a relatively short period of time. Pasture rejuvenation is also expected to cost less than establishing new pasture and lowers the risk of irreversible stand damage when the newly established pasture is grazed too soon.

The traditional method to improve depleted pasture or hay-land in the prairies is breaking up the whole stand and reseeding (Malhi et al., 2000). In this case, the land is ploughed and left fallow to ensure there is no leftover vegetation from the previous stand. The period of keeping land fallow may vary from a few months to over a year depending on location, soil type, available water and previous crop grown. After the old vegetation is completely removed a new seed bed is prepared that is suitable for planting the new species or mixtures. Although this method conserves moisture in the dry prairies, it leaves the land out of production and exposes soil to wind and water erosion. Extra investment in machinery and lack of productivity during the process increases the cost of using this traditional method (Waddington, 2017). Sometimes annual crops are grown before seeding perennial forages to reduce economic loss and control weeds. Although this method allows producers to use improved cultivars, different crop species or pasture mixtures, it is not true pasture rejuvenation, but complete replacement of a forage stand or reestablishment of pasture.

Some current practices for improving depleted pastures are fertilization, mechanical aeration of pastureland (Malhi et al., 2000) and direct seeding with or without vegetation suppression (Waddington, 2017). Fertilization enhances nutrient status of the soil and mechanical aeration ameliorates compacted soil. Both methods of rejuvenation focus on improving productivity of existing pasture without increasing plant density. Direct seeding with or without vegetation suppression increases plant density of the pastureland and in some cases may increase soil health while reducing possibility for erosion. Therefore, direct seeding with or without vegetation suppression would be advantageous over other methods of rejuvenation.

It is generally accepted that mixed pastures have higher yield and nutritive value than pure stands (Deak et al., 2007; Sturludóttir et al., 2012). Therefore, rejuvenating pasture by introducing new species into resident vegetation may improve above ground productivity and forage nutrient composition quickly and efficiently. Also it is accepted that legume introduction can recover nitrogen in the grassland soil without the use of expensive N fertilizer; this approach is also expected to increase productivity and biodiversity of the pastureland (Götsch, 1994).

The objective of this chapter was to collate current information on pasture rejuvenation methods, identify their strengths and weaknesses, benefits of forage legume inclusion, identify appropriate methods to utilize with the present state of knowledge and recent improvements in some bloat-free forage legumes, production systems and associated machinery.

2.2. Methods of rejuvenating pastures

2.2.1. Fertilization

Productivity losses of pastures are likely to be associated with nutrient deficiency (Malhi et al., 2000). Due to inadequate availability of nutrients, grass swards cannot maintain initial productivity and this necessitates stand renewal. Typically grass pastures show N and P deficiency over time when no nutrient management regime has been used (Delpino et al., 2016; Lardner et al., 2000). Among the major nutrients depleted from pasture land, low plant-available N in soil is responsible for reduced forage yield whereas, low availability of soil P with less mineralization of organic matter has been associated with poor herbage quality (Delpino et al., 2016). As a remedy fertilizers are added to the pasture based on routine soil tests. In most cases the residual soil N level is not taken into account when assessing soil quality instead the decision to apply N fertilizer and its levels are usually based on forage yield and farm profit (Silveria et al., 2016).

Application rates of N depend on types of forages and/or mixtures, soil type, topography and weather and are applied typically in a 4-5 split dose (Vogeler et al., 2016). Efficiency of N fertilization decreases with increasing N input (Feyter et al., 1985) through direct N leaching from poorly timed application and indirectly through leaching from urine patches (Ledgard et al., 2009; Oenema et al., 2005). Risk of direct N leaching can be minimized by timing N application as part of the best management practice, but minimizing risk of N leaching from urine patches is challenging (Vogeler et al., 2016). Dillard et al. (2015) through a detailed study conducted at Auburn, TX suggested that animal and forage management has a greater effect on forage characteristics and soil quality than addition of N fertilizer. At a plant community level, biomass yield, foliar P mass and P concentration of pasture plants did not show a response to varying rates of N fertilization, though effects were seen at an individual plant level. In that study, higher concentration of foliar P mass was observed from minimal N application in grass-legume pastures. This study also observed reduced soil extractable P thereby decreasing P loss from the soil (Dillard et al., 2015).

Overuse and/or repeated use of N fertilizer in improperly managed pastureland increases N accumulation, potential risk of N loss, NO3 accumulation and cost while impacting plant nutrient dynamics by lowering pH (Le Roux et al., 2003; Wedin and Tilman, 1996). Therefore, this costly practice has a potential impact on the environment. As an alternative, atmospheric N-fixing forage legumes can be efficiently used as a means to provide N while reducing both environmental and economic risk associated with use of inorganic N fertilizer (Silveria et al., 2016).

2.2.2. Mechanical aeration

Soil compaction limits pasture productivity. Intensive livestock activity can be detrimental to soil health by adversely influencing soil structure, air diffusion, water and nutrient movement, drainage, root exploration and chemical and biological processes in the soil that reduce pasture productivity and sustainability (Burgess et al., 2000; Butler et al., 2008; Cournane et al., 2011; Laurenson et al., 2015). Soil compaction can be remedied by mechanically aerating the soil (Burgess et al., 2000). Different topsoil looseners or aerators like use of the Aer-Way™ aerator (Malhi et al., 2000) or James soil aerator (Burgess et al., 2000) have been used to mechanically loosen the soil. Net profit from aerated forage crop paddocks was higher than non-aerated forage crop paddocks (Laurenson et al., 2015). Mechanical aeration decreases surface runoff and losses of soil P by allowing it to bind with minerals from a greater exposed surface area (Butler et al., 2008). However, Cournane et al. (2011) suggested that there is need for further research before recommending 'aeration as a means to reduce losses of soil P and surface sediment'. According to Burgess et al. (2000) and Cournane et al. (2011), changes in soil physical properties due to aeration are short-lived and the soil can revert back into a non-aerated state within 7-10 months. Malhi et al. (2000) from a study that compared pasture rejuvenation methods concluded that mechanical aeration is not consistently beneficial for pasture rejuvenation and recommended that improvement of nutrient status of pastureland especially N status is a better strategy for increasing pasture productivity. Although N application to pastureland was considered to be a better alternative for increasing productivity, Schmeer et al. (2014) noted that it had significantly higher N_2O emission when associated with compaction.

2.2.3. Direct seeding for introduction of new species

New species can be introduced in old pasture with or without vegetation suppression (Waddington, 2017). Vegetation suppression is necessary to create openings in dense resident vegetation to reduce competition for light, water and nutrients between seedlings and their neighbours (Goldberg and Werner, 1983). In highly depleted pasture, resident vegetation suppression may not be necessary particularly when resident plant density is very low. For such pastures, rejuvenation can be accomplished by direct seeding of more productive and nutritive species into the pasture.

2.2.3.1. Direct seeding after vegetation suppression

Death of the established plants opens up pastures which can be due to disturbances by animals, plant death due to environmental or frost heaving or can be induced anthropogenically (Goldberg and Werner, 1983; Schellenberg and Waddington, 1997).There are two ways to intentionally create openings in an established pasture; i.e., mechanically and chemically. For both, the objective is to suppress the existing pasture, create openings and reduce or stop competition for light, nutrients and water between desired species and the established pasture (Schellenberg and Waddington, 1997). The size of the openings in the existing stand can be an important factor for establishment of perennial crops from seed. Seeds of the desired species are drilled or broadcasted after suppressing part of the pasture (Waddington, 2017). For chemical suppression, herbicides are sprayed to kill or reduce the vigor of the existing vegetation prior to seeding. Forages established, without cultivation, by drilling into suppressed vegetation (known as sod seeding) have advantages over traditional ways of rejuvenation. Schellenberg and Waddington (1997) determined that sod-seeded alfalfa, in grass pastures suppressed with glyphosate, showed increased productivity and forage quality. No cultivation cost along with elimination of soil erosion risk in sod seeding can be favorably compared against the cost of chemical suppression of resident vegetation (Schellenberg and Waddington, 1997). For mechanical suppression, often ribbons of existing vegetation are removed making a slot. Seeds of desired species are seeded in bare soil slots, a process known as slot seeding. However in an experiment at Swift Current, SK aimed at comparing sod seeded and slot seeded alfalfa into established crested wheatgrass, productivity of sod seeded alfalfa was observed to be higher than slot seeded. The combination of fewer disturbances in the soil surface and adequate suppression of existing vegetation contributed to greater productivity obtained by sod seeding (Schellenberg and Waddington, 1997).

2.2.3.2. Direct seeding into pastures

Forage crops are mostly grown in rain fed conditions all over the world. In order to increase water use efficiency for such crops, significant reduction of tillage is recommended (Cociu et al., 2012). Minimum tillage also retains surface vegetation and crop residue which improves biological, physical and chemical properties of soil and reduces risk of water and wind erosion (Moraru et al., 2015). Efficient water utilization and improved soil health can be achieved by direct seeding of pasture for rejuvenation. Seed drills such as the Baker boot drill (McCartney et al., 2005) and pan drill can be used for direct seeding which may reduce cost of rejuvenation and equipment damage compared to cultivation methods. It is necessary to evaluate advantages of direct seeding in terms of soil moisture conservation, soil properties and cost for rejuvenation.

Broadcasting seed into existing pastures or scattering seed by feeding the seed to cattle do not normally help effective pasture rejuvenation and so is not recommended (Willms et al., 1995). Direct drilling of seed for pasture rejuvenation has been used in western

Canada with some success (Acharya, 2015). Some drills that can be used for seeding into pasture are as follows:

2.2.3.2.1. Pan drill

This drill is used by researchers in western Canada to establish forage plots in weed-free cultivated seed beds. They are not meant to be used for direct seeding into existing stands. Drill pans are not assisted by hydraulic pressure. They penetrate the soil by their weight and assistance from springs. This type of drill is not expected to deliver the seed under the soil surface in old pastures where the soil is normally compacted. The one used by forage researchers in western Canada for forage stand establishment was built in 1971 by Fabro Mfg^{TM} and is a self-propelled pan drill with hydraulics.

2.2.3.2.2. Baker boots drill

The drill name came from the fact that it has simple winged openers known as 'Baker boots' which are fitted to cultivator tynes with a coulter (disk knife). The coulter is expected to cut the sod which is followed by an inverted 'T' boot to deposit seed. The shaft of the upside-down 'T" runs in the knife cut and the cross part of the 'T' makes a small horizontal groove under the sod where the seed drops. This technology is suitable for no-tillage, reduced tillage and conventional cultivation in different agro-systems. It is suitable for a wide range of seeds from small sized grass, clover or herb seeds to large beans or maize seeds. The low power requirement and other environmental advantages due to minimal disturbance to soil by the Baker boots drill are notable (Stevens et al., 2004).

2.2.3.2.3. Air seeder or air drill

The air drill has been traditionally used for minimum tillage or direct seeding of cereal crops, pulses and oilseed. Measuring cup(s) fitted below a large tank deliver seed to various boots or seed openers through an air delivery system. Because of easy handling of seed and fertilizer this drill is used on most Canadian prairie farms (McCartney et al., 2005). However, this seeder had not been used for pasture as some of the grass seeds bridge above measuring cups in the tank. This results in uneven distribution of grass seed. Slight modification to its manufactured agitation system has helped to meter and distribute grass seed (pure meadow brome) by preventing bridging above the measuring cup (McCartney et al., 2005). Examples of air seeders suitable for grass seed are: the Bourgault 3165^{TM} air seeder and Flexi-coil 172^{TM} air seeder with minor modifications.

2.2.3.2.4. Great Plains drill

Great Plains drill is a no-till drill with a working width of 7.5m. It is a towed seeding implement with an opener disc for seed bed, seed tubes mounted between the discs to place seed in furrows followed by a press wheel to close the furrows. Adjustment handles are provided to control depth and seeding rate. Great Plains drills are used to address conventional or minimum tillage requirement for agriculture production and/or seed production (Great Plains Operator Manual, 2015).

2.3. Benefits of including legumes in a mixed pasture

Crop mixtures can contribute to increased light capture and better light availability over pure crop stands (Frankow-Lindberg and Wrage-Mönnig, 2015), which can improve efficiency of the pastureland. Mixing legumes with non-legume pastures means mixing species with contrasting leaf morphology that provide differing spatial arrangement of plants in addition to increasing biodiversity of pastureland. Also, legumes have been known for their role in “low-input livestock production system” (Rochon et al., 2004).

2.3.1. Improved productivity and nutritive value of mixed pasture

Diverse prairie plant mixtures produce a consistently high and stable plant yield while suppressing weed growth effectively (Bonin and Tracy, 2012). Selection of a species to reach transgressive overyielding depends upon the strength of interspecific competition between selected species (Trenbath, 1974). More species in the mixture and higher yield variation among species when grown in mixtures increases the strength of diversity effect required to reach transgressive overyielding (Schmid et al., 2008). A mixture of grass and legumes showed positive diversity effects and resulted in transgressive overyielding in an experiment designed to investigate yield and herbage nutritive value of grass and clover mixtures compared with their monocultures. The increased herbage yield was not accompanied by reduced herbage digestibility and crude protein (CP) concentration (Sturludóttir et al., 2012). Sleugh et al. (2000) in an experiment designed to compare binary legume-grass mixtures found that cumulative yield, CP and in-vitro dry matter digestibility of mixtures were higher than grass monocultures and this was accompanied by improved seasonal yield distribution. Similarly, cattle grazing in mixtures had average daily gain similar to legume monocultures and more than a grass monoculture (Villalba et al., 2015).

It is well known that CP concentration of grass monocultures are lower than that measured in grass/legume mixtures and legume monocultures. However, when CP concentrations of legume monocultures and binary mixtures were compared, CP concentrations of legume monocultures were higher than mixtures at first harvest but the differences were not significant in a later harvest (Sleugh et al., 2000; Sturludóttir et al., 2012). The authors concluded that this may be due to an increase in the proportion of legumes found in the mixtures in later harvest. Neutral detergent fiber (NDF) concentrations of grass-legume mixtures were higher than legume monocultures and lower than grass monocultures. The NDF concentration of mixtures was actually governed by the dominant species in the mixture which was increased by grasses and reduced by legumes (Brink et al., 2015).

Binary mixtures of two different legumes have been used as a pasture management strategy to reduce risk of legume bloat in cattle. Alfalfa (*Medicago sativa* L.) is one of the most common bloat-causing legumes grown in North America. It is known that condensed tannin-containing legumes such as sainfoin (*Onobrychis viciifolia* Scop.), when mixed with alfalfa, reduce the incidence of bloat (Berg et al., 2000; McMahon et al., 1999). In a three year study, presence of 35% sainfoin in alfalfa-sainfoin mix reduced incidence of bloat by 77%, but did not eliminate bloat in grazing steers (Wang et al., 2006). In another study, a mixture of alfalfa and 25% sainfoin planted in alternate rows prevented alfalfa pasture bloat by 90-98% when tested under bloat maximizing conditions (Sottie et al., 2014). This indicates that mixing two different but appropriate legume species may improve productivity of the pasture, improve feed efficiency through improved pasture quality, and prevent bloat in grazing cattle (Acharya et al., 2013).

Perennial herbaceous polycultures, especially legume-grass mixtures, out-yielded monocultures as well as more diverse polycultures or complex mixtures yielded more than less diverse ones (Picasso et al., 2008). Complex mixtures of plants can be more sustainable than simple forage mixtures due to their adaptation to variable environment and climatic conditions. Complex mixtures produce more dry matter yield evenly throughout the growing season than simple mixtures. However, nutritive value of a mixture depends upon the proportion of individual plant species, not the complexity of the mixture (Deak et al., 2007). Dominance of one species in a mixture is particularly apparent under adverse climatic conditions which can change with time. Hence the benefit of the mixture is best realized when it consists of relatively few species (2-4 species) selected for their adaptability to the environment (Brink et al., 2015). Effects on nutritive value of a mixture are more evident when nutritive values among species are highly different. Grass-grass mixtures have less nutritive value benefit than grass-legume mixtures because nutritive value difference between grass and legume is relatively higher than the nutritive value difference between two different grasses (Brink et al., 2007 and 2015).

Nutritive value of a pastureland is calculated from total DM yield which also includes weeds. Highly weed-invaded swards show a poorer nutritive value than non-invaded ones (Sturludóttir et al., 2012). However, mixtures created by mixing legumes and grasses have shown increased resistance to weed invasion relative to monocultures (Brink et al., 2015; Deak et al., 2007; Picasso et al., 2008; Sturludóttir et al., 2012). In two experiments of grass and legume mixtures, weeds made up around 5% of total DM herbage yield compared to 10-60% of herbage yield in monocultures and the abundance of weeds was lower at later harvest (Sanderson et al., 2012 and 2013; Sturludóttir et al., 2012).

2.3.2. Biological nitrogen fixation and Nitrogen (N) cycling

In a symbiotic relationship with rhizobia housed in nodules, legumes fix atmospheric N. It is estimated that the amount of atmospheric N fixed in a grass/legume pasture ranges from 13 to 682 kg N $ha^{-1}yr^{-1}$ (Ledgard and Steele, 1992) and that a large fraction (~ 80%) of fixed nitrogen is transferred to associated grass (Carlsson and Huss-Danell, 2003). Hence, legume introduction can be an alternative to fertilization with N fertilizers for increasing grassland productivity. However, in a grass/legume mixture, legume species and its relative proportion or the optimum grass-legume ratio for best economic return should be understood. It is also known that incorporation of perennial legumes in the grassland showed improved N cycling efficiency over annual legumes (Schipanski and Drinkwater, 2012). Alfalfa mixed with natural temperate grass in a ratio of 1:1 showed the greatest economic and ecological potential by enhancing soil water and nitrate-N availability (Li et al., 2015). This result is consistent with results from a study on nitrogen yielding by grass-legume mixture which showed that 40-60% of legumes in grass-legume mixtures efficiently transformed N into their biomass by stimulating N uptake from both symbiotic and non-symbiotic sources (Nyfeler et al., 2011). Increasing legume density (>

50%) in a mixture could shift the C:N ratio in soil by enhancing soil C and decreasing soil N storage (Li et al., 2016). In another study on plant diversity and nitrate leaching, total nitrate loss was highly dependent on species composition of the mixture and showed a positive correlation with a higher abundance of legumes. The same study suggested that increasing the proportion of grasses in the community could reduce the risk of nitrate leaching due to complementary uptake of nitrate by non-legumes (Lorenzen et al., 2003). Thus, incorporating perennial legumes in grasslands to bring the ratio to 1:1 can improve performance of the mixture at a reduced cost.

2.3.3. Lower N_2O emission and carbon footprint

N_2O emission from legume based cropping system or legume in rotation was lower than for fertilized annual crop systems (Gregorich et al., 2005; Mackenzie et al., 1997). Legumes have been recognized with offsetting 28% of sequestered carbon from N_2O emission but in the same study for N fertilization N_2O emission exceeded C sequestration (Henderson et al., 2015). There is variability in N_2O production between perennial forage crops like alfalfa and annual cash crop like soybean. Annual N_2O production from soybean was lower than alfalfa (Gregorich et al., 2005; Rochette et al., 2004). Nevertheless legume based pasture emitted lower N_20 than N fertilized system and through diversification increased biodiversity, improved soil structure, reduced annual energy consumption, soil erosion and usage of agrichemicals (Jensen and Nielsen, 2003; Jensen et al., 2012). These unique abilities of legumes justify the benefits of including legumes in pasture production.

In a study to determine carbon footprint of durum wheat grown in different rotations in Saskatchewan, the legume-durum system had a lower carbon footprint than a cereal-durum system (Gan et al., 2011). Similarly, in another study to determine carbon footprint of intercropped grass-forage legumes and a monocrop legume, pure stands of legumes without fertilization had lower carbon footprints than crops with high or low N input (Nielsen et al., 2016). Legume as a monocrop, intercrop or in rotation, resulted in lower carbon footprint values and less N_2O emission.

2.3.4. Carbon sequestration

Grass-legume association can play a restorative role by stabilizing the carbon cycle (Fisher et al., 1994) and tends to be more preservative through retention of soil C particularly from root inputs (Gregorich et al., 2001). Highly productive pasture species such as deep rooted grasses can sequester carbon deep in the soil thereby offsetting anthropogenic CO_2 emissions (Fisher et al., 1994). A long term study on biodiversity restoration of grasslands in Europe showed significant benefits to soil C and N storage when treatments were combined with forage legumes such as red clover (De Deyn et al., 2011). Legumes fix atmospheric N which is taken up and used by grasses thus increasing below-ground biomass which ultimately serves as an input for soil C and N (Fornara and Tilman, 2008). Soil organic carbon (SOC) accumulation was significantly higher in five species mixture including two legumes than in a two species grass-legume mixture (Skinner and Dell, 2016) and suggested that there is a positive relationship between productivity and SOC accumulation via roots (Rutledge et al., 2017a; Skinner and Dell, 2016). Also, Drinkwater et al. (1998) suggested that legume based cropping systems reduced loss of soil C and N. However, methods of seeding should also be considered for estimation of carbon balance. In a detailed study to compare net ecosystem carbon balance (NECB) of grass-legume pastures renovated by using direct drilling after spraying method and ploughing method showed that ploughing did not have more negative impact on NECB than drilling method (Rutledge et al., 2017b). Spraying before drilling may have reduced root biomass of the resident vegetation. A legume-based cropping system can sequester carbon and deep rooted forages can sequester carbon deeper into the soil.

2.3.5. Legumes and the below ground micro-biome ecosystem

Legume stands increased soil microbial biomass (Zhao et al., 2015). The consequence of loss of legume stands in grassland has been apparent in evaluation of above ground plant biomass as well as below ground microbial biomass. Studies (Baghdadi et al., 2016; Sleugh et al., 2000; Sturludóttir et al., 2012) have explored effect of legume incorporation in a cropping system on plant biomass and aboveground productivity. Few studies have addressed the relationship between legumes and soil biota. The biomass of soil microbes was highest in a legume monocrop, lower in a grass-legume mixed crop and lowest in grass monoculture because legumes increased the availability and accessibility of nitrogen-enriched litter for soil microbes (Zhao et al., 2015). Another study to investigate effect of anthropogenic N fertilization on the soil microbial community concluded that biomass of soil microbes was reduced by 15% under N fertilization which was more evident as load and duration of N fertilization increased (Treseder, 2008) because plant C input was altered by N fertilization (Allison et al., 2007). Also, accumulation of N inhibited microbial growth due to reduced release of greenhouse gases from soil to the atmosphere (Treseder, 2008). In a similar study with nitrate addition, microbial biomass was reduced by 18% although the microbial community composition did not change (DeForest et al., 2004). This suggests that absence of legumes in a cropping system and addition of N fertilizer may have a negative impact on the underground micro-biome by reducing their biomass. An unrelated benefit of introducing legumes would be to lower the water table due to the deep root system of legumes and thus reduce top soil salinization in problem areas.

2.3.6. Cattle preference for forage legumes

Regardless of forage species, cattle display partial preference to legumes over grasses. When given free choice between alfalfa and tall fescue monocultures in adjacent plots, beef steers displayed partial preference for alfalfa (62.5%) (Boland et al., 2011). Studies have also shown cattle preference for clover over grasses by approximately 70% (Rutter, 2006). In a study where cattle were given free choice of grazing in separate monocultures strips of tall fescue, sainfoin and alfalfa, tall fescue was least preferred over legumes (Villalba et al., 2015). In another experiment to study eating behaviour of dairy cows offered ensiled clovers and grass either separate or mixed, cows preferred clover silage and their mixtures over rye grass silage (Dorland et al., 2008). Higher intake of clover than grass was seen in cattle when both the species were grown in spatially separated monocultures than when mixed together (Rutter, 2006). Therefore, he suggested that additional research is needed to understand diet choice of ruminants grazing in a complex mixture before making any recommendations.

2.3.7. Adaptation under stresses

Factors responsible for a decrease in pasture quality could be continuous grazing, environmental stresses particularly drought which leads to overgrazing and changing climate regime (Bélanger et al., 2002; Brummer and Moore, 2000). Brummer and Moore (2000) showed grazing tolerance varied among plant species and cultivars. In addition researchers have developed cultivars that can adapt in challenging environments. For example AC Longview alfalfa developed at LRDC has excellent winter hardiness, can tolerate verticillium and bacterial wilt, show grazing tolerance and yield equal to the superior hay-type cultivars (Acharya and Huang, 2000). In a growth chamber study, increased temperature alone or in combination with elevated levels of CO_2 DM yield of alfalfa increased while timothy yield reduced. For both alfalfa and timothy, elevated CO_2 decreased CP concentrations while reducing digestibility of timothy but not that of alfalfa (Thivierge et al., 2016). This suggests N fixing species have shown more productive response to elevated atmospheric CO_2 than non-fixing species. Mixing perennial crops with different levels of winter tolerance can reduce risk of winter damage and determine their winter survival (Bélanger et al., 2002). Mixing alfalfa (legume) with timothy (grass) showed a slight increase in annual forage yield in colder areas when grown under simulated future climatic conditions characterized by increased temperature and precipitation (Thivierge et al., 2016). This suggests that grass-legume mixtures can adapt better than grass alone under stresses, and cultivar improvement is necessary to adapt to changing growing conditions.

2.3.8. Economics

Inclusion of legumes in resident pastures increased DM yield, and reduced the cost of N fertilizer. Both of these factors increased the economic returns from pasture. Although polycultures can contribute a higher return, their persistence over time has been a major determinant for farm profitability (Ludemann et al., 2015). Persistence of forage crops under different grazing systems is one of the most desirable traits for cattle producers. Environmental stress, particularly drought, leads to overgrazing and potential for stand damage. So, determining the relative grazing tolerance of different forages can guide us to make better species and/or varietal recommendations (Brummer and Moore, 2000). The LRDC forage breeding program developed new populations of sainfoin selected for their persistence in multiple years showed a higher persistence and tolerance to multiple cuts over the years than old cultivars (Acharya et al., 2013). Ludemann et al. (2015) recommended giving emphasis to increased duration of peak DM production rather than improving pasture persistence at late maturity to increase operating profits from grazing pastures. In other words it would be more profitable if the pasture produces its peak potential DM for a longer time or if more than 65% of peak DM production is maintained annually (Malcolm et al., 2014). They recommended introduction of perennial legumes that can persist longer at a peak level of annual DM production for rejuvenating pastures.

2.4. Direct seeding forage legumes for pasture rejuvenation: future prospects

On the basis of the information reviewed above; introduction of forage legumes by direct seeding into an existing pasture can be a profitable and sustainable technology for pasture rejuvenation in the future. Direct seeding of appropriate legumes to promote pasture rejuvenation may have additional advantages of making pasture bloat-safe.

Studies on rejuvenating grass and legume pastures by using other legumes have not been reported to date however studies on mixing two different legumes that were seeded together have been reported earlier. Studies at LRDC have successfully established mixed pastures of alfalfa and sainfoin starting with clean seed beds but seeding into existing pastures was not attempted. Researchers at LRDC have generated information on stand survival and productivity of alfalfa/sainfoin mixtures, animal productivity, bloat severity and animal preference in those mixtures. In a simulated grazing study to determine ability of different sainfoin populations to survive in mixed alfalfa stands, old sainfoin cultivars 'Nova' and 'Melrose', were found to be eliminated quickly from the stand and could not regrow at the same rate as alfalfa when used in a new mixed stand. In the same study, new populations of sainfoin developed at LRDC survived in alfalfa pasture for 4 years under multiple harvests (Acharya et al., 2013). This persisting ability of new multiple-cut-type sainfoin populations may be used to determine their ability to renovate old and/or depleted alfalfa, grass or alfalfa/grass mixed stands. Another study conducted at LRDC demonstrated that seeding new sainfoin populations in a mixed stand with alfalfa produced >20% DM at each harvest while preventing bloat in grazing animals (Sottie et al., 2014). This study recommended that some new populations of sainfoin can be used in pasture mixtures with alfalfa for higher DM yield and average daily gain in steers while preventing bloat (Sottie, 2014).

This suggests that new sainfoin populations have potential for transforming under-utilized alfalfa pastures with bloat potential to bloat-safe alfalfa pastures because a mixture of alfalfa and >20% sainfoin can reduce the incidence of bloat by 90-98% in grazing cattle (Sottie et al., 2014). Although sainfoin is not known to show autotoxicity as alfalfa producers have not been able to utilize the new populations of sainfoin for rejuvenating their pastures due to lack of experimental evidence and an appropriate establishment protocol. There is anecdotal evidence that bloat-free legumes such as sainfoin and cicer milkvetch (CMV) stands can be rejuvenated by allowing the plants to produce seed, indicating their ability to establish in existing stands. This indicates that pasture rejuvenation with bloat-free legumes has a great potential, but appropriate cultivar improvement will be major factor for its success.

2.5. Recommendation for future study

Future studies should evaluate the compatibility of various legume species for their survival in grass pastures in a required proportion to achieve best results with respect to animal performance, pasture productivity and soil parameters. For depleted legume pasture rejuvenation, studies should be conducted to identify appropriate legume species and cultivar that can give best complementary effects while reducing risk associated with legumes grown in pure stands. Since most forage cultivars available in the market were developed from germplasm selected under monoculture situations it may be necessary to initiate breeding programs aimed at selecting germplasm for their ability to perform in mixed cropping and to establish in existing stands may be useful in rapid rejuvenation of depleted pastures. Other associated considerations would be methods of introducing legumes in existing pasture and type of equipment most suitable for rejuvenation.

Along with pasture productivity and appropriateness of species and cultivars, new studies on pasture rejuvenation should generate information on economics and soil property dynamics for determining sustainability of the technology. Future studies should be done under different eco-climatic conditions so that appropriate protocols can be recommended for different eco-climatic zones and on identification of germplasm that can be used for developing improved cultivars.

2.6. Conclusion

Among methods of rejuvenation, direct seeding of legumes into an established pasture may be the best option for rapid improvement of pasture performance (Fig. 2.1). Inclusion of legumes in a mixed pasture results in increased N availability, higher nutritive value, reduced emission of greenhouse gases, increased persistence, higher cattle preference and increased biodiversity leading to high farm profitability. The rejuvenated pasture will also have positive environmental consequences both above and below ground. It can be said that direct reseeding of compatible legumes into a depleted pasture for rejuvenation can reduce cost of cattle production. Generation of research based information regarding the possibility of introducing legume in old depleted pastures and simultaneous identification of compatible cultivar suitable for different pasture species would improve producer adoption of this new technology. Rejuvenation as a pasture management strategy for rapid improvement of high performance grazing will have a significant positive impact on sustainability and competitiveness of the cattle industry. The prognosis for rapid pasture rejuvenation looks positive.

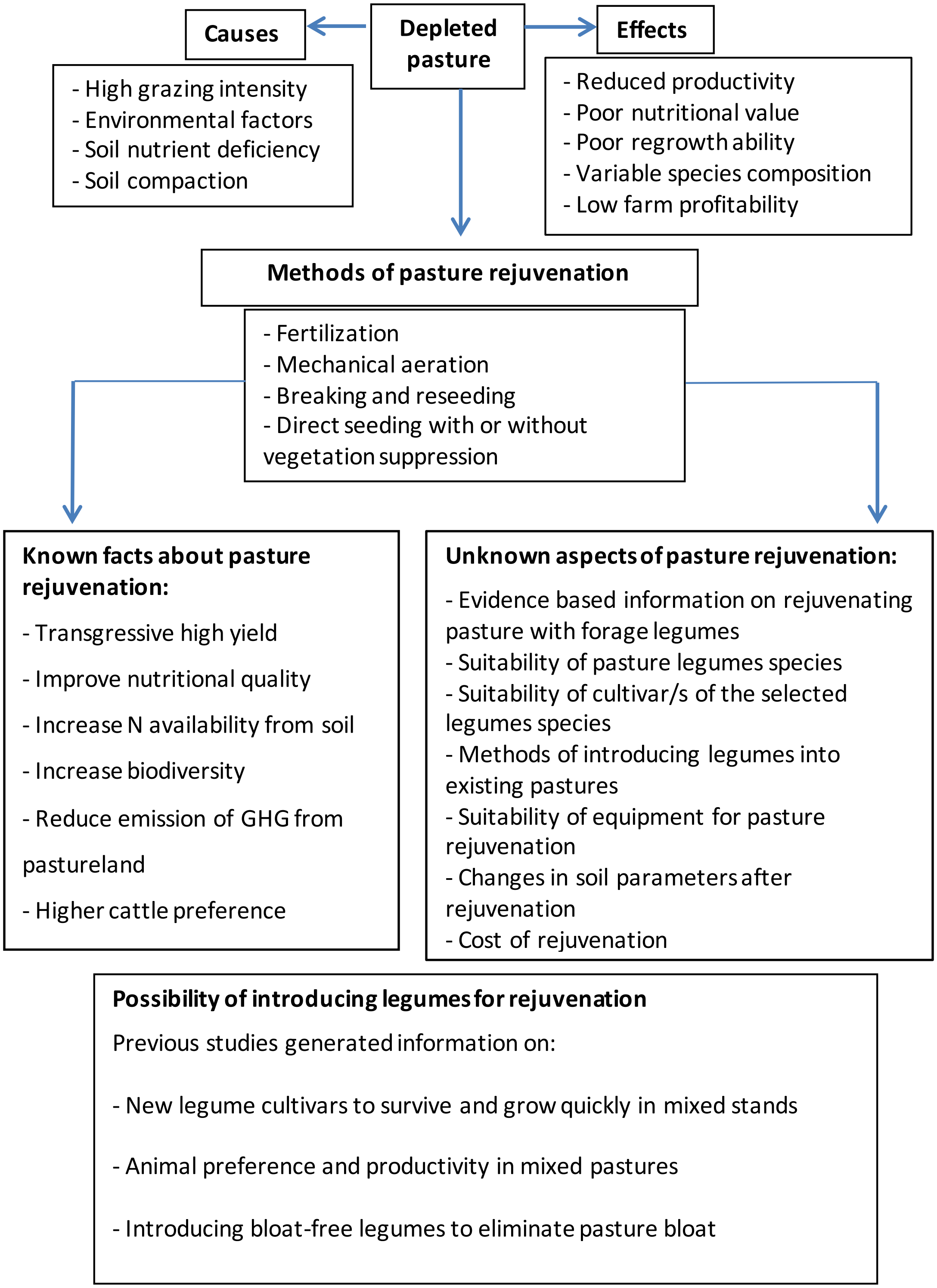


Figure 2.1. A snapshot of literature on perennial pasture rejuvenation

Chapter 3 : Ability of sainfoin and cicer milkvetch populations to rejuvenate existing pastures in Western Canada

3.1. Introduction

Rejuvenation can be a pasture management strategy for rapid improvement of existing and/or depleted pasture (Acharya, 2015). The traditional method of pasture rejuvenation is reseeding entire pastureland after completely breaking up the old stand (Malhi et al., 2000). Improvement of existing pasture without replacing resident vegetation is expected to take less time and be more economical method of pasture rejuvenation. Fertilization and mechanical aeration of pastureland has been shown to improve productivity of existing pastures rapidly (Malhi et al., 2000) but do not help increase plant stand density. For depleted pastures characterized by low plant density, rejuvenation method should consider improvement and maintenance of an ideal plant density for year-round yield distribution. Direct seeding of new species into existing pasture, a rejuvenation method proposed by Waddington (2017) is an important consideration for low-cost sustainable improvement of pasture productivity. An additional benefit of this approach is it allows us to introduce plant diversity while reducing risk associated with resident pasture such as alfalfa bloat.

Some of the causes for pasture depletion are nutrient deficiency (Malhi et al., 2000), soil compaction (Lardner et al., 2002; Schmeer et al., 2014), grazing pressure (Fanselow et al., 2011), climatic factors (Ren et al., 2015) and poor management. All the causes are prevalent for leguminous and non-leguminous pastures. For alfalfa (*Medicago sativa* L.) pasture the risk of pasture bloat may also play a role for rejuvenation with bloat-free legumes such as sainfoin or CMV. Alfalfa and its mixtures cover 65% (3,754,169 ha) of total land area (5,882,487 ha) covered by forages, tame hay, fodder crops and forage seed in Canada (Statistics Canada, 2016). However, this widely grown highly productive (Popp et al., 2000) pasture is not utilized to its full potential due to fear of bloat in grazing cattle and lack of experimental evidence that can convert existing alfalfa stands into bloat-safe improved pasture. Among several bloat control strategies, use of condensed tannin containing legumes with alfalfa has potential to be preferred for grazing cattle (Acharya et al., 2013). Ease of implementation and cost effectiveness of alfalfa-sainfoin (*Onobrychis vicifolia* Scop.) mixtures can make this approach the most logical one for bloat prevention (Sottie, 2014). Earlier studies have shown that presence of more than 20% sainfoin in alfalfa pasture can reduce bloat in grazing cattle by 90-98% (Sottie et al., 2014). New sainfoin populations developed at Lethbridge Research and Development Centre can be used to convert alfalfa pasture into a bloat-free pasture mixture by establishing new sainfoin/alfalfa mixed pasture in alternate rows. This ability of new sainfoin populations however have never been tested by drilling into existing alfalfa or any other resident pasture. Cicer milkvetch (CMV) (*Astragalus cicer* L.) is another non-bloating forage legume and LRDC has developed two CMV populations in recent years that can establish quickly and produce more biomass than the older cultivar Oxley (Acharya, 2001 and 2009). However, the ability of new CMV populations to survive in mixed alfalfa stands and bloat control in these mixtures in grazing cattle has not been tested. There is anecdotal evidence that sainfoin and CMV stands can be rejuvenated by allowing the plants to produce seed, indicating their ability to establish in existing stands.

Unlike alfalfa pasture, cattle do not bloat in grass pasture. However average daily gain of cattle in pure grass pasture was lower than growth on both legume monoculture and grass-legume mixtures (Villalba et al., 2015). Herbage yield, nutritive value and crude protein concentration of grass monocultures or grass mixtures were lower than legume alone or legume-grass mixtures (Brink et al., 2007 and 2015; Picasso et al., 2008; Schmid et al., 2008). In addition binary mixtures of legume and grass were accompanied by increased herbage digestibility (Sturludóttir et al., 2012) and improved seasonal yield distribution (Sleugh et al., 2000). Abundance of weed was up to 60% of total DM herbage yield in grass monocultures compared to 5% total DM yield in grass legume mixtures (Sanderson et al., 2012 and 2013). Higher weed invasion responsible for poor quality of pasture was apparent in grass monocultures (Deak et al., 2007; Picasso et al., 2008; Sturludóttir et al., 2012). Also cattle preferred legumes over grasses (Boland et al., 2011; Rutter, 2006). Studies suggest polycultures including perennial legumes had higher economic return over grass monocultures because of their ability to persist longer at a peak DM production level (Ludemann et al., 2015; Malcolm et al., 2014).

Earlier studies in Lethbridge Research and Development Centre (LRDC), Canada have generated information on ability of new multiple-cut-type sainfoin cultivars to survive in newly established mixed pasture with alfalfa and hay stands (Acharya et al., 2013). The authors concluded that newly established sainfoin-alfalfa mixed pasture is stable and is a sustainable approach for maximizing DM yield while preventing bloat in grazing cattle. Work at LRDC also concluded that new CMVs are better than old cultivars in biomass yield and rapid establishment (Acharya, 2001 and 2009).

The main objectives of this study were to determine if sainfoin and CMV populations will be able to establish in existing grass and legume pastures; determine differences among new and old populations of the two species for this purpose and type of equipment that can be used for successful establishment of new plants. For the purpose number of plants established, biomass contribution of each species and nutrient quality of mixed stands were compared by choosing old alfalfa or grass stands as existing pastures in different eco-climatic zones of Alberta.

3.2. Materials and Methods

3.2.1. Treatment structure

Four sainfoin and three cicer milkvetch populations (old and newly developed) were used to determine their ability to establish in existing alfalfa or grass pastures in Alberta. Population designation, parentage, development year, place of origin and DM yield contribution in mixed alfalfa pasture are presented in Table 3.1. All sainfoin populations belong to *Onobrychis vicifolia* subsp. *vicifolia* and all CMV populations belong to *Astragalus cicer* L. All the populations were used in trials for established alfalfa and existing grass pastures at 3 sites of Alberta. Two types of resident pastures were used in the study: one on established AC Blue J alfalfa pasture at Lethbridge (49°41'11.7"N 112°45'55.1"W) under irrigation and the other on existing grass pastures under rain-fed conditions at Ponoka (52°39'05.9"N 113°44'08.5"W) and Red Deer (52°18'09.8"N 113°19'14.7"W). The tests were established on 7-yr-old alfalfa pasture at Lethbridge and more than 10-yr-old grass pasture at Ponoka (bromegrass) and Red Deer (orchardgrass). Grass pasture at Ponoka had prominent clover and dandelion while they were not evident in Red Deer.

Table 3.1. Designation, parentage, year of development, origin and average total DM yield in pure stands grown under rain-fed conditions in western Canada.

Test Species	Population designation	Parent Population[+]	Year Developed	Origin	Yield potential[¥] kg/ha
Sainfoin	Nova	Cultivar	1980	Western Canada	5914
	AAC Mountainview	176 clone synthetic[£]	2014	Alberta, Canada	7671
	LRC 3432	Remont	2006	Montana, USA	7639
	LRC 3519	Splendid	2006	Romania	8026
Cicer milkvetch	Oxley	OA-408	1970	Former USSR	5934
	Oxley II	Oxley	2001	Alberta, Canada	8740
	Veldt	Oxley, Oxley II, Windsor, C15	2008	Alberta, Canada	7052

[£] Selected clones were LRC 3401 26%; LRC 3519 24%; Chinese accession 12%; Nova 9%; Remont 8%; Eski 7%; Melrose 6%; Perly 5%; and Kazakhstan 3% (Acharya et. al., 2013).

+ For more details regarding the parental population please see Acharya et al. 2013.

[¥] Values for sainfoin are 3-year mean total yearly dry matter (DM) yield in kg/ha when grown in pure stand at Lethbridge under rain-fed condition (Acharya et. al., 2013). Values for CMV are cited from cultivar description for respective cultivars.

The tests were established using a split plot design with three seeding methods as the main plot factor and eight plant treatments (4 sainfoin + 3 CMVs + 1 unseeded) as subplot factors. Three seeding methods included two types of seed drill (a Great Plains drill and a pan drill) (Figure 3.1) and one treatment (cultivated) where the old stand was sprayed, ploughed under and then seeded with AC Blue J alfalfa/sainfoin populations and AC Blue J alfalfa/CMV populations in alternate rows using pan drill. A Great Plains drill (Great Plains Manufacturing Inc.) is a towed seeding implement used for conventional or a minimum tillage requirement. It has opener discs and seeds are placed in furrows from seed tubes mounted between the opener discs followed by a press wheel which closes furrows (Great Plains Operator Manual, 2015). The pan drill (Fabro Mfg^{TM}) used in this study was self-propelled with hydraulics which have been used by forage researchers in western Canada for weed free cultivated plots (Acharya, 2015).

A total of 24 treatments were replicated four times making a total of 96 plots per location. Individual plot size was 2m × 8m. The eight plant treatments (sub plots) were randomized within each seeding method (main plot). All the seeding was done in 2015, reseeded in 2016 and then monitored in 2017. For Lethbridge drilled plots, seeds were drilled in between rows of established alfalfa pasture whereas for the other two locations seeds were drilled into the existing grass pastures as the rows were not visible.

Figure 3.1. Pictures of seed drills: Great Plains (A) and pan (B) used in the study.

At Lethbridge and Ponoka, cultivated plots were seeded immediately after cultivation with alternate rows of sainfoin cultivars and CMV cultivars. At Red Deer, cultivated plots were seeded the same way excepting that they were cultivated in 2014 and seeded to wheat immediately. In 2015 and 2016 they were seeded and reseeded as described for the other two locations.

3.2.2. Data Collection

Establishment success was determined by observing the unseeded treatment which was compared to the seeded area for plant counts and DM yield, over 2-years. A comparative method of stand establishment, proposed by Vogel and Masters (2001) was performed to determine plant density of each species. A 5×5 grid consisting of 25 squares that was 15 cm × 15 cm was randomly placed two-times in each plot. Area of the grid containing 25 squares (0.75 × 0.75) m^2 was 0.5625 m^2. All grids that contained 1 or more plants of a species for that plot were recorded, by species. The sum of two counts for each plot was adjusted (by doubling) to determine frequency of occurrence (in percentage). Frequency of occurrence (%) is the number of plants found in 100 squares or after placing the grid 4 times in the field covering a total area of (0.5625 × 4 m^2) 2.25 m^2. The plant count in 2.25 m^2 was converted to plant count per m^2 by multiplying with 0.44 (conservative estimation of plant density suggested by Vogel and Masters, 2001).

After completing the plant counts biomass contribution of each species was determined by collecting random samples from within each plot. Samples consisted of all the plant material growing within a 50×50 cm^2 area, clipped using a sickle at a height of 5 cm above the soil surface. Samples were immediately sorted by species, dried at 55^0C for 72 hours, weighed ($gm/0.25m^2$) and converted to dry matter yield (kg/ha) of each species. Forage nutritional quality of mixed stands under the test cutting regime was determined using dried forage samples collected from each plot. The dried forage samples were ground to pass through a 1-mm screen and then analyzed for crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) using Near Infrared Reflectance Spectroscopy (NIRS) (Norris et al., 1976). Relative Feed Value (RFV) for individual plots was calculated using the following formula (Pioneer Forage Manual, 1995).

$$RFV = (\%DDM \times \%DMI)/1.29$$

Where %DDM or %Digestible Dry Matter= 88.9-(0.779 × %ADF) and %DMI or %Dry matter Intake= 120/%NDF.

At each location data was collected two times in 2016 and 2017. For both the years, plant counts followed by cuts for each sample collection were done during late June and mid-August. After taking samples from each plot, the entire test plot was mowed in order to simulate grazing. Development stage of plants at each location was recorded before taking samples.

Weather data for each of the locations was collected from Alberta Agriculture and Forestry, Alberta Climate Information Service (ACIS) ‘https://agriculture.alberta.ca/acis’. Monthly average precipitation and temperature were extracted from historical weather data recorded at the closest weather stations to our test plots at each location. The 30 year average monthly precipitation and temperature were determined by taking the mean recorded for the respective month since 1987. Data for precipitation used in this study is a measure of liquid form of precipitation only.

3.2.3. Statistics

Data collected over multiple locations and years was analyzed using a SAS ANOVA MIXED procedure using replication as a random effect (SAS Institute, 2005). Means for all treatments were compared with unseeded pasture means. Comparisons of feed quality among the four major forages (alfalfa, grass, sainfoin and CMV) and mixed stands were determined for whole and separated samples. Means separation was made using analysis of variance followed by a least squares difference test (LSMEANS with the PDIFF procedure of SAS) when the treatment effect was significant. Treatment effects were considered significant at $p < 0.05$.

3.3. Results and Discussion

3.3.1. Weather data

In 2015 (a year before test establishment) total monthly precipitation was below the long-term average at Lethbridge and Red Deer while Ponoka received above the long-term average in the second half of 2015 (Fig. 3.2A). In 2016 and 2017 the annual precipitation pattern followed 30 year averages except for the month of June. For both years average monthly precipitation in June at all locations was below long-term monthly averages recorded for that month. Higher than long-term average precipitation was particularly noticeable in August of 2016 at Red Deer and in July, August and October of 2016 at Ponoka. Monthly average temperature in December 2016 appeared to be below the long-term average at all locations (Fig. 3.2B).

3.3.2. Legume seeded into legume pasture

Legume seeded into legume pasture at Lethbridge indicated that seeding method and year were significant for all parameters used to determine establishment success and biomass contribution of new plants in mixtures. However results varied for the interaction effect between seeding method and year (Table 3.2a). Number of new plants established using the Great Plains drill was similar to that seen for the cultivation method and significantly higher than the pan drilled method. It is known that plant density in the establishment year determines the efficiency of the seeding method used.

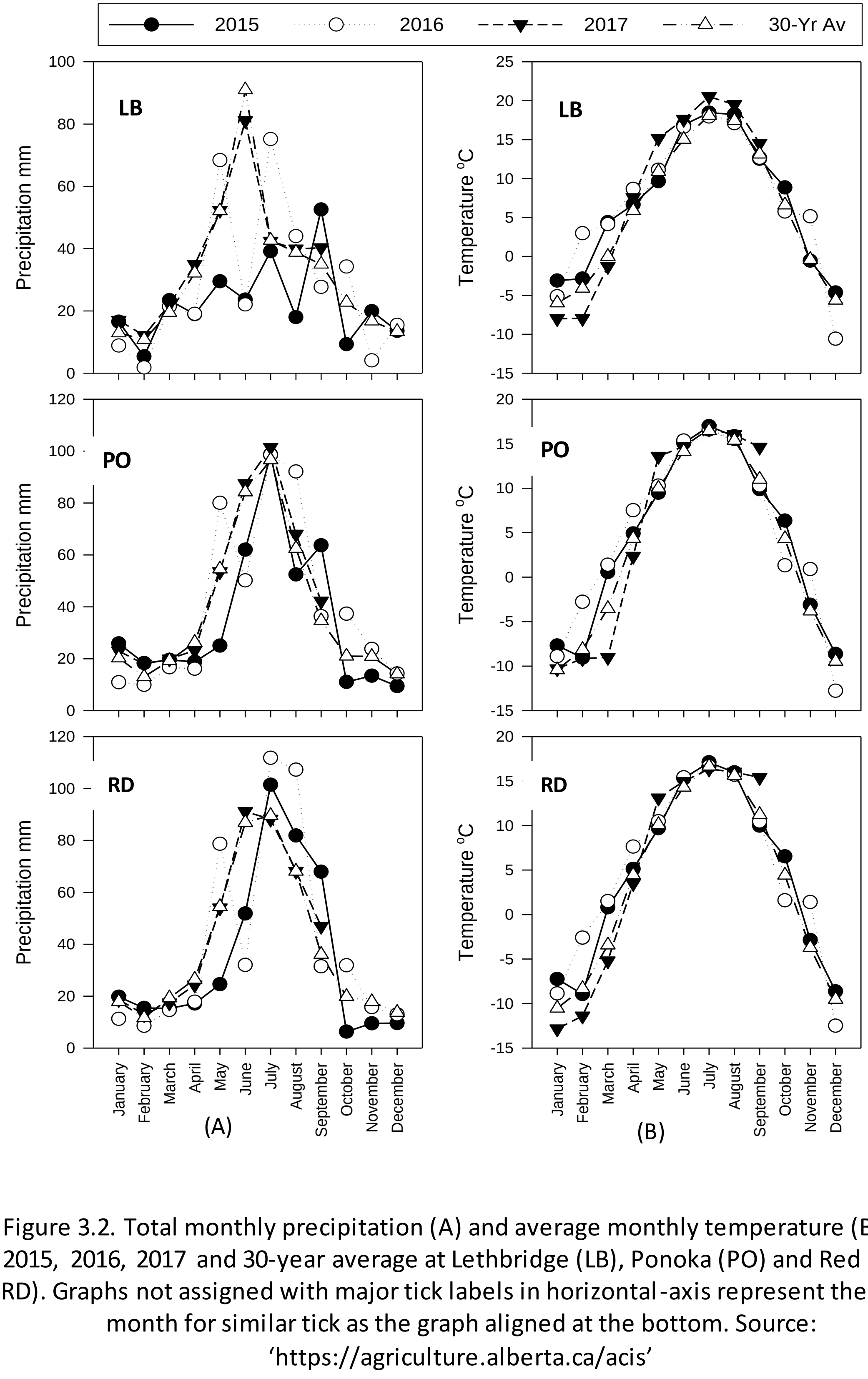


Figure 3.2. Total monthly precipitation (A) and average monthly temperature (B) for 2015, 2016, 2017 and 30-year average at Lethbridge (LB), Ponoka (PO) and Red Deer (RD). Graphs not assigned with major tick labels in horizontal-axis represent the same month for similar tick as the graph aligned at the bottom. Source: 'https://agriculture.alberta.ca/acis'

Note: 2017 Values are from January to September.

Interaction of method and year indicated that the Great Plains drill plots had a significantly higher number of introduced plants than the pan drilled plots in the year of introduction (Table 3.2b). Plant population sustained after year of introduction is determined by the persistence ability of introduced plants and relative dominance of resident stands but not by the drills used. Therefore, this test result suggests that efficiency of the Great Plains drill for seedling emergence was better than the pan drill and can be compared with cultivation approach for improving pasture. Although higher plant emergence was achieved from the Great Plains drilled plots than the pan drilled plots the percentage contribution of new plants to total DM yield from both drilled plots was similar and significantly lower than that seen in cultivated plots (Table 3.2a). In drilled plots higher plant numbers with a lower percentage contribution in mixtures suggested slow growth of new plants despite better seedling emergence.

Table 3.2a. Mean plant stand per meter^2, DM yield and percentage biomass contribution in mixtures of introduced plants in alfalfa pasture at Lethbridge. Data presented are means for three seeding method used, years and cuts.
P-values are given for each factor and their interactions.

	Plants/m^2	DM (kg/ha)	%DM contribution$^£$
Seeding Method			
Great Plains	12.3a	615b	17.6b
Pan	7.3b	456b	15.1b
Cultivate	13.8a	1112a	28.5a
Year			
2016	12.3a	561b	22.7a
2017	9.8b	895a	18.2b
Cut			
First	11.3a	945a	20.0a
Second	10.9a	510b	20.9a
p-value			
Method	<.0001	<.0001	<.0001
Year	<.0001	<.0001	0.003
Cut	ns	<.0001	ns
Method × Year	0.02	0.05	ns
Method × cut	ns	ns	ns
Year × cut	0.0001	ns	0.01

Means in a column with same lower case letter are not significantly different p=0.05, LSD=0.05

$^£$ % Biomass contribution= (mean DM yield of new seeded plants/mean total DM yield of mixed pasture for each cut) ×100

ns- not significant

Table 3.2b. Mean plant counts per meter^2 and %DM contribution in mixtures of new plants introduced using 3 seeding methods in 2016 and 2017 at Lethbridge.

Method ×Year	Cultivate		Great Plains		Pan	
	2016	2017	2016	2017	2016	2017
Plants/m^2	14.9a	12.8b	14.4ab	10.1c	7.6d	7.3d
% DM contribution[£]	30.4a	26.7a	20.2a	15.0a	17.4a	12.7a

Means in a row with same lower-case letter are not significantly different p=0.05, LSD=0.05

[£] % Biomass contribution= (mean DM yield of new seeded plants/mean total DM yield of mixed pasture for each cut) ×100

Table 3.2c. Mean plant counts per meter^2 and %DM contribution in mixtures of introduced plants for 2 cuts taken annually in 2016 and 2017 at Lethbridge.

Cut ×Year	2016		2017	
	Cut 1	Cut 2	Cut 1	Cut 2
Plants/m^2	11.5ab	13.0a	11.1b	8.6c
% DM contribution[£]	20.4b	25.0a	19.6b	16.7c

Means in a row with same lower-case letter are not significantly different p=0.05, LSD=0.05

[£] % Biomass contribution= (mean DM yield of new seeded plants/mean total DM yield of mixed pasture for each cut) ×100

Results also showed that the proportion and contribution of new plants in mixtures were higher in 2016 than 2017. However, DM yield from new plants was significantly higher in 2017 compared to 2016 (Table 3.2a). This was expected as the plants were a year older with well-established root systems. The higher DM yield seen in the second-production year was consistent with results obtained in another mixture test conducted in Lethbridge (Acharya et al., 2013).

Seeding method and year interaction showed that the mean plant count of introduced plants decreased significantly in cultivated and Great Plains drilled plots while it was same for the pan drilled plots over the years (Table 3.2b). The interaction effect of seeding method and year was not significant for biomass contribution of introduced bloat-free legumes. DM yield from new plants significantly decreased in the second cut, although proportion of new plants in mixtures did not differ between the two cuts and remained at 20% (Table 3.2a). This proportion particularly of sainfoin has been known to convert alfalfa pasture with bloat-potential into bloat-safe pasture (Sottie et al., 2014). Among cuts taken in 2 years, the number of new plants and their contribution in mixtures was lowest in the second cut of the second production year (Table 3.2c).

Proportion of alfalfa and introduced plants in the rejuvenated pasture is an important consideration. Either drill used to introduce bloat-free legumes can contribute 15% biomass in the mixed pasture. Plant mixture complexity in a mixed pasture may differ among cultivars and between species. Since the test population used in the study included two species with very different growth patterns, the results at a cultivar level would only make sense if the comparison is made within the species (Fig. 3.3).

3.3.2.1. Alfalfa in alfalfa stands

Mean DM yield of alfalfa in cultivated plots was similar to an established alfalfa stand in pan drill plots (Table 3.3). Generally seeding alfalfa on old alfalfa stands or recently broken alfalfa pasture is not recommended as the new plants suffer from ‘alfalfa sickness’, a term used to identify the autotoxicity effect that old alfalfa plants have on newly developing plants. In this study cultivated plots were established by breaking up a 2008 established AC Blue J alfalfa pasture in 2015 that was seeded immediately in the same year. Interaction of year, cut and seeding method was not significant which means that the DM yield of alfalfa in each harvest from the 3 seeding methods was similar in the two production years (Table 3.3). Our results suggest that AC Blue J can tolerate the autotoxicity effect of old alfalfa stands without compromising total biomass yield per unit area. The ability of AC Blue J to establish and grow in existing alfalfa stands is very different from what is generally known to most producers (Acharya, 2015). As per recommendation the producers generally wait for a year or two before establishing alfalfa again (Acharya, 2015). This means that the stand can be improved by seeding alfalfa again immediately after cultivating an old stand or that the stand can be rejuvenated by allowing it to go into seed. This result indicated that AC Blue J is capable of self-rejuvenating pasture and can be seeded or allowed to produce seed to maintain its stands. However, a study to confirm this observation is recommended before this type of rejuvenation is recommended to producers.

Table 3.3. Mean DM yield (kg/ha) of alfalfa in 3 differently seeded plots at Lethbridge, AB under irrigation. Interactions for year, cut and seeding method were not significant ($p>0.05$).

	2016		2017		2-yr mean[£]
	Cut 1	Cut 2	Cut 1	Cut 2	
	kg/ha				
Cultivate	2478	1212	4522	2368	2645b
Great Plains	2187	1257	6168	2995	3152a
Pan	2260	967	5419	2688	2834b

[£] 2-year mean followed by different letters are significantly different, p=0.05, LSD=0.05

Interaction between seeding method and test population for introduced plant density and their proportion in mixtures were significant (Figure 3.3). Plant densities of all cultivated sainfoin plots were significantly higher than drilled plots. Ability of sainfoin cultivars to survive and contribute to a pasture mixture was clearly higher than CMV's (Figure 3.3). All sainfoin populations contributed up to 18% in a mixture. Between drills used LRC3432 and Mountainview established well and survived better than other test populations. New sainfoin cultivars contributed higher (>51%) than the old cultivar Nova (38%) when cultivated.

Percentage contribution from cultivated Nova (38%) was similar to all Great Plains drilled sainfoin populations (29% to 34%) and pan drilled LRC3432 (31%) and Mountainview (30%). CMV populations were the same for their ability to contribute in alfalfa mixed pasture. It would be interesting to know how CMV plots do in the future as CMV is known to spread over time (Acharya, 2015).

Proportion of condensed-tannin containing sainfoin in an alfalfa stand is very important as 20% of the sainfoin in an alfalfa pasture can prevent bloat in grazing cattle by 90-98% (Sottie et al., 2014). Results of the alfalfa rejuvenation test at Lethbridge indicated the possibility of using both new and old populations of sainfoin to maintain a sufficient proportion in the stand to convert the alfalfa pasture with bloat-potential into a bloat-safe pasture in 2 years of production. In the third production year the proportion of Nova was below 10% when cultivated with alfalfa while Mountainview contributed above 20% in a study to determine persistence ability of new and old sainfoin populations when mixed with alfalfa (Acharya et al., 2013).

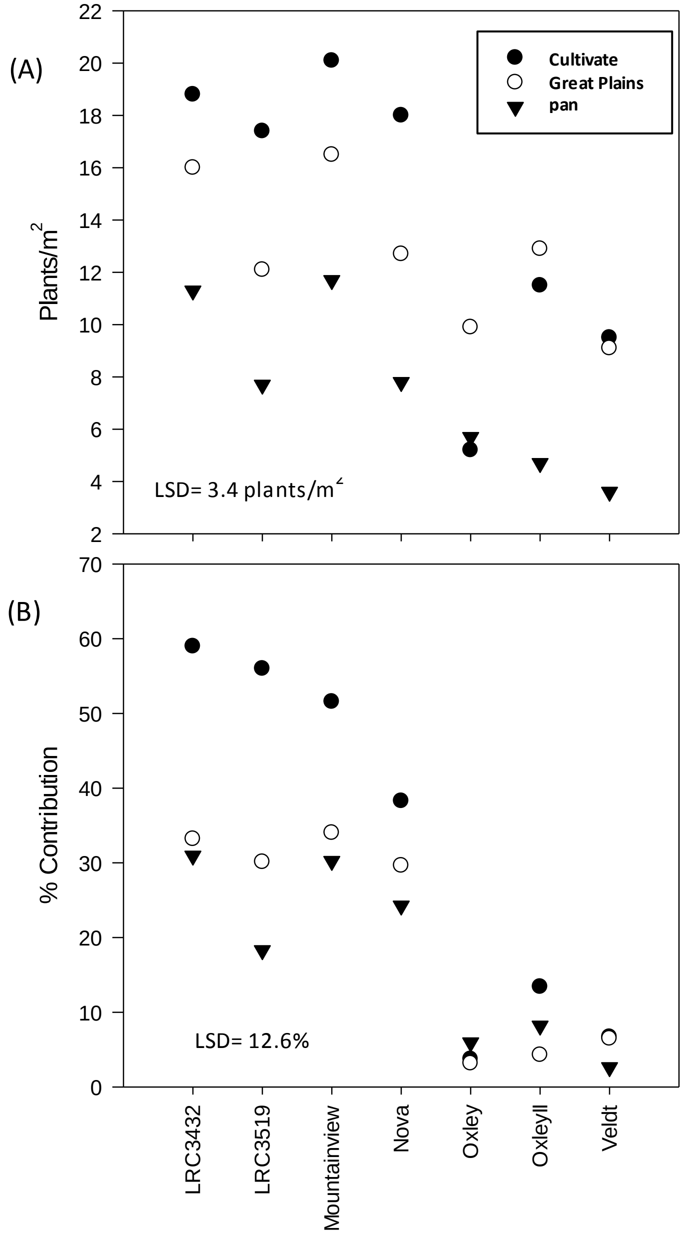


Figure 3.3. Mean plant stand per meter^2 (A) and biomass contribution (B) of introduced test cultivars seeded using the Great Plains drill, pan drill and cultivation method into alfalfa pasture at Lethbridge, AB. LSD restricted at p=0.05.

Cultivated sainfoin maintained a proportion well above 20% as expected. Except for LRC3519, use of both drills can be used to establish sainfoin stands able to contribute >20% DM in a pasture mixture.

The year and cultivar interaction showed that all new sainfoin cultivars contributed less in the second year of production (Figure 3.4). Values in the graph represent mean values of two cuts taken annually. Cumulative annual yield of cultivars and interaction of cut, year and cultivar were not significant. Three new populations of sainfoin contributed more than 40% in 2016 when Nova contributed 33% to the rejuvenated pasture. In the second production year the proportion of LRC3432 and Mountainview dropped down to 38% and 33% respectively while LRC3519 was similar to Nova (27%). This may have resulted from increased competition from higher alfalfa DM in 2017 (4027 kg/ha) compared to (1728 kg/ha) in 2016. The CMV cultivars contributed below 10% in both years and were not significantly different among each other.

All sainfoin populations showed ability to persist in alfalfa pasture when simulated grazing was done (cut twice annually). Ability to persist in this case means an introduced plant can grow and contribute at least 20% in total biomass yield. All test sainfoin cultivars contributed more than 20% in mixtures for both cuts in 2016 and 2017.

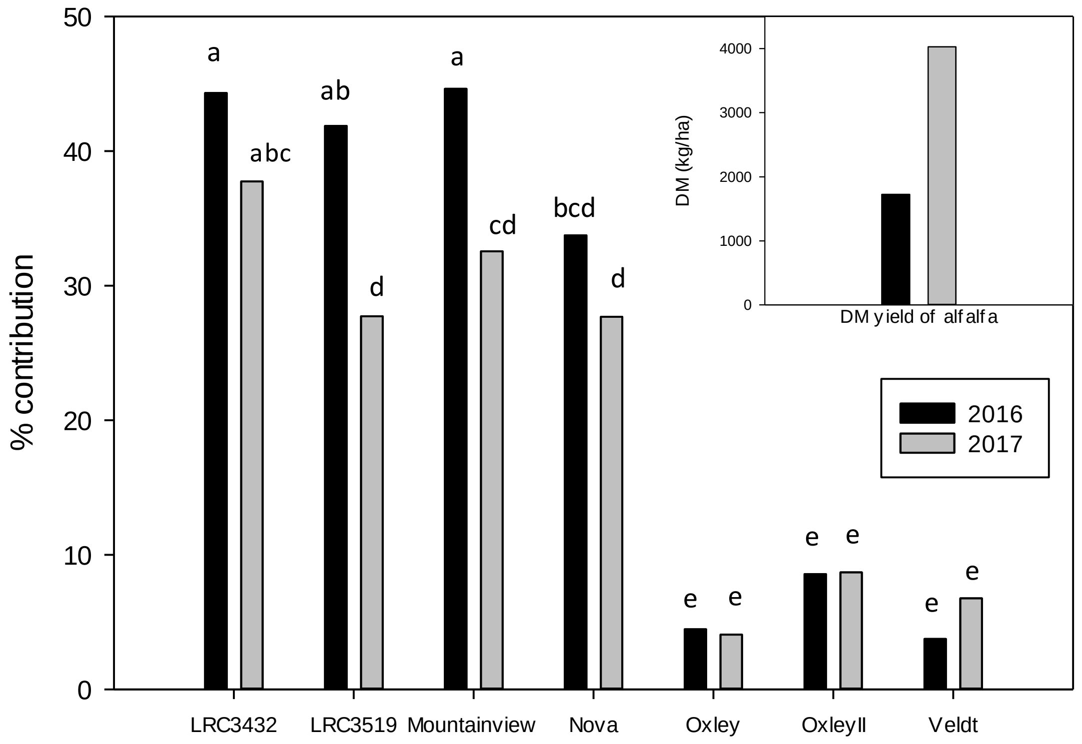


Figure 3.4. Average percentage contribution (LSD = 14.0%) of test cultivars in mixed total DM yield observed in 2016 and 2017 at established pasture at Lethbridge, AB. The small graph at the top right corner represents mean DM yield of alfalfa for each year. Bars represent the average value of cuts taken twice annually. Percentage contribution of test populations associated with the same letters are not significantly different. LSD restricted at p=0.05

3.3.3. Legumes in the established grass pasture test (Ponoka and Red Deer)

Since resident vegetation was different for drilled and cultivated plots tested in Ponoka and Red Deer, the results are presented separately. Results of the rejuvenation test in orchardgrass at Ponoka and bromegrass at Red Deer indicated that the Great Plains drill performed better than the pan drill at Ponoka while they were not different when used to establish new legumes in grasses at Red Deer (Table 3.4). Density of introduced plants was clearly higher in the year of seeding. Introduced legumes had a higher proportion in total biomass yield in the second cut at Red Deer and plant density improved in the second plant count at Ponoka. All possible interactions between method of seeding, years and cuts were not significant for drilled plots at the Red Deer and Ponoka locations.

At Ponoka, the Great Plains drill and pan drill were significantly different for new plant densities, DM yield and biomass contribution of new plants. The highest plant density of five plants per square metre was achieved with the Great Plains drill where a 2.3% contribution was achieved (a value that may not be sufficient to consider a success for pasture rejuvenation). At Red Deer, both drills were not significantly different for new plant density and DM yield. However, total biomass contribution from new plants at Red Deer was comparatively higher (up to 8% of legumes) than that seen for Ponoka plots. This indicates the possibility of using either drill to incorporate legumes into grass pasture in some parts of Alberta. A significant increase in percentage contribution of legumes in the second year of establishment at Red Deer suggests that certain cultivars can be drilled for introducing bloat-free legumes to improve grass pasture in this area.

Table 3.4. Mean plant stand per meter^2, DM yield and total biomass contribution (in mixtures) of introduced plants in grass pasture at Ponoka and Red Deer. Data is given as means for the three seeding methods used, years and cuts. P-values are given for each factor.

	Ponoka			*Red Deer*		
	Plants/m^2	DM (kg/ha)	% contribution	Plants/m^2	DM (kg/ha)	% contribution
Method						
Great plain	5.7a	29a	2.3a	5.9a	203a	7.3a
Pan	3.3b	12b	0.1b	6.0a	220a	8.2a
Year						
2016	7.6a	20.1a	1.2a	6.8a	210a	6.1b
2017	1.4b	20.3a	2.1a	5.2b	212a	9.6a
Cut						
First	4.0b	10.8b	0.3a	6.5a	255a	6.5b
Second	5.0a	29.6a	3.1a	5.4a	168a	9.1a
p-value						
Method	<.0001	0.001	0.04	ns	ns	ns
Year	<.0002	ns	ns	0.02	ns	0.01
Cut	0.02	0.0005	ns	ns	ns	0.05

[£] % contribution= (mean DM yield of new seeded plants/mean total DM yield of mixed pasture for each cut) ×100

Mean total DM yield of mixed pasture for each cut can be calculated by adding DM yield of alfalfa and new plants.

ns- not significant

Results of cultivated plots at Ponoka and Red Deer indicated that location, year, cut and their 2-way interactions were significant for contribution of bloat-free legumes in a mixed alfalfa pasture (Table 3.5). Proportion of bloat-free legumes cultivated with alfalfa significantly decreased in the second production year with a simultaneous increase in alfalfa production which is consistent with the result from established alfalfa pasture in Lethbridge.

In cultivated plots at Ponoka and Red Deer the proportion of CMV stands were significantly higher than sainfoin test cultivars (Fig. 3.5A). Cultivated Mountainview had more than a 20% contribution in mixture which was significantly higher than Nova (14.6%). Oxley and Veldt contributed 17.7% and 18.6% in a mixed alfalfa pasture while contribution of Oxley II was 13.5%.

Test cultivars used in this study were significantly different for their ability to establish and contribute in a grass pasture mixture (Fig. 3.5B). Plant count of Veldt in drilled plots at Red Deer was more abundant than sainfoin stands. However biomass contributions from sainfoin were higher than CMVs at both locations. In drilled plots at Red Deer Nova, CMV contributed highest (15%) followed by LRC3432 and Mountainview with 12% and 9% respectively. At Ponoka, and Mountainview stands in drilled plots contributed more than all other drilled legume cultivars with less than a 5% contribution in the mixture. All drilled CMV contributed less than 2% at Ponoka while Oxley II and Veldt contributed 7% and 8% at Red Deer compared with drilled LRC3519.

Table 3.5. Mean plant stand per meter^2, DM yield and total biomass contribution in mixtures of introduced plants cultivated with alternate rows of alfalfa at Ponoka and Red Deer. Data are given as means for the three seeding methods used, years and cuts. P-values are given for each factor and their interaction.

	Plants/m^2	DM (kg/ha)	DM contribution$^£$
Location			
Ponoka	7.9a	887a	22a
Red Deer	6.1b	377b	10b
Year			
2016	8.1a	723a	19a
2017	5.8b	541a	13b
Cut			
First	6.3b	460b	12b
Second	7.6a	803a	19a
p-value			
Location	0.0002	<.0001	<.0001
Year	<.0001	ns	0.0001
Cut	0.01	0.001	0.0003
Location*Year	0.02	ns	0.01
Location*cut	ns	ns	0.01
Year*cut	<.0001	<.0001	0.0003

$^£$ % Biomass contribution= (mean DM yield of new seeded plants/mean total DM yield of mixed pasture for each cut) ×100

Mean total DM yield of mixed pasture for each cut can be calculated by adding DM yield of alfalfa and new plants.

ns- not significant

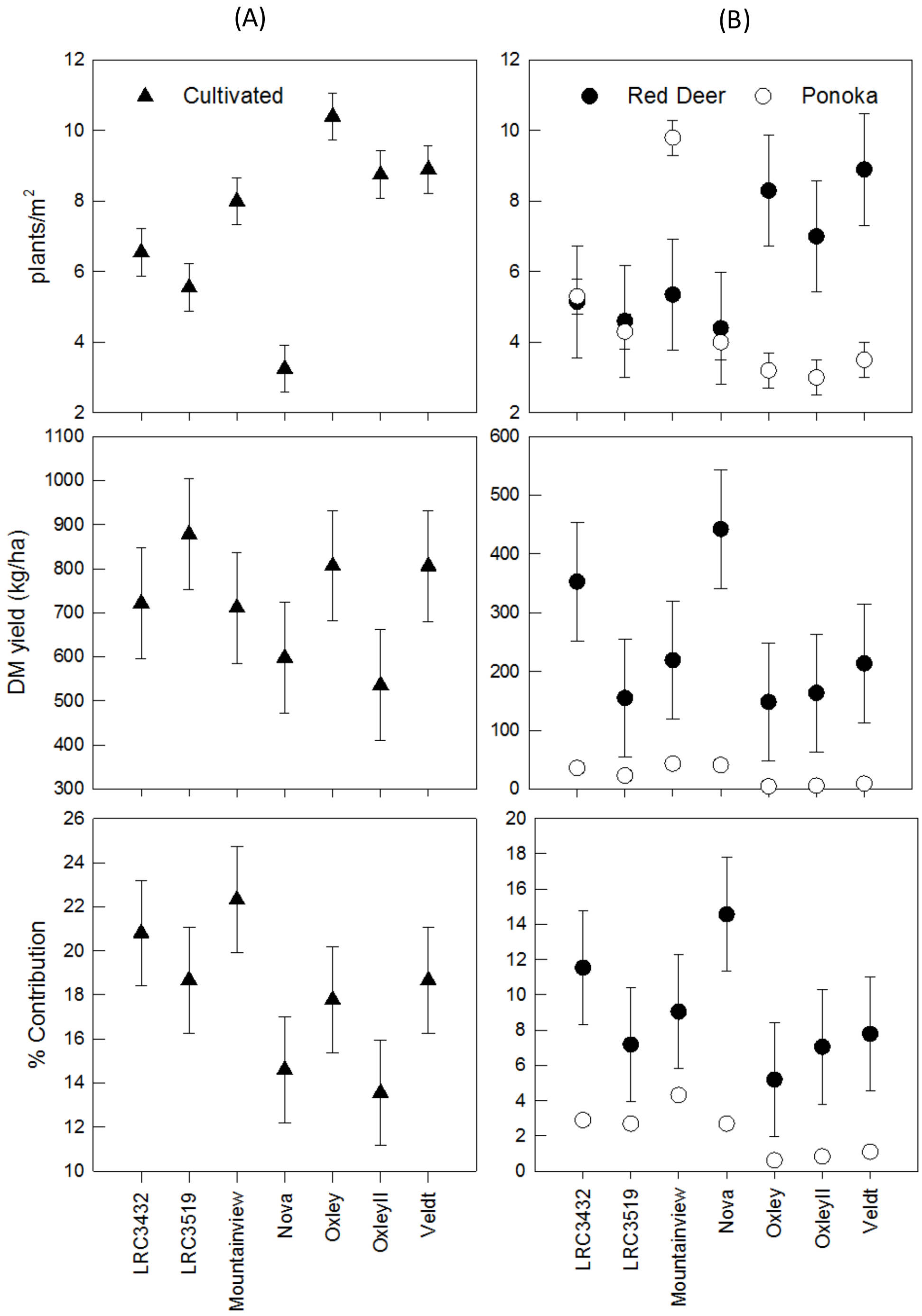


Figure 3.5. Mean plant count per meter^2, DM yield and biomass contribution of test cultivars introduced by cultivation method (A) and using drills (B) at Ponoka and Red Deer, AB. Figure 4(A) represents the average value ± SE for both locations and 4(B) represents the average value ± SE for both drills used at the same locations. Graphs not assigned with major tick labels in the horizontal axis represent the same cultivar as for a similar tick in the graph aligned at the bottom. Sigmaplot did not show SE bars for the point values with lower mean values.

A higher stand proportion of CMVs with a lower percentage contribution in the mixture indicates poor development of the CMV in spite of better seedling emergence. Insufficient growth of legumes in grass also explains the inability of introduced crops to overcome the relative dominance of resident grass pastures particularly in the case of Ponoka. LRC3432 and Nova on the other hand, despite lower plant stands contributed the highest of all indicating them as potential cultivars to improve resident grass pasture.

Results of cultivated plots at Ponoka showed that Mountainview can contribute >20% for both cuts in both production years (Fig. 3.6A). The other two new sainfoin cultivars maintained at least a 10% contribution in each cut while contribution of the old cultivar Nova dropped down to 4% in the second cut in the second year. Veldt grown in an alfalfa pasture at Ponoka contributed ≥20% in each cut. Cultivated plots at Red Deer showed that all cultivars contributed significantly higher in the second cut of the first production year (Fig. 3.6B) probably due to higher than the long-term average precipitation in August, 2016. The new sainfoin cultivar contributed consistently in both cuts in the second year while Nova contributed higher in the second cut, contrasting to the result for Ponoka. Oxley II and Veldt had a higher proportion in the mixture in the second cut. At both locations alfalfa DM yield increased from the first cut in the first production year to the first cut in the second production year and dropped down in the second cut in the second production year.

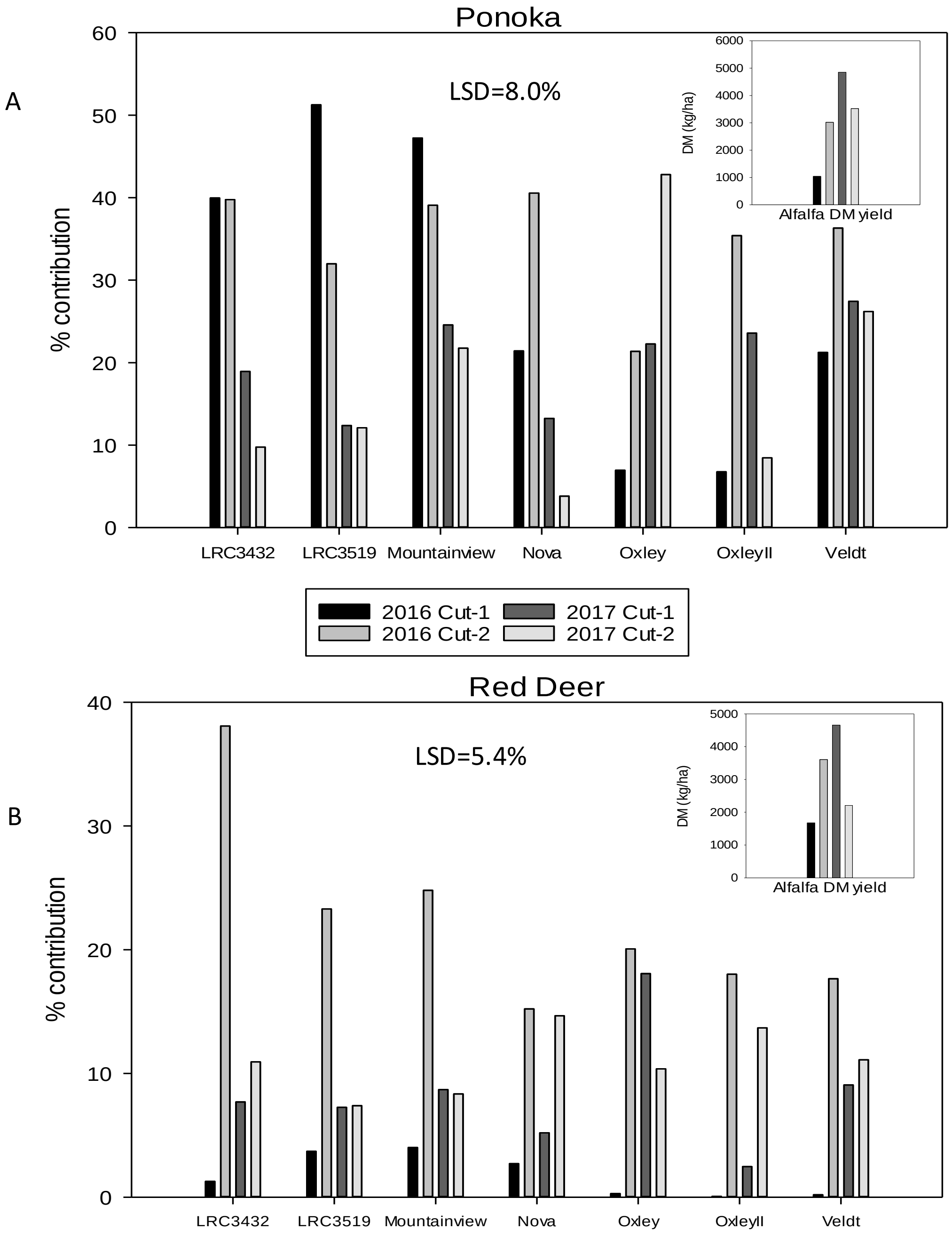


Figure 3.6. Year × cut × cultivar interaction effect on percentage contribution of test cultivars in cultivated plots established at Ponoka (A) and Red Deer (B). Small graph at top right corner represents mean DM yield of alfalfa for each year. Both graph represents average value of cuts taken twice annually. LSD restricted at p=0.05.

3.3.4. Quality of pasture mixture

3.3.4.1. Legume-legume mixtures

Results for the rejuvenated pasture test at Lethbridge with a legume-legume pasture mixture differed in all quality parameters and cuts (Table 3.6). Crude protein in the legume-legume mixtures significantly increased in the second cut while ADF and NDF content decreased. This positive attribute of the second cut simultaneously increased the relative feed value of the pasture mixture. However mixtures created by cultivating the legume mixture at Ponoka and Red Deer showed exactly the opposite results. CP and RFV of the new stands significantly decreased in the second cut. This suggests that CP content and RFV of the pastureland increased in the second cut when the majority of pastureland was old established alfalfa.

3.3.4.2. Grass-legume mixture

Quality of the grass-legume mixture at Red Deer and Ponoka differed in quality parameters between cuts (Table 3.6). CP content of the orchardgrass mixture at Red Deer decreased in the second cut which increased in the bromegrass mixture at Ponoka. An unusually high protein content of the grass at Ponoka was favoured by higher clover content in the pasture mixture. Pure clover stands from the test site had 23% CP content. At both locations ADF and NDF decreased during the second cut, consequently increasing relative feed value.

Table 3.6. Mean crude protein, acid detergent fibre, neutral detergent fibre and relative feed value of legume-legume mixture and grass legume mixture for each cut taken twice annually from test plots established in 2016 at respective location. Cultivated values represent average of Ponoka and Red Deer.

	Legume-legume mixture					Grass-Legume mixture			
	CP	ADF	NDF	RFV		CP	ADF	NDF	RFV
Lethbridge					Red Deer				
Cut 1	18.5	33.5	40.4	148	Cut 1	13.8	34.1	60.3	97.8
Cut2	26.6	22.2	24.1	282	Cut2	10.6	30.9	57.4	105.6
Cultivated (Red Deer and Ponoka)					Ponoka				
Cut 1	23.4	33.7	40.2	152	Cut 1	14.9	31.0	52.7	116.1
Cut2	18.3	31.8	43.1	141	Cut2	16.3	26.0	49.3	131.5

Values in each column for respective location and cultivated plots differs significantly between cuts (LSD=0.05, $p<0.05$).

3.4. Summary and conclusion

This study was initiated to determine if bloat-free legumes can improve existing grass and alfalfa pasture in western Canada and to evaluate the type of seeder that can be used for rejuvenating the depleted pastures. In this multilocation, two-year study, three different resident pastures were selected. At Lethbridge the resident pasture was an alfalfa pasture, at Red Deer and Ponoka the resident pastures were predominantly grass pasture. New sainfoin populations were drilled into the pasture and were observed to contribute more than 20% to the alfalfa pasture at Lethbridge whereas at Red Deer their contribution was only 10% to total biomass. In the grass pasture at Ponoka maximum biomass contribution from test populations were observed to contribute only 2.3% to the mixture. This proportion was not sufficient from a rejuvenation success point of view. This means that the old sainfoin cultivar Nova and the two new cultivars, Mountainview and LRC3432 can improve the existing alfalfa pasture and has the potential to improve grass pastures in some parts of western Canada. CMV cultivars at all locations contributed well below sainfoin cultivars, thus suggesting that CMV cultivars do not establish quickly and should not be used for quick pasture rejuvenation. Mixed alfalfa and bloat-free legumes can be used by completely breaking and reseeding (cultivation) a pasture with Mountainview, LRC3432 (two new sainfoin populations) or Veldt (a new CMV) when harvested twice annually.

From the test at Lethbridge it was clear that introducing LRC3432 and Mountainview into a resident alfalfa pasture can contribute >20% in total DM yield. Use of sainfoin cultivars not only adds up in total biomass production but can prevent pasture bloat in grazing cattle. This test also demonstrated that alfalfa cultivar AC Blue J used in this study can be planted immediately after breaking the old alfalfa stand. Results from this test suggest that direct drilling of some sainfoin cultivars into existing pasture can be a successful rejuvenation strategy for improving productivity and quality of non-leguminous pasture and, in addition can reduce bloat risk associated with use of legumes in most, but not all parts of western Canada.

Chapter 4 : Soil parameters before and after bloat-free legumes are introduced into alfalfa or grass pastures

4.1. Introduction

Pasture rejuvenation by introduction of bloat-free legumes can be a low-cost pasture improvement technique with positive environmental consequences (Acharya, 2015). Benefits of legumes in natural and/or depleted pasture on above ground pasture productivity are well known (Schmid et al., 2008; Sturludóttir et al., 2012). However, little is known about below ground complexity of mixed pasture particularly for mixtures created by later introduction of legumes. In recent years efforts have been made to understand some below ground dynamics of grass-legume mixtures. It is also important to understand the complexity of mixtures when both resident and introduced species are legumes.

It is known that inclusion of legumes benefits companion crops by increasing the soil N pool. N transfer to the non-legume crop in legume mixed pastures is due to root exudation of soluble N, legume root decomposition and growth of plant-associated mycorrhizae (Dhamala et al., 2017; Thilakarathna et al., 2016). Studies conducted to investigate N-transfer and compatibility testing of grass-legume mixtures indicate that certain grass-legume mixtures are suitable for intercropping however the N receiving capacity of grass might be a limiting factor (McElroy et al., 2016). Another study on multispecies (grass-legume-deep rooted non-legume) mixtures showed that grass relies mostly on legume derived shallow deposited N which is favored by its intermingling fibrous root system (Dhamala et al., 2017). Biological nitrogen fixation and soil N storage were highest in a 1:1 grass-legume mixed stand (Li et al., 2016; Nyfeler et al., 2011).

The importance of pasture as a global carbon sink has not been considered as good as forest because of its below-ground storage system (Post and Kwon, 2000). Forest is known to accumulate organic matter in its above ground biomass while pasture does so below the ground. Removal of above ground biomass of pastureland reduces its rate of organic matter deposition (Conrad et al., 2017). Productivity losses of pastureland can be maintained by N fertilization (Delpino et al., 2016; Malhi et al., 2000) or legume inclusion in pasture (Silveria et al., 2016). This suggests that introduction of legumes in a non-legume pasture add to the N fertility of the pastureland which can sequester more C than under fertilized pasture conditions.

Change in organic carbon alters stability of soil aggregates and biological activity (Haynes and Naidu, 1998). Unstable aggregates, mostly in cultivated fields are more prone to erosion (Whalen and Chang, 2002). Soil carbon is also vital for microbial growth; and their diversity affects biogeochemical properties of the soil (Insam and Goberna, 2004). Soil organic matter regulates microbial biomass carbon (MBC) and extracellular enzyme activity (EEA) (Burns et al., 2013). Extracellular enzymes analysed in this study are β-glucosidase (BG) for C-cycling, N-acetyl-β-glucosaminidase (NAG) for N-cycling, phosphomonoesterase (P) for P-cycling and arylsulfatase (S) for S-cycling (Jian et al., 2016). N fertilization increased the activity of BG and NAG (Cenini et al., 2016) while reducing MBC; therefore this discrepancy may be incorporated in future studies (Jian et al., 2016). Legume inclusion affects the C:N ratio (Li et al., 2016) so examination of microbial biomass carbon and extracellular enzyme activities may help us to understand below ground complexity of mixed stands.

The objective of this study was to generate information on soil property dynamics of a rejuvenated pasture production system. We presume that such information can determine sustainability of the technology. The intent was to generate information on changes in soil nutrient status, microbial population dynamics and carbon sequestration before and after pasture rejuvenation. We hypothesize that available N pool, sequestered C, water aggregate stability and enzyme activity of legume introduced plots differ 1) over years, 2) among seeding methods used and 3) between legume-legume and grass-legume mixtures.

4.2. Materials and methods

4.2.1. Site characteristics

A seven year old alfalfa pasture at Lethbridge and long existing grass pastures at Ponoka and Red Deer were chosen for rejuvenation. The Lethbridge site (49°41'11.7"N 112°45'55.1"W) was on black brown Chernozemic soil; the Ponoka site (52°39'05.9"N 113°44'08.5"W) and Red Deer site (52°18'09.8"N 113°19'14.7"W) were on black Chernozemic soils.

4.2.2. Experimental design

This study was an attempt to understand below-ground changes on pastureland before and after rejuvenation. Rejuvenation was assessed by introducing two types of bloat-free legumes into the resident pasture at all locations. Rejuvenation tests were done using a split-plot design with the three seeding methods as main plot factor and eight plant treatments (4 sainfoin + 3 CMVs + 1 unseeded) as sub plot factors. The eight plant treatments were randomised within each seeding method. A total of 24 treatments were replicated four times making a total number of 96 plots per location in 12 experimental blocks. Three seeding methods in the rejuvenation test included two types of seed drill (a Great Plains drill and a pan drill) and cultivation method where the old stand was terminated with herbicide, ploughed under and seeded with alfalfa and sainfoin and alfalfa and CMV in alternate rows.

The same test plots were used to study below-ground change due to rejuvenation effect. Both tests were simultaneously undertaken. For the purpose block analysis was carried out and plot were not analysed individually. For both the legumes upon their introduction into pasture, we predicted underground changes might be consistent within blocks. Rejuvenation effect as a whole on soil quality parameters was investigated. Each block included an equal (eight) number of 2 m × 8 m plots assigned for each set of 8 plant treatments. Three seeding methods as experimental factor were replicated four times and assigned randomly for 12 experimental blocks. Plant species composition was the same for all the blocks except for cultivated plots where resident pasture was replaced by alfalfa. Each block had also one unseeded check plot which were avoided during soil sampling. Seed of new plants were drilled in between 12 inches row spacing for alfalfa at Lethbridge and directly into the grass pastures at the other two locations.

4.2.3. Methods used for introducing bloat-free legumes

Three methods of introducing bloat-free legumes were used and evaluated for their ability to improve pasture productivity quickly and efficiently. Success of rejuvenation and suitability of a seeding method were determined by productivity and the relative proportion of introduced plants in a mixed pasture but, we presumed that sustainability of the technology can be based on a better understanding of below ground changes after rejuvenation. This study primarily focuses on below ground changes, but part of the aboveground changes was observed for evaluating nutrient transfer/pathways. The Great Plains drill is a no-till drill with a working width of 7.5 m. It is a towed seeding implement with an opener disc for seed bed, seed tubes mounted between the discs to place seed in furrows followed by a press wheel to close the furrows. Adjustment handles are provided for depth and seeding rate. A Great Plains drill is used when conventional or minimum tillage is required for agriculture production and/or seed production (Great Plains Operator Manual, 2015). The pan drill used in the study was self-propelled with hydraulics, built in 1971 by Fabro Mfg^{TM}. This drill has been used by forage researchers in western Canada in weed-free cultivated land and is not expected to deliver seed effectively in compacted soil with vegetation. The third method of seeding involved a complete plow down of existing pasture and reseeding of alfalfa and sainfoin and alfalfa and CMV cultivars in alternate rows.

4.2.4. Soil sampling and analysis

Baseline soil chemical quality parameters were established from soil samples collected in September 2014 (a year before establishment of rejuvenation test plots) from

Lethbridge and Red Deer (Table 4.1). For the Ponoka location soil chemical quality indicators analysed from 2015 samples are presented in Table 4.1. After first seeding in May 2015, annual soil samples were collected during the month of September for three years in a row at Lethbridge and Ponoka. Annual soil samples were collected from Red Deer in September of 2016 and 2017 only. Baseline soil samples were taken in three incremental layers (0-30, 30-60 and 60-90 cm). We assumed the bulk densities of the soil for 0-30 cm, 30-60 cm and 60-90 cm depth were 1.2 gm/cm^3, 1.3 gm/cm^3 and 1.4 gm/cm^3 respectively at all locations. A truck mounted hydraulic core sampler was used to take one soil core from each depth. Collected samples were air dried and ground to pass through a 2-mm screen. Subsamples were finely ground to analyze N and C concentration.

Soil samples collected in 2015 and 2016 from all depths were analyzed for extractable NO_3-N and NH_4-N (Auto analyzer components using Astoria Pacific 305D digital detector, Clackamas, Oregon, USA). Fine ground samples collected in 2015 and 2016 to a depth of 60 cm were analyzed for total N, total C and organic C by dry combustion using an automated analyzer (Carlo Erba NA 2100 Elemental Analyzer, Carlo Erba Strumentazione, Italy) (Larney et al., 2017). Top most layer samples collected in 2015, 2016 and 2017 were analyzed for water aggregate stability (Angers et. al., 2008).

For soil microbial properties, baseline mean values for enzyme activity and biomass activity were analyzed from fresh samples collected in 2015 and compared with samples collected in 2017. Samples from 0-7.5 cm depth were taken from 6 blocks (2 replicates for each seeding method) at each location and kept frozen until analysed. Microbial biomass was determined by using a substrate induced respiration procedure (Horwarth et al., 1994). Enzyme activity: β-glucosidase and β-N-acetyl-glucosaminidase (C and N cycling), acid phosphomonoesterase (P cycling) and arylsulphatase (S cycling) were determined by using microplate fluorimetric assays that utilize 4-methylumbelliferyl (MUF)-labelled substrates (Deng et al., 2011). Baseline microbial composition was determined from 2015 collected samples from all locations by extracting DNA using PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA) and pyrosequenced by MR DNA lab (www.mrdnalab.com, Shallowater, Texas) (Lupwayi et al., 2017). Treatment effects on soil microbial biomass were not analysed.

4.2.5. Statistics

A SAS ANOVA MIXED procedure was used to analyze data collected over multiple locations and years (SAS Institute., 2005). Means for all treatments were compared among locations. Comparisons of soil quality parameters among the three seeding methods were determined from samples collected from different depths. Least squares difference test was made after means separation when the treatment effect was significant (LSMEANS with the PDIFF procedure of SAS). Treatment effects were considered significant at $p < 0.05$.

4.3. Results and discussion

4.3.1. Weather data

At Lethbridge and Red Deer total monthly precipitation as recorded by Alberta Agriculture and Forestry, Alberta Climate Information Service (ACIS) 'https://agriculture.alberta.ca/acis' was below the long-term average in 2015 while Ponoka received an above the long-term average monthly precipitation in the second half of 2015 (Fig. 4.1). In 2015 and 2016, total monthly precipitation during the month of June was below the 30-year average at all three locations. In 2016 higher than long-term average precipitation was particularly noticeable in May and July at Lethbridge, in August at Red Deer and in July, August and October at Ponoka. Average monthly temperature followed the 30-year average pattern at all three locations.

4.3.2. Baseline soil C and N content

Comparison of mean values for total C, total N and organic C content from 2014 collected samples from Lethbridge and Red Deer showed no differences (based on standard error) for selected parameters between locations (Table 4.1). The only difference was observed between 7-year old legume pasture (Lethbridge) and long existing grass pasture (Red Deer) for the soil available N content. As expected the soil available N was significantly higher at all depths at Lethbridge than at Red Deer. All three depths were similar for available N content at Red Deer. The top soil layer was higher in total N content than the deeper layer within a location. Organic C content of grass and legume pasture indicate similar carbon sequestration potential for both types of pasture.

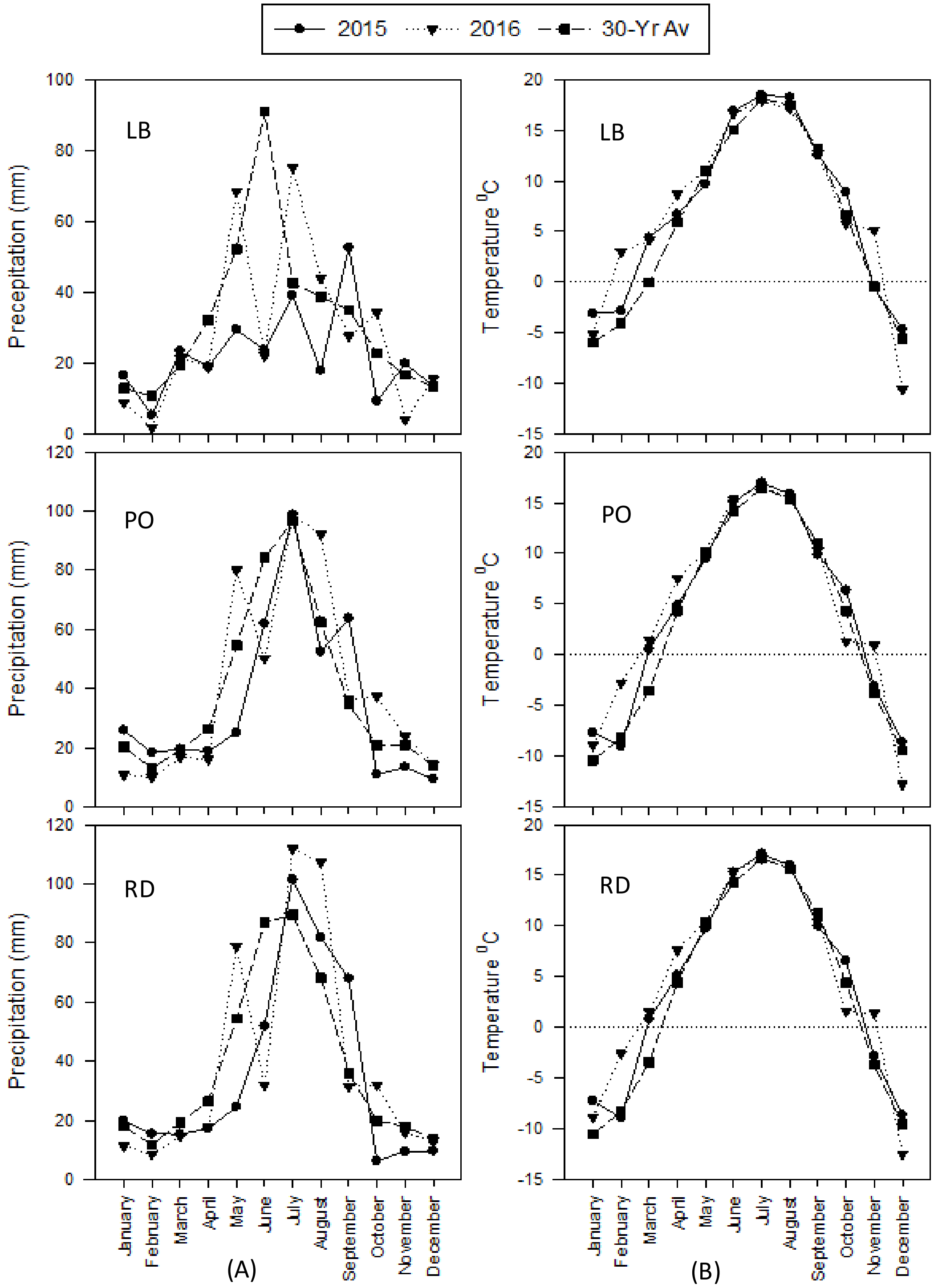


Figure 4.1. Total monthly precipitation (A) and average monthly temperature (B) for 2015, 2016 and 30-year average at Lethbridge (LB), Ponoka (PO) and Red Deer (RD). Graphs not assigned with major tick labels in X-axis represent the same month for similar tick as graph aligned at bottom. Source: 'https://agriculture.alberta.ca/acis'

Means with higher standard errors indicate a higher variability for selected parameters within a location. This was particularly reflected in the values from assigned plots for the three seeding methods before introduction of bloat-free legumes. Soil samples collected from assigned plots before a treatment application varied for all parameters except available N. Although baseline variability was evident within a location the ratio of baseline mean total in two depths (0-30 and 30-60 cm) for total C, N and organic C were similar. Total C, N and organic C content of the top layer were 2 and 1.5 fold greater than the deeper layer at Lethbridge and Red Deer respectively.

Soil samples collected from the top soil profile in Ponoka after test plot establishment showed a higher content for all parameters measured in cultivated plots compared to drilled plots. This may be from the combined effect of treatment and sampling depth differences between cultivated plots and drilled plots. Tillage in cultivated plots increased the surface elevation so sampling depth in such plots might not represent the actual depth and determination of soil chemistry and organic matter from those non-equivalent soil mass may have influenced the results (Ellert and Bettany, 1995). Drilled plots were similar for indicators examined at the same depth. In deeper depth total N and available N were similar in both cultivated and drilled plots. Carbon content of non-tilled plot was greater than for the tilled plots at a deeper layer. This indicates that a legume pasture mixture created by cultivation can sequester more carbon in the upper soil profile than pure or grass-legume mixture in the year of legume introduction. However, drilled plots with deep rooted grass can sequester more C in deeper profile than grass less mixed legumes plots.

Table 4.1. Baseline mean values and standard error of selected chemical properties of soil in assigned plots for three seeding methods at three sites.

	Total N (t/ha)		*Total C (t/ha)*		*SOC (t/ha)*		*Avail. N (kg/ha)*		
	0-30	30-60	0-30	30-60	0-30	30-60	0-30	30-60	60-90
Lethbridge site									
Cultivate	12.5 ± 2.1	5.5 ± 0.1	137.3 ± 14.2	52.6 ± 1.1	130.8 ± 26.9	48.1 ± 0.7	59.0 ± 5.2	41.0 ± 3.8	36.8 ± 1.3
Great Plain	7.2 ± 2.6	7.0 ± 2.8	96.2 ± 6.1	69.1 ± 29.6	68.3 ± 29.3	65.3 ± 27.2	60.2 ± 20.0	29.3 ± 1.9	36.7 ± 1.3
Pan	11.2 ± 2.2	4.2 ± 0.4	116.9 ± 14.9	73.2 ± 20.0	109.4 ± 21.2	35.8 ± 3.9	57.2 ± 17.9	26.6 ± 2.4	28.8 ± 1.0
Mean	10.3 ± 1.4	5.6 ± 0.9	116.8 ± 9.4	65.0 ± 10.1	102.8 ± 16.4	49.8 ± 8.9	58.8 ± 7.1	32.2 ± 3.0	34.1 ± 1.7
Ponoka site[£]									
Cultivate	14.3 ± 0.9	2.8 ± 0.1	168.9 ± 10.5	31.8 ± 7.0	164.3 ± 6.7	28.4 ± 6.5	81.7 ± 26.2	28.4 ± 8.0	24.4 ± 3.6
Great Plain	11.6 ± 1.6	2.8 ± 0.4	139.0 ± 22.1	40.9 ± 18.0	133.8 ± 20.9	39.1 ± 19.0	40.8 ± 8.2	21.7 ± 5.2	27.2 ± 6.4
Pan	9.9 ± 1.1	2.4 ± 0.1	116.2 ± 13.8	26.5 ± 2.0	113.4 ± 10.4	22.2 ± 3.1	31.5 ± 1.7	26.2 ± 3.6	21.7 ± 1.8
Mean	11.9 ± 0.7	2.6 ± 0.1	141.5 ± 8.8	33.0 ± 4.5	137.2 ± 8.1	30.0 ± 4.8	51.3 ± 8.8	25.4 ± 2.3	24.4 ± 1.7
Red Deer site									
Cultivate	7.4 ± 0.8	3.5 ± 0.3	83.3 ± 10.0	55.6 ± 9.1	73.9 ± 0.9	35.1 ± 2.8	22.1 ± 1.4	22.9 ± 1.2	16.6 ± 0.0
Great Plain	11.5 ± 3.4	5.4 ± 2.0	136.2 ± 41.8	83.6 ± 12.6	135.6 ± 43.9	60.2 ± 37.0	20.4 ± 3.9	20.3 ± 0.8	26.8 ± 7.1
Pan	10.6 ± 2.4	3.2 ± 0.2	138.8 ± 28.6	29.4 ± 5.0	131.4 ± 54.2	24.7 ± 1.0	29.8 ± 3.1	30.3 ± 9.1	20.8 ± 3.5
Mean	9.8 ± 1.4	6.2 ± 2.2	119.4 ± 16.3	83.5 ± 25.0	113.6 ± 25.5	70.5 ± 29.8	24.1 ± 2.2	24.4 ± 3.0	21.3 ± 2.7

[£] Values of Ponoka site are from 2015 collected samples after introduction of bloat-free legumes.

SOC- Soil Organic Carbon; Avail N- Available Nitrogen

4.3.3. Soil chemistry after rejuvenation

At Lethbridge, results of the rejuvenation test indicated that the seeding method, year and interaction effect were not significant for total N, total C and organic C (Table 4.2). Methods of seeding and year were significant for available N content however the interaction effect was not different. All selected parameters were different for sampling depth. Interactions of depth with other treatment factors were similar. Results of the Lethbridge test suggests that introduction of a legume into a legume pasture has no effect on total N and C content of the soil and did not result in accumulation of organic carbon over the two years of production (Appendix 1.0). Seeding method effect on available N showed that cultivated plots had higher N availability than drilled plots (Fig. 4.2A). Cultivated plots had 48.0 kg ha^{-1} of available N while drilled plots were not different for N availability (40.0 kg ha^{-1}). At Lethbridge year effect showed that N availability of the soil increased in the year of introduction which significantly decreased in the second year of production. Baseline available N content throughout the soil profile was 41.0 kg ha^{-1} which significantly increased to 56.0 kg ha^{-1} in 2015 and dropped down to 30.0 kg ha^{-1} in 2016 (Fig. 4.2B). The significant increase of available N in 2015 indicates that introduction of bloat-free legumes in an alfalfa pasture may have helped increase in available N promptly. This increasing trend did not last for long as there was a significant decrease in soil available N in 2016. This decrease was possibly due to N leaching. In 2016, six inches of irrigation (double of normal application) was applied in August (a month before soil sampling) as well as monthly precipitation recorded for July and August in 2016 was higher than the long-term average.

Table 4.2. Results of analysis of variance of selected soil parameters after 2 years of bloat-free legume introduction.

	Total N (kg/ha)	Total C (t/ha)	SOC (t/ha)	Avail. N (kg/ha)
		Lethbridge site		
T	ns	ns	ns	*
Y	ns	ns	ns	***
D	***	***	***	***
T × Y	ns	ns	ns	ns
T × D	ns	ns	ns	ns
Y × D	ns	ns	ns	ns
		Ponoka site		
T	*	*	ns	***
Y	*	*	ns	ns
D	***	***	***	***
T × Y	ns	ns	ns	ns
T × D	*	*	ns	**
Y × D	*	ns	**	ns
		Red Deer site		
T	ns	ns	ns	ns
Y	ns	ns	ns	***
D	***	ns	*	ns
T × Y	ns	ns	ns	ns
T × D	ns	ns	ns	ns
Y × D	ns	ns	ns	ns

*, **, *** significant at $P<0.05$, <0.01, <0.001 respectively, ns-not significant
SOC- Soil Organic Carbon, Avail. N- Available nitrogen, T- Method of seeding, Y-Year, D-Depth

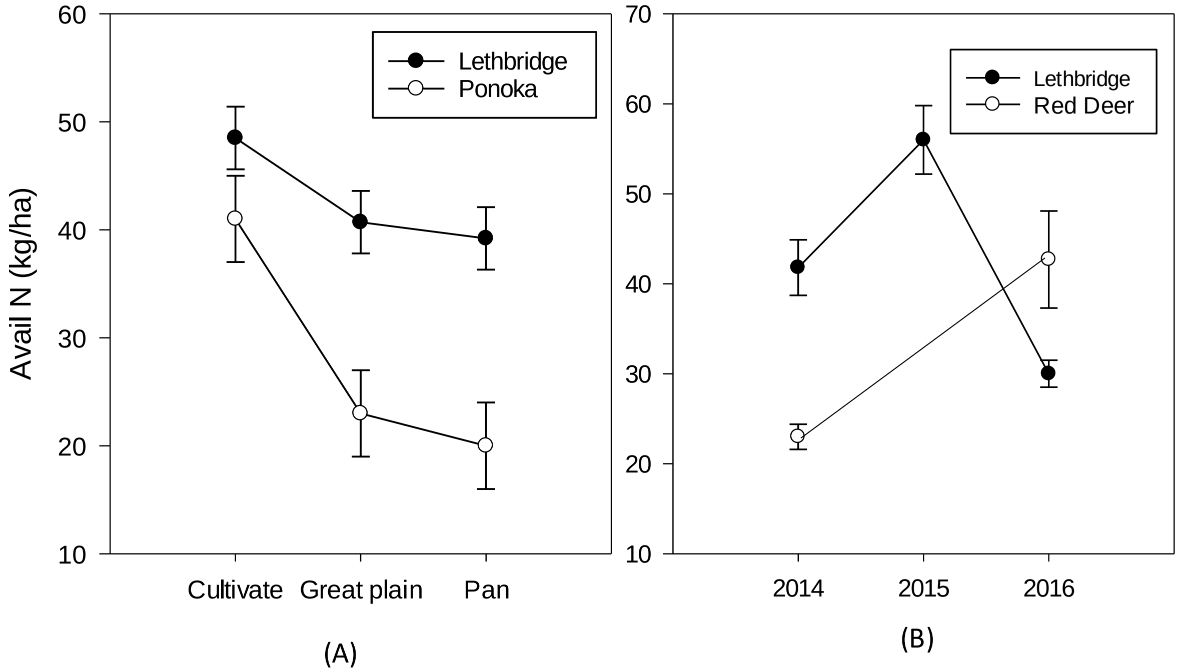


Figure 4.2. Available N content at selected site throughout the soil profile for 3 seeding methods (averaged over years)(A) and years (averaged over seeding methods) (B). Missing sites Red Deer in Figure 4.2A and Ponoka in Figure 4.2B is due to lack of significance for the respective treatment factors. 2015 data point for Red Deer in Figure 4B is missing because soil samples were not taken from that site in that year.

The effect of additional irrigation coupled with higher precipitation before sampling helped soil available N to leach down at Lethbridge. Higher number and proportion of bloat-free legumes in the pasture mixture can increase N availability.

Results of the Ponoka soil analyses showed that the method of seeding and year had effects on total N and C content of the soil (Table 4.2).Throughout the soil profile cultivated plots had 41.0 kg ha^{-1} available N while drilled plots had 21.0 ± 3.0 kg ha^{-1}(Fig. 4.2A). All selected parameters were different for each of the sampling depths (Appendix 2.0). Depth and year interaction was significant for total N and soil organic C.

Depth and year interaction effects showed that the highest organic C accumulated in the top soil profile in the year of legume introduction (137.0 t ha^{-1}) compared to the second year of production (82.6 t ha^{-1}). The 30-60 cm soil profile for all plots was similar in accumulated organic C. The same was true for total N content with the highest (12.0 t ha^{-1}) seen in 2015 relative to 9.7 t ha^{-1} in 2016. Total N content in the top soil layer in cultivated plots (13.0 t ha^{-1}) was significantly higher than that seen in drilled plots. The same was true for total C content with the highest (152.0 t ha^{-1}) being observed in the cultivated plot compared to (129.6 t ha^{-1}) seen in the drilled plots.

Seeding method and depth interaction on soil available N showed 81.0 kg ha^{-1} availability in the top soil layer of the cultivated plots and 36.0 kg ha^{-1} in drilled plots. Deeper depths were not different for N availability. Decreased soil total N, C and organic carbon accumulation over the production years corresponded with poor legume growth which in turn had non-significant effect on N availability.

Results of the Red Deer pasture showed that the year, method of seeding and their interaction have no significance on soil total C, N and organic C content (Table 4.2). Soil total N and organic C varied in depths (Appendix 2.0). Interactions of depth with other treatment factors were also not significant. However, legume availability over the years increased N availability. Baseline soil available N content throughout the soil profile increased from 23.3 kg ha^{-1} in 2014 to 42.8 kg ha^{-1} in 2016 (Fig. 4.2B). This suggests that legume introduction in grass pasture might not have immediate effect on soil N and C content in the first two years observed. However, legume growth in a grass legume mixed pasture is expected to increase N availability of the stand over years (Ledgard and Steele, 1992).

4.3.4. Relationship between soil available N and legume stands in grass

Further analysis of soil N availability for variable legume proportion in pasture indicated that soil available N increased with an increased legume proportion in a grass-legume mixture (Fig. 4.3). For this purpose soil samples were collected from shallow depth (0-15 cm). Regression analysis indicated a positive effect between soil available N and percentage of legume in pasture mixture (R^2 = 0.60, $p<0.02$ for 0-15 cm; R^2= 0.44, $p<0.07$). Soil available N decreased with increased soil depth. Soil N availability of the top (0-15cm) soil depth increased significantly as relative percentage of legume increased in the pasture ($p<0.02$). At $p<0.07$, N availability of 15-30 cm soil depth increased with increased proportion of legumes in the pasture. Soil samples from deeper soil profiles were no different for N availability.

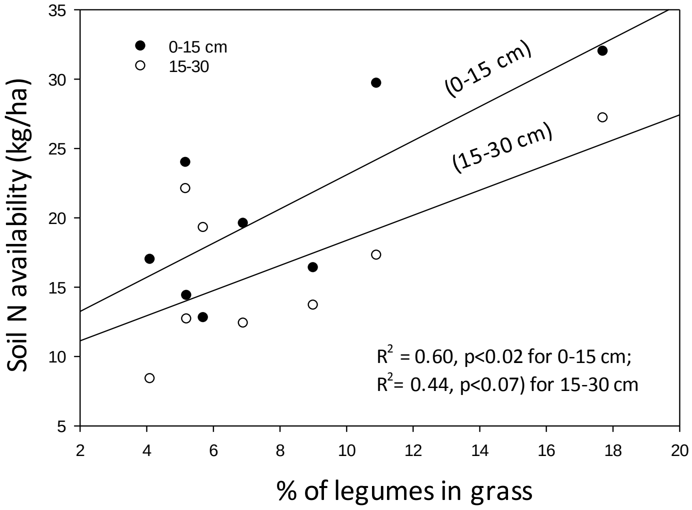


Figure 4.3. Soil N availability in relation to legume proportion in 0-15, 15-30 cm depths of grass pasture at Red Deer.

4.3.5. Water Aggregate Stability

A significant increase in water stable aggregate (WSA) over years was seen at Lethbridge and Red Deer (Fig. 4.4). Percentage of the >1 mm fraction of prewet aggregates significantly increased after introduction of legumes to a pasture compared to the baseline average.

Prior to the start of our study at Lethbridge and the Red Deer site, mean WSA percentage was 75% and 61% respectively. Presence of bloat-free legumes (sainfoin and CMV) in alfalfa pasture at Lethbridge and in grass pasture at Red Deer significantly increased WSA to 91% at both locations. WSA percentage significantly increased in the year of bloat-free legumes introduction in Lethbridge. Poor establishment of introduced legumes at Ponoka resulted in no significant effect on WSA in the >1 mm of soil fraction. Significant effect of year showed that a pure stand of alfalfa and grass pasture had a lower proportion of stable aggregates than a mixed stand. This indicates positive effect of number of species in pastureland on water stable aggregates.

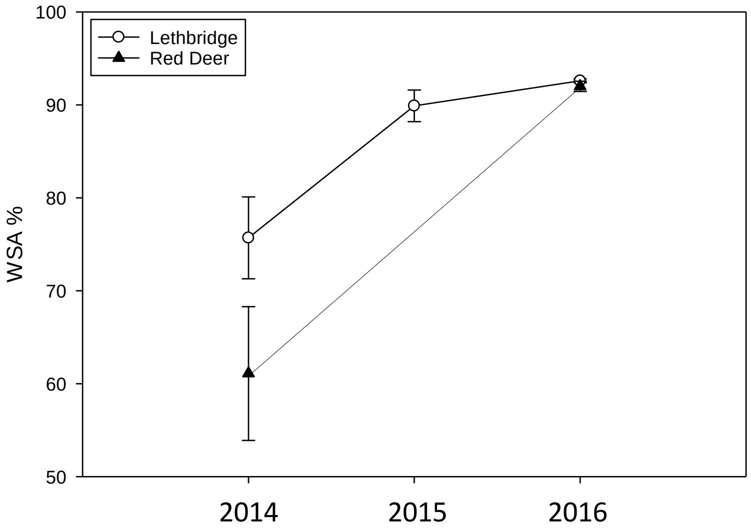


Figure 4.4. Percentage water stable aggregate in 0-7.5 cm depth observed in selected years at Lethbridge and Red Deer. 2015 data point for Red Deer in figure is missing because soil samples were not taken from that site in that year.

4.3.6. Microbial biomass carbon (MBC) and extracellular enzyme activity

Results for the base line soil data showed that MBC in a legume pasture at Lethbridge was significantly higher than for grass pasture at the other two locations (Fig. 4.5A). Location and year interaction showed a significant increase in MBC at Red Deer over years. MBC content at Lethbridge remained the same while Ponoka showed a significant decrease over the years.

Bloat-free legume introduction showed an effect on BG activity in grass pastures only (Fig. 4.5B). Significant reduction of BG activity was observed at Ponoka while it improved at Red Deer over years. However, BG activities were the same at both locations in 2017. P activity significantly increased in pasture soil at Lethbridge and Red Deer but decreased at Ponoka (Fig. 4.5C). In both years S activity at Ponoka was highest among all locations (Fig. 4.5D) although like other enzyme activities at Ponoka, S activity also decreased in 2017. S activity in Lethbridge significantly decreased in 2017 while it increased in Red Deer. NAG activity averaged over years was significantly different among locations which showed that Ponoka had the highest NAG followed by Lethbridge and Red Deer (Fig. 4.6).

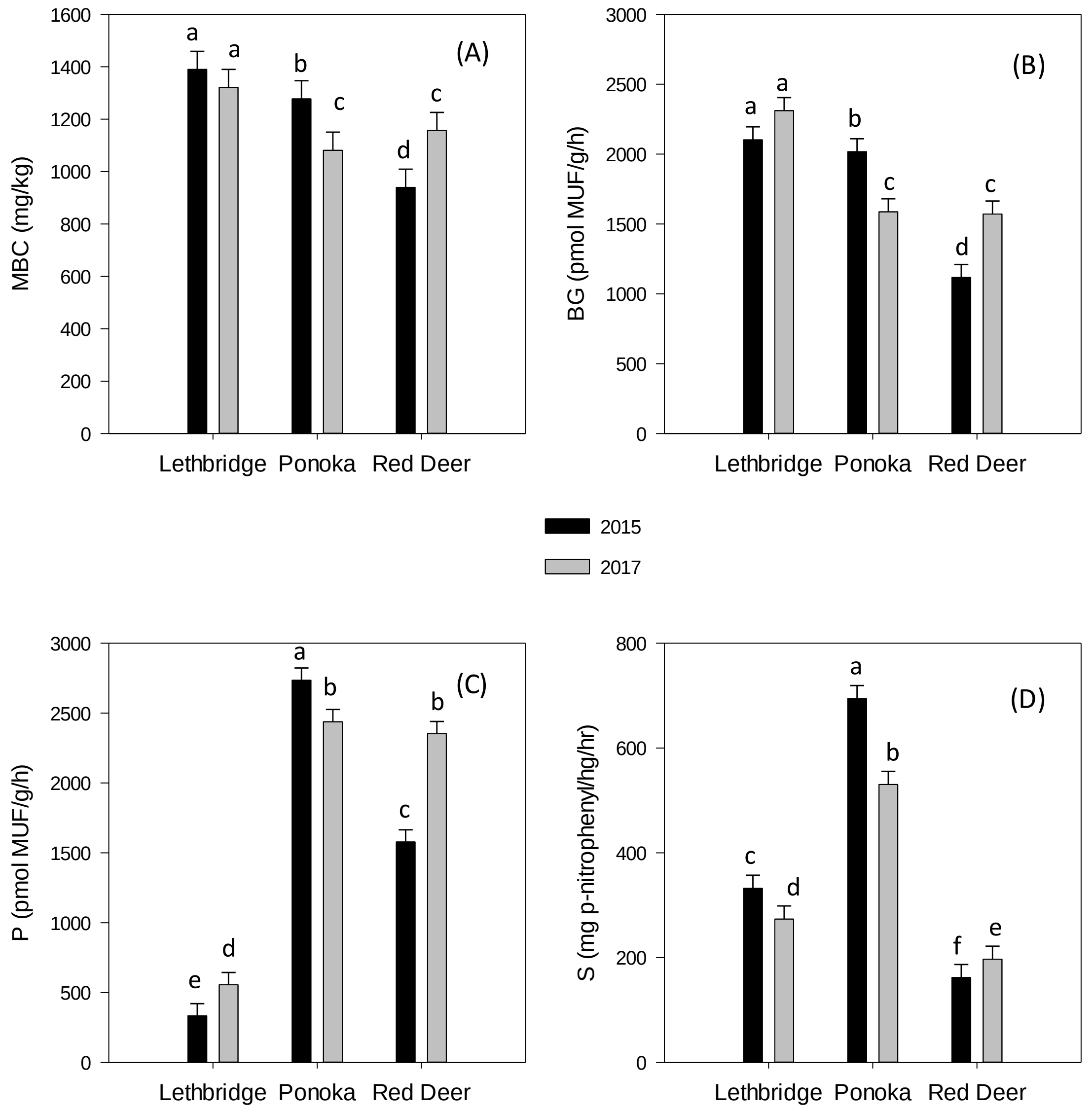


Figure 4.5. MBC: Microbial biomass carbon (A), BG: β-glucosidase (B), P: phosphomonoesterase (C) and S: arylsulfatase (D) activities at different locations. Values are mean ± SE. Mean bars with same letters are not significantly different.

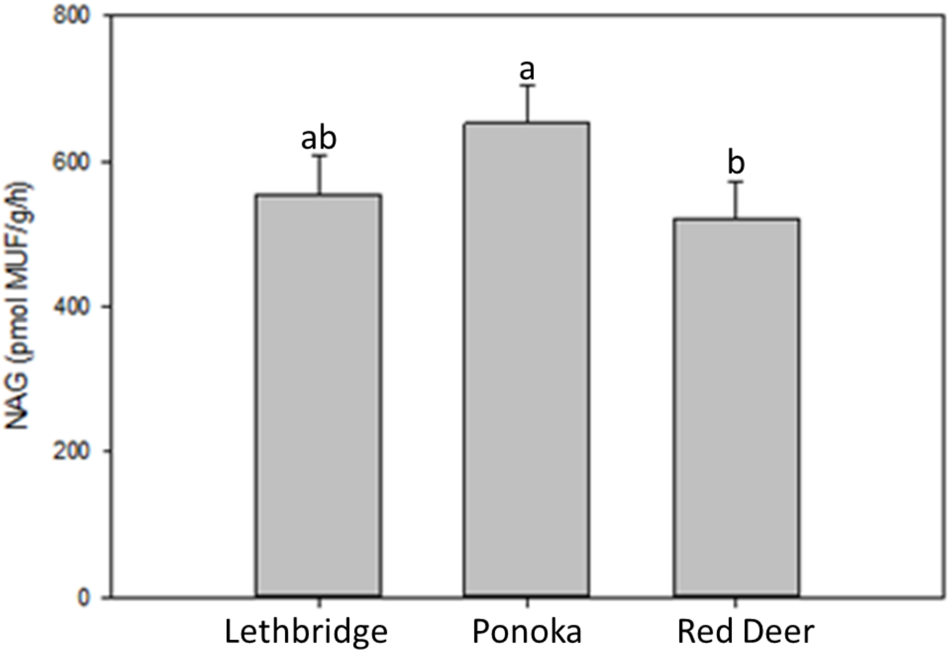


Figure 4.6. N-acetyl-β-glucosaminidase activity averaged over years at Lethbridge, Ponoka and Red Deer. Values are means ± SE. Mean bars with same letters are not significantly different.

We observed MBC and other enzyme activities significantly increased in the grass-legume mixture at Red Deer over years. These results suggest that as low as 10% legume in existing grass pasture can improve soil microbial C and extracellular enzyme activity. More than 20% of bloat-free legumes grown in the alfalfa pasture did not change microbial biomass of the pasture mixture but increased enzyme activities responsible for C and P cycling and decreased S-cycling enzyme activities. Legume incorporation in either legume or grass may not change NAG activity responsible for N-cycling. Clover mixed grass pasture at Ponoka showed higher NAG activity than legume-legume mixture suggesting that more sophisticated research to investigate NAG activity of mixtures at species level is needed.

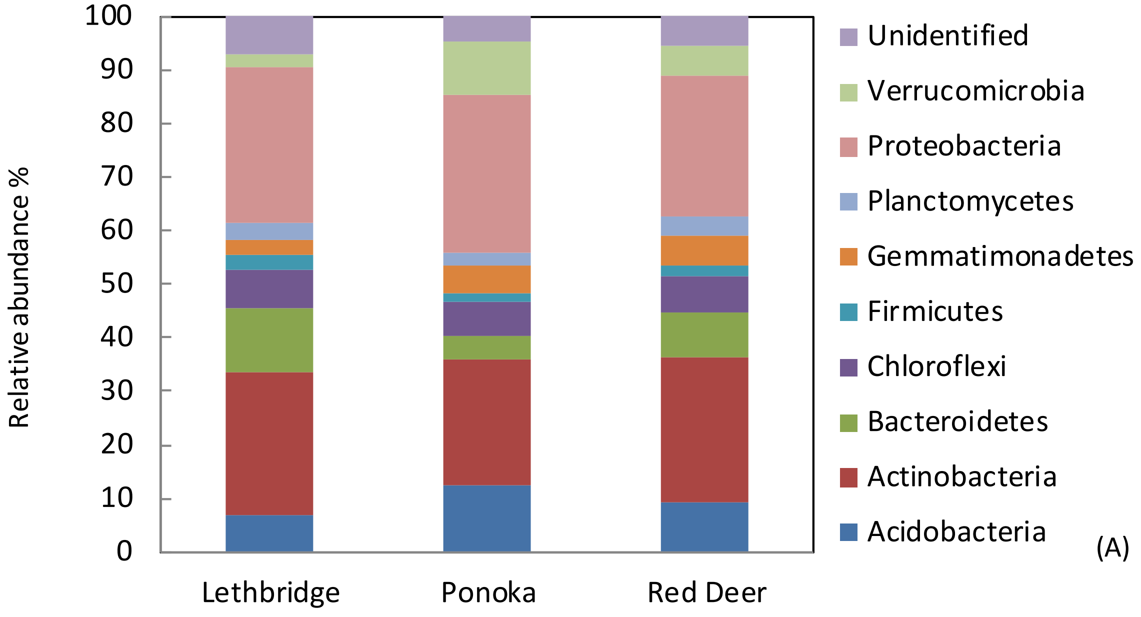
4.3.7 Microbial composition

For soil microbial composition we identified bacterial and fungal phyla in 2015 that were collected from fresh soil samples from three locations. Change effect on microbial composition due to rejuvenation was not analysed so a conclusion on effect of legume introduction into depleted legume and grass pastures cannot be drawn. Soil microbial compositions were compared among sites and in relation to other soil properties for respective locations.

4.3.7.1 Bacterial Taxonomy

For bacterial phyla at all three locations, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Gemmatimonadetes, Planctomycetes, Proteobacteria and Verrucomicrobia showed relative abundance of more than 1% (Fig. 4.7A). Actinobacteria and Proteobacteria were the most abundant phyla (relative abundance >20%). Relative abundance of Bacteroidetes was highest at Lethbridge (11.9%) > Red Deer > Ponoka whereas Acidobacteria and Verrucomicrobia were highest at Ponoka (12.6 and 9.7% respectively) >Red Deer> Lethbridge. Contribution of Firmicutes and Planctomycetes were similar at Lethbridge and Red Deer and more at Ponoka.

Proteobacteria dominated bacterial phyla constituting 29.3%, 29.7% and 26.5% at Lethbridge, Ponoka and Red Deer respectively. At the class level, Alphaproteobacteria dominated with more than 40% at all locations. Other classes Gammaproteobacteria and Deltaproteobacteria were more abundant in the alfalfa pasture at Lethbridge than in the grass pastures at the other 2 locations (Fig. 4.8A). In contrast presence of Betaproteobacteria was more abundant in grass pastures than in the legume pasture.



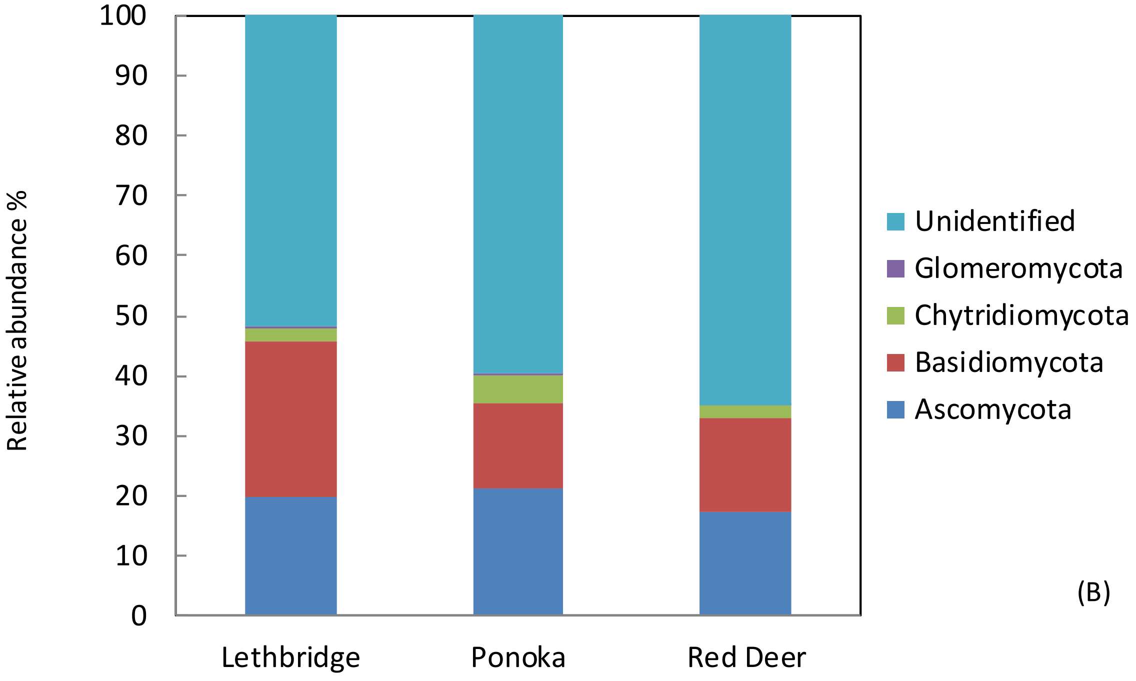


Figure 4.7. Relative abundance of Bacterial (A) and Fungal (B) Phyla present at Lethbridge, Ponoka and Red Deer sites in 2015.

Actinobacteria, the second major bacterial phyla, constituted 26.6%, 23.3% and 27.1% at Lethbridge, Ponoka and Red Deer respectively. At Ponoka, more than 50% Actinobacteria was Thermoleophilia which was least (31.5%) at Lethbridge among all locations. Other classes, Actinobacteria and Acidimicrobia were more abundant at Lethbridge than at the other two locations. Abundance of Rubrobacteria was similar at Lethbridge and Red Deer and more than Ponoka (Fig. 4.8B).

The bacterial community can be classified as copiotrophic (Proteobacteria, Firmicutes and Bacteriodetes) and oligotrophic (Acidobacteria and Plantomycetes) based on their response to organic substrates present in the soil (Fierer et al., 2007 and 2012). Relative abundance (Fig. 4.7A) of copiotrophic organisms are higher in C and/or N rich soil whereas oligotrophic organisms are slow growing and can survive in resource poor soil. In our study, although C and N cycling was poor in Red Deer relative abundance of Proteobacteria and Firmicutes (copiotrophic) were similar as in the other two locations. The only difference among locations for copiotrophic was for Bacteriodetes which was higher in Lethbridge. For the oligotrophic community, although Planctomycetes were similar among locations it was surprising that the Acidobacteria proportion was highest in the N and C rich soil found at Ponoka. Analysis at the phyla level may not address this discrepancy and demands further analysis at a genus level before coming to any conclusion.

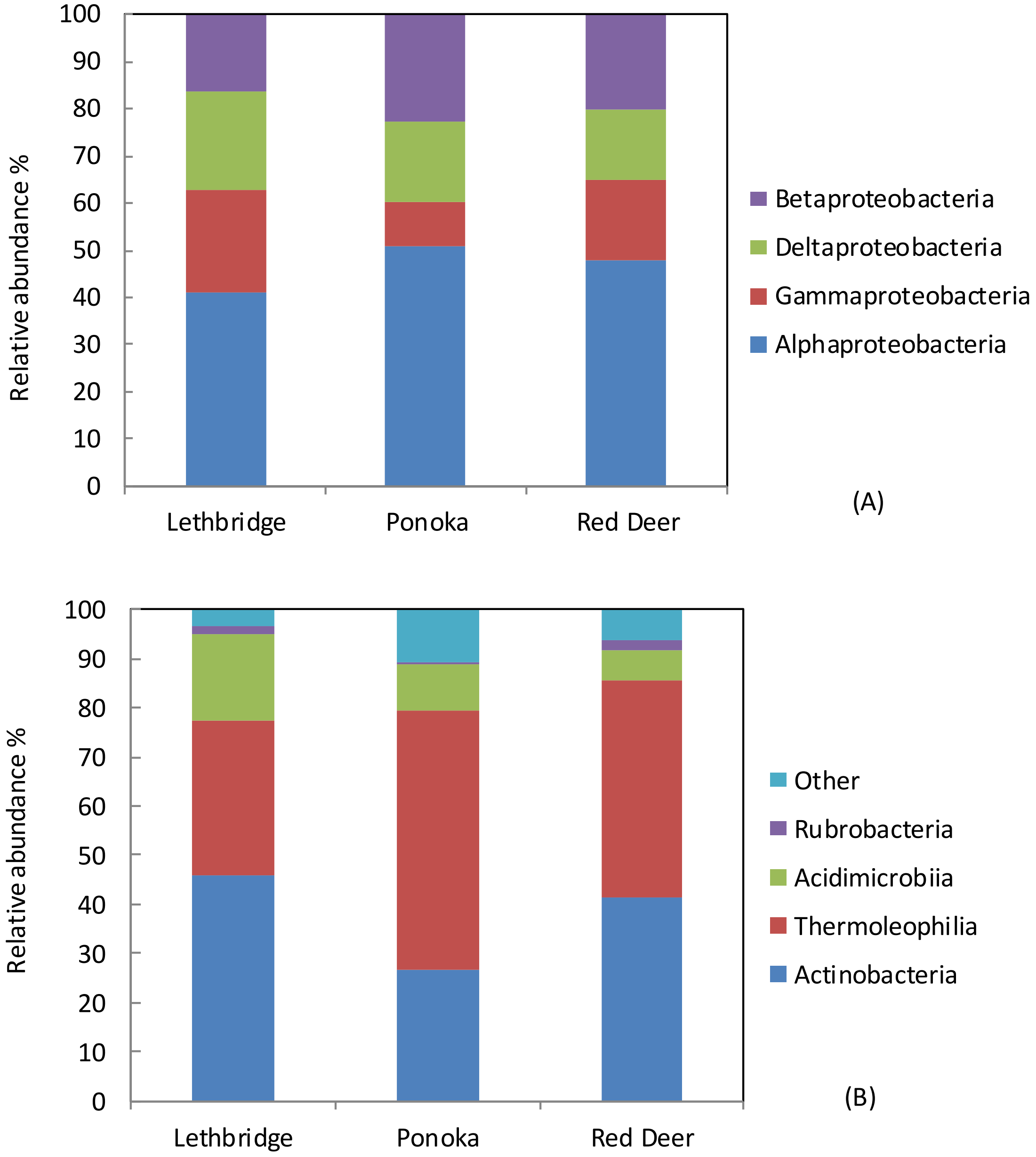


Figure 4.8. Relative abundance of selected classes of Proteobacteria (A) and Actinobacteria (B) at Lethbridge, Ponoka and Red Deer sites.

4.3.7.2 Fungal taxonomy

Among identified phyla, Ascomycota and Basidiomycota were more abundant at all pastureland (Fig. 4.7B). At class level more than 20% Sordariomycetes dominated Ascomycota at Red Deer and Ponoka while percentage of Dothideomycetes, Leotiomycetes and Sordariomycetes were similar and higher than Eurotiomycetes in legumes at Lethbridge (Fig. 4.9A). At all locations Agaricomycetes dominated the Basidomycetes phyla (Fig. 4.9B). Classification of soil fungi at a phyla and class level can give a rough guide to soil health however, the wide range of members within a phyla behave differently for their role in the soil such as decomposition of organic material and mycorrhizal association with plant roots. Hence long term study (> 3 years) would be expressive to compare relative abundance of the microbial community after a treatment effect which has been practised in another study (Lupwayi et al., 2017). Based on our baseline data for microbial composition and diversity, other studies of the pasture rejuvenation effect on soil microbial properties can be conducted in the future.

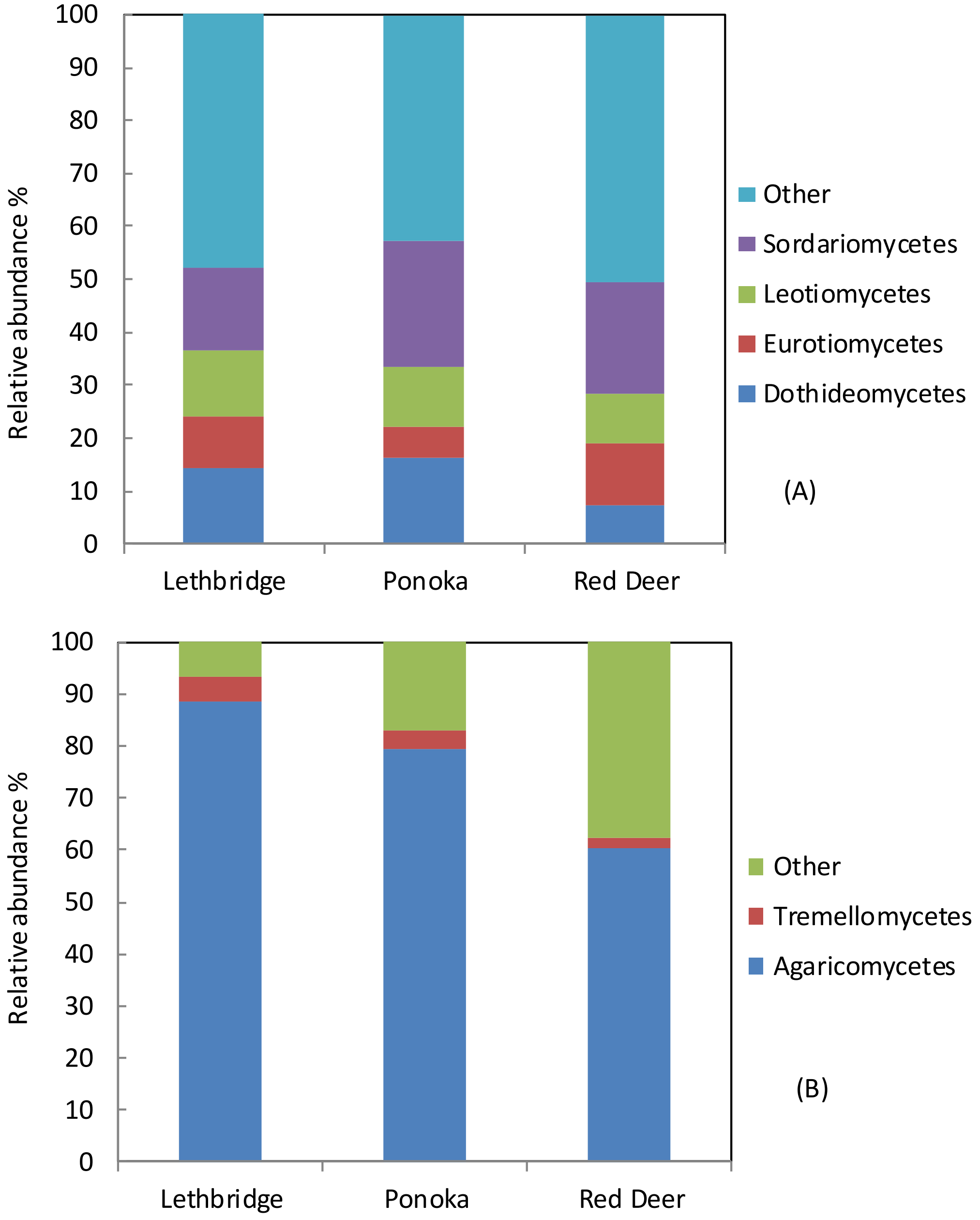


Figure 4.9. Relative abundance of selected classes of Ascomycota (A) and Basidiomycota (B) at Lethbridge, Ponoka and Red Deer sites.

4.4. Summary and Conclusion

This study was initiated to determine soil quality parameters before and after bloat-free legumes was introduced into alfalfa and grass pastures in western Canada. This 2-year and multilocation study showed an increased fraction of stable aggregates in pasture having more than >10% legume stands. Carbon was not sequestered over the two years at all three locations. However, a significant increase in microbial biomass carbon at Red Deer and C-cycling enzyme activity at both Red Deer and Lethbridge indicates a positive effect on the C substrate accumulation. Therefore, it might be considered that the immediate change identified in our study may have resulted from the addition of bloat-free legumes which in turn may result in sequestering carbon in the future. Two years of production likely were not enough to expect changes in the soil C and N pool. On the other hand, soil available N which can be readily available to neighboring plants increased over years in grass pasture at Red Deer and showed positive linkage with legume stands in pasture but not in Ponoka. This suggests overall pasture performance may be improved by introducing bloat-free legumes in some but not all grass pastures of western Canada. This study established a baseline microbial composition and diversity for all 3 test sites that can be useful in future research.

This study suggests that drilling legumes in grass pasture can improve some physical, chemical and biological properties of soil indicating that pasture rejuvenation approach is sustainable in some but not all parts of western Canada.

Chapter 5 : General Discussion

Three hypotheses tested in this study were that:

1. Sainfoin and CMV populations differ in their ability to establish in existing alfalfa and grass pastures.
2. Direct seeding for rejuvenation differs from the traditional method of plow down and establishment.
3. Soil parameters vary before and after rejuvenation.

The first and second hypotheses related to above ground changes were accepted. In the test conducted at Lethbridge, test populations in cultivated plots contributed almost one-third of the biomass found in the mixed stand. At this location, all sainfoin populations tested contributed more than 18% of the mixed biomass while CMV populations' contributions were less than 10%. At Ponoka and Red Deer maximum biomass contribution from test populations when drilled were 4.5% and 14.5% respectively. Low proportion of legumes in grass pasture of Ponoka and Red Deer may not be sufficient from the rejuvenation success point of view. It is important to note that success of pasture rejuvenation cannot be judged in two years as the observations may be very different in later years. The contribution of new legume populations in the cultivated plots at Ponoka and Red Deer was similar to the Lethbridge cultivated plots. Mountainview and LRC3432 consistently produced and contributed a higher proportion (>20%) of biomass in both years at Lethbridge while only Mountainview produced >20% DM in cultivated plots at Ponoka. Both legumes were similar in pasture quality parameters studied when mixed with alfalfa while they increased the amount of crude protein value and relative feed value of the grass.

The third hypothesis related to below-ground changes was accepted for some soil parameters. Pasture mixture where more than 10% of the stand consisted of introduced plants showed an increased fraction of stable aggregates in the soil. Introduction of bloat-free legumes in the pasture did not sequester carbon within the 2 years observed in this study. However, microbial biomass carbon at Red Deer and C-cycling enzyme activity at Lethbridge and Red Deer increased indicating a positive effect of introduced legumes in mixed stand. Presence of >10% legumes in grass increased ($p<0.05$) soil available N, P and S of pastureland. Proteobacteria dominated bacterial phyla constituting 29.3%, 29.7% and 26.5% at Lethbridge, Ponoka and Red Deer, respectively. At class level of Proteobacteria, gamma and delta Proteobacteria were more abundant ($p<0.05$) in alfalfa pasture at Lethbridge than grass pastures at other two locations.

The new sainfoin population Mountainview and LRC3432 can be drilled into alfalfa pasture in order to improve pasture and prevent bloat as they consistently contributed higher proportion of bloat decreasing legumes than other cultivars. Similarly, these sainfon populations can be drilled in some grass pastures of western Canada to improve above ground and below ground performance of pastureland.

5.1. Future studies could be done in order to:

1. Determine the ability of CMV to establish and persist in existing pasture followed by grazing trial.
2. Assess the effect of seeding alfalfa populations into established alfalfa pasture to determine their ability to survive in alfalfa pasture.
3. Determine carbon sequestration and other changes in soil quality parameters due to rejuvenation effect on pasture.
4. Study soil microbial composition and diversity of pastureland following use of different rejuvenation methods and management practices.

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Appendices

Appendix 1.0: Total N (TN), total C (TC), soil organic C (SOC) and available N (AN) of Lethbridge soil collected in 2015 and 2016.

				2015				***2016***			
Block	Type	Rep	Depth	TN (t/ha)	TC (t/ha)	SOC (t/ha)	AN (kg/ha)	TN (t/ha)	TC (t/ha)	SOC (t/ha)	AN (kg/ha)
1	Great Plains	2	0-30	12.1	164.2	132.4	100.0	7.8	108.3	79.7	37.8
1	Great Plains	2	30-60	6.5	80.9	68.4	45.5	6.1	79.4	55.9	30.6
1	Great Plains	2	60-90				34.9				19.7
2	Cultivate	2	0-30	12.0	135.0	121.6	133.3	7.9	109.6	80.8	35.7
2	Cultivate	2	30-60	3.9	94.1	35.4	61.6	4.1	58.3	36.0	19.1
2	Cultivate	2	60-90				64.9				27.0
3	Pan	2	0-30	11.2	127.3	123.1	98.3	8.8	109.5	85.5	25.6
3	Pan	2	30-60	4.7	65.0	45.3	27.4	4.3	58.9	36.7	17.0
3	Pan	2	60-90				25.8				19.6
4	Cultivate	1	0-30	6.9	82.9	64.5	46.5	8.6	91.8	82.9	44.0
4	Cultivate	1	30-60	4.1	47.1	38.8	49.1	3.7	39.7	30.1	30.3
4	Cultivate	1	60-90				36.2				26.4
5	Pan	1	0-30	9.4	103.4	98.4	63.3	9.3	102.2	90.6	36.1
5	Pan	1	30-60	5.1	58.0	50.2	39.7	3.9	32.9	32.6	20.7
5	Pan	1	60-90				71.4				23.7
6	Great Plains	1	0-30	10.6	115.4	112.1	61.8	9.2	98.3	85.9	45.4
6	Great Plains	1	30-60	5.1	65.9	48.8	35.0	4.2	39.3	35.7	16.0
6	Great Plains	1	60-90				68.0				19.4
7	Pan	4	0-30	10.0	110.9	99.6	71.1	9.5	99.1	88.0	49.4
7	Pan	4	30-60	5.0	87.2	44.4	27.4	4.9	69.3	40.0	17.0
7	Pan	4	60-90				45.2				32.0
8	Cultivate	4	0-30	10.9	134.4	103.7	74.4	10.5	118.7	98.6	52.5
8	Cultivate	4	30-60	4.2	56.4	35.9	82.4	4.5	47.5	38.6	43.7
8	Cultivate	4	60-90				35.2				26.2
9	Great Plains	4	0-30	11.0	125.5	100.9	55.3	10.4	111.8	96.2	34.4
9	Great Plains	4	30-60	4.6	62.2	36.2	25.7	4.6	102.0	40.7	18.7
9	Great Plains	4	60-90				28.1				20.9
10	Pan	3	0-30	12.2	136.4	113.1	86.9	8.7	111.2	81.8	27.9
10	Pan	3	30-60	5.0	64.3	44.6	45.6	4.9	96.3	43.8	22.6
10	Pan	3	60-90				28.1				37.8
11	Cultivate	3	0-30	9.4	115.1	88.6	67.0	9.4	105.3	88.6	43.0
11	Cultivate	3	30-60	5.1	85.7	47.0	68.5	4.2	56.4	35.7	35.6
11	Cultivate	3	60-90				60.8				34.6
12	Great Plains	3	0-30	9.9	113.5	102.1	68.6	11.1	130.4	107.2	52.5
12	Great Plains	3	30-60	5.5	63.3	50.0	36.0	5.2	71.6	46.1	26.2
12	Great Plains	3	60-90				48.0				33.1

Appendix 2.0: Total N (TN), total C (TC), soil organic C (SOC) and available N (AN) of Ponoka and Red Deer soil collected in 2016.

				Ponoka				***Red Deer***			
Block	Type	Rep	Depth	TN (t/ha)	TC (t/ha)	SOC (t/ha)	AN (kg/ha)	TN (t/ha)	TC (t/ha)	SOC (t/ha)	AN (kg/ha)
1	Great Plains	2	0-30	9.5	114.7	109.1	24.9	9.9	112.9	108.5	47.0
1	Great Plains	2	30-60	2.0	33.2	18.5	13.5	3.0	78.6	28.3	24.6
1	Great Plains	2	60-90				22.2				36.4
2	Cultivate	2	0-30	12.1	145.9	86.4	73.6	7.2	75.6	74.1	25.2
2	Cultivate	2	30-60	2.1	27.6	20.3	36.5	3.1	29.0	27.6	17.8
2	Cultivate	2	60-90				32.3				25.3
3	Pan	2	0-30	9.7	113.8	104.1	30.2	8.1	84.3	78.8	104.6
3	Pan	2	30-60	2.2	23.5	18.8	16.2	2.0	58.8	19.7	36.3
3	Pan	2	60-90				19.7				37.3
4	Cultivate	1	0-30	6.6	85.4	70.4	24.7	7.6	78.2	75.8	28.3
4	Cultivate	1	30-60	2.1	27.0	20.5	18.8	3.1	67.6	26.7	30.9
4	Cultivate	1	60-90				17.6				34.5
5	Pan	1	0-30	5.9	135.5	67.2	21.2	9.3	108.8	107.0	25.4
5	Pan	1	30-60	2.5	34.3	31.7	17.0	4.3	63.3	44.5	23.7
5	Pan	1	60-90				18.8				27.7
6	Great Plains	1	0-30	8.1	139.8	87.0	24.2	9.9	118.6	113.8	30.1
6	Great Plains	1	30-60	2.6	47.1	45.5	13.9	6.4	77.2	68.5	26.9
6	Great Plains	1	60-90				19.8				36.2
7	Pan	4	0-30	7.5	90.2	86.0	16.2	18.4	221.1	203.8	32.0
7	Pan	4	30-60	3.0	26.8	26.5	15.9	12.5	148.4	140.4	13.8
7	Pan	4	60-90				18.4				24.0
8	Cultivate	4	0-30	14.3	171.3	64.9	17.0	14.1	163.9	147.4	50.6
8	Cultivate	4	30-60	2.3	24.4	19.6	12.7	3.8	35.2	34.0	32.4
8	Cultivate	4	60-90				16.3				24.5
9	Great Plains	4	0-30	7.2	148.2	72.3	42.8	14.5	166.1	152.7	137.4
9	Great Plains	4	30-60	2.4	31.0	18.3	13.0	6.4	68.4	65.5	25.8
9	Great Plains	4	60-90				20.1				24.0
10	Pan	3	0-30	9.2	113.1	107.1	40.4	8.3	93.8	88.0	32.0
10	Pan	3	30-60	3.0	31.0	30.8	7.2	4.5	38.9	36.5	25.1
10	Pan	3	60-90								36.3
11	Cultivate	3	0-30	11.6	142.2	75.2	104.7	6.5	70.2	63.8	179.4
11	Cultivate	3	30-60	3.6	41.2	40.4	16.7	3.7	93.5	32.3	119.1
11	Cultivate	3	60-90				22.2				70.5
12	Great Plains	3	0-30	15.5	194.4	61.8	43.5	8.5	94.3	89.2	27.1
12	Great Plains	3	30-60	5.2	57.8	55.0	13.8	3.7	37.7	35.8	30.2
12	Great Plains	3	60-90				20.8				36.8