FORAGE PRODUCTION, WEED SEEDBANK AND ALLELOPATHIC POTENTIAL OF SELECTED GRASS AND LEGUME SPECIES NATIVE TO THE GREAT PLAINS REGION OF CANADA

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Abstract

The forage yield and quality, weed seedbank abundance and allelopathic potential of seven native grass and legume species were evaluated in field and greenhouse experiments conducted at the Agriculture and Agri-Food Canada (AAFC), Swift Current Research and Development Centre (SCRDC), Saskatchewan, Canada. Native perennial forage species were selected from three functional groups (C_3, C_4 grasses and legumes) and seeded in 2010 and 2014 in monocultures and mixtures, including: western wheatgrass (WWG) (*Pascopyrum smithii* (Rydb.) Barkworth & D.R. Dewey), bluebunch wheatgrass (BBW) (*Pseudoroegneria spicata* (Pursh) Á. Löve), nodding brome (NOB) (*Bromus porteri* (J.M. Coult.) Nash), little blue stem (LBS) (*Schizachyrium scoparium* (Michx.) Nash), side-oats grama (SOG) (*Bouteloua curtipendula* (Michx.) Torr.), purple prairie clover (PPC) (*Dalea purpurea* Vent.) and white prairie clover (WPC) (*Dalea candida* Willd.). Objectives of this thesis were to: 1) evaluate the long-term forage yield and quality of these forage species in monocultures and mixtures; 2) determine the weed seedbank density and aboveground weed populations in stands of these species; and 3) evaluate the allelopathic effect of these species on three problematic weeds: dandelion (*Taraxacum officinale* F.H. Wigg.), scentless chamomile (*Matricaria perforata* Mérat) and foxtail barley (*Hordeum jubatum* L.). Forage mixtures produced greater dry matter than monocultures at all harvesting times. Mixtures of which WWG was a component produced higher forage yield, and a mixture of WWG, BBW, LBS and legumes can provide sustainable forage yield and quality and can be suitable options for seeded pastures. In this study, the forage stands experienced one of the driest and wettest years in the history of the region. We observed no significant differences in forage production of each species from dry to wet year supporting the idea of high stability and productivity of native species during varying climate conditions. Mixtures of forage species also promoted lower weed densities in the seedbank and in the swards aboveground compared to monocultures. Among mixtures, those containing WWG had a significant lower abundance of weeds in the seedbank and aboveground weed populations compared to other forage species. The weed seedbank varied seasonally with the minimum number of weed seeds in early spring and maximum in late summer. The most abundant weeds in the seedbank were the least abundant weeds in aboveground population and vice versa. WWG showed promising results as a native forage species by demonstrating the potential to suppress weeds and reduce weed seed size when seeded in monocultures and mixtures. In the greenhouse, root leachate from WWG, LBS and SOG reduced the aboveground and belowground growth of weeds up to 90%. These findings suggest that the use of allelopathic species may provide weed control and management benefits in seeded pastures and native prairie restorations. In conclusion, forage mixtures produced greater dry matter and promoted lower weed densities in the seedbank and aboveground populations compared to monocultures. This demonstrates that increasing forage mixture diversity can increase forage yield and be an effective ecological and non-chemical weed control tactic in seeded pastures.

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CHAPTER 1

INTRODUCTION

1.1 Native species in Canadian Prairie

About 5.7 million hectares of the Canadian Prairie is covered by seeded pastures (Statistics Canada, 2010), primarily with introduced species like crested wheatgrass (*Agropyron cristatum* L. Gaertn.), smooth brome (*Bromus inermis* Leyss) and Russian wildrye (*Elymus junceus* Fisch.) (Otfinowski et al. 2007; Smoliak and Dormaar 1985). However, the use of native perennial forage species is increasing for seeded pastures and land reclamation projects in the Northern Great Plains. In this region, forage grasses are commonly seeded in monoculture or a binary mixture with legumes. The majority of seeded forage species are cool-season grasses of Eurasian origin and, while highly productive, the invasive behavior of some of the introduced species is a serious threat to native grasslands (Biligetu et al. 2014; DeKeyser et al. 2015; Otfinowski et al. 2007). Many native prairie grasses are adapted to a broad range of soil and climatic conditions and have great commercial potential for forage production, soil reclamation, and long-term sustainability under grazing (Schellenberg et al. 2012; Tilman et al. 2001; Willms et al. 2005). Locally adapted species or ecotypes can better cope with the changes in the local climates, have better tolerance to diseases, and have a long-term association with soil microorganisms and other species in the community (Dorner 2002).

1.2 Thesis objectives

This thesis is focused on the agronomy, quality, allelopathic potential and seedbank composition of Western wheatgrass, Bluebunch wheatgrass, Nodding brome, Little blue stem, Side-oats grama, Purple prairie clover and White prairie clover in mixtures. The intent of this thesis is to test the following general hypotheses: 1) That mixtures of forage species are more productive than monocultures; 2) Certain native forage species can better suppress weeds; and also mixtures of forage species may be more effective at decreasing weed population and seedbank abundance compared to monocultures; and 3) Lower weed density in some forage species especially western wheatgrass can be linked to allelopathic potential.

I have three major objectives in my thesis: 1) To evaluate the long-term forage yield and quality of these seven forage species in monoculture, binary mixtures, and a series of complex multispecies mixtures predicted to be high-yielding (Chapters 3 and 4); 2) To determine the weed seedbank density and aboveground weed populations of forage species in monocultures and mixtures (Chapter 5); and 3) To evaluate the allelopathic potential of selected forage species (Chapter 6).

The first major objective (forage production and quality) includes two separate sub-studies. In the first sub-study, I evaluate the long-term forage yield and quality of the aforementioned species in monoculture and mixture over a 6-year period in the semi-arid ecoregion of Saskatchewan, Canada (Chapter 3). This experiment was started in 2010 with 2011 data collected by Jenalee Mischkolz for her MSc thesis (Mischkolz 2013), 2012 and 2013 data by technicians under the supervision of Dr. Michael P. Schellenberg, and 2014-2016 data collected by me. The specific objectives of this sub-study were to: 1) Evaluate forage yield and quality of each species under a range of climate conditions; 2) Assess persistence of less competitive species in the mixtures; 3) Study the effects of functional group diversity on forage productivity and quality; and 4) Determine the effect of binary mixtures of forage species on productivity. In the second sub-study, I evaluated forage yield and quality of these seven species, in monoculture, binary mixtures and complex mixtures in two different ecoregions of Canadian Prairie, Mixed Grass Prairie Ecoregion (Swift Current, SK) and Tall Grass Prairie Ecoregion (Brandon, MB) (Chapter 4). The specific objectives of this sub-study were to: 1) Evaluate forage yield and quality of monoculture and simple mixtures vs. complex mixtures; 2) Evaluate whether the complex mixtures predicted to be more productive are actually more productive; and 3) Assess the changes in mixture composition with time.

The second major objective was to evaluate the weed seedbank density and aboveground weed populations of forage species (Chapter 5). There is a growing interest in the use of native perennial forage species for sustainable beef production systems. However, little research has been conducted on weed seedbank composition and aboveground populations in seeded pastures. The specific objectives of weed seedbank study were to evaluate: 1) The effects of different native forage species in monocultures on weed seedbank composition and aboveground weed populations; 2) The effect of forage mixtures on the weed seedbank and aboveground weed populations; and 3) The similarity between the weed seedbank and emerged weed populations in mixtures of native perennial forage species.

The third major objective (allelopathy) was to evaluate the allelopathic potential of these forage species (Chapter 6). There are many studies of the potential of allelopathic crops for weed control, but much less is known about the allelopathic potential of forage species in pastures. There are many benefits to using diverse mixtures of forage species in seeded pastures and moreover, identifying those species with high allelopathic properties in the mixtures could reduce plant-weed competition, increase forage productivity and decrease the cost of weed control in pastures. The specific objectives of allelopathy study were to evaluate: 1) The allelopathic potential of seven native forage species early and later in the first season of growth; and 2) The effect of multispecies leachate mixtures on weeds.

CHAPTER 2

LITERATURE REVIEW

In this section, the relationships between forage species in mixture will first be reviewed including complementarity, competition, and the effects of species mixtures on productivity. Secondly, the use of native forage species in Canadian Prairies and their role in forage productivity will be examined. In the next section, the literature on soil seedbanks, seed dormancy and the relationships between soil seedbank and aboveground population will be reviewd. Finally, allelopathy and the implications of allelopathy for weed control in perennial forages will be discussed.

2.1 Native species and their role in forage productivity

2.1.1 Complementarity and competition in the mixtures

In a plant community, each species may either compete with others or complement each other to capture resources. The type and intensity of these interactions determine the community productivity (Hooper et al. 2005a; Lamb et al. 2011; Miller 1997; Mischkolz et al. 2016; Sheehan et al. 2006; Trenbath 1974a). When mixtures produce greater yield than monocultures, the mixture is over-yielding (Trenbath 1974a) which is a sign of positive interactions between species. Positive or complementary interactions between species are because of combinations of characteristics or functional roles that are beneficial for mixtures to increase productivity (Brooker et al. 2008; Hooper et al. 2005a). Over-yielding is achieved when species in the community complement each other in their use of resources by occupying different niches or through facilitation (Brooker et al. 2008; Callaway 1995; Tracy and Sanderson 2004). Facilitation is another example of complementarity where at least one species is benefited in the interaction and harm is caused to neither. Facilitation is one of the most important plant-plant interactions with strong impacts on population and community ecology (Lortie 2007). Facilitation can influence communities through strong effects on plant growth rates, population distribution, species diversity and composition, and even landscape community dynamics (Bruno et al. 2003). Facilitation enables plants to exploit a greater portion of available resources like nutrients and light, and therefore can increase the utilization of the fundamental niche space (Bruno et al. 2003). The relationship between grasses and legumes can be an example of facilitation (Bertness and Callaway 1994; Bruno et al. 2003; Muir et al. 2011; Tilman et al. 1997). Legumes by fixing atmospheric nitrogen can provide sustainable source of nitrogen for grasses and other species in the mixture (Duchene et al. 2017). The ability of legumes to fix atmospheric nitrogen is linked to their symbiosis relationship with a *Rhizobium* bacteria, located in the root structures called nodules (Duchene et al. 2017). Facilitation can also play an important role in determining community structure, and maintaining the productivity in harsh conditions (Callaway and Howard 2007; Lortie et al. 2016).

Under-yielding, on the other hand, happens when negative interactions such as competition between species in the mixture occur (Trenbath 1974b). The competitive ability of a plant has two components, the "competitive effect" which is the ability of one species to suppress neighbours, and "competitive response" which is the ability of one species to tolerate suppression by neighbours (Goldberg 1996). The competitive ability of a species depends on its size and growth rate, whereas competitive response depends on persistence and avoidance of the species from the neighbours' damage (Keddy et al. 1998; Keddy et al. 1994; Wang et al. 2010). In plant communities when complementarity is maximum and competition is minimum, optimum forage yield is obtained (Brooker et al. 2008).

2.1.2 Species diversity and productivity

Increasing community diversity results in increased resource capturing, nutrient cycling, stability, and decreased community susceptibility to weed invasion or other pests (Knops et al. 1999; Loreau and de Mazancourt 2013; Sanderson et al. 2005). Many studies have shown a positive association between diversity and productivity, particularly in planted or artificial communities (Balvanera et al. 2006; Díaz and Cabido 2001; Hooper et al. 2005a; Kirwan et al. 2007; Lehman and Tilman 2000; Reich et al. 2004; Sheehan et al. 2006; Tilman 1996; Tilman et al. 2001; Walker 1995; Walker et al. 1999; Weigelt et al. 2009; Wight and White 1974). Communities that have higher species richness can be beneficial as more species traits join the community (e.g. different rooting depths), resources can be used more effectively as compared to monocultures, thus ecosystem productivity and stability can be improved (Picasso et al. 2008; Weigelt et al. 2009). Moreover, with increasing species richness there is a higher chance of including a highly productive species in the mixture. Combinations of different species or different functional groups may also show additivity or complementarity in resource use which can increase productivity and plant community stability (Brooker et al. 2008; Hooper et al.

2005a; Mischkolz et al. 2016; Picasso et al. 2008; Spehn et al. 2005; Tilman et al. 2001; Weigelt et al. 2009).

This is a controversial topic however, as the productivity-diversity relationship in natural communities can be affected by various factors including plant litter accumulation, plant morphology, disturbance, species composition and soil microbial community (Grace 1999). Similarly, Huston et al. (2000) emphasized that in ecological experiments there are many interactions between biotic and abiotic factors that complicate the design and interpretation of the results. He emphasized that ,in many cases, species diversity has no statistically or biologically significant effect on produvtivity. In his perspective, there are three type of “hidden treatments” that potentially affect biodiversity experiments: 1) biotic and abiotic fators like resource levels and predators, 2) non-random selection of species, and 3) the increased statistical probability of including a species with a dominant negative or positive effect on biomass and productivity. In these cases the results may be wrongly attributed to variation in plant diversity (Huston et al. 2000).

2.1.3 Native species' mixtures

This thesis deals with native species in mixtures. Native forage species have developed and existed naturally for many years within a given region or ecosystem with no human intervention, as opposed to tame forages which are introduced or non-native grass and legume species cultivated for feeding livestock (Barnes et al. 1995). Forage indicates plant material, often herbaceous in nature, utilized by grazing livestock (Fageria 1997). Forage mixtures composed of native species have the potential to be as productive as tame monocultures in a greater range of environmental conditions and may provide a more reliable source of forage yield even in years with different environmental conditions (Lehman and Tilman 2000; Schellenberg et al. 2012). Forage species diversity can provide stable yield and improve the nutritional quality and palatability of forages by providing a mixed diet throughout the growing season (Holechek et al. 2004; Wang and Schellenberg 2012). Diversity in native forage mixtures can also enhance ecosystem services like carbon sequestration and wildlife habitat quality, decrease in pathogen infection and reduce nutrient loss from soil (Hector et al. 1999; Hooper and Vitousek 1998; Knops et al. 1999; McNaughton 1977; Mischkolz et al. 2016; Symstad et al. 2003; Tilman and Downing 1994; Tilman et al. 1996; Vibart et al. 2016; Weigelt et al. 2009). In a diverse forage mixture, warm-season and cool-season species with different maturation times have the potential to provide high forage quality for livestock over a longer period of the growing season than a monoculture or simple cool-season mixtures (Jones and Wilson 1987; Tilman et al. 2001). In a seven-year study on 16 grassland species, Tilman et al. (2001) concluded that mixtures produced 2.7 times the biomass than monocultures. Positive effects of species diversity on productivity can be explained by different factors including: interspecific complementarity, increase in the use of available resources, nutrients cycling and potential reduction of herbivory and disease outbreaks (Tilman et al. 2014). Not all native species produce high forage yield, but forage mixtures including less productive species may bring beneficial characteristics like drought or grazing tolerance to the plant community. The diverse species mixtures guarantee the forage yield under good climate condition and more importantly, ensure the acceptable forage productivity under unpredictable harsh conditions. In the mixtures, low productivity of one species can be compensated by other species (Doak et al. 1998; Lehman and Tilman 2000; Lhomme and Winkel 2002; Mischkolz et al. 2013; Tilman 1999).

2.1.4 Selected native species

In native dry-mixed grasslands of the Canadian prairies, C_3 grasses are the dominant species and produce the bulk of the forage yield and provide most of the digestible energy (Muir et al. 2011; Schellenberg and Banerjee 2002). C_3 grasses start growing early in the season, whereas C_4 grasses initiate growth later in the season (Lehman and Tilman 2000; McGraw et al. 2004; Schellenberg et al. 2012; Tilman et al. 2001). Legumes are best known for their nitrogen fixation ability. Grasses can use legumes' nitrogen through the process of nitrogen fixation; thus, less fertilizer is needed in rangeland and pastures (Brooker et al. 2008; Callaway 1995; Muir et al. 2011; Oelmann et al. 2007; Temperton et al. 2007; Whitbread et al. 2009). Legumes also contain high protein concentrations which can increase the total crude protein concentration of the forage mixtures (McGraw et al. 2004; Muir et al. 2011).

In this study seven native perennial forage species from three functional groups (C_3, C_4 and legumes) were selected; Western wheatgrass (*Pascopyrum smithii* (Rydb.) Barkworth & D.R. Dewey), Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve), Nodding brome (*Bromus porteri* (J.M. Coult.) Nash), Little blue stem (*Schizachyrium scoparium* (Michx.) Nash), Side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.), Purple prairie clover (*Dalea purpurea* Vent.) and White prairie clover (*Dalea candida* Willd.). Since these species are native to the Canadian Prairie, they are expected more effectively to cope with environmental stresses in this region. These species have the potential to be agronomic crops in seeded pastures and native prairie restorations as they are available in the market, have sufficient nutritional quality, have the ability to work well with conventional machinery and are distributed broadly in the Prairie provinces.

These species were evaluated in the greenhouse studies (Mischkolz et al. 2016) and have continued to be evaluated in the field studies in Saskatoon and Swift Current, SK, (Biligetu et al. 2014; Mischkolz et al. 2013; Schellenberg et al. 2012), and ongoing field studies in Swift Current and Brandon, MB Canada. More details on the characteristics of these seven species are provided in Mischkolz (2013).

2.2 Soil seedbank

Seedbank can be defined as all dormant and non-dormant seeds in the soil and is a source of floristic diversity that contribute to plant population stability (Baskin and Baskin 1978; Harper 1977). The soil seedbank is also a legacy of past weed populations in a region and a source of plants that have the potential emerge following disturbance (Murphy et al. 2006; Sanderson et al. 2007; Sosnoskie et al. 2006). Although soil seedbank of pastures contains useful species, it is also a reservoir of undesirable weedy species (Rice 1989). There are many ways to define *weed*. Although each weed scientist has a clear understanding of the term weed, but there is no universal definition that is accepted by all scientists. In 1967 the Weed Science Society of America defined a weed as “a plant growing where it is not desired”. In 1989, it was changed to “any plant that is objectionable or interferes with the activities or welfare of man”. The European Weed Research Society defined a weed as “any plant or vegetation, excluding fungi, interfering with the objectives or requirements of people” (Zimdahl 2007). Since in this thesis the focus was on the quality and yield of seeded native species, we considered any non-seeded species as a weed. Knowledge of soil weed seedbank composition can be useful for pasture manager, as it may indicate which species most likely will emerge after a disturbance that opens a gap in the sward and successional processes in the pasture (Sanderson et al. 2014).

Soil microsites contain different humidity, temperature and oxygen that affect seed fate in the seedbank (Fenner 2000; Young et al. 2001). Other factors like solar radiation, CO_2, topography, longitude, latitude, slope, biotic factors like bacteria, predators and fungus can also have an impact on seeds in the seedbank (Dekker 2011). Different seeds have different longevity in the soil seedbank (Burnside et al. 1996; Telewski and Zeevaart 2002). Seeds at soil surface are more likely to deplete faster due to predation and other rapid changes in the environmental conditions (Roberts and Feast 1972). The composition of seedbank changes by seed rain and losses (Dekker 2011). The seed rain provides additions to the active and dormant seed bank (Harper 1977). Seed losses, on the other hand, occur due to mortality including predation, pathogenic decay, unsuccessful germination and physiological seed death (Cavers 1983; Zorner et al. 1984).

2.2.1 Seed dormancy

Seed dormancy is the phenomenon that seeds are unable to germinate in specific situations as compared with non-dormant seeds that are indeed able to germinate (Baskin and Baskin 2004). Dormancy is an important mechanism that prevents seed germination during unsuitable conditions when there is a low chance of seedling survival (Black et al. 2006); thus seed dormancy improves plants' ability to survive in natural situations (Grime 1981).

The soil seedbank can be classified as active and dormant. Active seeds are ready to germinate under favorable situations, whereas dormant seeds do not germinate even in favorable situations. The active seeds can be transformed to dormant seeds and vice versa (Dekker 2011).

Seed dormancy can be classified as primary and secondary. Primary dormancy is an innate dormancy present in the seeds at the first stages of seed formations, whereas secondary dormancy is a dormant state that is induced in non-dormant seeds when the conditions for germination are unfavorable (Benech-Arnold et al. 2000; Karssen 1982). Seed dormancy cycling from primary to secondary dormancy happens to many weed species (Baskin and Baskin 1998). Dormancy cycles in temperate environments, where there is an abundance of water, are most often influenced by soil temperature (Batlla and Benech-Arnold 2003). For example, in a summer annual species, dormancy is reduced by the low temperatures during winter, whereas high temperatures during summer increase the level of seed dormancy. In winter annual species, on the other hand, the high temperature of summer reduces the dormancy and low temperature of winter can induce secondary dormancy (Batlla and Benech-Arnold 2007). Many studies support the primary role of soil temperature on seed dormancy, but there are some studies that show seed dormancy might be regulated by soil moisture (Batlla and Benech-Arnold 2004; Benech-Arnold et al. 2000).

Seed dormancy is not an all-or-nothing trait (Batlla and Benech-Arnold 2007). Seed dormancy status can vary between some point of maximum and some point of minimum (Batlla and Benech-Arnold 2004). Seeds with a low level of dormancy can germinate in a wide range of environmental conditions until they reach a maximum level of germination. While seeds with a high level of dormancy show a narrow range of environmental conditions allow for germination, until germination is no longer possible at any temperature or water potential (Batlla and Benech-Arnold 2004; Benech-Arnold et al. 2000).

2.2.2 Seedbank dynamics

Weed seedbank composition changes over time (Warr et al. 1993). Many weed seeds are dormant in the seed rain, but over time some will lose their dormancy (Dekker 2011). Other seeds may remain non-dormant waiting for suitable conditions to germinate or enter secondary dormancy (Forcella et al. 1997; Karssen 1980; Taylorson 1982). Annual change in seed germinability in the soil seedbank has been reported for many weeds (Baskin and Baskin 1985). In dormant seeds, both endogenous and exogenous factors may affect cyclic physiological changes. This strategy is an important adaptation for weeds to survive in natural situations, and germinate in an appropriate time to avoid fatal germination (Dekker 2011).

2.2.3 Seedbank management strategies

Weeds can significantly reduce forage yield and quality, affecting livestock production qualities and increase rangeland management costs (DiTomaso 2000). Within the range of available weed control practices, mechanical and chemical are the most commonly used methods (Altieri and Liebman 1988). Understanding the processes affecting seedbank dynamics can help managers to select better weed control strategies (Buhler et al. 1997; Dekker 1997; Dekker 2011).

The weed seedbank can be easier to manage in soils containing more diverse communities of weed seeds compared to those that are dominated by a few problematic weeds (Dekker 2011). Occupying the soil seedbank by a few dominant weeds is an indication that the cropping systems are leaving some free niches to exploit (Dekker 2011). Diverse weed seedbank, on the other hand, is an indication that fewer opportunities are available for weeds, and resources are used by many species. Thus, communities that have few small unused resources are more likely to have the smaller, more diverse and more easily managed weed seed bank flora (Dekker 2011).

2.2.4 Relationships between the weed seedbank and aboveground communities

While some studies have found strong relationships between the weed seedbank and aboveground communities (Dessaint et al. 1997; Rahman et al. 2006; Rahman et al. 2001; Zhang et al. 1998), others have found low correlations (Cardina and Sparrow 1996; Tracy and Sanderson 2000; Webster et al. 2003). Generally, low similarity between aboveground plant community and seedbank has been reported for perennial species (Bakker et al. 1996; Milberg 1995; Rabinowitz 1981; Schenkeveld and Verkaar 1984; Thompson and Grime 1979), and greater similarity in annual communities (Chang et al. 2001; Moore 1980; Unger and Woodell 1993; Unger and Woodell 1996). Sanderson et al. (2014) also showed that permanent pasture can have a more stable soil seedbank than that of recently cultivated lands. They also found that annual weeds are more common in the seedbank of hayfields and recently seeded pastures, whereas the weed seedbank in older pastures tended to be dominated by perennial grasses.

2.3 Allelopathy

Allelopathy is the direct or indirect, negative or positive effect of species on other species by production and release of chemical materials (Inderjit and Callaway 2003; Rice 1984). The word ‘Allelopathy’ was first coined in 1937 by Hans Molisch, an Austrian scientist, and is derived from two Greek words: ‘*Allelo*’ (mutually) and ‘*Pathy*’ (suffering) (Fujii et al. 2004). Plants produce more than 100,000 primary and secondary chemical compounds, many of which can act as allelochemicals (Callaway and Howard 2007). Among them, phenolics and terpenoids have been studied more widely, but the role of many secondary compounds is still unclear (Reigosa et al. 1999). Phenolics are common in cool and humid climates, whereas terpenoids are frequent in dry climates (Reigosa et al. 1999).

Most allelochemicals are water soluble and can enter the environment and affect the adjacent plants in four ways: aboveground leaching, litter decomposition, shoot volatilization and root exudates (Bonanomi et al. 2006; Gawronska and Golisz 2006; Nishida et al. 2005; Reigosa et al. 1999). Litter decomposition has been recognized as the most important source of allelochemical materials in many ecosystems (González et al. 1997; Souto et al. 1995). Decomposition conditions of litter affect allelochemical production, where waterlogging and anoxia can increase the production of allelochemicals (Patrick 1971). There are few studies on root exudation, but it might be a very important process since it can directly affect soil microorganisms and other plant roots (Robinson 1972). Allelochemical production in the roots of plants can be affected by many factors like plant habitat, the age of root, temperature, water stress, etc. (Inderjit and Callaway 2003; Reigosa et al. 1999).

2.3.1 Effects of allelochemicals on ecosystem

Allelopathy plays an important role in natural ecosystems (Rice 1984). Allelopathy can affect plant species' diversity, distribution, abundance, dominance, succession, climax, community and agroecosystem productivity, weed invasion and the ecosystem structure and function dramatically (Bias et al. 2003; Callaway and Aschehoug 2000; Chou 1999; Grant et al. 2003; Hierro and Callaway 2003; Inderjit et al. 2008; Rice 1972; Rice 1979).

Allelochemicals can only be effective if they are released into the environment with adequate concentrations (Reigosa et al. 1999). Plants tend to produce more allelochemicals under stresses (Einhellig 1996; Tang et al. 1995). The production of allelochemicals can be influenced by many biotic and abiotic factors like light quality and quantity, nutritional deficits, water stress, extreme temperatures, use of herbicides and pesticides, plant diseases, plant age and genotype (Ahmed and Wardle 1994; Chung and Miller 1995; Einhellig 1996; Einhellig et al. 1970; Gerson and Kelsey 1998; Koeppe et al. 1976; Miller 1996; Mwaja et al. 1995).

2.3.2 Mode of action

Allelochemicals have different mode of actions (Seigler 1996). The most important modes of actions include: effect on cell division, cell elongation, cell structure, cell wall, ultrastructure of the cell, growth regulators (mostly inhibitors), membrane permeability, nutrient uptake, stomatal aperture, photosynthesis and respiration (Reigosa et al. 1999). The effect of allelochemicals on target plants is different from pesticides. Chemical pesticides are very target specific and have stronger effects than that of allelochemicals (Reigosa et al. 1999). However, Macías (1995) suggested that some allelochemicals can be as effective as commercial pesticides.

In natural conditions, allelochemicals are not produced and released in high enough concentrations to suppress other species, and they are also not very stable in the environment and are biodegraded easily like phenolic compounds (Blum 1998; Turner and Rice 1975). Juglone as an exception can keep its allelopathic potential in humid soils for more than 90 days (Fisher

1978). Unlike herbicides that have one or few mode of actions (like ALS inhibitors), allelochemicals can have different mode of actions simultaneously (Reigosa et al. 1999).

2.3.3 Allelopathic weed control

Allelopathy has long been recognized to influence plant–plant interactions and is a well-known mechanism of weed suppression in some crops (Kumar et al. 2009; Milchunas et al. 2011). Allelopathic compounds released by donor crop plants can reduce both emergence and growth of weeds (Zeng et al. 2008). A number of methods are available for weed control in pastures including grazing, mechanical, cultural, chemical, biological and allelopathic weed control (Bailey et al. 2010; Jabran et al. 2015). In some agricultural systems, especially organic systems, allelopathic weed control can be one of the most important tactics available for suppressing weeds (Jabran et al. 2015). There are many studies of the potential of allelopathic crops for weed control (Milchunas et al. 2011; Singh et al. 2003), but much less is known about the allelopathic potential of forage species in pastures. Allelopathic weed control through the selection of forage species with high allelopathic properties for seeded pastures can be a practical and sustainable way to suppress weeds. There are many benefits to using diverse mixtures of forage species in seeded pastures (Mischkolz et al. 2013; Mischkolz et al. 2016) and moreover, identifying those species with high allelopathic properties in the mixtures could reduce plant-weed competition, increase forage productivity and decrease the cost of weed control in pastures.

CHAPTER 3

MIXTURES OF NATIVE PERENNIAL FORAGE SPECIES PRODUCE HIGHER YIELD THAN PURE STANDS IN A LONG-TERM STUDY IN SEMI-ARID ECOREGION OF SASKATCHEWAN, CANADA

Abstract

To evaluate the forage yield and quality of seven perennial native species in monoculture and mixtures under a range of climate conditions, a 6-year field experiment was conducted at the Agriculture and Agri-Food Canada (AAFC), Swift Current Research and Development Centre (SCRDC), Saskatchewan, Canada. Seven native perennial forage species from three functional groups (C_3, C_4 grasses and legumes) were seeded in 2010, in monocultures and mixtures. Forage yield and quality (crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), phosphorus (P), calcium (Ca) and copper (Cu)) were measured during the first week of July and last week of August in 2011-2016. Mixtures that included western wheatgrass (*Pascopyrum smithii*) (WWG) produced greater yield, where 90% of the composition within these mixtures was WWG. Adding bluebunch wheatgrass (*Pseudoroegneria spicata*) (BBW), little blue stem (*Schizachyrium scoparium*) (LBS) and prairie clovers (*Dalea* spp.) to the mixtures can increase the positive aspects of species diversity on stability and productivity in seeded pastures. Among the grasses, WWG contained higher crude protein and lower ADF and NDF concentration. Mixtures of forage species produced higher forage yield compared to monocultures. Native forage species can produce stable forage yield across very different climate situations. In mixtures, WWG showed promising results in forage productivity and quality and can be a suitable option for seeded pastures.

3.1 Introduction

There is growing interest in the use of native perennial forage species for seeded pastures and land reclamation projects in the Northern Great Plains. In this region, forage grasses are commonly seeded in monoculture or in a mixture with legumes like alfalfa. The majority of seeded forage species are cool-season grasses of Eurasian origin and, while highly productive, the invasive characteristics of some of these introduced species is a serious threat to native grasslands (Biligetu et al. 2014; DeKeyser et al. 2015; Otfinowski et al. 2007). Many native prairie grasses are adapted to a broad range of soil and climatic conditions and have commercial potential for forage production, soil reclamation, and long-term sustainability under grazing (Schellenberg et al. 2012; Tilman et al. 2001; Willms et al. 2005).

Forage mixtures composed of native species have the potential to be as productive as tame monocultures in a greater range of environmental conditions and may provide a more reliable source of forage yield even in years with very different environmental conditions (Lehman and Tilman 2000; Schellenberg et al. 2012). An ideal mixture would provide nutritious and adequate forage throughout the growing season. Combinations of different species or different functional groups may show additivity or complementarity in resource use which can increase productivity (Brooker et al. 2008; Hooper et al. 2005a; Mischkolz et al. 2016; Picasso et al. 2008; Weigelt et al. 2009) and plant community stability (Spehn et al. 2005; Tilman et al. 2001). Moreover, species diversity provides a mixed diet which can improve the nutritional quality and palatability of forages (Holechek et al. 2004; Wang and Schellenberg 2012). In a diverse forage mixture, warm-season and cool-season species with differing maturities have the potential to provide higher forage quality for livestock over a longer period of the growing season than a monoculture or simple cool-season mixtures (Jones and Wilson 1987; Tilman et al. 2001).

A number of recent studies have documented the advantage of forage mixtures for western Canada. Schellenberg et al. (2012), for example, studied the forage production of 7-species and 14-species mixtures of native cool and warm-season grasses in a semi-arid ecoregion of Canada. Forage mixtures of cool-season grasses were more productive than a combination of warm and cool-season grasses, however, mixtures that included warm-season grasses had increased protein content in the late growing season which improve nutritive value of those mixtures. Similarly, Biligetu et al. (2014) evaluated mixtures of grass-legume or monocultures of grasses over a 7-year period where mixtures of alfalfa with cool-season grasses produced more forage yield compared to warm-season grasses. The mixture of alfalfa and WWG ranked the highest among other mixtures for forage quality and yield. Finally, Mischkolz et al. (2013) showed that, though western wheatgrass dominated productivity in two-species native mixtures, there were no negative effects of including other native species in the mixtures. Inclusion of less productive species with traits such as drought tolerance may provide insurance against productivity declines under sub-optimal conditions.

In this study, I evaluated the forage yield and quality of seven native perennial forage species, including C_3 and C_4 grasses and legumes, in monoculture and mixtures over a 6-year period in a semi-arid ecoregion of Saskatchewan, Canada. The objectives of this study were to: (1) evaluate forage yield and quality of species in monocultures over time; (2) assess persistence of less competitive species in the mixtures; (3) study the effects of functional group diversity on forage productivity and quality; and (4) determine the long-term relationship between forage mixtures and productivity.

3.2 Materials and Methods

This experiment was conducted at the Agriculture and Agri-Food Canada (AAFC), Swift Current Research and Development Centre (SCRDC) near Swift Current (latitude 50°25′N, longitude 107°44′W, 824 m elevation), Saskatchewan, Canada. This area is located in the Dry Mixed Grass Prairie ecoregion, which is the driest part of the province. This ecoregion has an Orthic Brown Chernozemic soil (Swinton loam) with a pH of 7.4 (Ayers et al. 1985; Bailey et al. 2010). The average annual temperature, annual precipitation and May-July precipitation is 4.1 °C, 327 mm and 153 mm, respectively (Bailey et al. 2010). Weather data were collected for 2011-2016 and compared to the 120-year average from the AAFC, SCRDC (Figure 3.1). In general, 2016 was the 4th wettest year on record (May-July precipitation was 347 mm), whereas 2015 was one of the driest years in the last 120 years in Swift Current (May-July precipitation was 125 mm).

3.2.1 Forage Species

Seven North American native perennial forage species from three functional groups were selected, including three C_3 grasses, two C_4 grasses and two legumes (Table 3.1). These species were evaluated in greenhouse studies (Mischkolz et al. 2016) and field studies in Saskatoon and Swift Current, SK, (Biligetu et al. 2014; Mischkolz et al. 2013; Schellenberg et al. 2012) and also they are under evaluation in ongoing field studies in Swift Current and Brandon, MB.

3.2.2 Experimental Design

Forage species were seeded in a split-plot block design with four replicates of 30 treatments in June 2010. Treatments in each block included seven 'monoculture' plots, 21 'two-species mixture' plots, one 'seven-species mixture' plot and one 'blank' or non-seeded plot.

Plots were summer fallowed for one year before starting the experiment. Weed control practices began in the spring of 2010 before planting the forage species with Roundup WeatherMAX® (Monsanto Canada Inc., Winnipeg, Canada) at a rate of 0.82 L ha^{-1}) and eleven days later with 2-4DB Cobutox® 625 (Interprovincial Cooperative Limited, Winnipeg, Canada) in the rate of 2.47 L ha^{-1}. Neither herbicide nor fertilizer was applied after seeding the forage species. Forage species were seeded with a press drill at the depth of 1.3 cm, in 4×8 m plots with 12 rows spaced 22.5 cm apart. Grass and legume species were planted at a rate of 100 and 200 pure live seeds m^{-1}, respectively. In two-species mixtures and seven-species mixtures, the seeding rate was reduced to half and one seventh of the monoculture plots, respectively. In the mixture plots, all species were seeded in the same row. Full details on the experimental design are provided in Mischkolz et al. (2013).

3.2.3 Forage Yield

Forage production was measured in the first week of July (mid-season) and last week of August (late-season) in 2011-2016 by clipping aboveground biomass at ground level from two separate square-meter quadrats from different spots in each plot. In 2014-2016, forage species in the mixture plots (two-species and seven-species mixture plots) were separated by hand to evaluate the proportion of each species in the stand. Clipped biomass of seeded forage species was dried in a forced-air oven at 60 °C to constant mass and weighed.

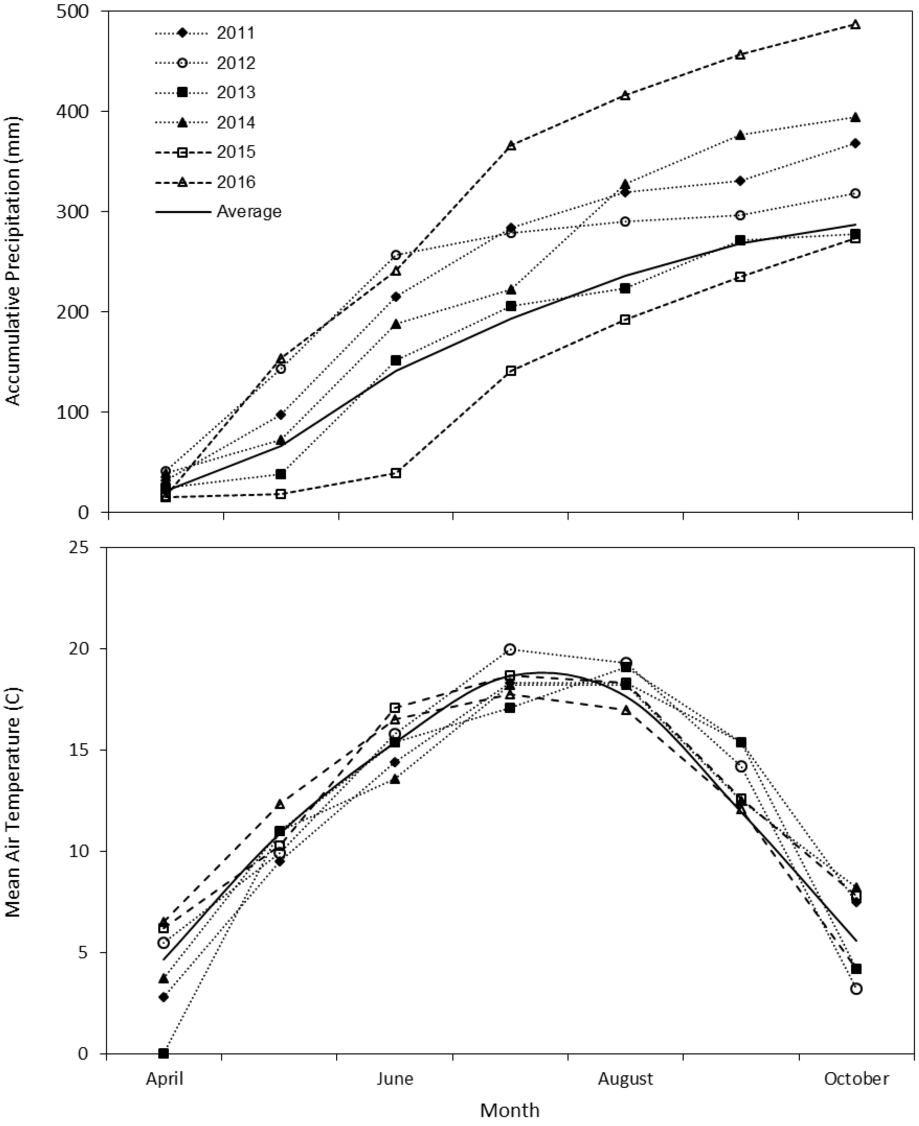


Figure 3.1 Accumulative precipitation (mm) and mean air temperature (°C) in 2011-2016 and average of 120 years at Swift Current SK Canada. Data were provided from AAFC in Swift Current SK. Precipitation in 2016 was higher than normal, whereas, 2015 was among the driest years in the region.

Table 3.1 Common name, Latin name, abbreviation and functional group of selected species.

Common and Latin Name	Abbreviation	Functional Group
Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve)	BBW	C_3
Nodding brome (*Bromus porteri* (J.M. Coult.) Nash)	NOB	C_3
Western wheatgrass (*Pascopyrum smithii* (Rydb.) Barkworth & D.R. Dewey)	WWG	C_3
Little blue stem (*Schizachyrium scoparium* (Michx.) Nash)	LBS	C_4
Side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.)	SOG	C_4
Purple prairie clover (*Dalea purpurea* Vent.)	PPC	Legume
White prairie clover (*Dalea candida* Willd.)	WPC	Legume

3.2.4 Forage Quality

Dried forage samples from each harvest were ground using a Thomas Scientific Wiley Mill (3379-K35 Variable Speed Digital ED-5 Wiley Mill, Swedesboro, NJ, USA). A maximum of 25 g of grounded materials were stored in 125 mL glass bottles for forage nutritive analysis. Total Nitrogen (N) concentration was determined according to Noel and Hambleton (1976). Crude protein (CP) concentration was calculated by multiplying total Kjeldahl N by 6.25. Acid detergent fiber (ADF) was determined according to Goering and Van Soest (1970). Neutral detergent fiber (NDF) was measured using the ANKOM200 fiber analyzer (Model 200; ANKOM; Fairport, New York) using the Filter Bag Technique. Calcium (Ca) and Cupper (Cu) analysis performed by ICP-OES (Inductively Coupled Plasma – Optical Emission Spectroscopy) on a Fisher Scientific iCAP6300 Duo according to Jones (1991). Phosphorous (P) Analysis performed by AAS (Atomic Absorption Spectroscopy) on a Hitachi Z-8200 according to the standard equipment operating setup according to Hamm et al. (1970).

3.2.5 Statistical analysis

The effect of different forage species and their mixtures on forage quantity and quality were analyzed as repeated measures via a mixed model (PROC MIXED; SAS Server Interface 2.0.4). Treatment, year and harvesting month were fixed effects and block was a random effect.

Since there were unequal periods between harvesting times (two months period from July to August in each year, and 10 months period from August to next July harvest), data was analyzed using a spatial power covariance structure. Denominator degrees of freedom were calculated using the BETWITHIN option (Appendix 9.1). Coefficient of variation (CV) for each treatment during the six-year study was calculated via mixed model to measure stability. A significance value of $P < 0.05$ was used and mean comparisons made using Fisher's Protected LSD test at $P = 0.05$. The data related to effect of each forage species on productivity in the mixtures (Figure 3.3) and forage productivity of different functional groups (Figure 3.5) were not analyzed statistically since each data point was used more than once (i.e. each bar contains data points that were used for making another bar as well), and thus were not statistically independent.

3.3 Results

3.3.1 Forage Production

Forage production significantly differed between the treatment, year, month and their interactions (Figure 3.2, Table 3.2, Appendix 9.2). In the monoculture plots, forage yield of WWG was the highest in 2011, decreased sharply in 2012 and stayed almost constant in the following years. Forage production of nodding brome (NOB) was among the highest in 2011, but decreased continuously thereafter and reached the lowest yield in 2016. Among C_4 grasses, production of LBS was higher than SOG across all years and harvest months. August harvest of LBS was significantly higher than July harvest in 2012, 2014 and 2016. Although LBS produced less dry matter in 2011 in relation to other C_3 species, in the following years a stable forage production within each harvest occurred. Forage production of legumes were the lowest in 2011 and then increased in 2012 and 2013. Legumes produced low yields in the dry year of 2015, but yield increased dramatically in the wet year of 2016, particularly late in the growing season.

Table 3.2 *F* statistics and *P* values indicating statistical significance for the treatment, year, month and their interactions on production, crude protein, ADF, NDF, P, Ca, Cu and CV of seven forage species in monoculture and mixtures.

Fixed Effects	*F* statistic and *P* value							
	Forage Production	Crude Protein	ADF	NDF	P	Ca	Cu	CV
Treatment	$F_{28,87}=35.91$ $P < 0.0001$	$F_{28,87}=117.73$ $P < 0.0001$	$F_{28,87}=60.69$ $P < 0.0001$	$F_{28,87}=141.08$ $P < 0.0001$	$F_{28,87}=21.03$ $P < 0.0001$	$F_{28,87}=88.60$ $P < 0.0001$	$F_{28,87}=16.41$ $P < 0.0001$	$F_{28,87}=5.20$ $P < 0.0001$
Year	$F_{5,435}=89.84$ $P < 0.0001$	$F_{5,435}=67.53$ $P < 0.0001$	$F_{5,430}=78.69$ $P < 0.0001$	$F_{5,430}=38.34$ $P < 0.0001$	$F_{5,428}=130.2$ $P < 0.0001$	$F_{3,256}=45.37$ $P < 0.0001$	$F_{3,256}=42.41$ $P < 0.0001$	---
Month	$F_{1,87}=265.15$ $P < 0.0001$	$F_{1, 87}=919.37$ $P < 0.0001$	$F_{1,87}=892.79$ $P < 0.0001$	$F_{1,87}=180.74$ $P < 0.0001$	$F_{1,87}=1831.44$ $P < 0.0001$	$F_{1,86}=1.45$ $P = 0.2311$	$F_{1,86}=64.02$ $P < 0.0001$	$F_{1,144}=1.77$ $P =0.1872$
Treatment ×Year	$F_{140,435}=6.47$ $P < 0.0001$	$F_{140, 435}=3.78$ $P < 0.0001$	$F_{140,430}=6.86$ $P < 0.0001$	$F_{140,430}=4.23$ $P < 0.0001$	$F_{140,428}=3.92$ $P = 0.0037$	$F_{84,256}=2.53$ $P < 0.0001$	$F_{84,256}=2.09$ $P < 0.0001$	---
Treatment ×Month	$F_{28,87}=2.79$ $P = 0.0001$	$F_{28, 87}=3.62$ $P < 0.0001$	$F_{28,87}=13.09$ $P < 0.0001$	$F_{28,87}=4.97$ $P < 0.0001$	$F_{28,87}=4.45$ $P < 0.0001$	$F_{28,86}=3.17$ $P < 0.0001$	$F_{28,86}=2.02$ $P = 0.0072$	$F_{28,144}=1.17$ $P =0.2856$
Year ×Month	$F_{5,435}=15.72$ $P < 0.0001$	$F_{5, 424}=41.44$ $P < 0.0001$	$F_{5,417}=63.80$ $P < 0.0001$	$F_{5,420}=18.22$ $P < 0.0001$	$F_{5,398}=279.66$ $P < 0.0001$	$F_{3,238}=7.43$ $P < 0.0001$	$F_{3,237}=16.61$ $P < 0.0001$	---
Treatment ×Year×Month	$F_{140,435}=1.45$ $P = 0.0024$	$F_{140, 424}=1.29$ $P = 0.0292$	$F_{140,417}=1.87$ $P < 0.0001$	$F_{140,420}=1.24$ $P = 0.0558$	$F_{139,398}=2.04$ $P < 0.0001$	$F_{84,238}=1.13$ $P = 0.2373$	$F_{84,237}=1.02$ $P = 0.4522$	---

3.3.2 Species Composition

The biomass ratio changed dramatically over time (Table 3.3). In Table 3.3 only proportion of seeded species are provided; weeds and non-seeded forage species in each plot were not included in the species composition. In mixtures of legumes and grasses (except WWG), more than 15% of the forage yield was from legumes, but in the mixture of WWG+legumes, only 1-11% of the yield was from legumes. In the mixtures of C_4+C_3 grasses (except WWG), the proportion of forage species in the mixture was dependent on forage species and harvesting time. Generally, the proportion of C_3 grasses was higher than C_4 grasses in July, and reversed in August. Whereas, the majority of forage yield in WWG+C_4 mixtures was from WWG at all sampling dates and years. In the mixtures of WWG and the other two C_3 grasses, WWG composed more than 97% of the stand. In the mixture of two C_4 grasses, the proportion of LBS was higher than SOG at all sampling dates.

3.3.3 Forage production of functional groups, monocultures vs. mixtures

Mixtures, in which WWG was a component, produced the highest forage yield at both harvesting times (Figure 3.3). In all years and harvesting times, forage production from mixture plots was higher than monoculture plots (Figure 3.4, Table 3.4). Mixtures of C_3+C_3 grasses produced the highest forage yield in 2011 (Figure 3.5). In general, mixtures containing C_3s followed by C_4s and legumes contributed the most in forage productivity.

There was significant effect of forage species on the temporal stability of forage production (Table 3.2; Table 3.6). Among monocultures, WWG had the lowest CV followed by LBS which indicated that temporal stability of these two forage species were greater than other studied species. Legumes, on the other hand, contained the highest CV among species. Mixtures containing WWG had the lowest CV, but mixtures containing legumes and NOB, on the other hand, had the highest CV compared to other mixtures.

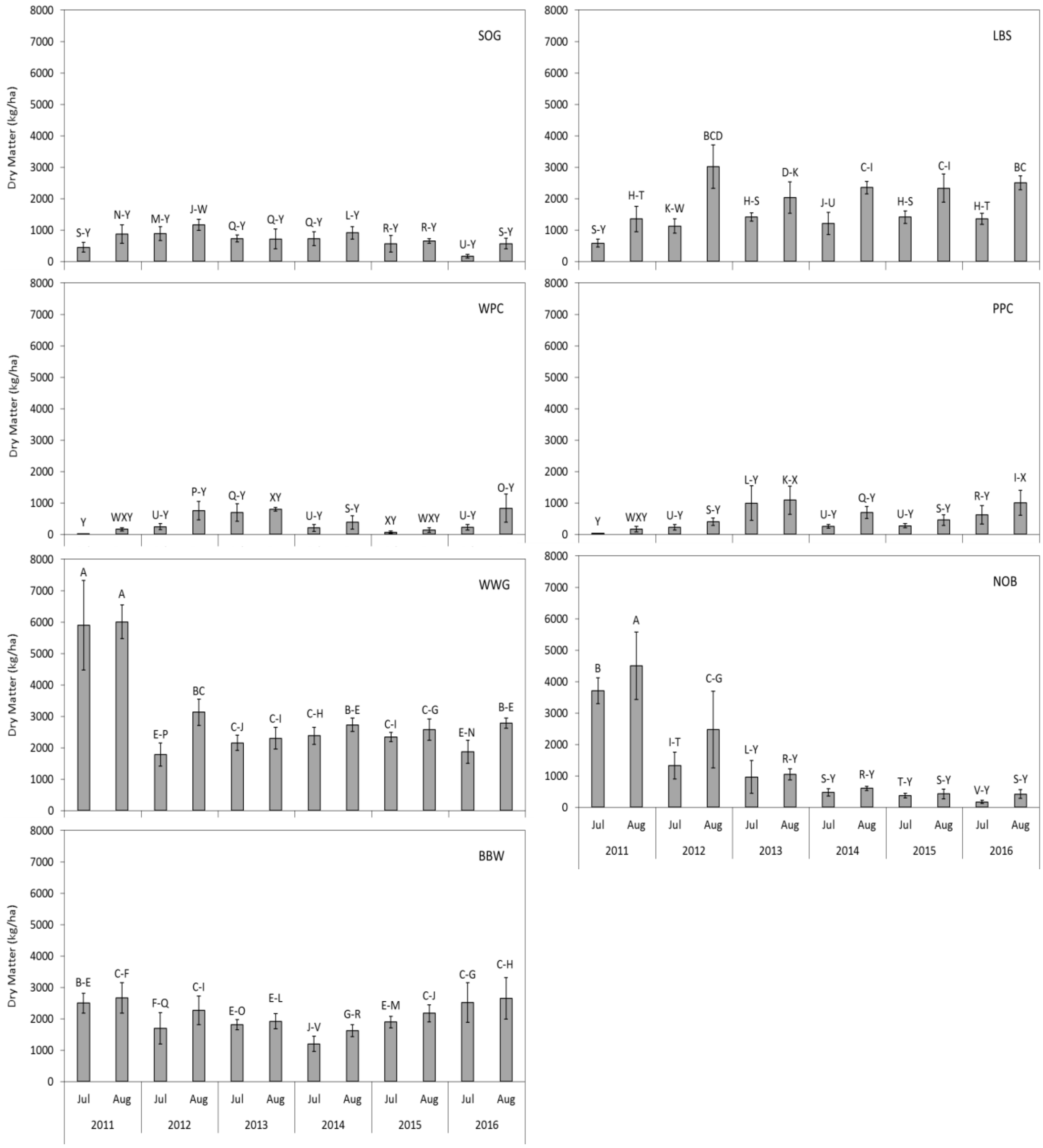


Figure 3.2 Forage production (kg/ha) in monoculture plots in July and August of 2011-2016. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; n = 4. Bars containing more than three significant letters are shown by the first and last letters, separated by dash. Bars containing the same letter in each graph are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

Table 3.3 Forage species composition in the mixture plots in July and August of 2014-2016. Proportion of each species in the mixture at the time of seeding was 50%. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover.

Functional Groups	Mixtures	Species	2014		2015		2016	
			Jul	Aug	Jul	Aug	Jul	Aug
			------------------------ % ------------------------					
C3+Legume	BBW+PPC	BBW	95	88	94	87	86	79
		PPC	5	12	6	13	14	21
	BBW+WPC	BBW	93	93	96	94	96	78
		WPC	7	7	4	6	4	22
	NOB+PPC	NOB	87	72	75	57	61	42
		PPC	13	28	25	43	39	58
	NOB+WPC	NOB	88	60	32	39	33	14
		WPC	12	40	68	61	67	86
	WWG+PPC	WWG	99	98	96	99	97	89
		PPC	1	2	4	1	3	11
	WWG+WPC	WWG	99	93	99	96	99	91
		WPC	1	7	1	4	1	9
C4+Legume	SOG+PPC	SOG	83	68	63	75	48	27
		PPC	17	32	37	25	52	73
	SOG+WPC	SOG	91	75	79	96	64	51
		WPC	9	25	21	4	36	49
	LBS+PPC	LBS	96	92	92	91	87	85
		PPC	4	8	8	9	13	15
	LBS+WPC	LBS	85	77	93	86	76	80
		WPC	15	23	7	14	24	20
C4+C3	LBS+BBW	LBS	34	68	41	55	27	42
		BBW	66	32	59	45	73	58
	LBS+NOB	LBS	71	64	77	74	85	89
		NOB	29	36	23	26	15	11
	SOG+BBW	SOG	25	23	14	23	14	27
		BBW	75	77	86	77	86	73
	SOG+NOB	SOG	88	64	93	50	52	91
		NOB	12	36	7	50	48	9
	LBS+WWG	LBS	13	8	3	3	5	32
		WWG	87	92	97	97	95	68
	SOG +WWG	SOG	3	> 1	> 1	> 1	> 1	2
		WWG	97	99	99	99	99	98
C3+C3	NOB+BBW	NOB	17	12	2	8	12	22
		BBW	83	88	98	92	88	78
	WWG+BBW	BBW	3	2	1	> 1	1	> 1
		WWG	97	98	99	99	99	99
	WWG+NOB	NOB	1	1	1	7	1	1
		WWG	99	99	99	93	99	99
C4+C4	SOG+LBS	SOG	29	7	37	38	39	2
		LBS	71	93	63	62	61	98
Legume+ Legume	PPC+WPC	PPC	33	75	40	80	58	54
		WPC	67	25	60	20	42	46

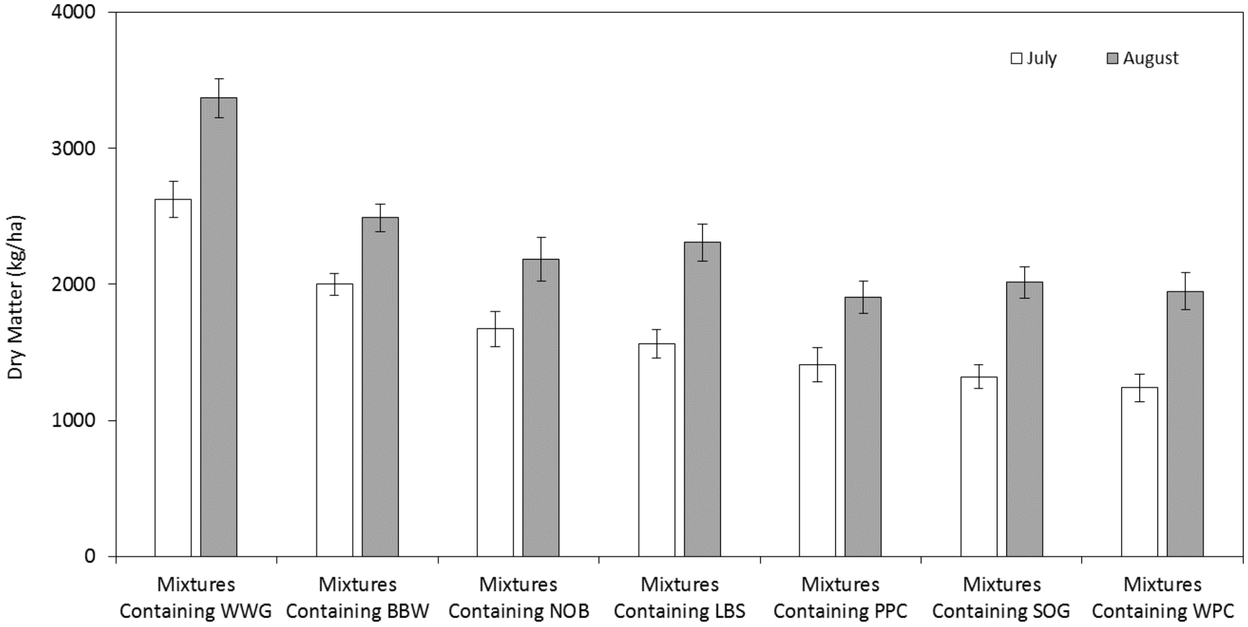


Figure 3.3 Forage production in mixture plots containing different forage species averaged across all years. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; *n* = 144.

3.3.4 Forage Quality

Crude protein was significantly different between treatment, year, month and their interactions (Figure 3.6, Table 3.2, Appendix 9.3). Crude protein concentrations ranged from 3-9% for C_3 grasses, 2-10% for C_4 grasses and 9-17% for legumes. In all forage species and all years, crude protein was higher in July compared to August. Among all grasses, WWG followed by BBW had the highest crude protein at all time. ADF and NDF concentration significantly differed between treatments, years, months and their interactions (Figure 3.7, Table 3.2). Among monocultures, PPC and WPC followed by WWG contained the lowest ADF and NDF concentrations. Ca and Cu concentrations significantly differed between different treatments, years and interactions including treatment by year, treatment by month and year by month (Table 3.2). Among forage nutrients, P and Ca are categorized in macronutrients and Cu in micronutrients. The average Ca concentration was higher in August than July in all species. Ca requirements for beef cattle ranges from 3100 to 5800 ppm depending on body size and milking ability (National Research Council 2001). Legumes contain higher Ca concentrations, however, plant species, maturity and tissue age can affect the concentrations of minerals in forages (Grings et al. 1996). In this study, the range of Ca varied between 1200-5000 ppm for grasses and 12000-21000 ppm for legumes. BBW contained the lowest range of Ca concentrations and legumes contained 2.4-17.5 times more Ca concentrations than grasses. The recommended level of Cu is 8 ppm for beef cattle (National Research Council 2001) which Karn and Hofmann (1990) found that Cu concentrations in forage species were below recommended levels in many North American pastures. In this study, legumes contained 1.5-6 times more Cu than grasses, and the average of Cu concentrations in legumes were higher in July. Cu concentrations in C_3 and C_4 grasses ranged between 1-4 ppm and in legumes ranged between 4-6 ppm. Cu concentrations in WWG increased from 1 ppm in 2011 to 4 ppm in 2014. Beef cattle require 0.21-0.26% P in their diets (National Research Council 2001). Concentration of P differed significantly between the different treatments, years, months and their interactions (Table 3.2). Generally, concentrations of P and Cu decreased whereas concentrations of Ca increased from July to August (Table 3.5).

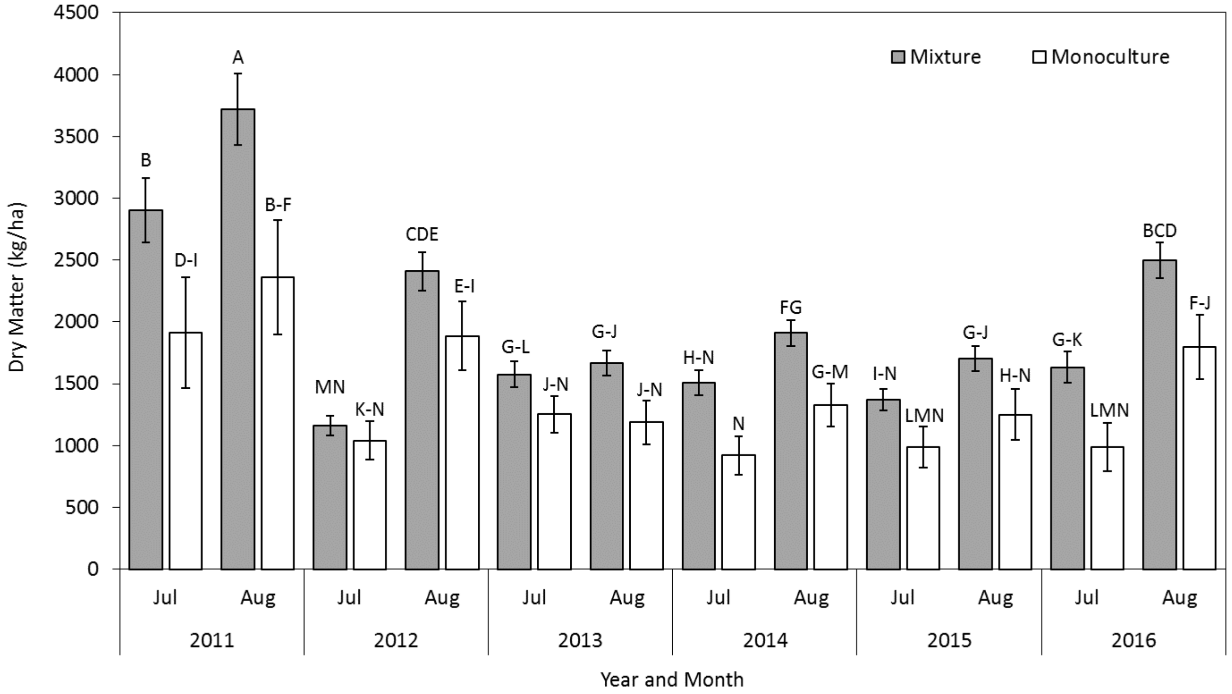


Figure 3.4 Forage production in mixtures vs. monoculture plots in July and August of 2011-2016. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; n = 84 (mixtures) and 28 (monocultures). Bars containing more than three significant letters are shown by the first and last letters, separated by dash. Bars containing the same letter in each graph are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

Table 3.4 *F* statistics and *P* values indicating statistical significance for forage production in monoculture vs. mixture treatments. Forage production of all monoculture plots were categorized in one group versus forage production of all mixture plots in another group.

	F statistic and *P* value
Fixed Effects	Monocultures vs. Mixtures
Treatment	$F_{1,110}=8.26$, $P = 0.0049$
Year	$F_{5,550}=10.62$, $P < 0.0001$
Month	$F_{1,110}=161.63$, $P < 0.0001$
Treatment×Year	$F_{5,550}=1.22$, $P = 0.2987$
Treatment×Month	$F_{1,110}=4.36$, $P = 0.0390$
Year×Month	$F_{5,550}=12.38$, $P < 0.0001$
Treatment×Year×Month	$F_{5,550}=0.65$, $P = 0.6633$

Table 3.5 P, Cu and Ca concentrations (ppm) of seven forage species in July and August of 2011-2014. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. One standard error around the mean is shown after ±; n = 4. Columns containing the same letter for each element are not significantly different (P ≤ 0.05) according to protected Fisher's LSD test. Columns containing more than three significant letters are shown by the first and last letters, separated by dash.

Forage		P (%)		Cu (ppm)		Ca (ppm)	
Species	Year	July	August	July	August	July	August
BBW	2011	0.16±0.005 h-r	0.14±0.005 p-w	0.966±0.140 v	2.048±0.263 q-v	1349±86 v	1510±340 q-v
	2012	0.19±0.019 e-i	0.14±0.005r-x	3.079±0.360 l-s	2.763±0.378 m-t	2326±219 l-s	2816±295 m-t
	2013	0.15±0.003 k-t	0.12±0.011s-y	3.375±0.118 i-q	3.304±0.339 j-r	2935±105 i-q	4064±356 j-r
	2014	0.17±0.006 g-q	0.12±0.007 s-y	3.932±0.156 d-m	3.003±0.345 l-s	2803±178 d-m	3219±222 l-s
LBS	2011	0.26±0.024 ab	0.19±0.022 d-i	3.606±0.489 g-o	2.418±0.364 n-u	3755±375 g-o	2301±558 n-u
	2012	0.21±0.012 c-g	0.12±0.007 t-y	3.320±0.282 i-r	1.627±0.158 tuv	2785±156 i-r	2219±234 tuv
	2013	0.15±0.009 k-t	0.13±0.011 s-y	3.374±0.185 i-q	2.201±0.196 p-v	3501±412 i-q	2840±157 p-v
	2014	0.18±0.004 g-p	0.15±0.005 o-v	3.044±0.206 l-s	2.201±0.062 p-v	3598±302 l-s	3724±321 p-v
NOB	2011	0.19±0.007 e-i	0.14±0.034 q-x	1.280±0.071 uv	1.625±0.265 tuv	1229±261 uv	1245±341 tuv
	2012	0.20±0.008 d-h	0.11±0.003 xy	2.724±0.102 mt	2.224±0.171 pv	1797±184 m-t	3009±514 p-v
	2013	0.17±0.005 h-q	0.14±0.011 r-x	3.748±0.306 f-n	2.870±0.148 m-t	2956±345 f-n	4504±230 m-t
	2014	0.24±0.013 abc	0.21±0.031 cde	3.604±0.111 g-o	3.889±0.391 e-m	3878±235 g-o	5174±235 e-m
PPC	2011	0.28±0.011 a	0.18±0.011 e-l	5.860±0.468 a-f	5.409±0.141 a-d	21330±2103 a-f	12786±4175 a-d
	2012	0.27±0.011 a	0.15±0.001 j-s	5.703±0.505 abc	4.239±0.401 c-l	21389±1345 abc	15584±2923 c-l
	2013	0.18±0.006 e-m	0.15±0.007 l-u	4.966±0.547 b-g	4.699±0.245 b-k	16919±4053 b-g	14554±932 b-k
	2014	0.23±0.012 bcd	0.14±0.004 r-x	6.082±0.186 a-b	4.841±0.397 b-g	16661±1769 a-b	17248±623 b-g
SOG	2011	0.24±0.016 abc	0.19±0.027 d-i	2.282±0.415 o-v	1.251±0.101 u-v	3196±178 o-v	2003±366 uv
	2012	0.24±0.011 abc	0.13±0.009 r-x	3.432±0.197 h-p	1.540±0.049 tuv	3696±213 h-p	2763±374 tuv
	2013	0.16±0.008 i-r	0.15±0.010 k-t	4.064±0.609 c-m	2.240±0.199 o-v	3928±250 c-m	4302±370 o-v
	2014	0.21±0.011 c-f	0.15±0.005 n-u	3.225±0.172 k-r	2.483±0.139 n-u	4268±151 k-r	5146±309 n-u
WPC	2011	0.28±0.013 ab	0.19±0.014 e-j	5.071±0.282 a-h	6.395±0.245 a	15073±1663 a-h	20364±1783 a
	2012	0.26±0.014 ab	0.15±0.004 m-u	5.715±0.026 abc	4.679±0.487 c-i	17017±137 abc	14246±2859 c-i
	2013	0.17±0.014 g-q	0.16±0.011 i-t	5.110±0.616 a-f	5.596±0.536 a-g	11693±3039 a-f	16025±1254 a-g
	2014	0.22±0.006 bcd	0.14±0.012 n-w	5.327±0.051 a-e	4.743±0.135 b-j	15839±1708 a-e	14926±1127 b-j
WWG	2011	0.18±0.008 f-o	0.12±0.011 s-y	0.986±0.132 v	1.970±0.127 r-v	1670±150 v	1746±469 r-v
	2012	0.22±0.003 bcd	0.11±0.007 u-y	2.780±0.076 m-t	1.754±0.072 s-v	2349±118 m-t	3279±137 s-v
	2013	0.15±0.009 j-s	0.09±0.004 y	3.012±0.154 l-s	2.120±0.162 p-v	3378±74 l-s	3899±163 p-v
	2014	0.19±0.006 e-i	0.11±0.004 wxy	3.155±0.264 l-r	3.816±0.284 b-g	3195±181 l-r	3284±88 b-g

3.4 Discussion

3.4.1 Productivity of forage species in monoculture

Among monoculture plots, WWG produced greater forage yield at all harvesting times. The high productivity of WWG compared to other grasses can be related to its rhizomatous growth behavior. Biligetu et al. (2014) found that rhizomatous C_3 grasses, regardless of the species' origin, produce greater dry matter than caespitose grasses. Forage production of WWG was the highest in 2011 and decreased in the following year. Increases in intraspecific competition and reduction in soil N levels might be explanations for this forage reduction. NOB performed well in 2011, but the forage yield decreased continuously thereafter and was among the lowest producers in 2016. Therefore, NOB doesn't seem to be a suitable option for stable and long-term forage productivity in the dry-mix ecoregion. BBW, on the other hand, produced more stable forage yield during the course of study and might be a good option for long-term stable forage productivity in seeded pastures. Forage production of LBS was the highest in August of 2012 and its yield stayed stable in August of the following years.

3.4.2 Mixtures produced greater forage yield than monocultures

In this study mixtures of forage species were consistently more productive than monocultures and demonstrated long-term benefits over monocultures. The mixture advantage was greatest in 2011 (first year after seeding forage species) followed by the wet year of 2016. Higher soil nutrients availability and lower intraspecific competition might explain the mixture advantage in the first year after seeding. Among mixture plots, those containing WWG followed by BBW, NOB and LBS had higher forage production. In the wet years, mixtures containing WWG even outperformed the monoculture of WWG. During the course of the study, 2015 was one of the driest years, whereas 2016 was one of the wettest years in the last 120 years in Swift Current. We observed no significant differences in forage production of WWG in monoculture and mixtures from dry to wet years. This yield stability across very different climate conditions is highly desirable. Native species may not be as productive as introduced species in some areas, but they can reduce the likelihood of yield failure in this semi-arid environment (Schellenberg et al. 2012). Among monocultures WWG and LBS had the highest temporal stability (lower CV) during the course of this study and NOB, PPC and WPC had the lowest temporal stability (higher CV). Drought tolerance of WWG and LBS (USDA 2016) and also rhizomatous growth behavior of WWG can be linked to higher stability of these species. However, regarding the high forage production of NOB in the second year after seeding and the sharp decrease thereafter, the low stability of NOB can be linked to sensitivity of this species to soil nutrients deficit or increasing in the intraspecific competition. Species composition in mixture plots was highly dependent on the growth behavior of each species in the mixture. WWG occupied more than 90% of the binary mixtures in 2014 and thereafter. WWG is a stronger competitor than the other species tested here in both seedling and maturity stages (Zhang and Lamb 2011). These results show the strong ability of WWG to occupy space, and limit the growth and survival of other species. WWG is a perennial grass that grows densely with sod-forming rhizomes that can occupy all the spaces between seeded rows (National-Research-Council 2001), and based on WWG's competitive ability, it may limit the presence of other species in the mixture. This competitive ability of WWG can be also implemented for weed control and management in seeded pastures, as many weeds may not find an empty niche to survive in a WWG sward (Chapter 5).

Among different functional groups, mixtures containing C_3 grasses consistently produced greater forage yield. C_3 grasses begin growth earlier than C_4 grasses, reduce light quality and quantity reaching C_4 grasses; therefore, they can be more competitive and productive than C_4 grasses in the Canadian Prairies (Jones 1992; Schellenberg et al. 2012). Among C_4 grasses, inclusion of LBS in the mixtures resulted in higher forage yields compared to SOG. LBS is a drought tolerant grass with broad adaptation to different ecoregions, whereas SOG is moderately drought tolerant (USDA 2016). In mixtures of C_3+C_4 grasses (except WWG), the proportion of each functional group in the mixture varied was based on the growth behavior of each species. We expected to see a higher proportion of C_3s in the early to mid-growing season and C_4s in the late growing season. This expected pattern was observed in the BBW+LBS mixture, however other possible patterns were also recorded from other C_3+C_4 mixtures.

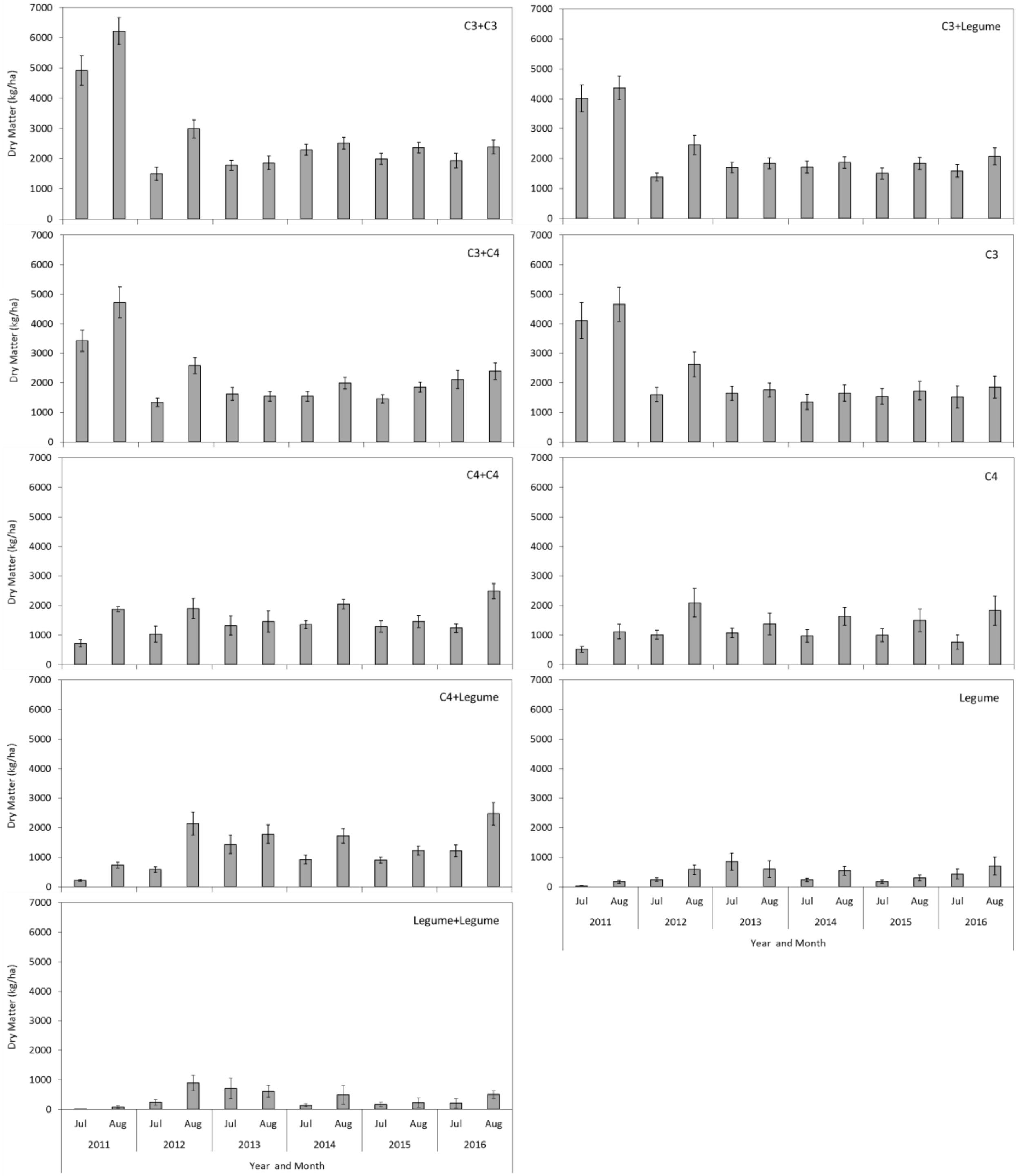


Figure 3.5 Forage production of different functional groups including C3, C4, legume and their mixtures in July and August of 2011-2016. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; *n* = 24 (C3+C4 and C3+legume), 16 (C4+Legume), 12 (C3+C3 and C3), 8 (C4 and Legume), 4 (C4+C4 and Legume+Legume).

Table 3.6 Coefficient of variation (CV) for each treatment in the month of July and August for the period of 2011-2016 (mean ±SE). Lower values represent higher temporal stability. Columns containing the same letter are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

Treatment	July CV %	August CV %
WWG+ SOG	18.1±1.9 k	27.4±6.0 h
WWG	25.2±6.3 ijk	27.4±4.8 h
ALL Species	25.3±6.0 ijk	28.8±7.3 h
LBS	26.8±6.9 hijk	31.4±3.9 fgh
SOG+LBS	28.3±8.3 hijk	31.0±3.6 fgh
NOB+WWG	28.4±6.7 hijk	32.0±7.2 fgh
WWG+PPC	30.3±6.7 ghij	40.1±6.3 fg
WWG+WPC	30.4±3.4 ghij	34.2±6.6 fgh
LBS+WWG	30.9±3.2 ghij	27.8±4.1 h
SOG+BBW	34.2±5.7 ghij	26.5±5.9 h
BBW+WWG	37.7±8.9 fghij	26.0±6.0 h
BBW+WPC	38.7±3.5 fghij	39.9±9.2 fgh
BBW+PPC	39.2±4.5 fghi	31.6±11.6 fgh
BBW	40.4±7.7 efghi	34.1±5.6 fgh
NOB+BBW	40.4±5.3 efghij	26.7±3.5 h
SOG+PPC	54.5±11.7 defghi	42.2±2.4 fg
LBS+PPC	55.4±9.1 defghi	67.1±16.1 bcdef
LBS+BBW	55.7±2.7 defghi	50.1±8.7 defg
SOG+WPC	59.2±12.0 cdefgh	92.4±9.2 ab
SOG	60.1±15.3 cdefgh	42.7±6.8 fg
NOB+PPC	65.7±6.8 bcdefg	45.8±11.2 efg
SOG+NOB	70.2±19.4 bcdef	56.1±25.4 cdefg
LBS+WPC	70.4±30.5 bcdef	52.8±18.1 cdefg
LBS+NOB	75.4±24.9 bcde	40.1±6.7 fg
NOB+WPC	82.2±12.1 bcd	83.5±7.5 abcd
PPC	88.6±27.1abcd	109.7±15.6 a
WPC	93.6±5.5 abc	81.5±16.6 abcde
NOB	100.7±11.0 ab	88.1±25.5 abc
PPC+WPC	118.8±22.3 a	112.8±36.9 a

In our study legumes had lower plant densities than the grasses in the mixtures and made up a small portion of the dry matter. PPC is slow to germinate which can affect its establishment when mixed with other forage species, especially grasses (Molano-Flores et al. 2011; Schellenberg and Banerjee 2002). Therefore, more studies are needed to enhance germination and establishment rates of PPC and WPC to facilitate their establishment in mixtures. Drought years can negatively affect the productivity of perennial legumes (Peterson et al. 1992). However, the rapid increase in yield of PPC and WPC in 2016 compared to the previous dry year suggests that these two legumes can successfully tolerate at least one dry year, and be rapidly productive again when conditions improve.

Many studies have identified positive relationships between plant diversity and productivity, ecosystem stability and function (Isbell and Wilsey 2011; Mischkolz et al. 2016; Picasso et al. 2011; Tilman et al. 2014; Tilman et al. 2001; Tilman et al. 2006a). Picasso et al. (2011) evaluated seven perennial forage species in monocultures and mixtures in two locations and concluded that forage species diversity provides sustainably higher productivity over time. In a 7-year study on 16 grassland species, Tilman et al. (2001) concluded that a mixture of species can increase the biomass 2.7 times more than monocultures. Positive effects of species diversity on productivity can be explained by different factors including interspecific complementarity, better use of available resources, better nutrients cycling and reduced chance of herbivory and disease outbreaks (Tilman et al. 2014). Not all native species produce high forage yield, but forage mixtures including less productive species may bring beneficial characteristics like drought or grazing tolerance to the plant community without incurring penalties to productivity (Mischkolz et al. 2013). The diverse species mixtures guarantee the forage sward under good climate condition and more importantly, ensure the forage productivity under unpredictable harsh conditions.

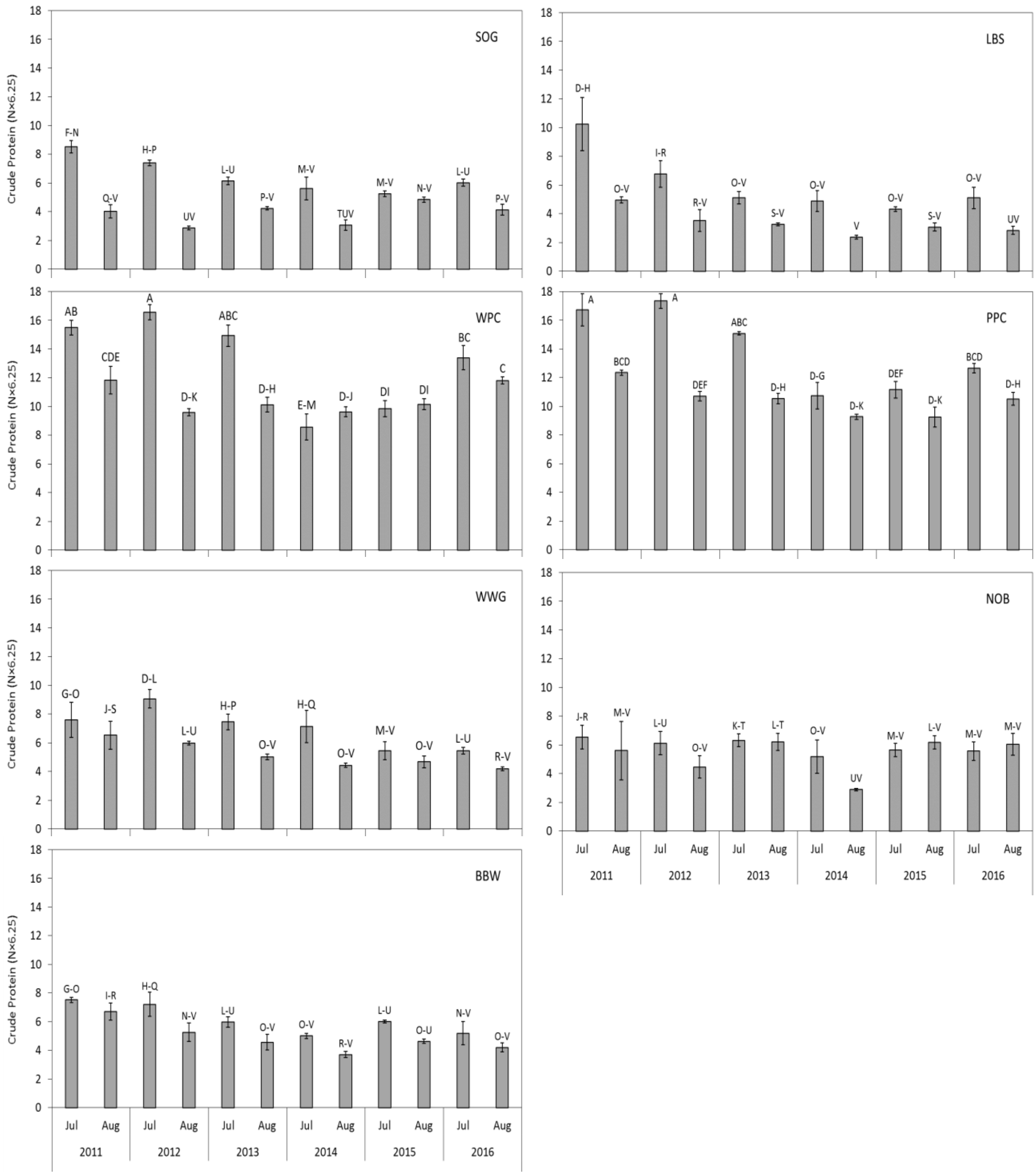


Figure 3.6 Crude protein (N×6.25) of forage species in July and August of 2011-2015. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; n = 4. Bars containing more than three significant letters are shown by the first and last letters, separated by dash. Bars containing the same letter in each graph are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

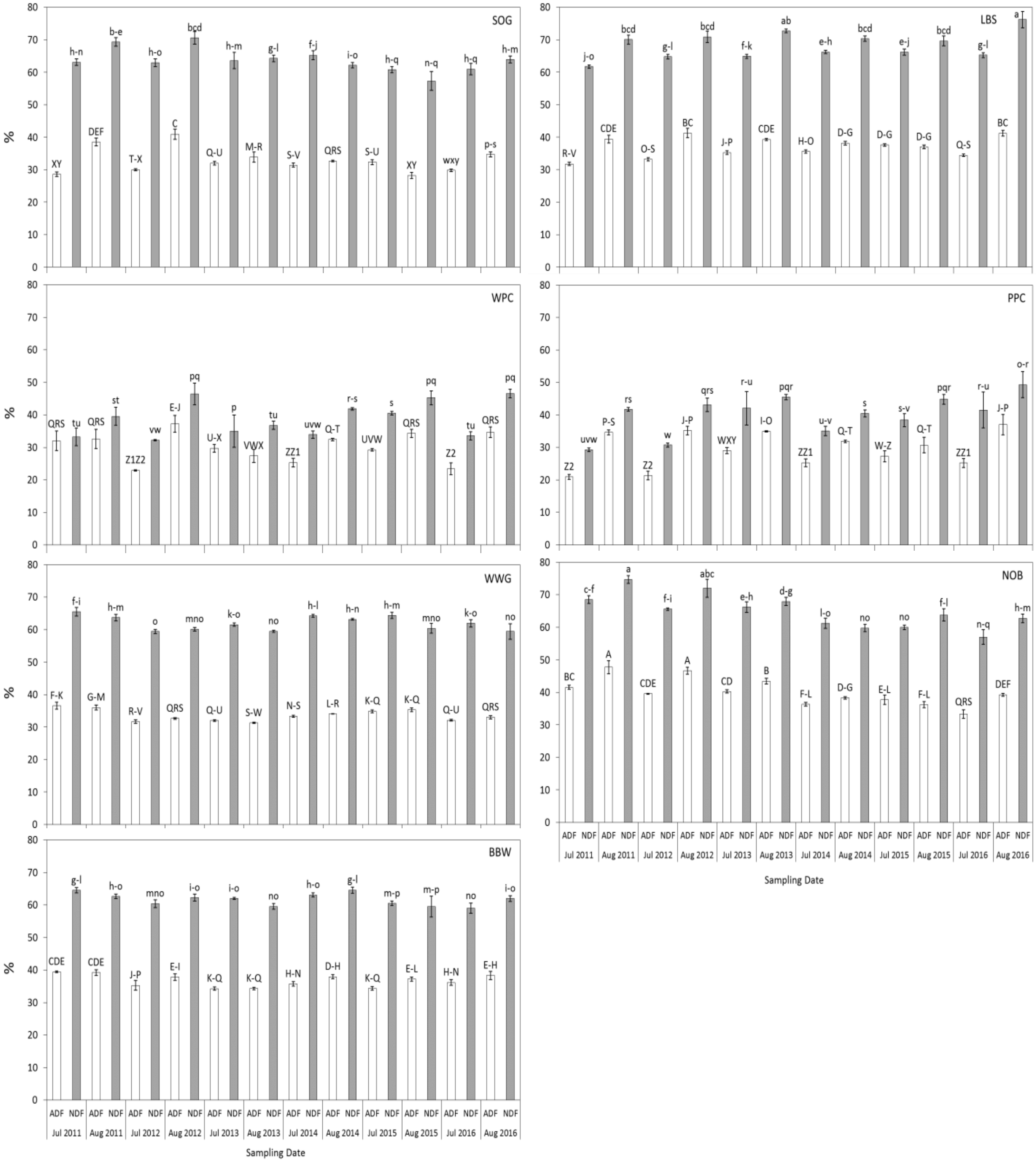


Figure 3.7 Acid detergent fiber (ADF) and neural detergent fiber (NDF) of seven forage species in July and August of 2011-2016. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; n = 4. Bars containing more than three significant letters are shown by the first and last letters, separated by dash. Bars containing the same letter in each graph are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

Side-oats grama was not as productive as LBS, and potentially indicating a lower tolerance for drought. Although SOG did not perform well in the dry-mixed ecoregion high forage productivity of SOG was recorded in an ongoing field experiment in Brandon, MB (Chapter 4). Brandon is located in the tall grass prairie ecoregion and has higher precipitation than Swift Current (Bailey et al. 2010). Therefore, SOG may be a more suitable as a forage candidate in the Tall-Grass Prairie ecoregion than in the dry-mixed ecoregion.

3.4.3 WWG contains the highest crude protein and lowest ADF and NDF among grasses

Among grasses, WWG contained the highest crude protein concentration which was in agreement with the data reported by Biligetu et al. (2014). Crude protein concentrations were higher in July compared to August in all species in both monoculture and mixtures. The result demonstrates that there is a negative relationship between plant maturity and the concentrations of crude protein. The decreases in leaf:stem ratio can be another reason for lower crude protein concentrations in late-season. PPC and WPC contained the higher crude protein concentration compared to grasses and adding legumes to the grasses increased the crude protein concentration of the mixtures.

Among all seven species, legumes contained the lowest ADF and NDF concentrations followed by WWG. These results are in agreement with Biligetu et al. (2014) and Jefferson et al. (2004). Jefferson et al. (2004) indicated that western wheatgrass contains the best potential forage digestibility for fall grazing. In this study, average ADF concentrations in C_4 grasses were similar to C_3 grasses but average NDF concentrations in C_4 grasses were higher than C_3 grasses. Warm-season or C_4 grasses tend to have more vascular tissue and thicker cell walls and more fiber concentrations than C_3 grasses (Van Soest 1994). However, short summer and average low temperature can limit the fiber concentrations in C_4 grasses of the Canadian Prairies (Jefferson et al. 2004). Since plants contain more structural compounds in late growing season (Bélanger et al. 2001), in our study ADF and NDF concentrations in all species were higher in August compared to July. Grasses in the late growing season tended to have a higher proportion of stem (structural tissue) and lower proportion of cell contents (metabolic compounds) (Bélanger et al. 2001).

The concentrations of P were greater in July, and legumes contained the highest P concentrations. Average P concentrations in WWG and BBW were the lowest among species. LBS and SOG provided higher P concentrations than WWG which is in agreement with Jefferson et al. (2004). Beef cattle require 0.21-0.26% P in their diets (National Research

Council 2001). In most years, P concentrations provided by grasses were lower than the minimum requirement, suggesting a need to provide P supplements under this production system. Biligetu et al. (2014) also indicated that P concentrations were lower than the requirement in forage mixtures.

Legumes can increase the Ca concentrations in the forage mixtures. Plant species, maturity and tissue age can affect the concentrations of minerals in forages (Grings et al. 1996). Ca requirements for beef cattle ranges from 3100 to 5800 ppm depending on body size and milking ability (National Research Council 2001). In our study, Ca concentrations in C_3 grasses did not meet this requirement in 2012 and 2013, but did in August of the following years. Grings et al. (1996) also found that Ca concentrations in C_3 grasses were lower than the minimum requirement. The results showed that perennial C_3 grasses may provide Ca requirement for beef cattle after 4 years of establishment.

Grings et al. (1996) reported an average of 2 ppm Cu concentrations in WWG. The recommended level of Cu is 8 ppm for beef cattle (National Research Council 2001), therefore, it seems that grasses and legumes and their mixtures cannot provide Cu requirements for beef cattle. Karn and Hofmann (1990) in North Dakota also found that Cu concentrations in forage species were below recommended levels. In this study, Cu concentration average of C_3 and C_4 grasses was the same, however, Grings et al. (1996) reported 7 ppm for C_3 grasses and 4 ppm for C_4 grasses.

3.5 Conclusion

This study was conducted over a six-year period to evaluate the forage yield and quality of seven native North American species in monoculture and mixtures. Forage mixtures produced greater dry matter than monocultures at all harvesting times. Mixtures of which WWG was a component produced higher forage yield, therefore, WWG may be a key species to increase the mixture productivity. In this study, a mixture of C_3+C_3 grasses produced higher forage yield, however, adding LBS to the mixture can guarantee the productivity in late-season. Among monoculture plots, WWG ranked the highest in forage productivity, but NOB doesn’t seem to be a suitable option for stable and long-term forage productivity. Among grasses, WWG contained the highest crude protein concentrations and the lowest ADF and NDF concentrations, indicating WWG can be a nutritious forage species for seeded pastures. In this study, the forage species experienced one of the driest and wettest years in the history of the region. We observed no significant differences in forage production of all native species from dry to wet year. The results support the idea of highly stability and productivity of native species during varying climate conditions. In conclusion, a mixture of WWG, BBW, LBS and legumes can provide sustainable forage yield and quality in varied climate conditions and can be suitable options for seeded pastures.

CHAPTER 4

EVALUATION OF BINARY AND COMPLEX MIXTURES OF NATIVE FORAGE SPECIES FOR THE DRY-MIXED AND TALL-GRASS ECOREGION OF CANADIAN PRAIRIE

Abstract

This study was carried out to evaluate the forage yield and quality of binary and complex mixtures of seven native North American species in two different ecoregions of Canadian Prairie. The relative abundance of each species in the complex mixtures was calculated based on a greenhouse study. Forage species were seeded in 2014 and 2016 in Swift Current and Brandon, Canada, respectively. Forage yield was measured in early July and late-August in 2015-2016 in Swift Current and mid-August in 2016 in Brandon. Species composition changed dramatically from 2015 to 2016 in Swift Current. Monocultures of western wheatgrass (*Pascopyrum smithii*) (WWG) and complex mixtures E (71% WWG, 5% noding brome (NOB), 5% little blue stem (LBS), 9% side oats-grama (SOG), 5% white prairie clover (WPC) and 5% purple prairie clover (PPC)), and mixture C (57% WWG, 5% bluebunch wheatgrass (BBW), 19% SOG and 19% PPC) produced the highest dry matter; whereas, in Brandon, monoculture of side-oats grama (*Bouteloua curtipendula*) and binary of SOG+purple prairie clover (*Dalea purpurea*) produced the greatest yield. In conclusion, WWG and SOG strongly affect the productivity of mixtures in dry-mixed and Tall-Grass Ecoregions, respectively. Western wheatgrass occupied more than 87 % of the forage mixture in 2016 regardless of the initial seeding rate. The results suggest that in the Dry-Mixed Ecoregion WWG is a key species for forage productivity, however, mixtures containing more than 57% WWG had no positive effect on productivity.

4.1 Introduction

There is a growing interest in using diverse plant communities in seeded pastures, agriculture and agroforestry, however, identifying optimal species combinations and species relative abundances is challenging (Kelty 2006; Malézieux et al. 2009; Mischkolz et al. 2016). There is a great deal of evidence that forage productivity can improve with diverse community composition, species richness, functional group richness and species evenness (Balvanera et al. 2006; Hooper et al. 2005b; Kirwan et al. 2007; Loreau et al. 2001; Mischkolz et al. 2016; Reiss et al. 2009). Further, forage mixtures containing different functional groups can improve nutritional quality and palatability (Holechek et al. 2004; Mischkolz et al. 2013; Wang and Schellenberg 2012) increase primary production (Loreau et al. 2001) and enhance ecosystem services like wildlife habitat quality and carbon sequestration (Symstad et al. 2003; Weigelt et al. 2009). It is also shown that increasing biodiversity can decrease the variability of ecosystem function, therefore, insure the ecosystem against environmental fluctuations because of the differential ability of each species to response to a changing environment (Loreau et al. 2001; Tilman et al. 2006b; Yachi and Loreau 1999).

Mixtures of warm and cool-season species with differing maturities have the potential to provide higher forage quality for livestock over a longer period of the growing season than a monoculture or mixtures of only cool-season species (Jones and Wilson 1987; Tilman et al. 2001). Combinations of different species or different functional groups may show additivity or complementarity in resource use which can increase productivity and plant community stability (Brooker et al. 2008; Hooper et al. 2005a; Mischkolz et al. 2016; Picasso et al. 2008; Spehn et al. 2005; Tilman et al. 2001; Weigelt et al. 2009).

A number of studies have evaluated the productivity of simple and complex forage mixtures. Schellenberg et al. (2012) studied the forage production of seven-species and 14-species mixtures in semiarid Swift Current, SK. They found that seven-species mixtures had greater dry matter and lower crude protein than the 14-species mixtures. They also concluded that cool season grasses are more competitive than other species. Foster et al. (2014) also evaluated forage yield of eleven treatments including monocultures of alfalfa and four grasses, binary mixtures of grasses+alfalfa, five-species mixture and ten-species mixtures in more mesic Melfort, SK. Alfalfa was included in all five-species and ten-species mixtures. They found that five-species and ten-species mixtures produced greater dry matter than the monoculture of grasses but lesser than binary mixtures or monoculture of alfalfa. Finally, Deak et al. (2007) conducted a three-year study of forage productivity of simple (binary or three-species mixtures) and complex (six-species mixtures) and very complex mixtures (nine-species mixtures) with different relative abundance for each species. They found that six-species mixtures produced the highest forage yield among other mixtures. However, at the end of the experiment only three species of orchardgrass (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.), and white clover (*Trifolium repens* L.) predominate the forage mixtures, regardless of the initial seeding rate.

In this study, I evaluated forage yield and quality of seven native perennial forage species, including C_3, C_4 and legumes, in monoculture, binary and complex mixtures in two different ecoregions of the Canadian Prairie, Dry-Mixed Grass (Swift Current, SK) and Tall-Grass Prairie (Brandon, MB). Mischkolz et al. (2016) developed a screening approach to identify optimal mixtures, and this field study was conducted to test the greenhouse results. The objectives of this study were to: (1) evaluate forage yield and quality of monocultures and simple mixtures vs. complex mixtures; (2) determine the effect of each species in mixtures productivity; and (3) assess the changes in mixture composition by time.

4.2 Materials and Methods

This experiment was conducted at the Agriculture and Agri-Food Canada (AAFC), Swift Current Research and Development Centre (SCRDC) near Swift Current SK. A second trial was conducted at the Brandon Research and Development Centre, Brandon MB, Canada. Only first year data are available for the Brandon site, however, so all result from that site are reported in the appendix. Swift Current is located in Dry-Mixed Grass Prairie ecoregion which is the driest part of the province. The annual temperature, annual precipitation and May-July precipitation are 3.7 °C, 322 mm and 159 mm, respectively (Bailey et al. 2010). This ecoregion has an Orthic Brown Chernozem soil (Swinton loam) and pH of 7.4 (Ayers et al. 1985; Bailey et al. 2010). In general, 2016 was the 4th wettest in 120 years; whereas 2015 was one the driest years in the last 120 years in Swift Current.

4.2.1 Forage Species

Seven native perennial forage species from three functional groups were selected, including three C_3 grasses, two C_4 grasses and two legumes (Table 4.1). These species were evaluated in greenhouse studies by Mischkolz et al. (2016) and their results were evaluated in the field situation in the present study. These species have also continued to be evaluated in field studies in Saskatoon and Swift Current, SK (Biligetu et al. 2014; Mischkolz et al. 2013; Schellenberg et al. 2012).

Table 4.1 Common name, Latin name, abbreviation and the functional group of selected species.

Common and Latin Name	Abbreviation	Functional Group
Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve)	BBW	C_3
Nodding brome (*Bromus porteri* (J.M. Coult.) Nash)	NOB	C_3
Western wheatgrass (*Pascopyrum smithii* (Rydb.) Barkworth & D.R. Dewey)	WWG	C_3
Little blue stem (*Schizachyrium scoparium* (Michx.) Nash)	LBS	C_4
Side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.)	SOG	C_4
Purple prairie clover (*Dalea purpurea* Vent.)	PPC	Legume
White prairie clover (*Dalea candida* Willd.)	WPC	Legume

4.2.2 Experimental Design

Forage species were seeded in a split-plot block design with four replicates of 15 treatments in May 2014 in Swift Current and 16 treatments in May 2016 in Brandon. In Swift Current the treatments in each block included a monoculture of WWG, six ‘binary mixture’ plots, and five “complex mixture” plots. Monoculture of SOG and binary mixtures of SOG+WPC and SOG+PPC did not establish in Swift Current. The proportion of each species in the complex mixture plots were calculated based on optimal mixtures identified by Mischkolz et al. (2016) (Table 4.2). The plot areas were summer fallowed one year prior to seeding and sprayed with Roundup WeatherMAX® (Monsanto Canada Inc., Winnipeg, Canada) in the rate of 0.82 L ha^{-1} before seeding forage species at both locations. Neither herbicide nor fertilizer was applied thereafter. Forage species were seeded with a press drill at a depth of 1.3 cm, in 2×8 m plots with six rows spaced 22.5 cm apart. Grasses in monocultures were planted at a rate of 100 pure live seeds m^{-2}. In binary plots, seeding rate was reduced to half of the monoculture plots for grasses and 100 pure live seeds m^{-2} for legumes. In the mixture plots, all species were seeded in the same row.

Table 4.2 The contribution of each species (%) at the seeding rate in complex mixtures, planted in Swift Current SK and Brandon MB.

	Forage Species						
	WWG	BBW	NOB	LBS	SOG	PPC	WPC
Mixtures	%						
Mixture A	14	14	48	9	5	0	10
Mixture B	14	62	0	5	14	5	0
Mixture C	57	5	0	0	19	19	0
Mixture D	38	19	24	0	0	0	19
Mixture E	71	0	5	5	9	5	5

4.2.3 Forage Yield

Forage yield was measured in the first week of July (mid-season) and last week of August (late-season) in 2015-2016 in Swift Current, and August 2016 (seeding year) in Brandon by clipping aboveground of species at ground level with two square-meter quadrats from different spots within each plot. Unlike in Swift Current, some forage species performed very well in the seeding year in Brandon, therefore, forage yield in Brandon was measured in 2016. Species in the mixture plots (binary and complex mixture plots) were separated by hand to evaluate the proportion of each species in forage production. Clipped biomass was dried in a forced-air oven at 60 °C to constant mass and weighed. In the October of each year the plots were mowed to mimic haying; plant materials were removed from the plots.

4.2.4 Forage Quality

Dried forage samples from each harvest were ground using a Thomas Scientific Wiley Mill (3379-K35 Variable Speed Digital ED-5 Wiley Mill, Swedesboro, NJ, USA). Total Nitrogen (N) concentration was determined according to Noel and Hambleton (1976). Crude protein (CP) concentration was calculated by multiplying total Kjeldahl N by 6.25. Acid detergent fiber (ADF) was determined according to (Goering and Van Soest 1970). Neutral detergent fiber (NDF) was measured using the ANKOM200 fiber analyzer (Model 200; ANKOM; Fairport, New York) using the Filter Bag Technique. Phosphorous (P), and analysis was performed by AAS (Atomic Absorption Spectroscopy) on a Hitachi Z-8200 (Hamm et al. 1970).

4.2.5 Statistical analysis

The effect of different forage species and their mixtures on forage quantity and quality in Swift Current were analyzed as repeated measures via a mixed model (PROC MIXED; SAS Server Interface 2.0.4). Treatments, years and months considered fixed effects and block as a random effect. Since there were unequal periods between harvesting times, a spatial power covariance structure selected. In Brandon, the effect of different forage species and their mixtures on forage quantity and quality were analyzed as randomized complete block design (RCBD) via a mixed model with a block as a random effect. Denominator degrees of freedom for both experiments were calculated using BETWITHIN option. A significance value of $P < 0.05$ was used and mean comparisons made using Fisher's Protected LSD test at $P = 0.05$.

4.3 Results

4.3.1 Forage Production

Forage production significantly differed between the treatment, year, month and year by month interaction in Swift Current (Figure 4.1). Forage yields of all treatments increased from 2015 to 2016, and monoculture of WWG produced the highest dry matter in August 2016. Among complex mixtures (Mixtures A-E) those of which the proportion of WWG was higher (Mixture E and Mixture C) produced higher dry matter.

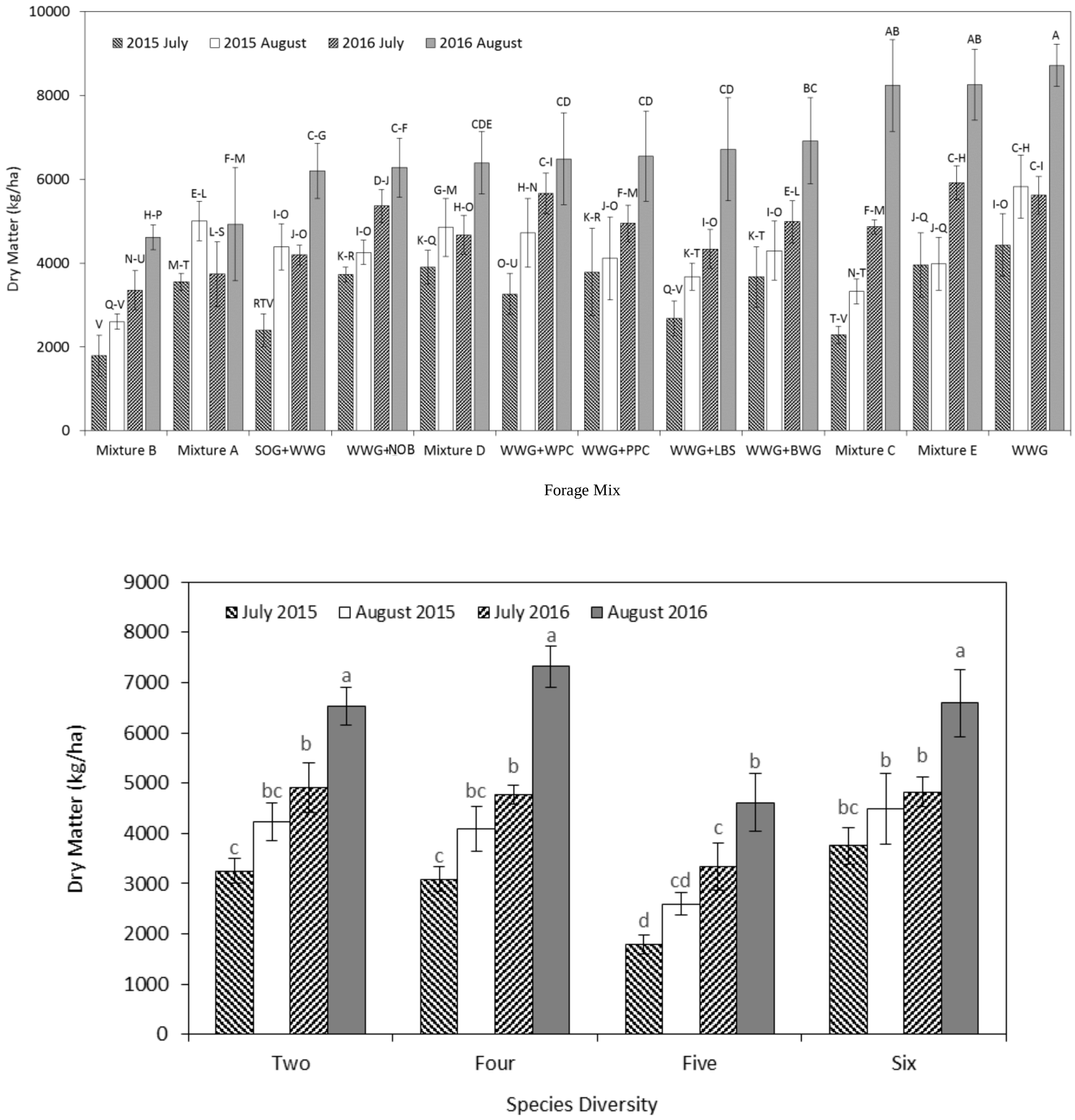


Figure 4.1 Forage production (kg/ha) in a monoculture of WWG and mixture plots (above graph) and forage production of mixtures with different number of species diversity (below graph) in July and August of 2015-2016 in Swift Current SK. The species composition of Mixtures A-E are provided in Table 4.2. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; $n = 4$. Bars containing more than three significant letters are shown by the first and last letters, separated by a dash. Bars containing the same letter are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

Table 4.3 *F* statistics and *P* values indicating statistical significance for the treatment, year, month and their interactions on production, crude protein, Acid detergent fiber (ADF), Neutral detergent fiber (NDF) and Phosphorus of seven forage species in Swift Current, SK.

Fixed Effects	*F* statistic and *P* value				
	Forage Production	Crude Protein	ADF	NDF	P
Treatment	$F_{11,16}$=4.23	$F_{11,16}$=6.24	$F_{11,16}$=17.87	$F_{11,16}$=3.93	$F_{11,16}$=1.82
	$P = 0.0005$	$P < 0.0001$	$P < 0.0001$	$P = 0.0009$	$P = 0.0871$
Year	$F_{1,36}$=89.79	$F_{1,36}$=19.68	$F_{1,36}$=4.03	$F_{1,36}$=0.32	$F_{1,36}$=92.03
	$P < 0.0001$	$P < 0.0001$	$P = 0.0521$	$P = 0.5772$	$P < 0.0001$
Month	$F_{1,16}$=117.51	$F_{1,16}$=70.75	$F_{1,16}$=23.50	$F_{1,16}$=60.37	$F_{1,16}$=164.06
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Treatment	$F_{11,36}$=1.58	$F_{11,36}$=2.99	$F_{11,36}$=10.81	$F_{11,36}$=3.57	$F_{11,36}$=1.30
×Year	$P = 0.1456$	$P = 0.0063$	$P < 0.0001$	$P = 0.0019$	$P = 0.2658$
Treatment	$F_{11,16}$=1.19	$F_{11,16}$=1.26	$F_{11,16}$=3.99	$F_{11,16}$=1.59	$F_{11,16}$=1.62
×Month	$P = 0.3251$	$P = 0.2886$	$P = 0.0008$	$P = 0.1437$	$P = 0.1357$
Year	$F_{1,36}$=12.27	$F_{1,34}$=57.57	$F_{1,34}$=14.29	$F_{1,34}$=0.00	$F_{1,34}$=136.25
×Month	$P = 0.0012$	$P < 0.0001$	$P = 0.0006$	$P = 0.9964$	$P < 0.0001$
Treatment	$F_{11,36}$=1.11	$F_{11,34}$=1.97	$F_{11,34}$=2.18	$F_{11,34}$=0.76	$F_{11,34}$=2.82
×Year×Month	$P = 0.3845$	$P = 0.0648$	$P = 0.0404$	$P = 0.6789$	$P = 0.0100$

4.3.2 Species Composition

Species composition in binary and complex mixtures changed dramatically over time in Swift Current (Table 4.4). SOG, LBS and legumes did not germinate in Swift Current, but successfully germinated and established in Brandon (Appendix 9.6). In mixtures containing WWG, more than 87% of the forage yield came from WWG in August of 2016 in Swift Current.

4.3.3 Forage quality

Crude protein was significantly different between the treatment, year, month and their interactions except treatment by month in Swift Current (Figure 4.2, Table 4.3). In Swift Current, Crude protein concentrations were generally higher in 2015 compared to 2016, and higher in

July rather than August of both years. Crude protein concentrations ranged from 4-6% in Swift Current, and was higher in July compared to August (Figure 4.2, Appendix 9.7). ADF and NDF concentrations significantly differed between treatments, months and treatment by month interactions in Swift Current (Table 4.5, Table 4.3). P concentrations significantly differed between treatments, months, years, year by month, and treatment by year by month interactions in Swift Current (Table 4.6, Table 4.3).

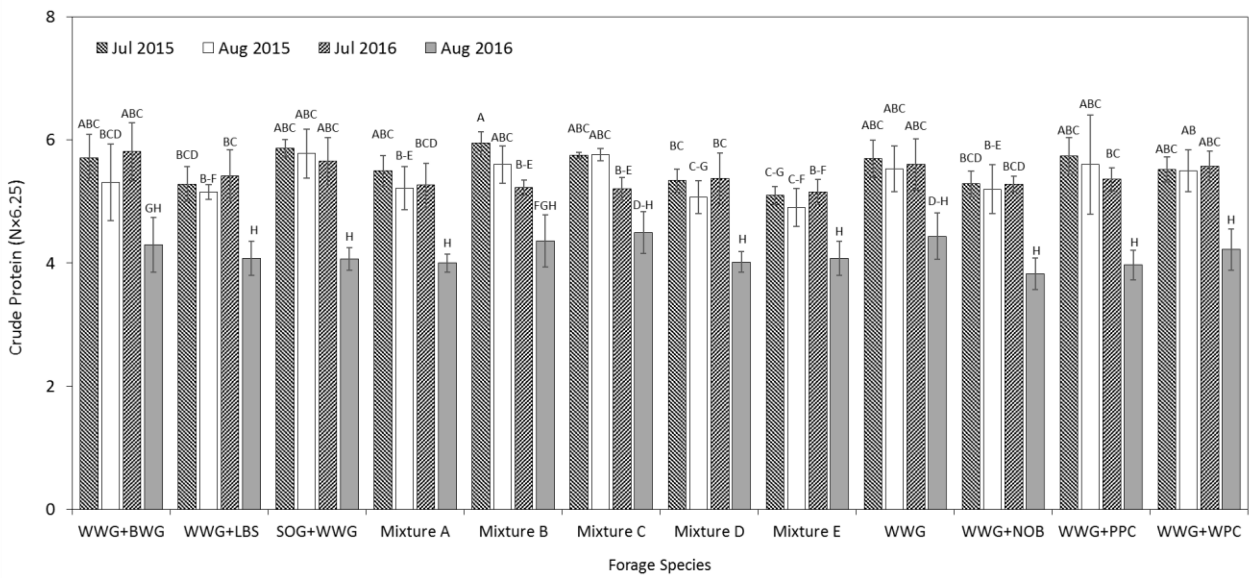


Figure 4.2 Crude protein (N×6.25) in a monoculture of WWG and mixture plots in July and August of 2015-2016 in Swift Current SK. The species composition of Mixtures A-E is provided in Table 1. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; n = 4. Bars containing more than three significant letters are shown by the first and last letters, separated by a dash. Bars containing the same letter are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

Table 4.4 Species composition in the mixture plots in July and August of 2015 and 2016 in Swift Current SK. Species composition was measured two and three years after seeding (2015 and 2016). Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover.

			Swift Current			
Mixture	Species	Seeding Rate %	Jul 2015	Aug 2015	Jul 2016	Aug 2016
			---------------------	%	---------------------	
Mixture A	WWG	14	6	20	61	87
Mixture A	WPC	10	0	0	0	0
Mixture A	SOG	5	0	0	0	0
Mixture A	NOB	48	94	76	39	13
Mixture A	LBS	9	0	0	0	0
Mixture A	BWG	14	0	5	0	0
Mixture B	WWG	14	81	96	100	100
Mixture B	SOG	14	0	0	0	0
Mixture B	PPC	5	0	0	0	0
Mixture B	LBS	5	0	0	0	0
Mixture B	BWG	62	18	4	0	0
Mixture C	WWG	57	96	93	100	100
Mixture C	SOG	19	0	0	0	0
Mixture C	PPC	19	0	0	0	0
Mixture C	BWG	5	4	7	0	0
Mixture D	WWG	38	29	56	92	89
Mixture D	WPC	19	0	0	0	0
Mixture D	NOB	24	71	44	8	11
Mixture D	BWG	19	0	0	0	0
Mixture E	WWG	71	71	90	97	100
Mixture E	WPC	5	0	0	0	0
Mixture E	SOG	9	0	0	0	0
Mixture E	PPC	5	0	0	0	0
Mixture E	NOB	5	29	10	3	0
Mixture E	LBS	5	0	0	0	0
WWG+BBW	WWG	50	95	95	100	100
WWG+BBW	BWG	50	5	5	0	0
WWG+LBS	WWG	50	100	100	100	100
WWG+LBS	LBS	50	0	0	0	0
WWG+NOB	WWG	50	27	46	86	96
WWG+NOB	NOB	50	73	54	14	4
WWG+PPC	WWG	50	100	100	100	100
WWG+PPC	PPC	50	0	0	0	0
WWG+SOG	WWG	50	100	100	100	100
WWG+SOG	SOG	50	0	0	0	0
WWG+WPC	WWG	50	100	100	100	100
WWG+WPC	WPC	50	0	0	0	0

4.4 Discussion

4.4.1 Forage yield

Our results showed that mixtures containing a higher proportion of WWG (e.g. mixture E and mixture C) produced a higher yield in 2016 in Swift Current. Mixture E and C contained 29% and 43% less WWG compared to monoculture, but forage yield did not differ significantly in 2016 from the monoculture of WWG. In other words, mixtures containing 57% or greater percentage WWG all produced the same amount of dry matter. Therefore, increasing the proportion of WWG higher than 57% in seed mixes does not seem to have any positive effects on forage productivity. Mixtures A and B (both of which contained 14% WWG) produced the lowest dry matter in 2016 that showed that 14% WWG was not high enough to achieve maximum productivity. Mischkolz et al. (2013) also found that even when WWG is seeded at half of the seeding rate, the final forage yield was still the same as WWG in monoculture. Forage yield increased in 2016 in almost all mixtures; filling of the spaces between seeded rows by WWG rhizomes and high precipitation in 2016 can explain this trend. Our study showed that the rhizomatous growth behavior of WWG might strongly affect the forage productivity of mixtures. Biligetu et al. (2014) found that rhizomatous C_3 grasses, regardless of the species' origin, produce greater dry matter than non-rhizomatous grasses. Mixture A which contained 48% NOB performed well in August of 2015, but was not as productive as mixtures E and C in the following year. In chapter 3, I also found that the forage yield of NOB decreased continuously from year to year, therefore, NOB does not seem to be a suitable option for stable and long-term forage productivity in the dry-mix ecoregion. In this study, legumes did not germinate successfully in Swift Current. PPC is slow to germinate which can affect establishment when mixed with other forage species, especially grasses (Molano-Flores et al. 2011; Schellenberg and Banerjee 2002). Therefore, given the benefits of legumes in the mixtures more studies are needed to identify ways to enhance germination and establishment rates of PPC and WPC to facilitate their establishment in mixtures with other species, especially in dry ecoregions.

In Brandon, SOG strongly affected the productivity of mixtures. Monoculture of SOG and binary mixtures containing SOG produced the highest forage yield in the seeding year of 2016. Among complex mixtures, mixture C followed by mixture E produced the highest forage yield. Monoculture of SOG produced about three-times more dry matter than that of WWG, however a longer study is needed to evaluate the forage yield of SOG over time in the more mesic Tall-Grass Prairie Ecoregion.

Many studies have identified positive relationships between plant diversity and productivity, ecosystem stability and function (Isbell and Wilsey 2011; Mischkolz et al. 2016; Picasso et al. 2011; Tilman et al. 2014; Tilman et al. 2001; Tilman et al. 2006a). Positive effects of species diversity on productivity such as those shown in Chapter three can be explained by different factors including interspecific complementarity, better use of available resources, better nutrients cycling and reduced chance of herbivory and disease outbreaks (Tilman et al. 2014). Not all native species produce high forage yield, but forage mixtures including less productive species may bring beneficial characteristics like drought or grazing tolerance to the plant community. The diverse species mixtures guarantee forage yield under good climate conditions and more importantly ensure stable forage productivity under unpredictable harsh conditions (Mischkolz et al. 2013).

Table 4.5 ADF and NDF concentrations in a monoculture of WWG and mixture plots in July and August of 2015-2016 in Swift Current SK. The species composition of Mixtures A-E is provided in Table 1. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. One standard error around the mean is shown after ±; *n* = 4.

	ADF (%)			
	2015		2016	
Species	July	August	July	August
Mixture A	38.89±1.28	43.85±1.05	34.80±0.77	34.27±0.95
Mixture B	32.22±0.17	32.85±0.84	34.61±0.39	34.15±0.76
Mixture C	32.39±0.99	32.10±0.67	33.28±0.55	32.60±0.81
Mixture D	36.03±0.87	40.38±0.88	33.85±0.86	35.08±0.58
Mixture E	33.62±0.56	34.82±1.11	33.62±0.57	33.65±0.52
SOG+WWG	31.44±0.83	31.58±0.29	34.01±0.92	33.65±0.66
WWG	31.20±0.72	31.88±0.63	32.46±0.55	32.75±0.32
WWG+BWG	32.66±1.15	33.19±1.42	32.64±0.52	33.99±0.82
WWG+LBS	31.96±0.50	32.46±0.93	33.08±0.56	33.90±0.60
WWG+NOB	36.71±0.72	41.95±0.94	33.45±0.68	34.80±0.85
WWG+PPC	31.74±0.72	32.54±1.16	32.36±0.81	32.81±0.62
WWG+WPC	32.18±0.37	32.16±0.60	34.70±0.84	33.54±0.84
	NDF (%)			
Mixture A	66.61±0.92	69.06±2.78	60.71±1.65	62.10±11.80
Mixture B	59.12±0.35	60.15±1.18	61.00±1.14	60.84±3.12
Mixture C	60.29±1.44	61.00±0.66	61.97±1.41	61.12±1.14
Mixture D	63.89±0.89	70.60±0.87	62.37±1.66	63.50±1.66
Mixture E	61.83±0.39	63.13±0.93	62.17±0.94	61.47±5.17
SOG+WWG	59.33±1.02	62.73±2.45	62.46±1.76	60.01±3.67
WWG	59.24±1.20	59.17±1.31	60.69±0.65	60.39±3.87
WWG+BWG	59.28±0.34	63.06±1.60	61.86±1.36	64.49±3.97
WWG+LBS	59.32±0.66	60.53±1.25	55.99±5.00	58.34±2.71
WWG+NOB	64.71±1.22	68.58±1.58	58.04±3.16	58.55±1.62
WWG+PPC	60.29±1.07	61.28±1.35	60.21±1.17	62.91±2.23
WWG+WPC	60.85±0.95	59.76±0.37	63.78±1.23	62.44±0.25

Table 4.6 P concentrations (%) in a monoculture of WWG and mixture plots in July and August of 2015-2016 in Swift Current SK. The species composition of Mixtures A-E is provided in Table 1. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. One standard error around the mean is shown after ±; n = 4.

	2015		2016	
Species	July	August	July	August
	----------------------------	Swift Current	----------------------------	
Mixture A	0.119±0.006	0.084±0.004	0.160±0.007	0.118±0.012
Mixture B	0.130±0.008	0.134±0.012	0.168±0.003	0.113±0.003
Mixture C	0.116±0.006	0.127±0.001	0.160±0.004	0.113±0.007
Mixture D	0.103±0.003	0.084±0.003	0.155±0.016	0.125±0.010
Mixture E	0.106±0.015	0.109±0.014	0.145±0.005	0.113±0.003
SOG+WWG	0.105±0.009	0.110±0.006	0.155±0.006	0.120±0.004
WWG	0.108±0.011	0.103±0.011	0.165±0.006	0.113±0.003
WWG+BWG	0.099±0.007	0.109±0.009	0.150±0.004	0.120±0.004
WWG+LBS	0.124±0.006	0.125±0.005	0.163±0.006	0.113±0.005
WWG+NOB	0.110±0.006	0.095±0.007	0.150±0.006	0.115±0.006
WWG+PPC	0.118±0.019	0.125±0.015	0.178±0.005	0.110±0.006
WWG+WPC	0.105±0.005	0.116±0.010	0.163±0.003	0.113±0.005

4.4.2 Species composition

Binary mixtures were seeded at the same rate (50-50%) at both locations, however, in August 2016, WWG occupied more than 96% of the mixtures in Swift Current. In complex mixtures (mixtures A-E), WWG occupied more than 87% of the mixtures, regardless of initial seeding rate. The results showed that WWG can aggressively occupy the majority of forage mixture in the third year after seeding. WWG is a stronger competitor than the other species in these mixtures at both the seedling and mature stages (Zhang and Lamb 2011). These results show the strong ability of WWG to occupy the spaces, and limit the growth and survival of other species. WWG is a perennial grass that grows densely with sod-forming rhizomes that can fill all the spaces between seeded rows (National-Research-Council 2001), and based on WWG's competitive ability, it may limit the presence of other species in the mixture. This competitive ability of WWG can also be implemented for weed control and management in seeded pastures, as many weeds may not find an empty niche to thrive beside WWG.

4.4.3 Forage quality

The result of this study demonstrates that there is a negative relationship between plant maturity and the concentrations of crude protein. In Brandon Mixture D (contained 19% WPC) followed by WWG+legumes contained the highest crude protein concentrations. Among grasses, WWG contained the highest crude protein concentration which agrees with the data reported by Biligetu et al. (2014).

In Swift Current, the mixtures of WWG+NOB and mixture A (containing 48% NOB) had the highest amount of ADF and NDF concentration, whereas monoculture of WWG contained the lowest amount of ADF and NDF concentrations in 2015. Carbohydrates are the main source of energy and should include 60-70% of the total diet (National Research Council 2001). In Chapter 3 I also found that the ADF and NDF concentrations of NOB were higher than WWG. In 2016, since most of the plots were occupied by WWG, the fiber concentration of all treatments ranged only between 32 and 34%. Jefferson et al. (2004) indicated that among grasses western wheatgrass showed the best potential forage digestibility for fall grazing of stockpiled, reseeded native grasses.

The concentrations of P were generally greater in July and mixtures containing legumes had higher P concentrations. Beef cattle require 0.21-0.26% P in their diets (National Research Council 2001). In both years at Swift Current, P concentrations provided by grasses were lower than the minimum requirement. Biligetu et al. (2014) also indicated that P concentrations were lower than the requirement in forage mixtures. However, in Brandon in the seeding year, P concentrations provided by forage mixtures met the minimum requirement.

4.5 Conclusion

In summary, the results showed that WWG is a suitable option for forage productivity in the Dry-Mixed ecoregion of Saskatchewan, and mixtures containing the higher proportion of WWG, up to 57%, can produce greater dry matter. The other 43% can be filled with legumes, NOB, BBW, LBS and SOG; and the proportion of SOG can be higher than other species in the Tall-Grass Ecoregion. Mixtures that contained NOB performed well in 2015 however, its yield decreased in the following year. NOB may be a good option when short-term forage productivity is targeted, but does not appear to be suitable for long-term use in pastures. In Brandon, on the other hand, SOG performed well in the seeding year and produced three times more dry matter than WWG, suggesting that that species may be preferred more mesic ecoregions. WWG is an aggressive perennial grass that has occupied more than 87% of the forage mixture in 2016 in Swift Current regardless of the initial seeding rate.

CHAPTER 5

MULTI-SPECIES FORAGE MIXTURES REDUCE WEED SEEDBANK AND ABOVEGROUND POPULATION

Abstract

To evaluate the effect of forage species in monoculture and mixtures on weed seedbank and aboveground density, seven native perennial forage species were seeded in the Dry-Mixed Grass Prairie Ecoregion of Saskatchewan, Canada. Four and five years later, weed seedbank and aboveground weed populations were measured. Weed seedbank and aboveground populations were significantly affected by the different seeding mixtures. The most abundant weeds in the soil seedbank were the least abundant weeds in the aboveground population and vice versa. About 57%, 37% and 6% of germinated seeds had biennial, annual and perennial life cycles, respectively. Biennial wormwood (*Artemisia biennis*), stinkweed (*Thlaspi arvense*), purslane (*Portulaca oleracea*) and flixweed (*Descurainia sophia*) comprised 86% of the total germinated seeds. We show that forage mixtures can reduce the abundance of weeds in the seedbank and aboveground weed population compared to monocultures. Among mixtures, those containing western wheatgrass had the lowest abundance of weeds in the seedbank and aboveground population. In conclusion, mixtures of forage species can reduce the number of weed seeds in the seedbank and size of the aboveground weed population. Inclusion of strong competitors like western wheatgrass in the mixtures can also increase the suppressive potential on weeds.

5.1 Introduction

The soil seedbank is a legacy of past weed populations and a source of potential emergents following disturbance (Murphy et al. 2006; Sanderson et al. 2007; Sosnoskie et al. 2006). Although the soil seedbank may contain useful pasture species, it also is a reservoir of undesirable weedy species (Rice 1989). Knowledge of soil weed seedbank composition can be useful for pasture manager, as it can indicate both which species may emerge after a disturbance that opens a gap in the sward and successional processes operating in the pasture (Sanderson et al. 2014).

Weeds can significantly reduce forage yield and quality, affecting livestock production qualities and increase rangeland management costs (DiTomaso 2000). More diverse aboveground plant communities tend to be more resistant to weeds compared to less complex plant communities (Elton 1958; Picasso et al. 2008). This phenomenon can be explained in two ways. The first perspective, ‘resource use complementarity’, suggests that plants in a diverse community use resources more efficiently leaving few unoccupied niches for the weeds (Knops et al. 1999; Naeem et al. 2000). The second perspective, ‘sampling effect’, theorizes that in more complex mixtures, there is at least one strong and productive species that can outcompete weeds and reduces weed presence in the mixtures (Huston 1997). Other benefits of forage mixtures include an increase in forage productivity, yield stability in different climate conditions, nutrient loss prevention from soil, a decrease in pathogen infection and provision of diverse nutrients for grazing animals (Hector et al. 1999; Hooper and Vitousek 1998; Knops et al. 1999; McNaughton 1977; Mischkolz et al. 2016; Tilman and Downing 1994; Tilman et al. 1996; Vibart et al. 2016).

A number of studies have examined the benefits of forage mixtures for weed suppression. For example, Picasso et al. (2008) evaluated 49 combinations of seven species including all monocultures and selected two to six species mixtures and found that as forage species richness increased, weed biomass decreased. Sanderson et al. (2007) similarly found a smaller proportion of aboveground weeds in six- and nine-species mixtures compared to two- and three-species mixtures, but no effect of different mixtures on the weed seedbank. Most of the weed seedbank was composed of annual forbs and there was only a minor relationship between weed seedbank composition and aboveground weed populations. Sanderson et al. (2014) also showed that a permanent pasture can have a more stable soil seedbank than that of recently cultivated land. They also found that annual weeds are more common in the seedbank of hayfields and recently seeded pastures, whereas the weed seedbank in older pastures tended to be dominated by perennial grasses.

About 5.7 million hectares of the Canadian Prairie is covered by seeded pastures (Statistics Canada, 2010), primarily introduced species like crested wheatgrass (*Agropyron cristatum* [L.] Gaertn.), smooth brome (*Bromus inermis* Leyss) and Russian wildrye (*Elymus junceus* Fisch.) (Otfinowski et al. 2007; Smoliak and Dormaar 1985). Although there is a growing interest in the use of native perennial species for sustainable beef production from seeded pastures, little research has been conducted on weed seedbank composition and aboveground populations in pastures seeded to native forages. In this study, we hypothesized that certain native forage species may better suppress weeds and reduce the weed seedbank, and mixtures of forage species may be more effective at excluding weeds and decreasing weed seedbank compared to monocultures. The objectives of this study were to (1) evaluate the effects of different native forage species on weed seedbank composition and size and aboveground weed populations; (2) investigate the effect of forage mixtures on weed seedbank and aboveground weed populations and; (3) evaluate the similarity between the weed seedbank and emerged weed density in mixtures of native perennial forage species.

5.2 Material and Methods

5.2.1 Study Site

This experiment was conducted at the Agriculture and Agri-Food Canada (AAFC), Swift Current Research and Development Centre (SCRDC) near Swift Current (latitude 50°25′N, longitude 107°44′W, 824 m elevation) Saskatchewan, Canada. This area is located in the dry mixed-grass Prairie ecoregion which is the driest part of the province (Bailey et al. 2010). The annual temperature, annual precipitation and May-July precipitation are 4.1 °C, 327 mm and 153 mm, respectively (Bailey et al. 2010). This ecoregion has an Orthic Brown Chernozemic soils (Swinton loam) with a pH of 7.4 (Ayers et al. 1985; Bailey et al. 2010). Weather data was collected from AAFC Swift Current for 2014, 2015 and the average of 120 years (Figure 3.1). In general, precipitation in 2014 was close to average; whereas, 2015 was one of the driest years in the history of Swift Current.

Seven native perennial forage species from three functional groups were selected in this experiment: three C_3 grasses, two C_4 grasses and two legumes (Table 5.1). These species were evaluated in greenhouse studies (Mischkolz et al. 2016) and have continued to be evaluated in breeding studies in Saskatoon and Swift Current, SK, (Biligetu et al. 2014; Mischkolz et al. 2013; Schellenberg et al. 2012) and ongoing field studies in Swift Current and Brandon, MB Canada. These native forage species have significant agronomic potential as they are broadly distributed, seeds are readily available, have acceptable nutritional quality profiles and do not require specialized machinery for seeding.

Table 5.1 Common name, Latin name, abbreviation and functional group of selected native forage species seeded in 2010 in Swift Current SK.

Common and Latin Name	Abbreviation	Functional Group
Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve)	BBW	C_3
Nodding brome (*Bromus porteri* (J.M. Coult.) Nash)	NOB	C_3
Western wheatgrass (*Pascopyrum smithii* (Rydb.) Barkworth & D.R. Dewey)	WWG	C_3
Little blue stem (*Schizachyrium scoparium* (Michx.) Nash)	LBS	C_4
Side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.)	SOG	C_4
Purple prairie clover (*Dalea purpurea* Vent.)	PPC	Legume
White prairie clover (*Dalea candida* Willd.)	WPC	Legume

5.2.2 Experimental Design

A randomized complete block design (RCBD) was seeded with four replicates of 30 treatments in June 2010. Full details on this experiments were provided in Mischkolz et al. (2013). Treatments in each block included seven ‘monoculture’, 21 ‘two-species mixture’, one ‘seven-species mixture’ and one ‘blank’ or non-seeded. Summer fallow were applied to plots one year before seeding the experiment. Before planting, weeds were controlled by the application of Roundup WeatherMAX® (Monsanto Canada Inc., Winnipeg, Canada) (0.82 L ha^{-1}) followed eleven days later with 2-4DB Cobutox® 625 (Interprovincial Cooperative Limited, Winnipeg, Canada) (2.47 L ha^{-1}). Neither herbicide nor fertilizer were applied thereafter. Forage species were seeded with a press drill at the depth of 1.3 cm, in 4 m wide×8 m long plots with 12 rows spaced 22.5 cm apart. Grass and legume species were planted at the rate of 100 and 200 pure live seeds/m^2, respectively. In two-species mixtures and the seven-species mixture, the seeding rate was reduced to half and one seventh of the monoculture plots, respectively.

5.2.3 Aboveground Weed Population

Emerged weed population composition and density were measured in the first weeks of May and July and the last weeks of August in 2014 and 2015. Two quarter-meter quadrats were randomly placed in each plot; weeds in the quadrats were identified to species and individuals directly counted.

5.2.4 Weed Seedbank Sampling

The weed seedbank was assessed early (first week of May) and late (last week of August) in the growing season in both 2014 and 2015 (four and five years after seeding the forage species). Soil sampling was done in a W-shaped pattern using a 35mm diameter hand probe. Ten spots in each plot were sampled at three depths: 0-5, 5-10 and 10-15 cm. Soil from each depth in each plot were kept in separate trays, broken up by hand, bulked and poured into 26.8 cm× 27.3 cm plastic bags. Across all four sampling dates, a total of 4800 soil samples were taken. Soil samples were dried at room temperature, sieved through a 2mm mesh and stored in -20 °C freezer in darkness until further processing. In preliminary testing, three different methods for weed seedbank measurement were evaluated (Mesgaran et al. 2007; Sanderson et al. 2014; Simard et al. 2011) to determine the best method of estimating weed seedbank. Based on the results, the direct greenhouse germination procedure (Sanderson et al. 2014; Thompson and Grime 1979) was selected. In the greenhouse, 120 g of soil from each depth was mixed with 150 mL of sterile growing mix (Sunshine Professional Growing Mix, Seba Beach, AB., Canada) and spread to a depth of 1cm on clear plastic trays (24×16×5.5 cm). Trays were kept at a temperature of 25-20 °C day/night with a 16-8 h light/dark cycle, and were watered by tap water once a day. Germinated seeds were identified to species, counted and removed every week for a period of one month. Unidentified seedlings were transplanted to other trays to allow growth until identification was possible. Seedlings that emerged but died before identification was possible, were counted and categorized as unknown species. At the end of the month, trays were air dried for three days to prevent the growth of algae, watered with 50 mL tap water and transferred to +2 °C fridge for a period of one month to break seed dormancy before being returned to the greenhouse. The soil in each tray was stirred by hand to stimulate germination of remaining weed seeds. The process of 'fridge-to-greenhouse' was repeated five times, as this time frame was considered adequate to reveal the majority of germinated seed in the soil by Tracy and Sanderson (2000). During our study, 64% of seedlings emerged in the first greenhouse period and more than 94% emergences were recorded in the first, second and third greenhouse periods. Less than 1% seedlings observed were from the fifth greenhouse period (Appendix 9.9).

5.2.5 Statistical Analysis

The effect of different forage species, forage mixtures, soil depth and sampling date on the weed seedbank and aboveground weed populations were analyzed as a split plot design via a mixed model (PROC MIXED; SAS Server Interface 2.0.4). Each model included the main effect of forage species, sub effect of sampling date and their interactions; block was a random effect. Forage species were grouped into monocultures and mixtures for statistical analysis. Weed seedbank and aboveground weed population in all treatments, monocultures and monocultures vs. mixtures were analyzed separately. Normality of residuals and equality of variance were examined using Shapiro-Wilk *W* test. Data were log (x+1) transformed to meet normality assumption if needed. Graphs are presented based on untransformed data, but significance tests were performed on analysis of transformed data. A significance value of $P < 0.05$ was used and mean comparisons made using Fisher's Protected LSD test at $P = 0.05$. The data related to weed seedbank and aboveground weed population in mixtures containing different forage species (Figure 5.6) were not analyzed statistically since each data point was used more than once (i.e. each category (mixture containing a particular species) contained data points that contributed to another category as well), and thus were not statistically independent.

5.3 Results

5.3.1 Aboveground Weed Population and Soil Seedbank

Aboveground weed populations were significantly affected by forage species and the interaction of forage species by year (Table 5.2). A total of 20 different weed species from 6 plant families were identified (Table 5.3); 11 annual and biennial dicots, eight perennial dicots and one perennial grass. Dicot weeds were the most abundant and more than half of the species were Asteraceae. The most abundant aboveground weeds were narrow-leaved hawk's beard, dandelion, foxtail barley, horseweed and Canada thistle.

In the soil seedbank a total of 8994 seedlings were identified for a total of 28 species from 11 plant families (Table 5.3). The soil seedbank was significantly affected by forage species, depth, sampling date and their interactions (Table 5.2). There were 19 annual or biennial dicots, eight perennial dicots, one perennial grass, one annual grass and one gymnosperm. Dicotyledonous weeds were the most common group, particularly species of Asteraceae. Biennial wormwood was the most abundant species in the soil seedbank and contributed 63% of all germinated seeds (Figure 5.3C). In addition to biennial wormwood, the three other dominant weeds were stinkweed, purslane and flixweed which accounted for an additional 23% of seeds. Seed density of biennial wormwood, purslane and stinkweed did not significantly vary with sampling date, whereas the density of flixweed was significantly higher in September of both years.

Thirteen weed species were present in both the weed seedbank and aboveground weed population, and seven species were exclusively recorded from the aboveground population including six species of Asteraceace (Table 5.3). Although flixweed seeds were present in many soil samples, flixweed was only present aboveground in the blank or non-seeded plots. Canada thistle, on the other hand, was recorded in aboveground measurements, but no seeds were recorded in the seedbank. The most abundant weeds in the soil seedbank were the least abundant weeds in the aboveground population and vice versa (Table 5.4).

In monoculture plots, forage species significantly influenced the composition and abundance of aboveground weed populations. Monocultures of WWG had the lowest weed density, legumes the highest, and among C_4 species LBS had a lower density of aboveground weeds than SOG (Figure 5.1). The number of aboveground weeds was lower in the dry year of 2015 compared to the wet year of 2014 (Figure 5.1); in both years weed populations were significantly lower in mixtures compared to monocultures (Table 5.2, Figure 5.2).

Table 5.2 *F* statistics and *P* values indicating statistical significance for the weed seedbank of all treatments, monocultures and monocultures vs. mixtures in both weed seedbank and aboveground population in Swift Current SK.

	F ratio and *P* value					
Source	Weed Seedbank			Aboveground Weed Population		
	All Treatments	Monocultures	Monocultures vs. Mixtures	All Treatments	Monocultures	Monocultures vs. Mixtures
Treatments	$F_{29,87} = 3.78$, $P < 0.0001$	$F_{6,18} = 13.55$, $P < 0.0001$	$F_{1,3} = 12.92$, $P = 0.0369$	$F_{29,87} = 11.62$, $P < 0.0001$	$F_{6,18} = 6.23$, $P = 0.0011$	$F_{1,3.53} = 6.64$, $P = 0.0697$
Sampling date	$F_{3,957} = 1818$, $P < 0.0001$	$F_{3,231} = 4.34$, $P = 0.0053$	$F_{3,314} = 5.80$, $P = 0.0006$	$F_{1,90} = 3.38$, $P = 0.0692$	$F_{1,21} = 2.73$, $P = 0.1134$	$F_{1,214} = 0.14$, $P = 0.7109$
Treatments ×Sampling date	$F_{87,957} = 1.41$, $P = 0.0089$	$F_{18,231} = 2.06$, $P = 0.0080$	$F_{3,314} = 1.03$, $P = 0.3792$	$F_{29,90} = 1.76$, $P = 0.0227$	$F_{6,21} = 0.41$, $P = 0.8667$	$F_{1,214} = 0.06$, $P = 0.8077$
Depth	$F_{2,957} = 1738.86$, $P < 0.0001$	$F_{2,231} = 437.74$, $P < 0.0001$	$F_{2,314} = 655.61$, $P < 0.0001$			
Treatments ×Depth	$F_{58,957} = 5.68$, $P < 0.0001$	$F_{12,231} = 14.92$, $P < 0.0001$	$F_{2,314} = 19.93$, $P < 0.0001$			
Sampling date ×Depth	$F_{6,957} = 2.58$, $P = 0.0174$	$F_{6,231} = 2.60$, $P = 0.0186$	$F_{6,314} = 3.04$, $P = 0.0059$			
Treatments ×Sampling date×Depth	$F_{174,957} = 1.26$, $P = 0.0219$	$F_{36,231} = 1.98$, $P = 0.0014$	$F_{6,314} = 0.67$, $P = 0.6745$			

Table 5.3 Latin name, common name and plant family of germinated seeds in soil seedbank and aboveground weed population.

Latin Name	Common Name	Family	Life Cycle	Morphotype
Only in weed seedbank				
Amaranthus retroflexus L.	Redroot pigweed	Amaranthaceae	A[*]	D[**]
Artemisia biennis Willd.	Biennial wormwood	Asteraceae	A, B	D
Brassica kaber (DC.) L.C. Wheeler	Wild mustard	Brassicaceae	A	D
Capsella bursa-pastoris (L.) Medik.	shepherd's-purse	Brassicaceae	A	D
Chenopodium album L.	Lamb's quarters	Chenopodiaceae	A	D
Echinochloa crusgalli (L.) Beauv	Barnyard grass	Poaceae	A	M
Kochia scoparia (L.) Schrad.	Kochia	Amaranthaceae	A	D
Lepidium densiflorum Schrad.	Common pepper-grass	Brassicaceae	A, B	D
Monolepis nuttalliana (Schult.) Greene	Goosefoot	Chenopodiaceae	A	D
Pinus sylvestris L.	Scots pine	Pinaceae	P	G
Portulaca oleracea L.	Purslane	Portulacaceae	A	D
Rumex pseudonatronatus (Borbás) Borbás ex Murb.	Field dock	Polygonaceae	P	D
Salsola pestifer A. Nels.	Russian thistle	Amaranthaceae	A	D
Solanum triflorum Nutt.	Wild tomato	Solanaceae	A	D
Thlaspi arvense L.	Stinkweed	Brassicaceae	A	D

Table 5.3 Continued. Latin name, common name and plant family of germinated seeds in soil seedbank and aboveground weed population.

Latin Name	Common Name	Family	Life Cycle	Morphotype
In both weed seedbank and aboveground population				
Conyza Canadensis (L.) Cronquist	Horseweed	Asteraceae	A	D
Crepis tectorum L.	Narrow-Leaved Hawk's Beard	Asteraceae	A	D
Descurainia sophia (L.) Webb ex Prantl	Flixweed	Brassicaceae	A	D
Hordeum jubatum L.	Foxtail barley	Poaceae	P	M
Lactuca scariola L.	prickly lettuce	Asteraceae	A, B	D
Melilotus albus Medik.	White sweet clover	Fabaceae	A, B, P	D
Melilotus officinalis (L.) Lam.	Yellow sweet clover	Fabaceae	A, B, P	D
Polygonum aviculare L.	Prostrate knotweed	Polygonaceae	A, P	D
Polygonum convolvulus L.	Wild buckwheat	Polygonaceae	A	D
Potentilla norvegica L.	Rough cinquefoil	Rosaceae	A, B, P	D
Solidago Canadensis L.	Canadian goldenrod	Asteraceae	P	D
Solidago missouriensis Nutt.	Missouri goldenrod	Asteraceae	P	D
Taraxacum officinale F.H. Wigg.	Dandelion	Asteraceae	P	D

Table 5.3 Continued. Latin name, common name and plant family of germinated seeds in soil seedbank and aboveground weed population.

Latin Name	Common Name	Family	Life Cycle	Morphotype
Only in aboveground population				
Achillea millefolium L.	Yarrow	Asteraceae	P	D
Artemisia frigida Willd.	Pasture sage	Asteraceae	P	D
Cirsium arvense (L.) Scop.	Canada thistle	Asteraceae	P	D
Lactuca pulchella (L.) C.A. Mey.	Blue lettuce	Asteraceae	B, P	D
Medicago lupulina L.	Black medic	Fabaceae	A, P	D
Sonchus asper (L.) Hill	Spiny annual sow-thistle	Asteraceae	A	D
Tragopogon dubius Scop.	Goat’s-beard	Asteraceae	A	D

[*] A: Annual; B: Biennial; P: Perennial

[**] D: Dicotyledonous; M: Monocotyledon; G: Gymnosperm

5.3.2 Distribution of Seeds in the Soil Layers

Soil depth, sampling date and their interactions had significant effects on the weed seedbank (Figure 5.3A, Table 5.2). This effect was greatly dependent upon the third sampling date, as indicated by the significant interaction. Weed abundance varied significantly with sampling date in depth 0-5 cm; remained stable at an average of 450 seeds m^{-2} in depth 5-10 cm; and decreased significantly from 178 seeds m^{-2} in May 2014 to 85 seeds m^{-2} in September 2015 in depth 10-15 cm. About 77%, 18% and 5% of germinated seeds were counted from depth 0-5, 5-10 and 10-15 cm, respectively. The maximum number of germinated seeds came from samples taken in September after most plants had dispersed their seeds.

5.3.3 Annual, Biennial and Perennial Weeds in the Seedbank

There were significant differences in the density of biennial, annual and perennial weeds between sampling dates. This effect was dependent upon sampling date, as indicated by the significant interaction between date and life cycles ($F_{6,1419} = 5.29$, $P < 0.0001$).

Table 5.4 Percentage of the most abundant weeds in the soil seedbank and aboveground population.

Common and Latin Name	Weed seedbank	Aboveground population
Abundant in weed seedbank	-------------- %	--------------
Biennial wormwood (*Artemisia biennis* Willd.)	63	0
Purslane (*Portulaca oleracea* L.)	10	0
Flixweed (*Descurainia sophia* (L.) Webb ex Prantl)	8	<1
Stinkweed (*Thlaspi arvense* L.)	5	0
Abundant in aboveground weed population		
Narrow-Leaved Hawk's Beard (*Crepis tectorum* L.)	<1	31
Dandelion (*Taraxacum officinale* F.H. Wigg.)	<1	29
Foxtail barley (*Hordeum jubatum* L.)	<1	17
Horseweed (*Conyza canadensis* (L.) Cronquist	1	4
Canada thistle (*Cirsium arvense* (L.) Scop.)	0	2

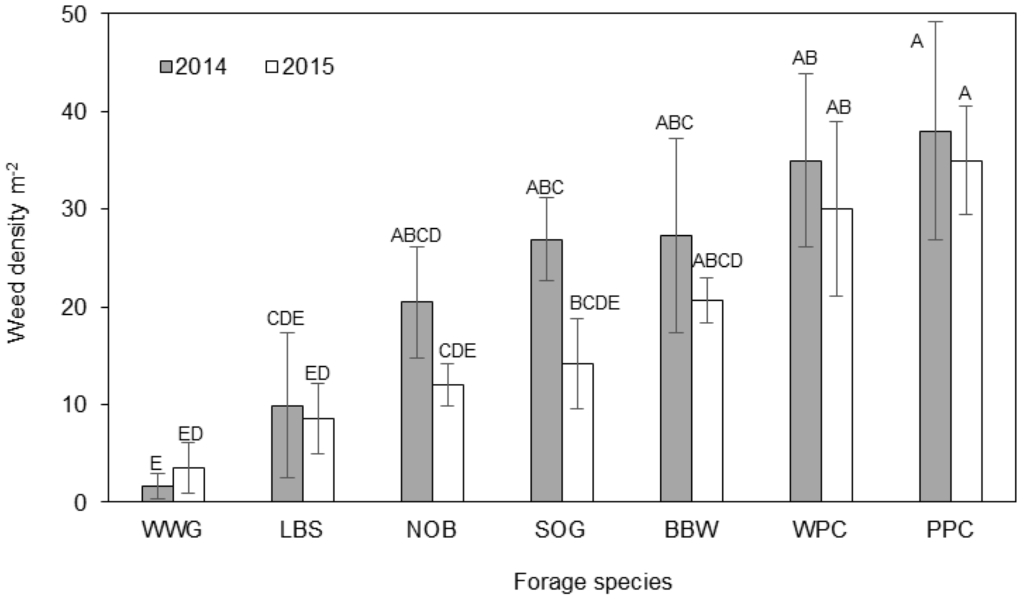


Figure 5.1 Aboveground weed density in monoculture plots in 2014 and 2015. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; *n* = 4. Bars with the same letter are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

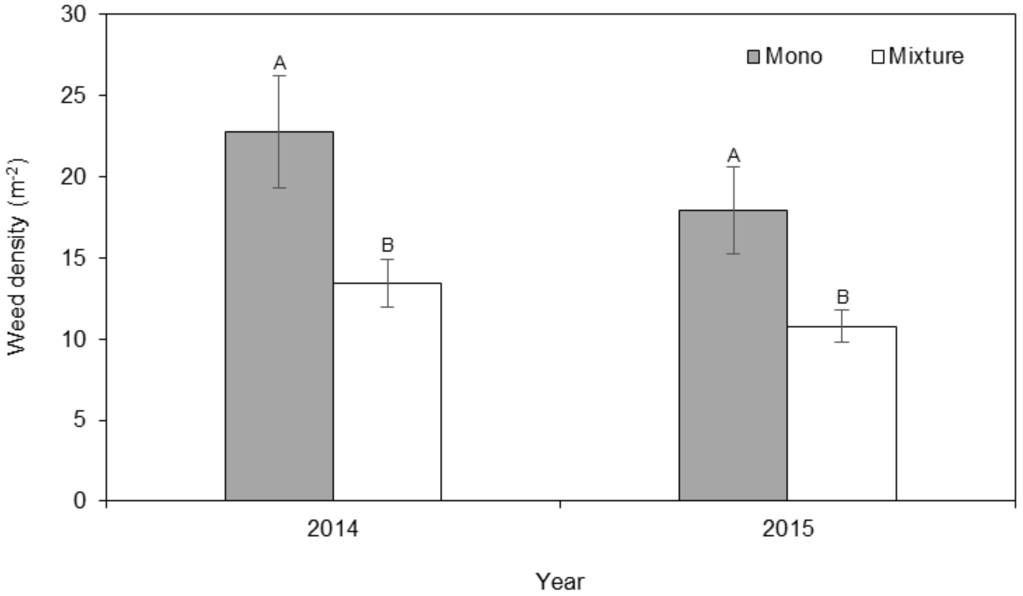


Figure 5.2 Aboveground weed density in monoculture and mixture plots in 2014 and 2015. The density of weeds between monoculture and mixture plots were statistically different in 2014 and 2015. Error bars represent one standard error around the mean; *n* = 28 and 84 for monoculture and mixture, respectively. Bars with the same letter are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

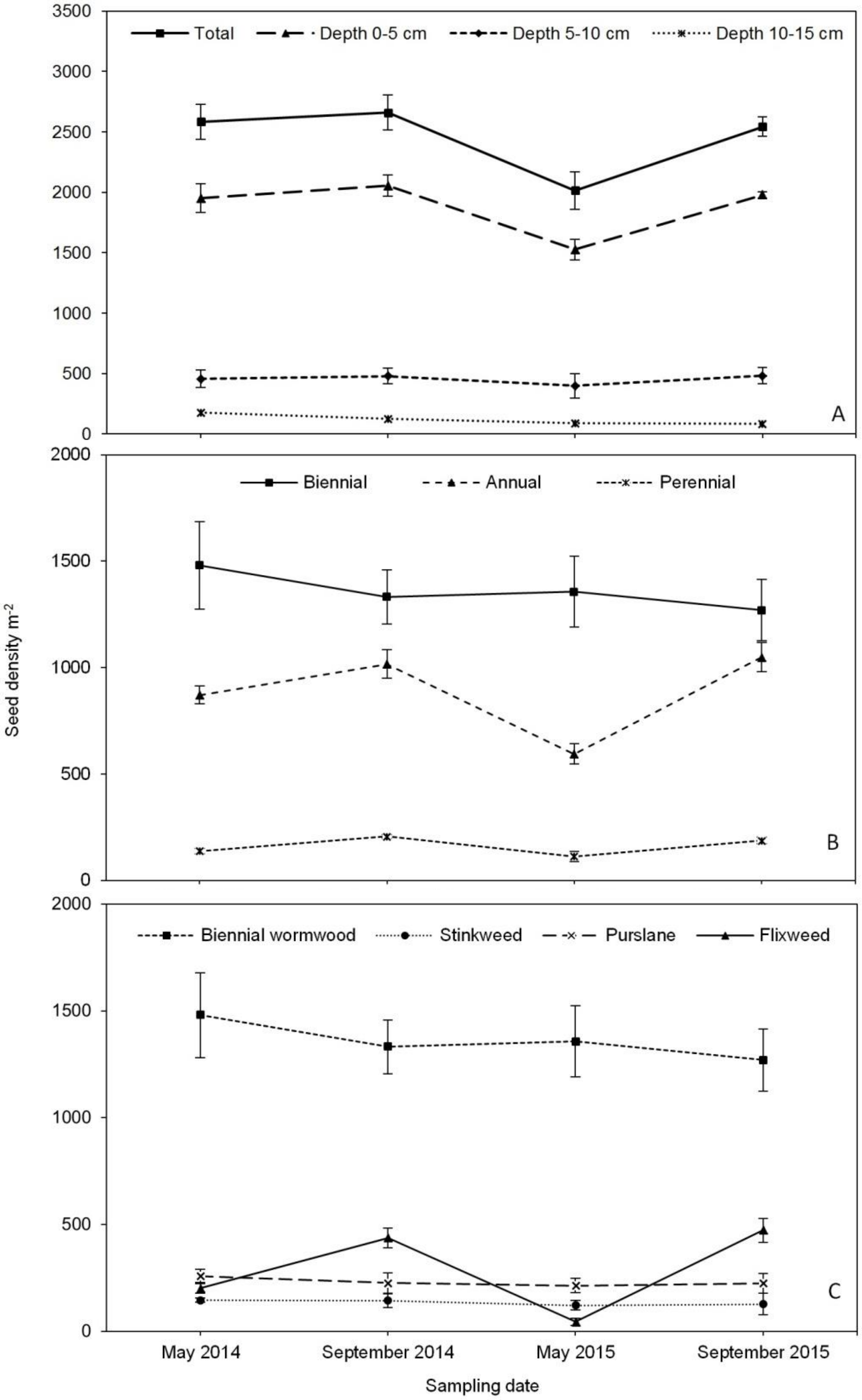


Figure 5.3 (A) Number of seeds m^{-2} from all weed species in depth 0-5, 5-10 and 10-15 cm and in total (0-15 cm) at four sampling dates; (B) Density of biennial, annual and perennial weeds in all treatments at four sampling dates; (C) Density of the most abundance species: biennial wormwood, stinkweed, purslane and flixweed in all treatments at four sampling dates. Error bars represent one standard error around the mean; *n*=120.

This interaction reflects the number of germinated seeds, particularly annuals. About 57% of recorded species had a biennial life cycle, 37% were annuals and 6% perennials (Figure 5.3B). Biennial wormwood, flixweed and dandelion were the dominant biennial, annual and perennial weeds, respectively. Seeds of these species were present in all treatments. The total density of biennials, annuals and perennials varied with sampling date.

5.3.4 Weed Seedbank in Monocultures and Mixtures

There were significant differences between monoculture plots (Figure 5.4, Table 5.2). Sampling date and sampling date by treatment interaction were not significant. Monocultures of WWG contained the lowest number of weeds in the seedbank (1100 seeds m^{-2}), but were not significantly different from two other C_3 grasses, whereas PPC and WPC contained the highest number of weed seeds (3200 and 3400 seeds m^{-2}, respectively). Between the two C_4 species, LBS contained significantly lower numbers of seeds compared to SOG.

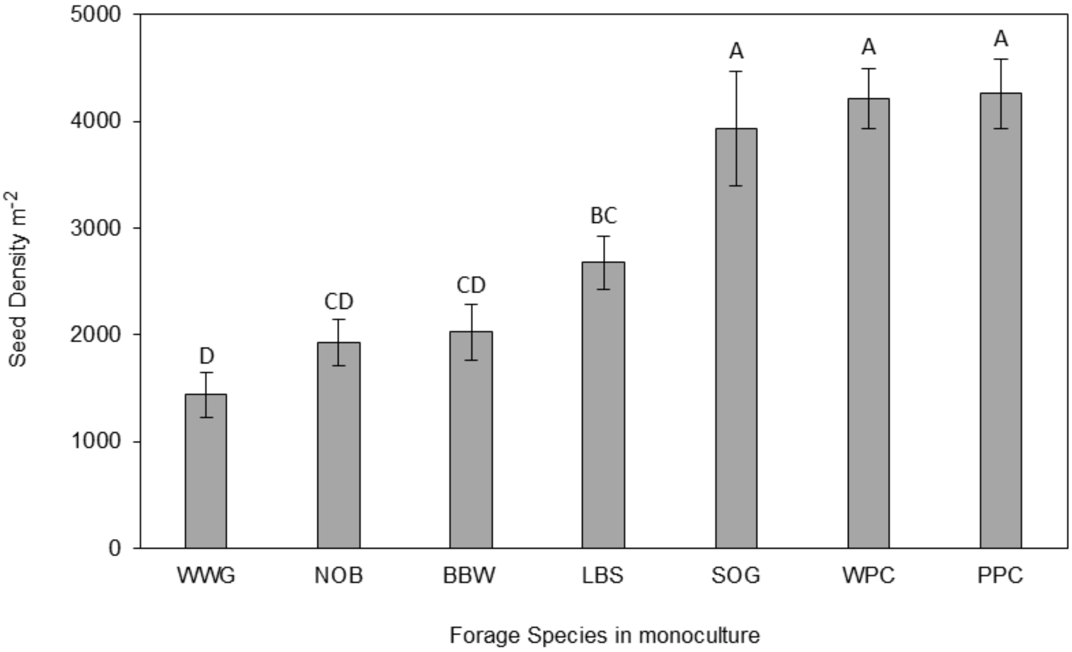


Figure 5.4 Average of weed seed bank density in monoculture plots from four sampling dates. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; *n* = 16. Bars with the same letter are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

The abundance of weeds in the seedbank was lower in mixtures compared to monocultures at all depths and sampling dates (Figure 5.5). The density of seeds in the weed seedbank between monoculture and mixture plots were significantly different in the depth 0-5 cm but not at depths 5-10 and 10-15 cm. Among mixtures, those that included WWG had the lowest seed density m^{-2}, whereas those that included legumes contained the highest density (Figure 5.6).

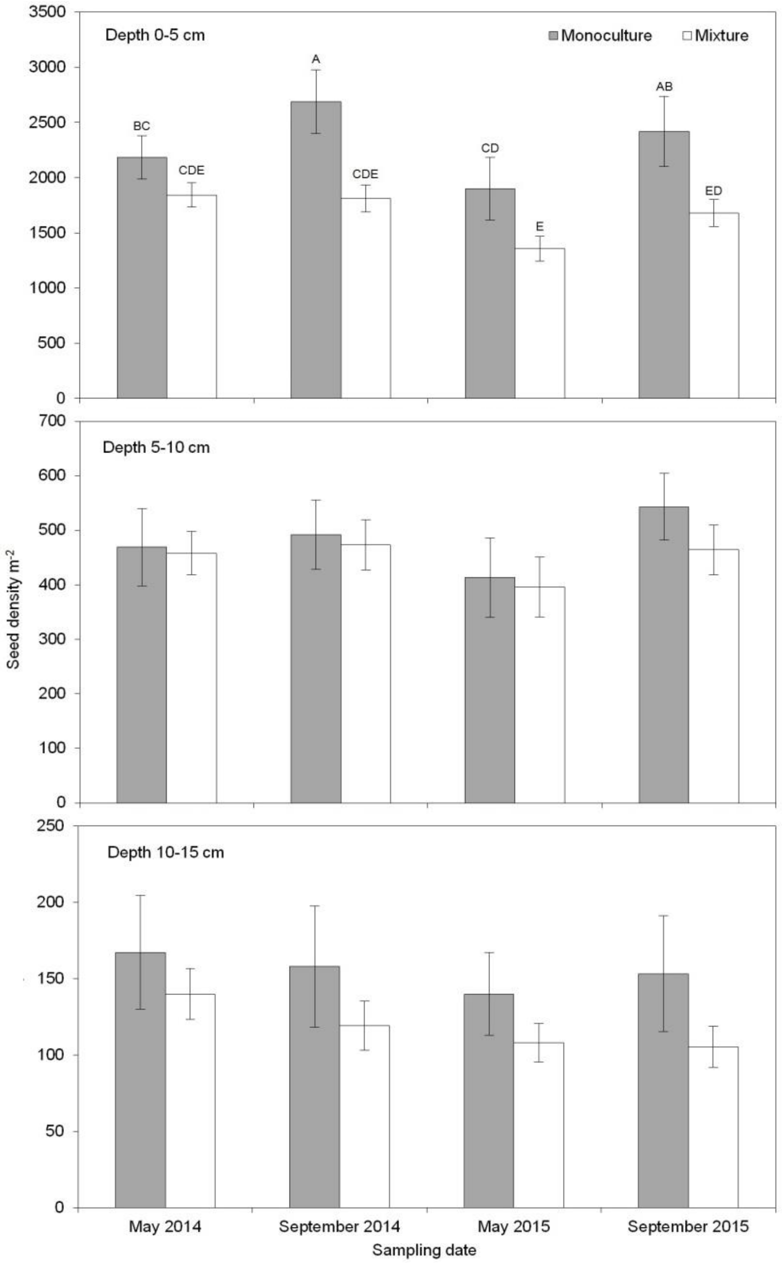


Figure 5.5 Weed seed bank density in monoculture and mixture plots in three depths: 0-5, 5-10 and 10-15 cm. The density of weed seed bank between monoculture and mixture plots were statistically different in depth 0-5 cm but not statistically different in depth 5-10 and 10-15 cm. Error bars represent one standard error around the mean; *n* = 28 and 84 for monoculture and mixture, respectively. Bars with the same letter are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

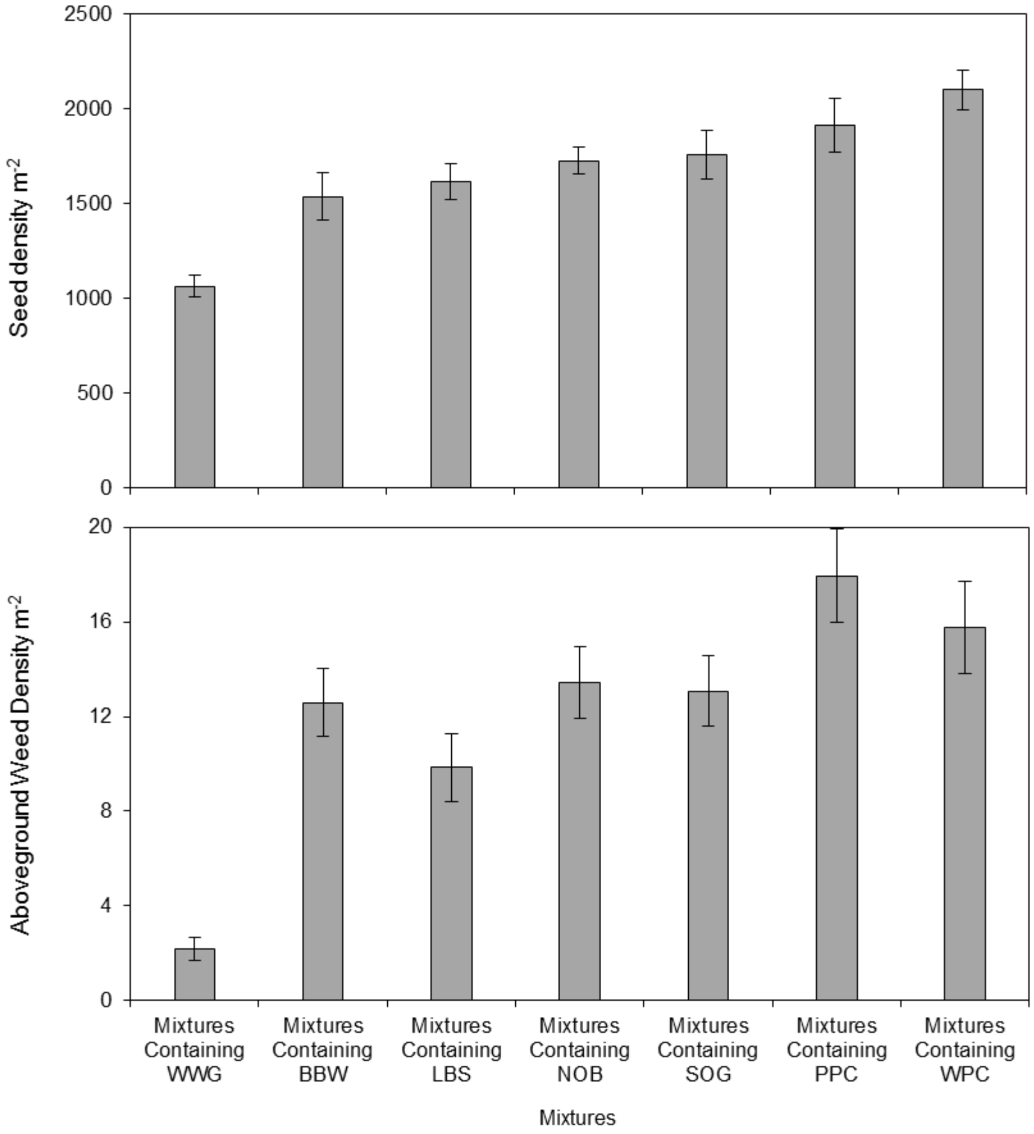


Figure 5.6 Weed seedbank and aboveground weed population in mixture plots containing different forage species. The data related to these graphs was not statistically analyzed since each data was used more than once to make the bars. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; *n* = 96 for weed seedbank and *n*=48 for aboveground weed population.

5.4 Discussion

5.4.1 Effect of Forage Mixtures on Weed Seedbank and Aboveground Population

Mixtures of forage species contained lower number of weeds in the seedbank and aboveground weed populations compared to monocultures. This demonstrates that increasing forage mixture diversity can be an effective ecological and non-chemical weed control tactic in seeded pastures. Among mixtures, those of which WWG was a component contained lower abundance of weeds in the seedbank and aboveground population. In the present study, the results of forage yield also showed higher forage productivity of mixtures containing WWG compared to other mixtures (Chapter 3). Thus, inclusion of highly productive and strong competitor forage species like WWG in the mixtures, seems to have both positive effect on forage productivity and strong negative effect on weed seedbank and aboveground populations.

Lower weed populations in more diverse plant communities have been reported in many studies (Crawley et al. 1999; Hector et al. 2001; Knops et al. 1999; Lyons and Schwartz 2001; Naeem et al. 2000; Pfisterer et al. 2004; Picasso et al. 2008). This phenomenon can be explained in two ways (Wardle 2001): (1) multiple species exhibit complementarity in resource use limits the ability of other species to enter the community and (2) a sampling effect that makes it more likely that a diverse mixture will contain a highly productive or competitive species that monopolizes resources. In this study, it seems that the ‘sampling effect’ is a more likely explanation in the reduction of weed densities in the seedbank and aboveground population in the mixtures containing WWG. WWG is a sod-forming rhizomatous grass and was the strongest competitor among the species in this study (Zhang and Lamb 2011). WWG dominated (more than 87% of the biomass) forage mixtures in the second year after seeding and thereafter (Chapter 3). Crawley et al. (1999) similarly found that the sampling effect from including *Alopecurus pratensis* in the mixture reduced invasive species’ biomass much more effectively than other species in the mixture. *A. pratensis* is an aggressive rhizomatous perennial grass with rapid growth in spring and dense canopy that can grow to a maximum of 1.2 m (Crawley et al. 1999). However, other studies have identified complementarity as a primary driver of mixture effects on weed populations. For example, Naeem et al. (2000) concluded that resource use complementarity best explained the lower narrow-leaved hawk's beard (*Crepis tectorom*) density in diverse forage communities, where higher species diversity decreased available light and nutrients to *C. tectorom* and decreased its success to invade a new area.

5.4.2 Aboveground Weed Population vs. Weed Seedbank

Among the 35 identified weed species aboveground and in the seedbank, only 13 weeds were common in both populations. The most abundant weed in the seedbank was biennial wormwood followed by purslane, both of which were absent in aboveground weed populations. Conversly, narrow-leaved hawk's beard followed by dandelion were the most abundant weeds aboveground, but least abundant in the seedbank. While some studies have found strong relationships between the weed seedbank and aboveground communities (Dessaint et al. 1997; Rahman et al. 2006; Rahman et al. 2001; Zhang et al. 1998), others have found low correlations (Cardina and Sparrow 1996; Tracy and Sanderson 2000; Webster et al. 2003). Generally, low similarity between aboveground plant community and seedbank has been reported for perennial-dominated plant communities (Bakker et al. 1996; Milberg 1995; Rabinowitz 1981; Schenkeveld and Verkaar 1984; Thompson and Grime 1979), and greater similarity in annual communities (Chang et al. 2001; Moore 1980; Unger and Woodell 1993; Unger and Woodell 1996).

5.4.3 Temporal Variability of Weed Seedbank and Aboveground Population

Weed seedbank varied seasonally, reached a minimum in early spring and maximum in late summer. Seed mortality during the fall and winter can explain the reduced weed seedbank in April, while the seed rain from existing weeds during the growing season will increase the seedbank reservoir in late season. Changes in weed seedbank size from 2014 to 2015 were also observed. Aboveground weed populations were generally low in 2015 compared to 2014. Since 2015 was one the driest years on record for the region, a decrease in weed growth and weed seed production is not surprising. In a related study, we found that the productivity of these native forage species was not significantly affected by the dry conditions in 2015 (Chapter 3), suggesting that germination conditions may be a stronger driver of aboveground weed community dynamics than the perennial plant community in this system.

5.5 Conclusion

Our results demonstrated that mixtures of forage species promote lower weed densities in the seedbank and aboveground compared to monocultures. Among mixtures, those containing WWG had lower abundance of weeds in the seedbank and aboveground weed populations. Species composition of mixtures had an effect on weed seedbank and aboveground weed populations. WWG, a strong perennial rhizomatous grass, limited the presence of weedy species in the mixtures stronger than other grasses. The weed seedbank varied seasonally with the minimum weed seeds in early spring and maximum in late summer. The most abundant weeds in the seedbank were the least abundant weeds in aboveground population and vice versa. Among monocultures, WWG contained the lowest weed densities in the seedbank and aboveground. WWG showed promising results as a native forage species by demonstrating the potential to suppress weeds and reduce weed seed size when seeded in monocultures and mixtures.

CHAPTER 6

THE POTENTIAL OF SEVEN NATIVE NORTH AMERICAN FORAGE SPECIES TO SUPPRESS WEEDS THROUGH ALLELOPATHY

Abstract

We conducted a series of three greenhouse studies to study the allelopathic effects of seven native perennial North American forage species alone and in mixture on three problematic weeds including dandelion (*Taraxacum officinale*), scentless chamomile (*Matricaria perforata*) and foxtail barley (*Hordeum jubatum*). Shoot dry weight and root:shoot ratio of weeds were affected by leachate from forage species in all three experiments. In the first experiment, leachate from little blue stem (*Schizachyrium scoparium)* reduced the shoot dry weight of weeds up to 58%. In the second experiment, leachate of little blue stem, western wheatgrass (*Pascopyrum smithii*) and side-oats grama (*Bouteloua curtipendula*) reduced shoot dry weight of weeds up to 72% and this number increased up to 90% in the third experiment. In the last experiment, no synergistic effects of mixed leachate from different forage species on shoot dry weight of weeds were observed. In this study dandelion and foxtail barley allocated less to roots and shoots, respectively. In conclusion, the results showed that root leachate from western wheatgrass, little blue stem and side-oats grama can reduce the aboveground and belowground growth of weeds. These findings suggest that the use of allelopathic species may provide weed control and management benefits in seeded pastures and native prairie restorations.

6.1 Introduction

Pasture weeds can significantly reduce forage yield and quality, affecting livestock production qualities and increase pasture management costs (DiTomaso 2000). The economic cost of weeds in pastures can be more than that of insects and pathogens combined (Quimby et al. 1991). In addition to mechanical, cultural, chemical and biological weed control, selection of highly allelopathic species can be an effective strategy in pastures (Bailey et al. 2010; Jabran et al. 2015). Allelopathic weed control through the selection of forage species with high allelopathic properties for seeded pastures can be a practical and sustainable way to suppress weeds. In some agricultural systems, especially organic systems, allelopathic weed control can be one of the most important tactics available for suppressing weeds (Jabran et al. 2015). The allelopathic relationship between crops and weeds is a reciprocal relationship. Not only agricultural crops produce allelochemicals, but allelopathic potential of invasive weedy species in the process of invasion, may play an important role in displacing the native species (Inderjit et al. 2008; Mitchell et al. 2006). Allelopathy is the direct or indirect effect of the species on others by producing and releasing chemical compounds (Inderjit et al. 2005). Plants produce more than 100,000 primary and secondary chemical compounds, many of which can act as allelochemicals (Callaway and Howard 2007). Allelochemicals can affect the soil microbial community and chemical and physiological properties of the soil (Pedrol et al. 2006). Most allelochemicals are water soluble and can enter the environment through aboveground leaching, litter decomposition, shoot volatilization and root leachate (Reigosa et al. 1999). Allelochemical production in the roots of perennial plants can be affected by many factors like plant habitat, age of root, temperature, water stress, etc. (Inderjit and Callaway 2003; Reigosa et al. 1999).

There are many studies of the potential of allelopathic agricultural crops for weed control (Milchunas et al. 2011; Singh et al. 2003), but much less is known about the allelopathic potential of forage species in pastures. There are many benefits to using diverse mixtures of forage species in seeded pastures (Mischkolz et al. 2013; Mischkolz et al. 2016) and moreover, identifying those species with high allelopathic properties in the mixtures could reduce plant-weed competition, increase forage productivity and decrease the cost of weed control in pastures. A number of studies have investigated the allelopathic potential of forage species. For example, Ghebrehiwot et al. (2013) examined the allelopathic potential of root and leaf extracts of five native grassland species in South Africa on lettuce seeds, concluding that the dominance of a small number of grass species in South African grasslands can be linked to the allelopathic properties of those species. San Emeterio et al. (2004) found that the Mediterranean forage grass *Lolium rigidum* exerts allelopathic effects on some pasture plants including *Lolium multiflorum* Lam., *Dactylis glomerata* L., and *Medicago sativa* L. In another study done by Bokhari (1978) allelopathic potential of western wheatgrass (*Pascopyrum smithii*) on seed germination of blue grama (*Bouteloua gracilis*) and buffalo grass (*Bouteloua dactyloides*) was reported. Bokhari (1978) found that western wheatgrass extract inhibited seed germination of blue grama and buffalo grass up to 40%, and also extracts collected at an early growth stage of western wheatgrass was more allelopathic than a later growth stage. Western wheatgrass, one of the study species in this paper, is a perennial cool season grasses, native to North America and has a shallow root system (Monsen et al. 2004). In an ongoing field experiment, started by Mischkolz et al. (2013) anecdotal observations of lower weed populations in western wheatgrass plots and mixture of forage species were recorded. Therefore, we hypothesized that lower weed density in western wheatgrass plots might be linked to allelopathic activity of western wheatgrass. Root age can affect allelochemical production (Reigosa et al. 1999), thus in the second experiment, we hypothesized that our perennial forage species may produce more allelochemicals when they get older and are cut. In the third experiment, we hypothesized that leachate mixtures might show synergistic allelopathic effects on weeds. The objectives of this study were to evaluate (i) the allelopathic potential of seven native forage species in the third month of growth, (ii) the allelopathic potential of seven native forage species in the fifth month of growth and after cutting, and (iii) the effect of multispecies leachate mixtures on weeds.

6.2 Materials and methods

A series of three greenhouse experiments was conducted at the Agriculture and Agri-food Canada (AAFC), Swift Current Research and Development Centre (SCRDC) near Swift Current, Saskatchewan, Canada. We investigated the allelopathic effects of seven perennial native North American forage species with high agronomic potential as donor plants (Table 6.1). These species have been evaluated in greenhouse studies (Mischkolz et al. 2016) and have continued to be evaluated in field studies in Saskatoon and Swift Current, SK (Biligetu et al. 2014; Mischkolz et al. 2013; Schellenberg et al. 2012). Forage seeds were from the same seedlots as used in the aforementioned studies. Three problematic weeds with a wide geographic distribution were collected from Swift Current area and selected as target plants: dandelion (*Taraxacum officinale* F.H. Wigg.), scentless chamomile (*Matricaria perforata* Mérat) and foxtail barley (*Hordeum jubatum* L.). Seeds of dandelion and foxtail barley were collected from SCRDC farm, and scentless chamomile seeds were collected from open areas in Swift Current, SK in the spring and fall of 2014, respectively.

Table 6.1 Common name, Latin name, abbreviation and functional group of selected species.

Common and Latin Name	Abbreviation	Functional Group
Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve)	BBW	C_3 grass
Nodding brome (*Bromus porteri* (J.M. Coult.) Nash)	NOB	C_3 grass
Western wheatgrass (*Pascopyrum smithii* (Rydb.) Barkworth & D.R. Dewey)	WWG	C_3 grass
Little blue stem (*Schizachyrium scoparium* (Michx.) Nash)	LBS	C_4 grass
Side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.)	SOG	C_4 grass
Purple prairie clover (*Dalea purpurea* Vent.)	PPC	Legume
White prairie clover (*Dalea candida* Willd.)	WPC	Legume

In the first experiment, the allelopathic potential of perennial native forage species at the third month of growth was studied. The second experiment investigated the allelopathic potential of these forage species at the fifth month of growth, and the last experiment investigated multispecies effects of root leachate on weed growth. Because of the importance of aboveground and belowground competition in plant communities, shoot dry weight and root:shoot ratio were measured as response variables. In these experiments, specific allelopathic chemicals were not identified or measured. The three experiments are outlined in detail below. For all experiments, greenhouse conditions were maintained with day and night temperatures of 25 and 20°C respectively and a 16-h light 8-h dark cycle. The greenhouse temperature exceeded 27 °C on some hot days in summer.

6.2.1 Allelopathic Properties of Perennial Forages in the third month of growth (Experiment 1)

The first experiment was conducted to examine the allelopathic effects of five forage species in the third month of growth on weed growth and development. Purple prairie clover (PPC) and white prairie clover (WPC) were not included as these two species did not stablish properly. Treatments were arranged in a completely randomized 6×3 factorial design with four replicates. Treatment combinations included a control (leachate from unseeded pots) and leachate from five forage species, western wheatgrass (WWG), little bluestem (LBS), bluebunch wheatgrass (BBW), nodding brome (NOB) and side-oats grama (SOG) crossed with three weed species (dandelion, foxtail barley and scentless chamomile). One hundred seeds of each forage species were seeded in trays filled with a mixture of silica sand and sterile growing mix (2:1 v/v) (Sunshine Professional Growing Mix, Seba Beach, AB Canada). After two months, 20 uniform plants of each forage species (Donor Plants) were transplanted into conical shaped pots (6.5 cm diameter×25 cm depth) (one plant per pot) filled with silica sand (20-40 grit, Target® Filter Sand, Morinville, AB Canada). A control treatment of twenty conical pots with no plants was filled with silica sand and watered with tap water the same way as other conical pots. Forage species were watered daily with 25 mL, 40 mL and 60 mL tap water in the first month, second month and thereafter, respectively. As root biomass increased over time, plant water uptake increased, therefore the amount of tap water applied was also increased in order to capture the same amount of leachate every day. Since we grew plants in silica sand, control and forage species pots were fertilized once a week with 1g/L of 20-20-20 NPK fertilizer solution (Winter "Plus" Formula, with chelated trace elements; The Professional Gardener Co. LTD. Calgary, AB Canada). Conical pots were arranged so that leachate from all 20 pots of each forage species was collected every day after each watering, in a single tray underneath, and weeds were watered by the collected leachate on the same day.

Three months after seeding the forage species, 100 seeds of each weed species were germinated in trays filled with the mixture of silica sand and growing mix (2:1 v/v). Three weeks after planting, uniform weed seedlings were transplanted into square pots (6.5×6.5×8.5 cm) filled with silica sand (one plant per pot) and then watered with leachate collected from forage species. Each weed species was watered with 20 ml of forage species leachate every day for a period of 30 days. The control weeds were watered with water that had passed through conical pots absent of forage species. At the end of the first experiment, weeds were harvested and roots were washed, and dried in 60 °C oven for 72 hours and then shoot and root dry weights were measured.

6.2.2 Allelopathic Properties of Forage Species in the fifth month of growth and after cutting (Experiment 2)

In the second experiment the increasing effect of allelopathic material in the root of forage species in the fifth month of growth and after cutting was studied. The cutting was included to simulate normal defoliation expected in the pastures and hayfields. Treatments were arranged in a 7×3 factorial structure using a completely randomized design with four replications. Treatment combinations included the control and leachate from six forage species (WWG, LBS, NOB, SOG, PPC, WPC) and a control crossed with three weeds (dandelion, foxtail barley and scentless chamomile). In this experiment and the next one, BBW was removed as that species was infested with disease after the first experiment; PPC and WPC were added to the experiments as donor plants. The legumes were seeded at the same time as the other species but since they were slow in growth and development, they were not included in the first experiment. The process for preparing pots containing PPC and WPC were the same as other species described for the first experiment. Forage species grown for the first experiment were cut before flowering stage at the end of the first experiment to a height of 2.5 cm and cut biomass was removed from the pots. Forage species were cut to simulate harvesting where forage species are cut either by animal or machinery. Pots containing weeds were prepared as in the first experiment. One month after cutting the forage species, weeds began to be watered with 20 ml of forage species leachate every day for a period of 30 days. At the end of the second experiment, weeds were harvested and roots were washed, dried in 60 °C oven for 72 hours and then shoot and root dry weights measured.

6.2.3 Root Leachate Mixtures (Experiment 3)

In the third experiment dandelion and scentless chamomile were watered with a mixture of forage species leachate. Foxtail barley was not included in the third experiment as there was not enough leachate available to water three weed species. In an ongoing field experiment started by Mischkolz et al. (2013), anecdotal observations of lower weed density in mixture plots prompted us to hypothesize that multispecies mixtures of leachate may have synergistic effects on weed suppression. This experiment was a completely randomized 22×2 factorial design with four replications. Treatment combinations included control and leachate from six forage species (WWG, LBS, NOB, SOG, PPC, WPC and control) and crossed with two weeds (dandelion and scentless chamomile). Forage species established for the first experiment were cut for a second time at the end of the second experiment before flowering stage to a height of 2.5 cm and cut biomass was removed from the pots. Pots containing dandelion and scentless chamomile were prepared the same as the first experiment. One month after cutting forage species, weeds were watered every day using leachate of forage species for a period of one month. Treatments included leachate from each forage species alone and all possible two-species combinations. As in previous experiments, leachate of forage species were collected and two-species mixtures were made using a 1:1 ratio of leachate from each, and then weeds were watered with 20 ml of either mixed or single species leachate. As with previous experiments, control weeds were watered using water passed through conical pots without plants. At the end of the third experiment, weeds were harvested and roots were washed, and dried in 60 °C oven for 72 hours and then shoot and root dry weights were measured.

6.2.4 Statistical analysis

The data were analyzed with analysis of variance (ANOVA) via a general linear model (PROC GLM; SAS Server Interface 2.0.4) to examine the effects of leachate treatments and weed species on weed shoot and root dry weight. Each model included the main effects of leachate source, weed species, and their interaction. Normality of residuals were examined using Shapiro-Wilk W test and homogeneity of variance were evaluated using Levene's test; based on the results no data transformations were needed. A significance value of $P < 0.05$ was used and mean comparisons made using Fisher's Protected LSD test at $P = 0.05$.

6.3 Results

6.3.1 Allelopathic Potential of Perennial Forages in the third month of growth (Experiment 1)

In the first experiment, leachate from LBS reduced significantly the shoot dry weight of dandelion, foxtail barley and scentless chamomile by 52, 46 and 58%, respectively compared to the control (Figure 6.1, left graphs, Table 6.2). Leachate released by WWG, SOG and NOB decreased the shoot dry weight of weeds by 33-53% compared to the control. Leachate from NOB and BBW reduced the root:shoot ratio of dandelion and foxtail barley significantly; but, the root:shoot ratio in scentless chamomile increased compared to the control after applying leachate (Figure 6.1). The average percentage shoot reductions of dandelion, foxtail barley and scentless chamomile from all leachates were 41, 34 and 51%, respectively.

Table 6.2 *F* statistics and *P* values indicating statistical significance for the weeds, forage species leachate and their interactions on the root and shoot dry weight of dandelion, foxtail barley and scentless chamomile.

		Weed	Forage Species	Weed×Forage Species
First Experiment	Shoot Dry Weight	$F_{2,54}=119.13$, $P < 0.0001$	$F_{5,54}=60.67$, $P < 0.0001$	$F_{10,54}=7.53$, $P < 0.0001$
	Root:Shoot Ratio	$F_{2,54}=43.35$, $P < 0.0001$	$F_{5,54}=3.65$, $P = 0.0064$	$F_{10,54}=5.74$, $P < 0.0001$
Second Experiment	Shoot Dry Weight	$F_{2,63}=85.46$, $P < 0.0001$	$F_{6,63}=45.76$, $P < 0.0001$	$F_{12,63}=2.88$, $P = 0.0032$
	Root:Shoot Ratio	$F_{2,63}=8.90$, $P = 0.0004$	$F_{6,63}=20.42$, $P < 0.0001$	$F_{12,63}=2.21$, $P = 0.0219$
Third Experiment	Shoot Dry Weight	$F_{1,132}=64.44$, $P < 0.0001$	$F_{21,132}=44.99$, $P < 0.0001$	$F_{21,132}=1.83$, $P = 0.0213$
	Root:Shoot Ratio	$F_{1,132}=85.18$, $P < 0.0001$	$F_{21,132}=2.84$, $P = 0.0002$	$F_{21,132}=1.81$, $P = 0.0230$

6.3.2 Allelopathic Properties of Forage Species in the fifth month of growth and after cutting (Experiment 2)

In the second experiment, suppression effects of WWG, SOG and LBS leachate on target weeds were up to 72% compared to the control (Figure 6.2, Table 6.2). The largest suppressive effect was from WWG leachate on scentless chamomile. Leachate from WPC and PPC had lesser effects and did not perform as well as other forage leachate on target weeds in the second experiment. Root:shoot ratios in dandelion were reduced by WWG, SOG and LBS leachate compared to the control, but increased in foxtail barley and scentless chamomile (Figure 6.2).

6.3.3 Root Leachate Mixtures (Experiment 3)

In the third experiment, suppressive effects of leachate from LBS, SOG and WWG on shoot dry weight of target weeds were up to 88% compared to the control (Figure 6.3, Table 6.2). Similar to the second experiment, leachate of PPC and WPC were not as suppressive as other leachate. Leachates from each forage species alone reduced the shoot dry weight of dandelion and scentless chamomile more than two-species mixtures; no synergistic effects of mixed leachate on shoot dry weight of dandelion and scentless chamomile were thus observed (Figure 6.3). Leachate of LBS significantly reduced the root:shoot ratio of scentless chamomile compared to leachate from SOG and WWG, but in dandelion the root:shoot ratio was similar among different non-mixed leachate. The results in the third experiment showed that dandelion was more susceptible to leachate from all treatments than that of scentless chamomile. Average shoot weight of dandelion and scentless chamomile from all treatments reduced up to 67 and 49%, respectively.

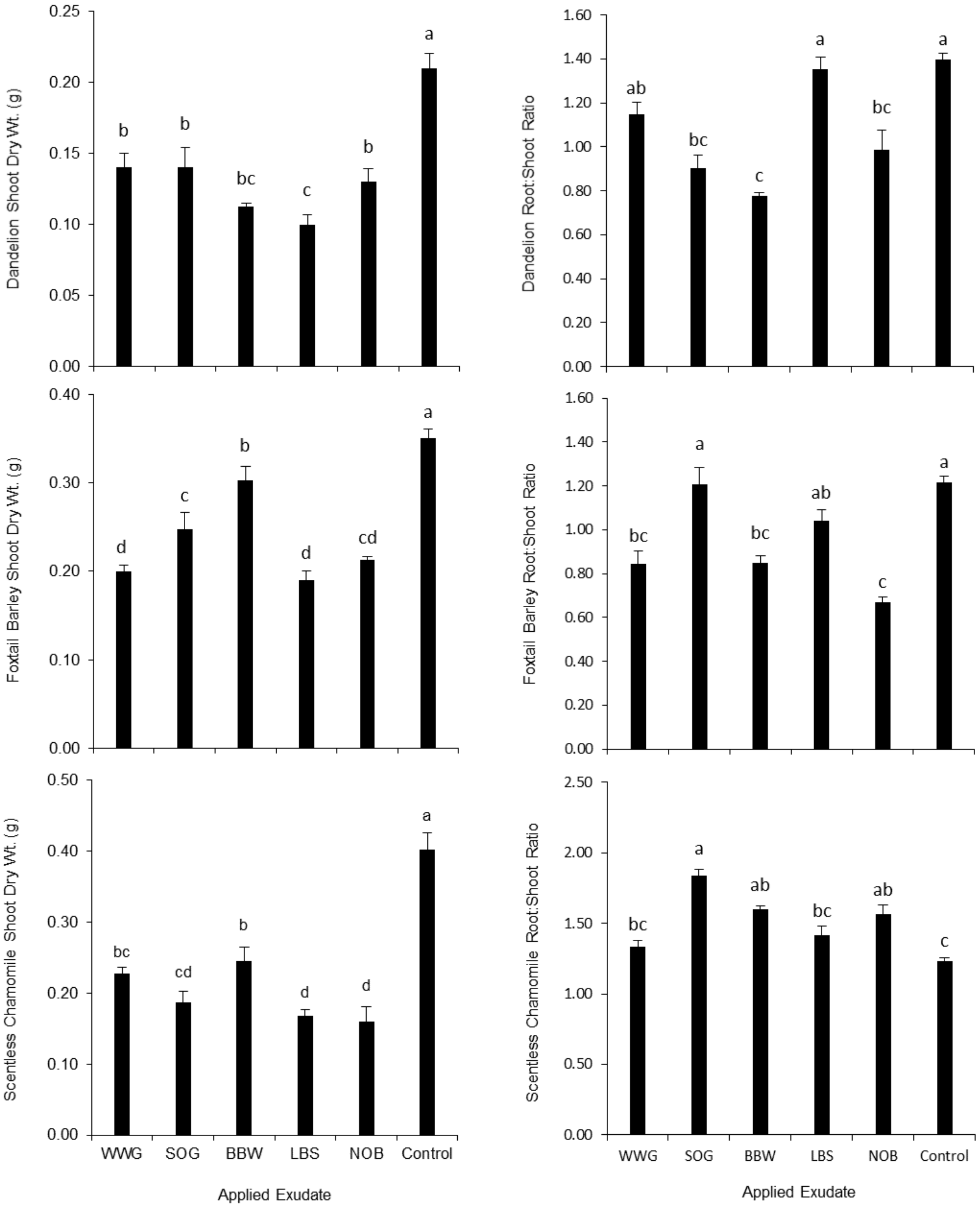


Figure 6.1 Shoot dry weight (left graphs) and root:shoot ratio (right graphs) of dandelion (upper graphs), foxtail barley (middle graphs) and scentless chamomile (lower graphs) in the first experiment, watered with leachate from five forage species including: western wheatgrass (WWG), side-oats grama (SOG), bluebunch wheatgrass (BBW), little blue stem (LBS) and nodding brome (NOB). Error bars represent + or - one standard error around the mean; *n*=4. Bars with the same letter in each graph are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

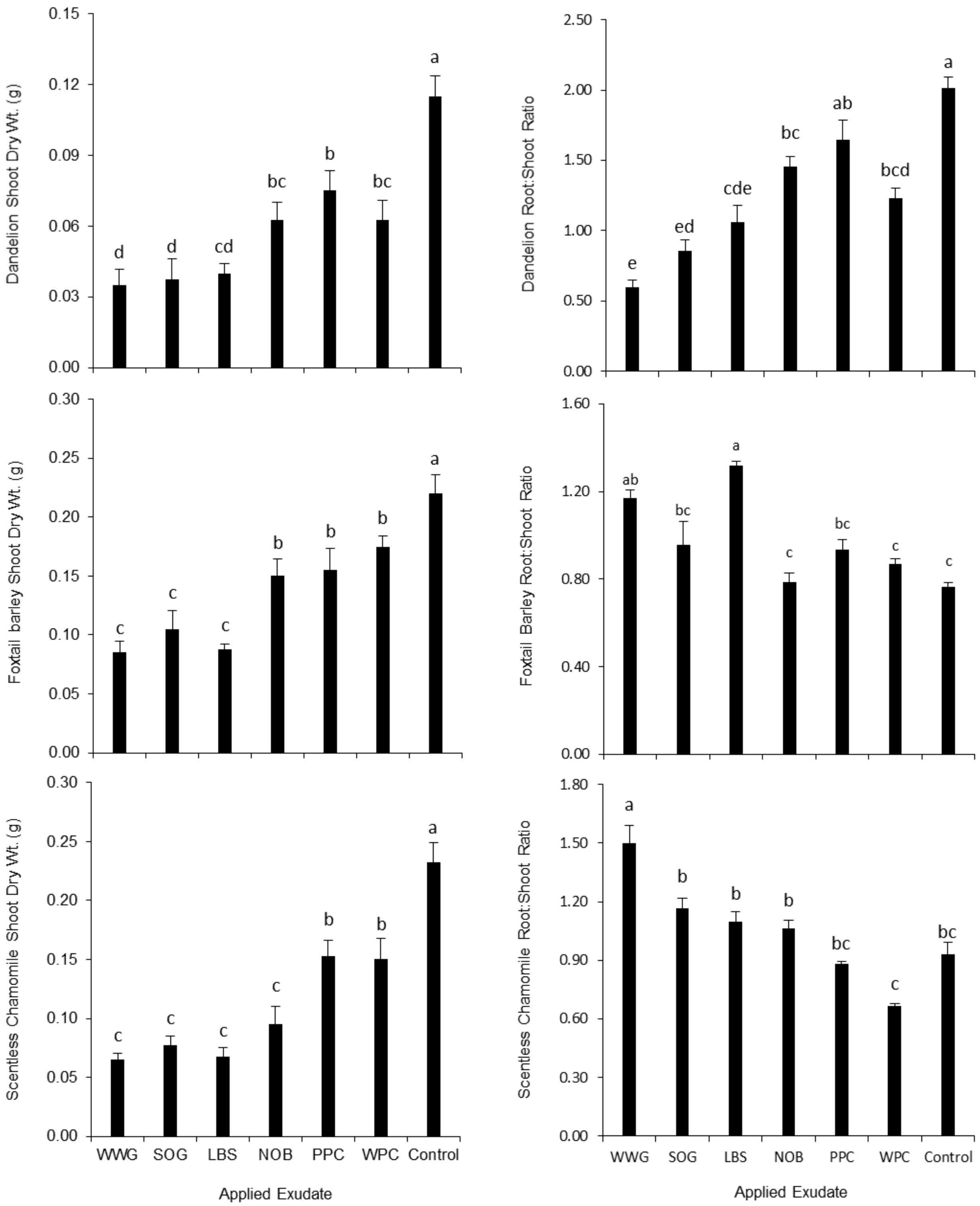


Figure 6.2 Shoot dry weight (left graphs) and root:shoot ratio (right graphs) of dandelion (upper graphs), foxtail barley (middle graphs) and scentless chamomile (lower graphs) in the second experiment (after first cut of forage species) watered by leachate from six forage species including: western wheatgrass (WWG), side-oats grama (SOG), little blue stem (LBS), nodding brome (NOB), purple prairie clover (PPC) and white prairie clover (WPC). Error bars represent one standard error + or - around the mean; *n*=4. Bars with the same letter in each graph are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

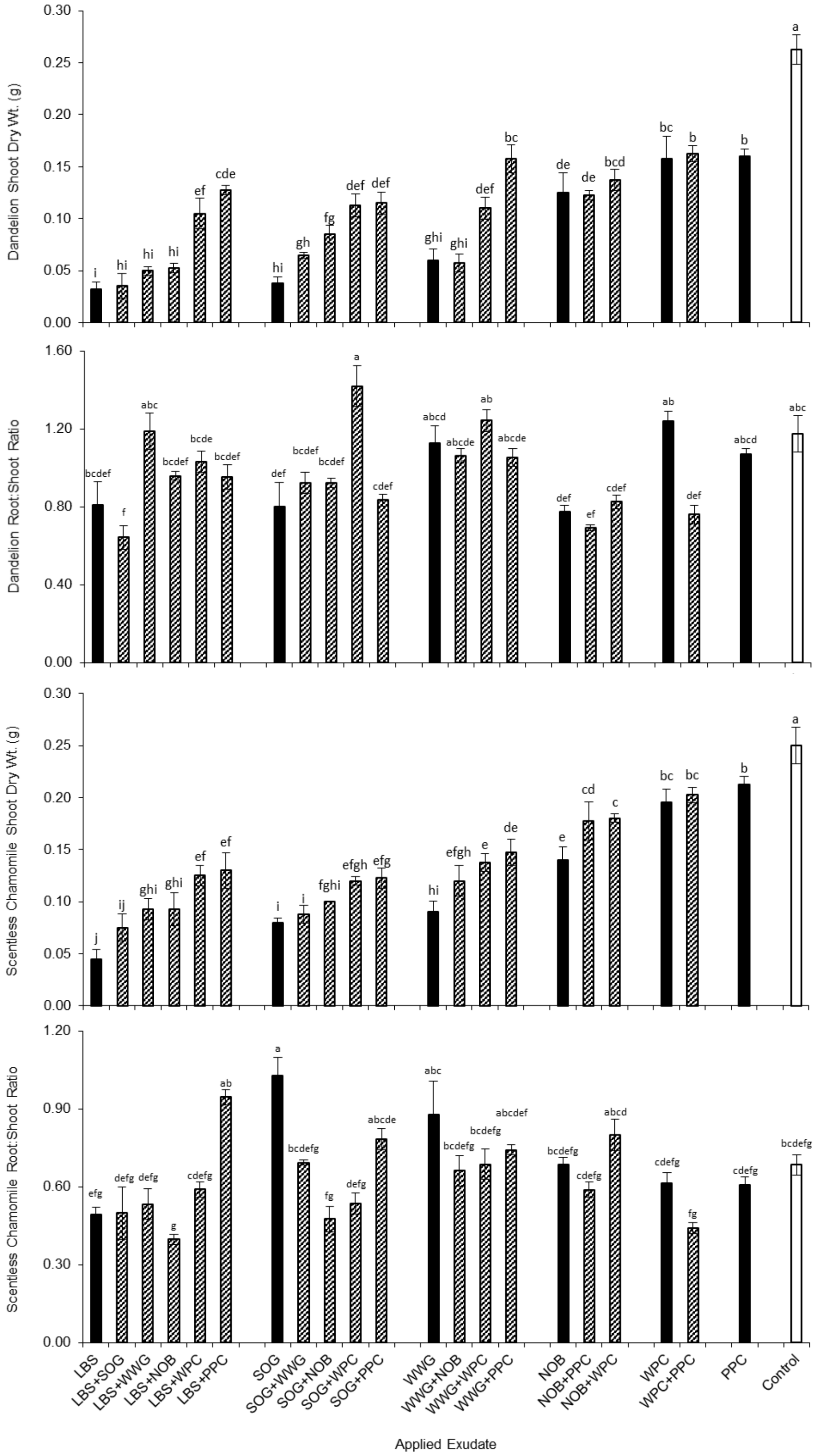


Figure 6.3 Shoot dry weight and root:shoot ratio of dandelion and scentless chamomile watered by leachate from six forage species including: western wheatgrass (WWG), side-oats grama (SOG), little blue stem (LBS), nodding brome (NOB), purple prairie clover (PPC) and white prairie clover (WPC). Leachate were applied alone and in a 1:1 mixture. Error bars represent one standard error + or - around the mean; *n*=4. Bars with the same letter in each graph are not significantly different (*P*≤0.05) according to protected Fisher's LSD test.

6.4 Discussion

This study showed that root leachate from all seven native forage species likely contain allelochemicals that can reduce shoot dry weight of dandelion, foxtail barley and scentless chamomile, but leachate from LBS, WWG and SOG had the strongest allelopathic effects on selected weeds. These results are consistent with our anecdotal observations of lower density and populations of weeds in WWG plots than seen for other forage species in an ongoing field experiment. LBS is a warm season grass and might be an ideal option for the southern part of the Great Plains, whereas WWG is a cool season grass and performs well in the Canadian Great Plains where there are shorter summers (Mischkolz et al. 2013). This result may be general as in another study WWG growth was not affected by knapweed in either the greenhouse or field (Grant et al. 2003). WWG is a strong competitor (Zhang and Lamb 2011), and its dense root system can occupy large soil volumes, potentially exposing neighboring plants to more allelochemicals. More studies are needed on WWG to definitively separate resource competition effects from allelopathy. The results also showed an interaction between allelochemicals from forage species and weeds. Differential sensitivity to allelochemicals between target weeds was observed in this study. Plants can present different degrees of sensitivity to allelochemicals (Jensen and Ehlers 2010; Viard-Crétat et al. 2012); this may explain why our studied weeds showed different reactions to allelochemicals. Different degrees of co-occurrence and interactions between donor and target plants during the process of evolution might be an explanation for differential sensitivity of target plants to allelochemicals (Viard-Crétat et al. 2012). In this study, dandelion allocated less biomass to roots compared to foxtail barley and scentless chamomile which might reduce the overwinter survival rates of dandelion in natural situation. Dandelion roots are viable during the winter and act as a nutrient reservoir for spring regrowth (Cyr and Bewley 1990). Foxtail barley, on the other hand, allocated less to shoots which can make it a weak competitor in aboveground competition in pastures. Dandelion is a perennial weed present in many Canadian farms and pastures (Wilson and Michiels 2003), and is among the top six most abundant weeds in areas with no tillage (Stewart-Wade et al. 2002). Foxtail barley is a native perennial grass common in pastures and rangelands of the Canadian Prairies that is problematic because it is not palatable once the head is formed (Best et al. 1978). Scentless chamomile is a noxious invasive weed in that grows in both native prairie and tame pasture (Woo et al. 1991). Alternative control measures for scentless chamomile are important as many herbicides do not provide efficient control (Graham et al. 2006).

Allelochemicals can be either water soluble or water insoluble and based on the design of our experiment, all the suppression effects from root leachate can be linked to water soluble compounds. Allelochemicals have multiple modes of action including effects on cell division, elongation, structure, wall, cell ultrastructure, growth regulators, membrane permeability, nutrient uptake, stomatal aperture, photosynthesis and respiration (Reigosa et al. 1999; Seigler 1996). Unlike herbicides that have one or a few modes of action, allelochemicals can have multiple modes of action simultaneously (Reigosa et al. 1999). The suppression effect of root leachate varied in different experiments. Although the effects of allelochemicals are not highly predictable (Reigosa et al. 1999), in our experiment the allelopathic potential of forage species generally appeared to increase with age. The increase in root mass and increase in aboveground material as a source of allelochemicals might be an explanation for increasing effect of allelopathic potential with age in perennial forage species.

Many factors like soil microorganisms, temperature, light intensity, etc. can affect allelochemical fate and transform them to less or more harmful compounds (Kobayashi 2004). The results of this experiment open a promising perspective for more field studies on WWG, LBS and SOG to investigate the effects of environmental factors in natural conditions on allelochemicals production and fate. Growing weeds in soil collected from pastures occupied by WWG, LBS and SOG can be another possible experiment to determine the presence of allelochemicals in the soils of these species. Future field research will provide wider view on allelopathic potential of these forage species for weed control and management in seeded pastures.

6.5 Conclusion

In conclusion, the results showed that root leachate from WWG, LBS and SOG can affect the aboveground and belowground growth of three problematic weeds in the region. Compared to LBS and SOG which are warm season (C_4) grasses, WWG is a cool season (C_3) grass with a dense rhizomatous growth behavior and one of the strongest forage competitors, which makes it one of the best options for seeded pastures, both for high forage production and weed suppression in the Canadian Prairie (Mischkolz et al. 2016; Zhang and Lamb 2011). Leachate from these three forage species showed promising results regarding the suppression of scentless chamomile, one of the most important invasive weeds in the region (Woo et al. 1991). Allelopathic potential of some forage species on other pasture species have been reported (Miller 1996; San Emeterio et al. 2004), but our selected native forage species have been grown successfully in mixture in a long-term study started by Mischkolz et al.

(2013). Planting native forage species with high allelopathic potential in seeded pastures can reduce the cost of weed controls, prevent or eliminate the spread of invasive weeds and provide a sustainable and environmentally friendly approach for weed control and management practices.

CHAPTER 7

GENERAL CONCLUSION

In this thesis seven native perennial forage species were evaluated from three perspectives: 1) forage yield and quality, 2) weed seedbank and aboveground weed population and 3) allelopathic potential to suppress weeds. In Chapters three and four, forage yield and quality of forage species in monoculture, binary mixtures and complex mixtures were evaluated in two ecoregions. Several general conclusions arise from this work, the most important being the consistently higher productivity of mixtures over monocultures.

Mixtures of forage species were consistently more productive and supported a lower weed seedbank density and aboveground weed population than that of monocultures, demonstrating the long-term benefits of perennial forage mixtures over monocultures (Chapter 3, 4 and 5). Mixture composition effects were region and year specific. In the Tall-Grass Ecoregion, SOG strongly affected the productivity of the binary and complex mixtures in the seeding year (Chapter 4). In the Dry-Mixed Ecoregion, however, mixtures containing WWG had higher forage production and lower weed seedbank density and aboveground weed population (Chapter 3 and 5). Complex mixtures that contain 57% or more WWG, produced the same amount of dry matter, therefore increasing the proportion of WWG higher than 57% doesn’t seem to have any positive effects on forage productivity (Chapter 4). Mischkolz et al. (2013) similarly found that even when WWG is seeded at half of the full seeding rate, the final forage yield was still as same as WWG in monoculture. Therefore, I recommend that seed companies consider mixtures containing a maximum of 57% WWG with the remainder depending on the ecoregion. For example, more LBS should be included in the mixture for the Dry-Mixed Ecoregion and more SOG for in the Tall-Grass Ecoregion. Seed mixtures containing WWG, BBW and LBS or SOG (depends on the region) have a potential to provide higher forage yield, suppress weeds based on their allelopathic potential, and reduce the weed seedbank abundance.

Long-term trends in species composition in mixture plots were highly dependent on the growth behavior of each species in the mixture. Binary mixtures were initially seeded at the same rate (50-50%), however WWG occupied more than 90% of the mixtures in the latest years of the experiment (Chapter 3). WWG is a stronger competitor than the other species tested here as both a seedling and at maturity (Zhang and Lamb 2011). These results show the strong ability of WWG to occupy space, and limit the growth and survival of other species.

This competitive ability of WWG can also be implemented for weed control and management in seeded pastures, as many weeds may not find an empty niche to survive beside WWG (Chapter 5).

The strong agronomic benefits of mixtures identified in this study are consistent with many studies that have identified positive relationships between plant diversity and productivity, ecosystem stability and function (Isbell and Wilsey 2011; Mischkolz et al. 2016; Picasso et al. 2011; Tilman et al. 2014; Tilman et al. 2001; Tilman et al. 2006a). More diverse plant communities tend to be more productive than less complex plant communities (Elton 1958; Picasso et al. 2008). This phenomenon can be explained in two ways: 1) resource use complementarity, and 2) sampling effect (Knops et al. 1999; Naeem et al. 2000; Huston 1997). In the ‘sampling effect’ perspective, more complex mixtures contain at least one strong and productive species that highly influences the productivity (Huston 1997). In this study, regarding rhizomatous growth behavior of WWG and also its strong competitive ability, it seems that the ‘sampling effect’ is a more likely explanation for increased forage productivity of the mixtures compared to monocultures.

Positive effects of species diversity on productivity can be explained by many factors including interspecific complementarity, better use of available resources and reduced chance of herbivory and disease outbreaks (Tilman et al. 2014). Not all native species produce high forage yield, but forage mixtures including less productive species may bring beneficial characteristics like drought or grazing tolerance to the plant community. The diverse species mixtures not only provide good forage yields under favorable climate conditions, but ensure the maintenance of forage productivity under unpredictable harsh conditions (Mischkolz et al. 2013).

Among monoculture plots, WWG produced greater forage yield at all harvesting times and contained the lowest weed seedbank and aboveground weed populations (Chapter 3, 4 and 5). Lower weed population in WWG can also be linked to its allelopathic potential (Chapter 6). Although WWG is a strong competitor (Zhang and Lamb 2011), its dense root system can also occupy more soil volume and potentially exposes neighboring plants to more allelochemicals. Therefore, more studies are needed on WWG to separate competition from allelopathy. A suggested experiment following the design of Duke (2015) would be to examine the growth of WWG and target plants under conditions whereby both competition and allelopathy can be manipulated. This experiment should contain six treatments: 1) WWG and target species grown under conditions whereby both competition and allelopathy occur, 2) using semipermeable sheets to separate roots, and still allowing allelochemicals to move easily, 3) using non-permeable sheets to separate roots from competition and allelopathy, 4) using non-permeable sheets to separate shoots from competition, 5) using non-permeable sheets to separate shoots from competition, and using semipermeable sheets for roots, 6) using non-permeable sheets for both roots and shoots. Using activated charcoal in these experiments can provide further support for an allelopathy hypothesis. Results of these six experiments can separate the effect of allelopathy from competition.

During the course of the study, 2015 was one of the driest years, whereas 2016 was one of the wettest years in the last 120 years in Swift Current. We observed no significant differences in forage production of WWG between dry and wet years (Chapter 3). This yield stability across very different climate conditions is highly desirable in a forage species. The high productivity of WWG compared to other grasses can be related to its rhizomatous growth behavior. Biligetu et al. (2014) found that rhizomatous C_3 grasses, regardless of the species' origin, produce greater dry matter than caespitose grasses. NOB performed well in 2011, but the forage yield decreased continuously thereafter and was among the lowest producers in 2016. Therefore, NOB doesn't seem to be a suitable option for stable and long-term forage productivity in the dry-mix ecoregion (Chapter 3). BBW, on the other hand, produced more stable forage yield during the course of study and might be a good option for long-term stable forage productivity in seeded pastures. Forage production of LBS was among the highest in August of all years in Swift Current (Chapter 3). Although SOG did not perform well in the Dry-Mixed Ecoregion, the species did perform well in the Tall-Grass Prairie Ecoregion (Chapter 4). In the Tall-Grass Prairie Ecoregion, SOG in monoculture produced about three-times more dry matter than that of WWG in the seeding year, however a longer study is needed to evaluate the long-term forage yields of this species in this ecoregion.

In the study on weed seedbank and aboveground weed population, among the 35 identified weed species aboveground and in the seedbank, only 13 weeds were common in both aboveground and belowground populations. The most abundant weeds in the seedbank were the least abundant weeds in aboveground population and vice versa (Chapter 5). Generally, low similarity between aboveground plant community and seedbank has been reported for perennial species, thus seedbank measurements in perennial crops seem necessary to avoid dominance of non-desirable species after disturbance (Bakker et al. 1996; Milberg 1995; Rabinowitz 1981; Schenkeveld and Verkaar 1984; Thompson and Grime 1979).

The results of the allelopathy study (Chapter 6) showed that root leachate from WWG, LBS and SOG can reduce the aboveground and belowground growth of three problematic weeds in the region. Leachate from these three forage species showed promising results regarding the suppression of scentless chamomile, one of the most important invasive weeds in the region. Different sensitivity to allelochemicals between target weeds was also observed (Chapter 6). Different degrees of co-occurrence and interactions between donor and target plants during the process of evolution might be a possible explanation for the different sensitivity of target plants to allelochemicals from some donor plants (Viard-Crétat et al. 2012). In this study, dandelion allocated less biomass to roots compared to foxtail barley and scentless chamomile which may reduce the overwinter survival rates of dandelion in a natural situation. Foxtail barley, on the other hand, allocated less to shoots which can make it a weak competitor in aboveground competition in pastures. Although the effects of allelochemicals are not highly predictable (Reigosa et al. 1999), in our experiment the allelopathic potential of forage species generally appeared to increase with age (Chapter 6). The increase in root mass and an increase in aboveground material as a source of allelochemical production might be an explanation for increasing effect of allelopathy with age in perennial forage species. The results of this experiment initiated the promising perspective for more studies on WWG, LBS and SOG under field conditions. Planting forage species with high allelopathic potential in seeded pastures can reduce the cost of weed controls, prevent or eliminate the spread of invasive weeds and provide a sustainable and environmentally friendly approach for weed control and management practices.

In summary, the overall goal of this thesis was to provide a wider view on forage productivity, weed seedbank and allelopathic potential of some native species. Among the studied species, WWG showed promising results as a native forage species by demonstrating the potential to produce high forage yield and quality, produce allelopathic compounds, suppress weeds and reduce weed seedbank density. The inclusion of BBW, LBS and legumes to the mixtures with WWG can provide sustainable forage yield and quality in varied climate conditions and can be suitable options for seeded pastures. In this study we observed no significant differences in forage production of all native species from dry to wet year. The results showed high stability and productivity of native forage species during very diverse climate conditions. Forage mixtures produced greater dry matter and promoted lower weed densities in the seedbank and aboveground populations compared to monocultures. This demonstrates that an increase in forage mixture diversity can increase forage yield and can provide ecological and non-chemical weed control techniques in seeded pastures.

CHAPTER 8

LITERATURE CITED

Ahmed, M. and Wardle, D. A. 1994. Allelopathic potential of vegetative and flowering ragwort (*Senecio jacobaea* L.) plants against associated pasture species. Plant and Soil 164(4):61-68.

Altieri, M. A. and Liebman, M. Z. 1988. Weed Management in Agroecosystems: Ecological Approaches. CRC Press, Boca Rato´n.

Ayers, K. W., Acton, D. F. and Ellis, J. G. 1985. The soils of the Swift Current map area 72J Saskatchewan. Saskatchewan Institute of Pedology, Saskatoon

Bailey, A. W., McCartney, D. and Schellenberg, M. P. 2010. Management of Canadian prairie rangeland. Agriculture and Agri-Food Canada, Ottawa. v, 58 p. pp.

Bakker, E. S., Rosén, E., Verweij, G. L., Bekker, R. M. and Bakker, J. P. 1996. Soil seed bank composition along a gradient from dry alvar grassland to Juniperus shrubland. Journal of Vegetation Science 7(2):165 - 176.

Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J. S., Nakashizuka, T., Raffaelli, D. and Schmid, B. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol Lett 9(10):1146-56.

Baskin, C. C. and Baskin, J. M. 1998. Seed Dormancy and Germination: Ecology, Biogeography and Evolution. Academic Press, San Diego.

Baskin, J. M. and Baskin, C. C. 1978. The seed bank in a population of an endemic plant species and its ecological significance. Biological Conservation 14(2):125-130.

Baskin, J. M. and Baskin, C. C. 1985. The annual dormancy cycle in buried weed seeds:a continuum. Bioscience 35(18):492-498.

Baskin, J. M. and Baskin, C. C. 2004. A classification system of seed dormancy. Seed Science Research 14(1):1-16.

Batlla, D. and Benech-Arnold, R. L. 2003. A quantitative analysis of dormancy loss dynamics in *Polygonum aviculare* L. seeds. Development of a thermal time model based on changes in seed population thermal parameters. Seed Science Research 13(1):55-68.

Batlla, D. and Benech-Arnold, R. L. 2004. Seed dormancy loss assessed by changes in population hydrotime parameters. Seed Science Research 14(3):277-286.

Batlla, D. and Benech-Arnold, R. L. 2007. Predicting changes in dormancy level in weed seed soil banks: Implications for weed management. Crop Protection 26(3):189-197.

Bélanger, G., Michaud, R., Jefferson, P. G., Tremblay, G. F. and Brégard, A. 2001. Improving the nutritive value of timothy through management and breeding. Canadian Journal of Plant Science 81(4):577-585.

Benech-Arnold, R. L., Sa´ nchez, R. A., Forcella, F., Kruk, B. C. and Ghersa, C. M. 2000. Environmental control of dormancy in weed seed banks in soil. Field Crops Research 67(2):105-122.

Bertness, M. D. and Callaway, R. M. 1994. Positive interactions in communities. Trends in Ecology & Evolution 9(5):191-193.

Best, K. F., Banting, J. D. and Bowes, G. G. 1978. The biology of Canadian weeds. 31. *Hordeum jubatum* L. Canadian Journal of Plant Science 58:699-708.

Bias, H. P., Vepachedu, R., Gilroy, S., Callaway, R. M. and Vivanco, J. M. 2003. Allelopathy and Exotic Plant Invasion: From Molecules and Genes to Species Interactions. Science 301(5638):1377-1380.

Biligetu, B., Jefferson, P. G., Muri, R. and Schellenberg, M. P. 2014. Late summer forage yield, nutritive value, compatibility of warm- and cool-season grasses seeded with legumes in western Canada. Canadian Journal of Plant Science 94(7):1139-1148.

Black, M., Bewley, J. D. and Halmer, P. 2006. The encyclopaedia of seeds: science, technology and uses. CABI International, Wallingford, UK.

Blum, U. 1998. Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interactions. Journal of Chemical Ecology 24(4):685-708.

Bokhari, U. G. 1978. Allelopathy among prairie grasses and its possible ecological significance. Annals of Botany 42(1):127-136.

Bonanomi, G., Sicurezza, M. G., Caporaso, S., Esposito, A. and Mazzoleni, S. 2006. Phytotoxicity dynamics of decaying plant materials. New Phytologist 169(3):571-578.

Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielborger, K., Travis, J. M. J., Anthelme, F. and others. 2008. Facilitation in plant communities: the past, the present, and the future. Journal of Ecology 96(1):18-34.

Bruno, J. F., Stachowicz, J. J. and Bertness, M. D. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology & Evolution 18(119-125).

Buhler, D. D., Hartzler, R. G. and Forcella, F. 1997. Implications of weed seed and seedbank dynamics to weed management. Weed Science 45(3):329-336.

Burnside, O. C., Wilson, R. G., Weisberg, S. and Hubbard, K. G. 1996. Seed Longevity of 41 Weed Species Buried 17 Years in Eastern and Western Nebraska. Weed Science 44(1):74-86.

Callaway, R. M. 1995. Positive interactions among plants. The Botanical Review 61(4):306-349.

Callaway, R. M. and Aschehoug, E. T. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290(5491):521-523.

Callaway, R. M. and Howard, T. G. 2007. Competitive networks, indirect interactions, and allelopathy a microbial viewpoint on plant communities. *in* K. E. Bochum, U. L. Darmstadt, W. B. Bielefeld, J. M. Tokyo, eds. Progress in Botany. Springer-Verlag Berlin Heidelberg New York.

Cardina, J. and Sparrow, D. H. 1996. A comparison of methods to predict weed seedling populations from the soil seedbank. Weed Science 44(1):46-51.

Cavers, P. B. 1983. Seed demography. Canadian Journal of Botany 61:3578-3590.

Chang, E. R., Jefferies, R. L. and Carleton, T. J. 2001. Relationships between vegetation and soil seed banks in arctic coastal marsh. Journal of Ecology 89:367-384.

Chou, C. H. 1999. Roles of allelopathy in plant biodiversity and sustainable agriculture. Critical Reviews in Plant Sciences 18(5):609-636.

Chung, I. M. and Miller, D. A. 1995. Differences in autotoxicity among seven alfalfa cultivars. Agronomy Journal 87(3):596-600.

Crawley, M. J., Brown, S. L., Heard, M. S. and Edwards, G. R. 1999. Invasion-resistance in experimental grassland communities: species richness or species identity? Ecology Letters 2(3):140-148.

Cyr, D. R. and Bewley, J. D. 1990. Proteins in the roots of the perennial weeds chicory (*Cichorium intybus* L.) and dandelion (*Taraxacum officinale* Weber) are associated with overwintering. Planta 182(3):370-374.

Deak, A., Hall, M. H., Sanderson, M. A. and Archibald, D. D. 2007. Production and Nutritive Value of Grazed Simple and Complex Forage Mixtures. Agronomy Journal 99(3):814.

DeKeyser, E. S., Dennhardt, L. A. and Hendrickson, J. R. 2015. Kentucky Bluegrass (Poa pratensis) invasion in the northern Great Plains: A story of rapid dominance in an endangered ecosystem. Invasive Plant Science and Management 8(3):255-261.

Dekker, J. 1997. Weed diversity and weed management. Weed Science 45:357-363.

Dekker, J. 2011. Soil Weed Seed Banks and Weed Management. Journal of Crop Production 2(2):139-166.

Dessaint, F., Chadoeuf, R. and Barralis, G. 1997. Nine years' soil seed bank and weed vegetation relationships in an arable field without weed control. Journal of Applied Ecology 34(1):123-130.

Díaz, S. and Cabido, M. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends in Ecology & Evolution 16(11):646–655.

DiTomaso, J. M. 2000. Invasive weeds in rangelands: Species, impacts, and management. Weed Science 48(2):255-265.

Doak, D. F., Bigger, D., Harding, E. K., Marvier, M. A., O'Malley, R. E. and Thomson, D. 1998. The statistical inevitability of stability diversity relationships in community ecology. The American Naturalist 151(3):264-276.

Dorner, J. 2002. An introduction to using native plants in restoration projects. *in* B. o. L. M. Plant Conservation Alliance, US Department of Interior, ed., Center for Urban Horticulture, University of Washington.

Duchene, O., Vian, J. F. and Celette, F. 2017. Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. Agriculture, Ecosystems and Environment 240:148-161.

Duke, S. O. 2015. Proving Allelopathy in Crop–Weed Interactions. Weed Science 63(Special Issue):121-132.

Einhellig, F. A. 1996. Interactions involving allelopathy in cropping systems. Agronomy Journal 88(6):886-893.

Einhellig, F. A., Rice, E. L., Risser, P. G. and Wender, S. H. 1970. Effects of scopoletin on growth, CO_2 exchange rates, and concentration of scopoletin, scopolin, and chlorogenic acids in tobacco, sunflower, and pigweed. Bulletin of the Torrey Botanical Club 97(1):22-23.

Elton, C. S. 1958. The Ecology of Invasions by Animals and Plants. Methuen, London.

Fenner, M. 2000. Seeds: the ecology of regeneration in plant communities. CAB International, Wallingford, UK. 410 pp.

Fisher, R. F. 1978. Juglone inhibits pine growth under certain moisture regimes. Soil Science Society of America journal 42:801-803.

Forcella, F., Wilson, R. G., Dekker, J., Kremer, R. J., Cardina, J., Anderson, R. L., Alm, D., Renner, K. A., Harvey, R. G., Clay, S. and others. 1997. Weed seed bank emergence across the corn belt. Weed Science 45(1):67-76.

Foster, A., Vera, C. L., Malhi, S. S. and Clarke, F. R. 2014. Forage yield of simple and complex grass-legume mixtures under two management strategies. Canadian Journal of Plant Science 94(1):41-50.

Fujii, Y., Shibuya, T., Nakatani, K., Itani, T., Hiradate, S. and Parvez, M. 2004. Assessment method for allelopathic effect from leaf litter leachates. Weed Biology and Management 4(1):19-23.

Gawronska, H. and Golisz, A. 2006. Allelopathy and biotic stresses. Pages 211-227 *in* M. J. Reigosa, N. Pedrol, L. Gonzalez, eds. Allelopathy: A Physiological Process with Ecological Implications. Springer, Netherlands.

Gerson, E. A. and Kelsey, R. G. 1998. Variation of piperidine alkaloids in ponderosa (*Pinus ponderosa*) and lodgepole pine (*P. contorta*) foliage from central Oregon. Journal of Chemical Ecology 24(5):815-827.

Ghebrehiwot, H. M., Aremu, A. O. and Van Staden, J. 2013. Evaluation of the allelopathic potential of five South African mesic grassland species. Plant Growth Regulation 72(2):155-162.

Goering, H. K. and Van Soest, P. J. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). U.S. Agricultural Research Service; for sale by the Supt. of Docs., U.S. Govt. Print. Off., Washington. iv, 20 p. pp.

Goldberg, D. E. 1996. Competitive ability: Definitions, contingency and correlated traits. Philosophical Transactions: Biological Sciences 351(1345):1377-1385.

González, L., Souto, X. C. and Reigosa, M. J. 1997. Weed control by *Capsicum annuum*. Allelopathy Journal 4:101-109.

Grace, J. B. 1999. The factors controlling species density in herbaceous plant communities: an assessment. Perspectives in Plant Ecology, Evolution and Systematics 2:1-28.

Graham, G. L., Peng, G., Bailey, K. L. and Holm, F. A. 2006. Interactions of Colletotrichum truncatum with Herbicides for Control of Scentless Chamomile (*Matricaria perforata*). Weed Technology 20(4):877-884.

Grant, D. W., Peters, D. P. C., Beck, G. K. and Fraleigh, H. D. 2003. Influence of an exotic species, *Acroptilon repens* (L.) DC. on seedling emergence and growth of native grasses. Plant Ecology 166(2):157-166.

Grime, J. P. 1981. The role of seed dormancy in vegetation dynamics. Annals of Applied Biology 98:555-558.

Grings, E. E., Haferkamp, M. R., Heitschmidt, R. K. and Karl, M. G. 1996. Mineral dynamics in forages of the Northern Great Plains. Journal of Range Management 49(3):234-240.

Hamm, J. W., Radford, F. G. and Halstead, E. H. 1970. The simultaneous determination of nitrogen, phosphorus and potassium in sodium bicarbonate extracts of soils. Pages 346 Advances in automated analysis Technicon International Congress 1969 Volume 2 Industrial analysis. Medied Inc., 188 E. Post Rd., White Plains, N.Y.10601.

Harper, J. L. 1977. Population Biology of Plants. Academic Press, London. 892 pp.

Hector, A., Dobson, K., Minns, A., Bazeley-White, E. and Lawton, J. H. 2001. Community diversity and invasion resistance: An experimental test in a grassland ecosystem and a review of comparable studies. Ecological Research 16(5):819-831.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M. C., Diemer, M., Dimitrakopoulos, P. G., Finn, J. A., Freitas, H., Giller, P. S., Good, J. and others. 1999. Plant diversity and productivity experiments in european grasslands. Science 286(5442):1123-7.

Hierro, J. e. L. and Callaway, R. M. 2003. Allelopathy and exotic plant invasion. Plant and Soil 256(1):29-39.

Holechek, J. L., Pieper, R. D. and Herbel, C. H. 2004. Range animal nutrition. Pages 265-316 Range management: Principles and practices. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S. and others. 2005a. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75(1):3-35.

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S. and others. 2005b. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75(1):3-35.

Hooper, D. U. and Vitousek, P. M. 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs 68(1):121-149.

Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110(4):449-460.

Huston, M. A., Aarssen, L. W., Austin, M. P., Cade, B. S., Fridley, J. D., Garnier, E., Grime, J. P., Hodgson, J., Lauenroth, W. K., Thompson, K. and others. 2000. No consistent effect of plant diversity on productivity. Science 289(5483):1255.

Inderjit and Callaway, R. M. 2003. Experimental designs for the study of allelopathy. Plant and Soil 256(1):1–11.

Inderjit, Seastedt, T. R., Callaway, R. M., Pollock, J. L. and Kaur, J. 2008. Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. Biological Invasions 10(6):875-890.

Inderjit, Weston, L. A. and Duke, S. O. 2005. Challenges, achievements and opportunities in allelopathy research. Journal of Plant Interactions 1(2):69-81.

Isbell, F. I. and Wilsey, B. J. 2011. Increasing native, but not exotic, biodiversity increases aboveground productivity in ungrazed and intensely grazed grasslands. Oecologia 165(3):771-81.

Jabran, K., Mahajan, G., Sardana, V. and Chauhan, B. S. 2015. Allelopathy for weed control in agricultural systems. Crop Protection 72:57-65.

Jefferson, P. G., McCaughey, W. P., May, K., Woosaree, J. and McFarlane, L. 2004. Forage quality of seeded native grasses in the fall season on the Canadian Prairie Provinces. Canadian Journal of Plant Science 84(2):503-509.

Jensen, C. G. and Ehlers, B. K. 2010. Genetic variation for sensitivity to a thyme monoterpene in associated plant species. Oecologia 162(4):1017-25.

Jones, D. I. H. and Wilson, A. 1987. Nutritive quality of forages. Pages 65-90 *in* J. B. Hacker, J. H. Ternouth, eds. The nutrition of herbivores. Academic Press, Sydney, Australia.

Jones, H. G. 1992. Plants and microclimate: A quantitative approach to environmental plant physiology. 2nd ed. Cambridge University Press, Cambridge, UK.

Jones, J. B. 1991. Plant Tissue Analysis in Micronutrients. *in* J. J. Mortvedt, F. R. Cox, L. M. Shuman, R. M. Welch, eds. Micronutrients in Agriculture Second Edition. Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Karn, J. F. and Hofmann, L. 1990. Comparison of the Copper and Molybdenum Status of Yearling Steers Grazing Reclaimed Mined-Land and Native Range. Journal of Range Management 43(1):69-72.

Karssen, C. M. 1980. Environmental conditions and endogenous mechanisms involved in secondary dormancy of seeds. Israel Journal of Botany 29(1-4):291-300.

Karssen, C. M. 1982. Seasonal patterns of dormancy in weed seeds. Pages 243-270 *in* A. A. Khan, ed. The Physiology and Biochemistry of Seed Development, Dormancy and Germination. Elsevier, Amsterdam.

Keddy, P., Fraser, L. H. and Wisheu, I. C. 1998. A comparative approach to examine competitive response of 48 wetland plant species. Journal of Vegetation Science 9(6):777-786.

Keddy, P. A., Twolanstrutt, L. and Wisheu, I. C. 1994. Competitive effect and response rankings in 20 wetland plants - Are they consistent across 3 environments. Journal of Ecology 82(3):635-643.

Kelty, M. J. 2006. The role of species mixtures in plantation forestry. Forest Ecology andManagement 233(2-3):195-204.

Kirwan, L., Luescher, A., Sebastia, M. T., Finn, J. A., Collins, R. P., Porqueddu, C., Helgadottir, A., Baadshaug, O. H., Brophy, C., Coran, C. and others. 2007.

Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. Journal of Ecology 95(3):530-539.

Knops, J. M. H., Tilman, D., Haddad, N. M., Naeem, S., Mitchell, C. E., Haarstad, J., Ritchie, M. E., Howe, K. M., Reich, P. B., Siemann, E. and others. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundance and diversity. Ecology Letters 2(5):286-293.

Kobayashi, K. 2004. Factors affecting phytotoxic activity of allelochemicals in soil. Weed Biology and Management 4(1):1-7.

Koeppe, D. E., Southwick, L. M. and Bittell, J. E. 1976. The relationship of tissue chlorogenic acid concentrations and leaching of phenolics from sunflowers grown under varying phosphate nutrient conditions. Canadian Journal of Botany 54(7):593-599.

Kumar, V., Brainard, D. C. and Bellinder, R. R. 2009. Suppression of Powell Amaranth (*Amaranthus powellii*) by Buckwheat Residues: Role of Allelopathy. Weed Science 57(1):66-73.

Lamb, E., Kennedy, N. and Siciliano, S. 2011. Effects of plant species richness and evenness on soil microbial community diversity and function. Plant and Soil 338(1):483-495.

Lehman, C. L. and Tilman, D. 2000. Biodiversity, Stability, and Productivity in Competitive Communities. The American Naturalist 156(5):534-552.

Lhomme, J. P. and Winkel, T. 2002. Diversity stability relationships in community ecology: Re-examination of the portfolio effect. Theoretical Population Biology 62(3):271-279.

Loreau, M. and de Mazancourt, C. 2013. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. Ecology Letters 16(1):106-115.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B. and others. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294(5543):804-8.

Lortie, C. J. 2007. An ecological tardis: the implications of facilitation through evolutionary time. Trends in Ecology & Evolution 22(12):627-630.

Lortie, C. J., Filazzola, A., Welham, C. and Turkington, R. 2016. A cost–benefit model for plant–plant interactions: a densityseries tool to detect facilitation. Plant Ecol 217:1315-1329.

Lyons, K. G. and Schwartz, M. W. 2001. Rare species loss alters ecosystem function – invasion resistance. Ecology Letters 4(4):358-365.

Macías, F. A. 1995. Allelopathy in the Search for Natural Herbicide Models. ACS Symposium Series 582:310-329.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., de Tourdonnet, S. and Valantin-Morison, M. 2009. Mixing plant species in cropping systems: concepts, tools and models. A review. Agronomy for Sustainable Development 29(1):43-62.

McGraw, R. L., Shockley, F. W., Thompson, J. F. and Roberts, C. A. 2004. Evaluation of Native Legume Species for Forage Yield, Quality, and Seed Production. Native Plants Journal 5(2):152-159.

McNaughton, S. J. 1977. Diversity and stability of ecological communities: a comment on the role of empiricism in ecology. The American Naturalist 111(979):515-525.

Mesgaran, M. B., Mashhadi, H. R., Zand, E. and Alizadeh, H. M. 2007. Comparison of three methodologies for efficient seed extraction in studies of soil weed seedbanks. Weed Research 47(6):472-478.

Milberg, P. 1995. Soil seed bank after eighteen years of succession from grassland to forest. Oikos 72(1):3-13.

Milchunas, D. G., Vandever, M. W., Ball, L. O. and Hyberg, S. 2011. Allelopathic Cover Crop Prior to Seeding Is More Important Than Subsequent Grazing/Mowing in Grassland Establishment. Rangeland Ecology & Management 64(3):291-300.

Miller, D. A. 1996. Allelopathy in forage crop systems. Agronomy Journal 88(6):854-859.

Miller, T. E. 1997. Direct and indirect species interactions in an early old-field plant community. The American Society of Naturalists 143:1007-1025.

Mischkolz, J. M. 2013. Selecting and evaluating complex native forage mixtures for the mixed grass prairie. University of Saskatchewan, Saskatoon, SK, CA. 112 pp.

Mischkolz, J. M., Schellenberg, M. P. and Lamb, E. G. 2013. Early productivity and crude protein content of establishing forage swards composed of combinations of native grass and legume species in mixed-grassland ecoregions. Canadian Journal of Plant Science 93(3):445-454.

Mischkolz, J. M., Schellenberg, M. P. and Lamb, E. G. 2016. Assembling productive communities of native grass and legume species finding the right mix. Applied Vegetation Science 19:111-121.

Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Hufbauer, R. A., Klironomos, J. N., Maron, J. L., Morris, W. F., Parker, I. M., Power, A. G. and others. 2006. Biotic interactions and plant invasions. Ecol Lett 9(6):726-40.

Molano-Flores, B., Coons, J. M. and Cunningham, J. B. 2011. Germination of Two Seed Types from Three Dalea Species. Castanea 76(3):266-271.

Monsen, S. B., Stevens, R., Shaw, N. L. and Rocky Mountain Research Station (Fort Collins Colo.). 2004. Restoring western ranges and wildlands. United States Dept. of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO.

Moore, P. D. 1980. Soil seed banks. Nature 284:123-124.

Muir, J. P., Pitman, W. D. and Foster, J. L. 2011. Sustainable, low-input, warm-season, grass-legume grassland mixtures: mission (nearly) impossible? Grass and Forage Science 66(3):301-315.

Murphy, S. D., Clements, D. R., Belaoussoff, S., Kevan, P. G. and Swanton, C. J. 2006. Promotion of weed species diversity and reduction of weed seedbanks with conservation tillage and crop rotation. Weed Science 54(3):606-606.

Mwaja, V. N., Masiunas, J. B. and Weston, L. A. 1995. Effects of fertility on biomass, phytotoxicity and allelochemical content of cereal rye. Journal of Chemical Ecology 21(1):81-96.

Naeem, S., Knops, J. M. H., Tilman, D., Howe, K. M., Kennedy, T. and Gale, S. 2000. Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. Oikos 91(1):97-108.

National-Research-Council. 2001. Nutrient requirements of beef cattle. 7th rev. ed. 1996 (updated 2001). Subcommittee on Beef Cattle Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council. National Academic Press, Washington, DC.

Nishida, N., Tamotsu, S., Nagata, N., Saito, C. and Sakai, A. 2005. Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: Inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. Journal of Chemical Ecology 31(5):1187-1203.

Noel, R. J. and Hambleton, L. G. 1976. Collaborative study of a semiautomated method for the determination of crude protein in animal feeds. J Assoc Off Anal Chem 59(1):134-40.

Oelmann, Y., Wilcke, W., Temperton, V. M., Buchmann, N., Roscher, C., Schumacher, J., Schulze, E. D. and Weisser, W. W. 2007. Soil and plant nitrogen pools as related to plant diversity in an experimental grassland. Soil Science Society of America Journal 71(3):720-729.

Otfinowski, R., Kenkel, N. C. and Catling, P. M. 2007. The biology of Canadian weeds. 134. *Bromus inermis* Leyss. Canadian Journal of Plant Science 87(1):183-198.

Patrick, Z. A. 1971. Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Science 111(1):13-18.

Pedrol, N., González, L. and Reigosa, M. J. 2006. Allelopathy and abiotic stress. Pages 171-206 *in* M. J. Reigosa, N. Pedrol, L. González, eds. Allelopathy: A Physiological Process with Ecological Implications. Springer, Netherlands.

Peterson, P. R., Sheaffer, C. C. and Hall, M. H. 1992. Drought effects on perennial forage legume yield and quality. 84(5):774-779.

Pfisterer, A. B., Joshi, J., Schmid, B. and Fischer, M. 2004. Rapid decay of diversity-productivity relationships after invasion of experimental plant communities. Basic and Applied Ecology 5(1):5-14.

Picasso, V. D., Brummer, E. C., Liebman, M., Dixon, P. M. and Wilsey, B. J. 2008. Crop Species Diversity Affects Productivity and Weed Suppression in Perennial Polycultures under Two Management Strategies. Crop Science 48(1):331-342.

Picasso, V. D., Brummer, E. C., Liebman, M., Dixon, P. M. and Wilsey, B. J. 2011. Diverse perennial crop mixtures sustain higher productivity over time based on ecological complementarity. Renewable Agriculture and Food Systems 26(04):317-327.

Quimby, P. C., JR., Bruckart, W. L., Deloach, C. J., Knutson., L. and Ralphs, M. H. 1991. Biological control of rangeland weeds. Pages 83-102 *in* L. F. James, J. Evans, M. H. Ralphs, R. D. Child, eds. Noxious Range Weeds. Westview Press, San Francisco.

Rabinowitz, D. 1981. Buried viable seed in a North American tall-grass prairie: The resemblance of their abundance and composition to dispersing seeds. Oikos 36(2):191-195.

Rahman, A., James, T. K. and Grbavac, N. 2006. Correlation between the soil seed bank and weed populations in maize fields. Weed Biology and Management 6(4):228-234.

Rahman, A., James, T. K., Mellsop, J. M. and Grbavac, N. 2001. Weed seedbank dynamics in maize under different herbicide regimes. New Zealand Plant Protection, Vol 54 54:168-173.

Reich, P. B., Tilman, D., Naeem, S., Ellsworth, D. S., Knops, J., Craine, J., Wedin, D. and Trost, J. 2004. Species and functional group diversity independently influence biomass accumulation and its response to CO_2 and N. Proceeding of the National Academy of Sciences of the United States of America 101(27):10101-10106.

Reigosa, M. J., Sánchez-Moreiras, A. and González, L. 1999. Ecophysiological Approach in Allelopathy. Critical Reviews in Plant Sciences 18(5):577-608.

Reiss, J., Bridle, J. R., Montoya, J. M. and Woodward, G. 2009. Emerging horizons in biodiversity and ecosystem functioning research. Trends in Ecology & Evolution 24(9):505–514.

Rice, E. L. 1972. Allelopathic effects of Andropogon virginicus and its persistence in old fields. American Journal of Botany 59(7):752-755.

Rice, E. L. 1979. Allelopathy—An update. The Botanical Review 45(1):15-109.

Rice, E. L. 1984. Allelopathy. 2nd ed. Academic Press, Orlando. xi, 422 p. pp.

Rice, K. J. 1989. Impacts of seed banks on grassland community structure and population dynamics. Pages 211-230 *in* M. A. e. a. Leck, ed. Ecology of soil seed banks. Academic Press, New York.

Roberts, H. A. and Feast, P. M. 1972. Fate of seeds of some annual weeds in different depths of cultivated and undisturbed soil. Weed Research 12(4):316-324.

Robinson, R. K. 1972. The production by roots of Calluna vulgaris of a factor inhibitory to growth of some mycorrhizal fungi. Journal of Ecology 60(1):219-224.

San Emeterio, L., Arroyo, A. and Canals, R. M. 2004. Allelopathic potential of *Lolium rigidum* Gaud. on the early growth of three associated pasture species. Grass and Forage Science 59(2):107-112.

Sanderson, M. A., Goslee, S. C., Klement, K. D. and Soder, K. J. 2007. Soil seed bank composition in pastures of diverse mixtures of temperate forages. Agronomy Journal 99(6):1514-1520.

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H. and Goslee, S. C. 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agronomy Journal 97:1465-1471.

Sanderson, M. A., Stout, R., Goslee, S., Gonet, J. and Smith, R. G. 2014. Soil seed bank community structure of pastures and hayfields on an organic farm. Canadian Journal of Plant Science 94(4):621-631.

Schellenberg, M. P. and Banerjee, M. R. 2002. The potential of legume-shrub mixtures for optimum forage production in southwestern Saskatchewan: A greenhouse study. Canadian Journal of Plant Science 82(2):357-363.

Schellenberg, M. P., Biligetu, B. and Iwaasa, A. D. 2012. Species dynamic, forage yield, and nutritive value of seeded native plant mixtures following grazing. Canadian Journal of Plant Science 92(4):699-706.

Schenkeveld, A. J. and Verkaar, H. J. 1984. The ecology of short- lived forbs in chalk grasslands: distribution of germinative seeds and its significance for seedling emergence. Journal of Biogeography 11(3):251-260.

Seigler, D. S. 1996. Chemistry and mechanisms of allelopathic interactions. Agronomy Journal 88(6):876-885.

Sheehan, C., Kirwan, L., Connolly, J. and Bolger, T. 2006. The effects of earthworm functional group diversity on nitrogen dynamics in soils. Soil Biology and Biochemistry 38:2629-2636.

Simard, M. J., Rouane, S. and Leroux, G. D. 2011. Herbicide Rate, Glyphosate/Glufosinate Sequence and Corn/Soybean Rotation Effects on Weed Seed Banks. Weed Science 59(3):398-403.

Singh, H. P., Batish, D. R. and Kohli, R. K. 2003. Allelopathic Interactions and Allelochemicals: New Possibilities for Sustainable Weed Management. Critical Reviews in Plant Sciences 22(3-4):239-311.

Smoliak, S. and Dormaar, J. F. 1985. Productivity of Russian Wildrye and Crested Wheatgrass and Their Effect on Prairie Soils. Journal of Range Management 38 (5):403-405.

Sosnoskie, L. M., Herms, N. P. and Cardina, J. 2006. Weed seedbank community composition in a 35-yr-old tillage and rotation experiment. Weed Science 54(2):263-273.

Souto, X. C., González, L. and Reigosa, M. J. 1995. Allelopathy in forest environment in Galicia, NW Spain. Allelopathy Journal 2:67-78.

Spehn, E. M., Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, M., Bazeley-White, E., Beierkuhnlein, C., Caldeira, M. C., Diemer, M., Dimitrakopoulos, P. G. and others. 2005. Ecosystem Effects of Biodiversity Manipulations in European Grasslands. Ecological Monographs 75(1):37-63.

Stewart-Wade, S. M., Neumann, S., Collins, L. L. and Boland, G. L. 2002. The biology of Canadian weeds. 117. Taraxacum officinale G. H. Weber ex Wiggers. Canadian Journal of Plant Science 82(4):825-853.

Symstad, A. J., Chapin, F. S., Wall, D. H., Gross, K. L., Huenneke, L. F., Mittelbach, G. G., Peters, D. P. C. and Tilman, D. 2003. Long-term and large-scale perspectives on the relationship between biodiversity and ecosystem functioning. BioScience 53(1):89-98.

Tang, C.-S., Cai, W.-F., Kohl, K. and Nishimoto, R. K. 1995. Plant Stress and Allelopathy. ACS Symposium Series 582:142–157.

Taylorson, R. B. 1982. Anesthetic effects on secondary dormancy and phytochrome responses in Setaria faberi seeds. Plant Physiology 70(3):882-886.

Telewski, F. W. and Zeevaart, J. A. D. 2002. The 120-yr period for Dr. Beal's seed viability experiment. American Journal of Botany 89(8):1285-1288.

Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B. and Buchmann, N. 2007. Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. Oecologia 151(2):190-205.

Thompson, K. and Grime, J. P. 1979. Seasonal-Variation in the Seed Banks of Herbaceous Species in 10 Contrasting Habitats. Journal of Ecology 67(3):893-921.

Tilman, D. 1996. Biodiversity: Population versus ecosystem stability. Ecology 77(2):350-363.

Tilman, D. 1999. The ecological consequences of changes in biodiversity: A search for general principles. Ecology 80(5):1455-1474.

Tilman, D. and Downing, J. A. 1994. Biodiversity and stability in grasslands. Nature 367:363-365.

Tilman, D., Isbell, F. and Cowles, J. M. 2014. Biodiversity and Ecosystem Functioning. Annual Review of Ecology, Evolution, and Systematics 45:471-493.

Tilman, D., Lehman, C. L. and Thomson, K. T. 1997. Plant diversity and ecosystem productivity: theoretical considerations. Proc Natl Acad Sci U S A 94(5):1857-61.

Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T. and Lehman, C. 2001. Diversity and productivity in a long-term grassland experiment. Science 294(5543):843-5.

Tilman, D., Reich, P. B. and Knops, J. M. 2006a. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441(7093):629-32.

Tilman, D., Reich, P. B. and Knops, J. M. 2006b. Biodiversity and ecosystem stability in a decadelong grassland experiment. Nature 441(7093):629-632.

Tilman, D., Wedin, D. and Knops, J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystem. Nature 379:718-720.

Tracy, B. F. and Sanderson, M. A. 2000. Seedbank diversity in grazing lands of the Northeast United States. Journal of Range Management 53(1):114-118.

Tracy, B. F. and Sanderson, M. A. 2004. Forage productivity, species evenness and weed invasion in pasture communities. Agriculture, Ecosystems and Environment 102(2):175-183.

Trenbath, B. R. 1974a. Biomass productivity of mixtures. Advances in Agronomy 26:177-210.

Trenbath, B. R. 1974b. Neighbour Effects in the Genus *Avena*. II. Comparison of Weed Species. Journal of Applied Ecology 11(1):111-125.

Turner, J. A. and Rice, E. L. 1975. Microbial decomposition of ferulic acid in soil. Journal of Chemical Ecology 1(1):41-58.

Unger, I. A. and Woodell, S. R. J. 1993. The relationship between the seed bank and species composition of plant communities in two British salt marshe. Journal of Vegetation Science 4(4):531-536.

Unger, I. A. and Woodell, S. R. J. 1996. Similarity of seed bank to above ground vegetation in grazed and ungrazed salt marsh communities on the Gower Peninsula, South Wales. International Journal of Plant Sciences 157(6):746-749.

USDA. 2016. Natural Resources Conservation Service Plant Materials Program https://www.nrcs.usda.gov/wps/portal/nrcs/main/plantmaterials/pmc/. [Online].

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Cornell University Press.

Viard-Crétat, F., Baptist, F., Secher-Fromell, H. and Gallet, C. 2012. The allelopathic effects of *Festuca paniculata* depend on competition in subalpine grasslands. Plant Ecology 213(12):1963-1973.

Vibart, R. E., Vogeler, I., Dodd, M. and Koolaard, J. 2016. Simple versus Diverse Temperate Pastures: Aspects of Soil–Plant–Animal Interrelationships Central to Nitrogen Leaching Losses. Agronomy journal 108(6):2174-2188.

Walker, B. 1995. Conserving Biological Diversity through Ecosystem Resilience. Conservation Biology 9(4):747-752.

Walker, B., Kinzig, A. and Langridge, J. 1999. Plant attribute diversity, resilience, and ecosystem function: The nature and significance of dominant and minor species. Ecosystems 2(2):95-113.

Wang, P., Stieglitz, T., Zhou, D. W. and Cahill Jr, J. F. 2010. Are competitive effect and response two sides of the same coin, or fundamentally different? Functional Ecology 24(1):196-207.

Wang, Z. and Schellenberg, M. P. 2012. Drought and N addition in a greenhouse experiment: blue grama and western wheatgrass. Journal of Agricultural Science and Technology B(2):29-37.

Wardle, D. A. 2001. Experimental demonstration that plant diversity reduces invasibility – evidence of a biological mechanism or a consequence of sampling effect? Oikos 95(1):161-170.

Warr, S. J., Thompson, K. and Kent, M. 1993. Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. Progress in Physical Geography 17(3):329-347.

Webster, T. M., Cardina, J. and White, A. D. 2003. Weed seed rain, soil seedbanks, and seedling recruitment in no-tillage crop rotations. Weed Science 51(4):569-575.

Weigelt, A., Weisser, W. W., Buchmann, N. and Scherer-Lorenzen, M. 2009. Biodiversity for multifunctional grasslands: equal productivity in high-diversity low-input and low-diversity high-input systems. Biogeosciences 6:1695-1706.

Whitbread, A. M., Hall, C. A. and Pengelly, B. C. 2009. A novel approach to planting grass-legume pastures in the mixed farming zone of southern inland Queensland, Australia. Crop & Pasture Science 60(12):1147-1155.

Wight, J. R. and White, L. M. 1974. Interseeding and pitting on a sandy range site in eastern Montana. Journal of Range Management 27(3):206-210.

Willms, W. D., Ellert, B. H., Janzen, H. H. and Douwes, H. 2005. Evaluation of Native and Introduced Grasses for Reclamation and Production. Rangeland Ecology & Management 58(2):177-183.

Wilson, R. G. and Michiels, A. 2003. Fall herbicide treatments affect carbohydrate content in roots of Canada thistle (*Cirsium arvense*) and dandelion (*Taraxacum officinale*). Weed Science 51(3):299-304.

Woo, S. L., Harms, V. L., Thomas, A. G., Peschken, D. P., Bowes, G. G., Douglas, D. W. and McClay, A. S. 1991. The biology of Canadian weeds. 99. *Matricaria perforate* Merat (Asteraceae). Canadian Journal of Plant Science 71(4):1101-1119.

Yachi, S. and Loreau, M. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. Proceedings of the National Academy of Sciences of the USA 96:1463-1468.

Young, T. P., Chase, J. M. and Huddleston, R. T. 2001. Community succession and assembly: comparing: contrasting and combining paradigms in the context of ecological restoration. Ecological Restoration 19(1):5-18.

Zeng, R. S., Mallik, A. U. and Luo, S. M. 2008. Allelopathy in Sustainable Agriculture and Forestry. Springer.

Zhang, J., Hamill, A. S., Gardiner, I. O. and Weaver, S. E. 1998. Dependence of weed flora on the active soil seedbank. Weed Research 38(2):143-152.

Zhang, S. and Lamb, E. G. 2011. Plant competitive ability and the transitivity of competitive hierarchies change with plant age. Plant Ecology 213(1):15-23.

Zorner, P. S., Zimdahl, R. L. and Schweizer, E. E. 1984. Sources of viable seed loss in buried dormant and non-dormant populations of wild oat (*Avena fatua* L.) seed in Colorado. Weed Research 24(2):143-150.

CHAPTER 9

Appendix

Appendix 9.1 Example SAS code used for analyzing data in chapter 2. The effect of different forage species and their mixtures on forage quantity and quality were analyzed as repeated measures via a mixed model. Treatment, year and harvesting month were fixed effects and block was a random effect. Since there were unequal periods between harvesting times (two months period from July to August in each year, and 10 months period from August to next July harvest), data was analyzed using a spatial power covariance structure. Denominator degrees of freedom were calculated using BETWITHIN option.

```
proc mixed;
class plot rep trt year month;
model dry=trt year month trt*year trt*month year*month trt*year*month/DDFM= BETWITHIN;
random rep;
repeated / subject=plot type=SP(LINL)(space) r rcorr;
run;
```

Appendix 9.2 Forage production (kg/ha) in mixture plots in July and August of 2011-2016. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. One standard error around the mean is presented after ± (n = 4).

	2011		2012		2013		2014		2015		2016	
Mixtures	July	August	July	August	July	August	July	August	July	August	July	August
All Species	3184±912	5621±1559	1623±360	3163±603	1897±304	1919±482	2010±170	2198±321	1893±137	2008±321	2129±361	2939±343
BBW+PPC	2639±589	2338±460	1977±333	2924±236	1592±426	2302±373	2386±453	1949±416	1864±173	2164±184	1927±478	1576±366
BBW+WPC	2514±315	2956±569	1297±115	1786±175	2067±209	1982±431	1489±303	2421±345	1874±198	2365±213	2440±643	1606±774
LBS+BBW	1630±662	1983±441	853±221	3454±527	2473±695	1734±524	1504±272	2934±482	1437±170	2434±296	2940±244	3633±121
LBS+NOB	3385±518	6065±1403	847±175	1329±243	794±332	917±153	1130±557	1222±270	1007±211	1106±48	2798±163	1871±339
LBS+PPC	128±36	582±57	695±190	1785±372	1554±268	1559±465	1417±293	1866±449	1281±236	1423±237	1662±555	3311±113
LBS+WPC	192±23	740±311	654±176	2105±485	2353±111	2556±710	1011±288	2222±601	1082±168	1385±479	1288±444	3716±724
NOB+BBW	3459±394	5696±738	1616±394	2122±304	1678±470	1497±183	1854±218	1719±136	1372±268	1827±254	1137±127	1832±380
NOB+PPC	4477±975	4455±816	1196±214	1577±152	674±205	980±316	689±236	1182±225	387±136	874±258	507±205	1925±126
NOB+WPC	2765±729	4446±899	525±105	959±42	1196±431	1216±518	779±156	446±87	212±93	444±155	875±330	2185±564
PPC+WPC	11±5	80±45	233±102	886±265	713±352	609±204	131±51	490±318	165±79	223±154	204±154	1316±442
SOG+BBW	1705±430	2598±281	1920±193	2189±515	2621±461	2079±264	1579±474	2001±218	1650±275	1745±215	2300±560	2344±387
SOG+LBS	711±123	1869±84	1033±271	1897±340	1315±327	1458±360	1345±137	2042±161	1289±189	1458±208	1231±150	2484±254
SOG+NOB	3583±379	5197±1250	813±109	1453±455	620±308	520±139	928±313	884±236	484±130	857±303	626±210	945±116
SOG+PPC	285±48	890±235	529±255	1662±278	907±76	1339±498	837±258	2059±438	648±100	1274±264	1246±302	2584±592
SOG+WPC	245±79	708±29	432±100	2995±146	914±407	1659±869	414±115	756±162	586±43	803±234	651±178	2652±617
WWG+BBW	4453±463	5594±579	1023±339	3324±399	1714±167	2156±472	2294±207	2831±161	2352±119	2861±222	2442±497	2643±375
WWG+LBS	5649±539	7138±950	1842±497	3323±598	1254±236	1932±219	2208±301	2434±377	2113±180	2512±360	2130±114	2617±307
WWG+NOB	6851±492	7374±789	1849±324	3506±604	1941±188	1928±470	2719±380	2985±226	2248±280	2397±148	2211±289	2677±359
WWG+PPC	7099±1207	5845±837	1739±371	3994±121	2582±256	2067±342	2397±337	2338±269	2081±69	2290±198	1834±550	3889±805
WWG+SOG	4577±762	5378±887	1732±200	3793±485	1973±68	2074±217	1914±342	2470±430	2035±220	2445±66	1880±217	2978±625
WWG+WPC	4588±918	6137±971	1555±403	3493±993	2097±127	2496±298	2565±544	2860±133	2593±153	2871±477	1942±299	3655±592

Appendix 9.3 Crude protein (N×6.25) in mixture plots in July and August of 2011-2015. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. One standard error around the mean is presented after ± (n = 4).

	2011		2012		2013		2014		2015		2016	
Mixtures	July	August	July	August	July	August	July	August	July	August	July	August
All Species	8.19±0.58	5.40±0.51	9.40±0.52	5.98±0.35	7.06±0.48	5.15±0.21	6.70±0.56	4.16±0.31	5.66±0.14	4.80±0.14	6.94±1.45	6.38±1.02
BBW+PPC	6.99±0.24	6.88±0.72	7.32±0.32	6.55±0.51	6.36±0.73	5.26±0.39	6.66±0.32	4.12±0.30	6.40±0.22	6.55±0.83	6.64±1.01	5.22±0.49
BBW+WPC	8.45±0.45	6.87±0.83	7.49±0.29	6.52±0.61	7.28±0.54	4.88±0.61	6.41±0.17	4.65±0.22	6.13±0.28	4.91±0.12	6.66±1.70	6.54±0.98
BBW+WWG	8.02±1.14	6.29±1.35	9.21±0.36	6.27±0.78	6.71±0.53	5.55±0.48	6.11±0.19	4.47±0.14	5.47±0.45	4.69±0.55	6.23±0.58	4.19±0.25
LBS+BBW	8.16±1.05	6.13±0.33	7.52±0.61	3.31±0.36	5.95±0.24	3.33±0.25	5.45±0.49	2.43±0.21	5.70±0.43	4.03±0.30	4.58±0.50	3.19±0.09
LBS+NOB	7.70±0.39	4.20±0.22	7.29±0.33	3.89±0.39	6.20±0.33	4.86±0.41	5.80±0.85	2.88±0.26	4.91±0.31	5.19±0.77	5.23±0.09	3.39±0.22
LBS+PPC	10.98±1.07	5.91±0.73	8.53±0.19	4.70±0.84	6.42±0.06	4.48±0.24	5.95±0.41	3.98±0.35	5.54±0.41	5.84±1.30	6.42±0.02	4.90±0.75
LBS+WPC	9.70±0.25	6.66±0.86	8.38±0.38	3.46±0.31	7.44±0.93	5.98±0.80	4.92±0.17	3.31±0.89	6.25±1.20	6.06±1.76	8.03±1.88	4.11±1.34
LBS+WWG	8.93±0.82	5.41±1.23	9.37±0.75	5.57±0.22	6.16±0.33	4.89±0.17	6.45±0.43	4.05±0.25	5.38±0.22	4.52±0.04	5.61±0.26	3.83±0.18
NOB+BBW	6.85±0.34	4.28±0.38	7.25±0.53	4.71±0.31	5.40±0.30	4.48±0.29	6.14±0.49	4.08±0.29	6.33±0.19	5.32±0.36	4.45±0.23	3.77±0.16
NOB+PPC	6.94±0.33	4.12±0.82	7.04±0.17	4.26±0.69	8.73±1.91	7.02±0.71	7.84±1.33	6.31±0.41	7.10±0.67	7.65±0.50	10.23±2.06	9.06±1.14
NOB+WPC	7.91±0.20	4.69±0.60	8.21±0.43	5.60±0.94	7.70±0.91	8.63±0.76	8.40±0.55	8.67±1.03	8.45±1.05	7.71±0.81	9.56±1.55	11.45±1.74
NOB+WWG	8.48±0.52	5.02±1.06	10.57±0.95	5.64±0.31	7.34±0.62	5.59±0.23	7.40±0.21	5.44±0.69	5.66±0.31	5.82±0.74	5.39±0.04	4.17±0.19
PPC+WPC	14.75±0.75	11.71±0.38	15.50±0.86	9.27±1.25	13.78±1.80	10.93±0.30	11.34±0.56	9.79±0.16	11.04±0.52	9.10±0.35	14.13±1.43	11.33±0.67
SOG+BBW	8.53±0.51	7.06±0.66	8.20±0.96	4.73±0.48	6.08±0.62	4.09±0.14	6.41±0.50	3.43±0.15	6.31±0.34	4.66±0.14	4.52±0.40	3.91±0.25
SOG+LBS	9.09±0.63	3.98±0.16	6.84±0.30	3.08±0.17	5.48±0.46	3.31±0.15	4.29±0.13	2.56±0.06	4.55±0.14	3.58±0.37	5.38±0.18	3.19±0.22
SOG+NOB	6.95±0.18	4.70±0.50	6.47±0.41	3.80±0.56	6.15±0.17	4.91±0.14	5.94±0.67	3.04±0.10	5.62±0.12	5.60±0.63	6.31±0.59	5.05±0.51
SOG+PPC	9.67±0.58	4.39±0.27	11.37±0.36	4.35±0.69	8.35±0.68	6.19±0.88	7.07±0.77	6.35±1.18	7.99±0.81	6.10±0.25	9.86±1.22	8.42±0.20
SOG+WPC	8.74±0.15	6.15±0.33	9.81±0.66	5.21±0.44	8.41±1.19	5.97±0.58	7.23±0.66	5.02±1.05	6.76±0.73	5.38±0.21	8.52±1.35	6.44±0.97
SOG+WWG	8.06±0.81	5.20±0.50	9.63±0.12	6.03±0.66	6.82±0.30	5.16±0.35	6.92±0.43	4.56±0.63	5.62±0.23	4.76±0.27	5.59±0.27	4.22±0.13
WWG+PPC	9.32±0.86	5.56±0.79	11.95±1.42	6.36±0.09	8.13±0.59	5.49±0.38	8.49±1.01	4.78±0.69	6.07±0.50	5.51±0.36	6.86±0.39	6.13±0.68
WWG+WPC	8.89±0.69	5.66±0.77	10.20±0.38	6.05±0.25	6.89±0.47	6.13±0.88	6.94±0.46	5.19±1.01	5.52±0.20	5.20±0.39	5.73±0.24	5.31±0.66

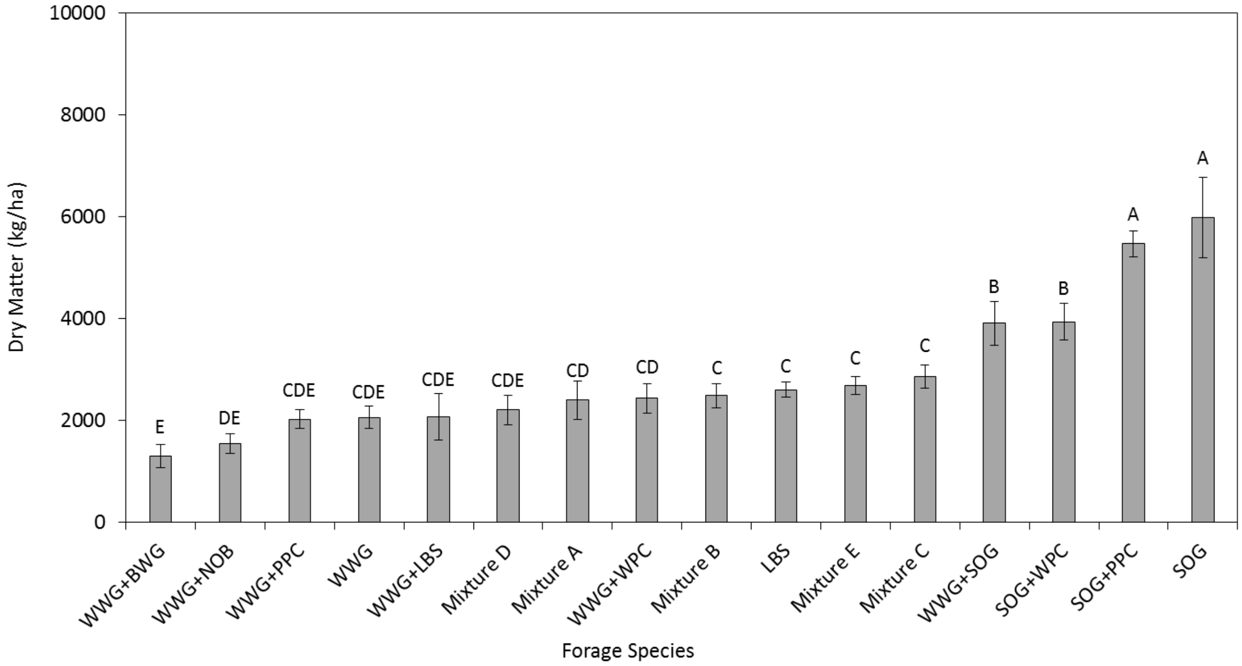
Appendix 9.4 *F* statistics and *P* values indicating statistical significance for the treatment, year, month and their interactions on production, crude protein, Acid detergent fiber (ADF), Neutral detergent fiber (NDF) and Phosphorus of seven forage species in Brandon MB.

	F statistic and *P* value				
Fixed Effects	Forage Production	Crude Protein	ADF	NDF	P
Treatment	$F_{15,45}$=16.39 $P < 0.0001$	$F_{15,45}$=9.68 $P < 0.0001$	$F_{15,45}$=4.45 $P < 0.0001$	$F_{15,45}$=18.09 $P < 0.0001$	$F_{15,45}$=2.31 $P = 0.0153$

Plots in Swift Current and Brandon were first seeded at the same time (May 2014) but the Brandon plots were heavily infested by perennial weeds and thus were reseeded in 2016. In Brandon, monocultures of LBS were added based on the results of the allelopathy experiments (Chapter 6) and forage yield was measured in the August 2016 (seeding year).

Forage production significantly differed between treatments (Appendix 9.5). Monoculture of SOG performed better than other monocultures and mixtures in the seeding year. Binary mixtures containing SOG produced higher forage yield, and among complex mixtures, mixture E and C provided greater dry matter in the establishment year of 2016.

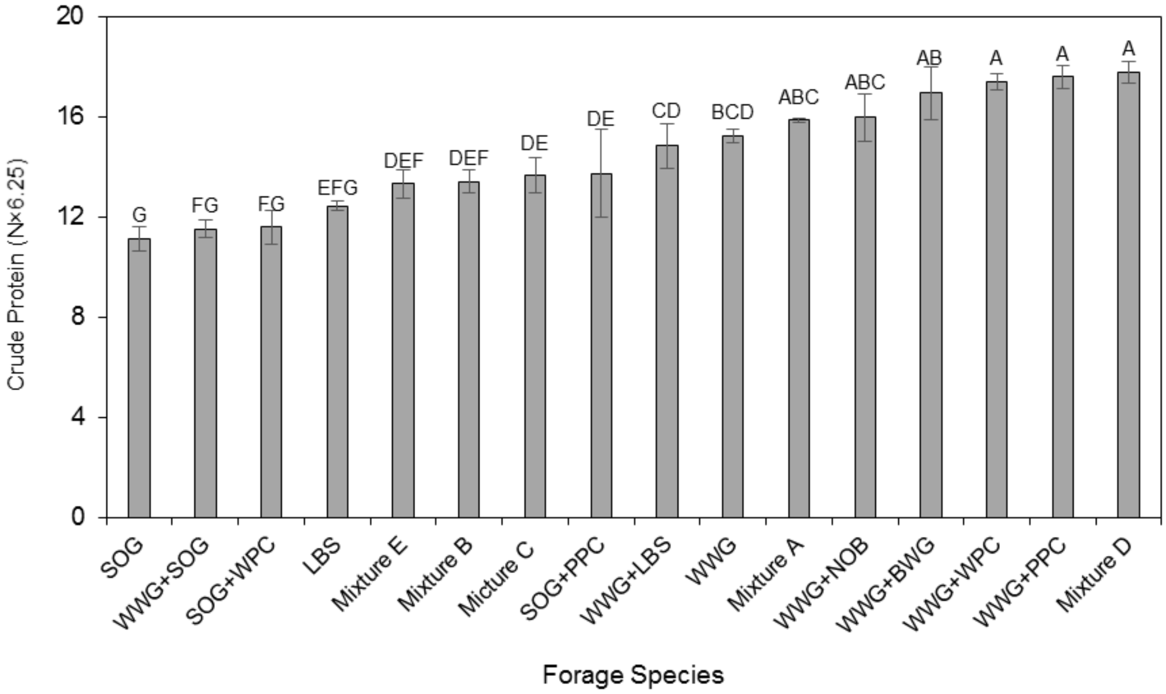
Crude protein was significantly different between treatments and its concentrations ranged from 11-18% (Appendix 9.7). Binary mixtures of WWG+legumes and mixture D (contained 19% WPC) contained the lowest ADF and NDF concentrations (Appendix 9.8). Thus, adding legumes to mixtures may reduce the fiber concentration of the forage mixtures.



Appendix 9.5 Forage production (kg/ha) in monoculture and mixture plots in August 2016 in Brandon MB. The species composition of Mixture A-E is provided in Table 4.2. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; n = 4. Bars with the same letter are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

Appendix 9.6 Species composition in the mixture plots in August of 2016 in Brandon MB. Species composition was measured the seeding year. The species composition of Mixtures A-E is provided in Table 4.2. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover.

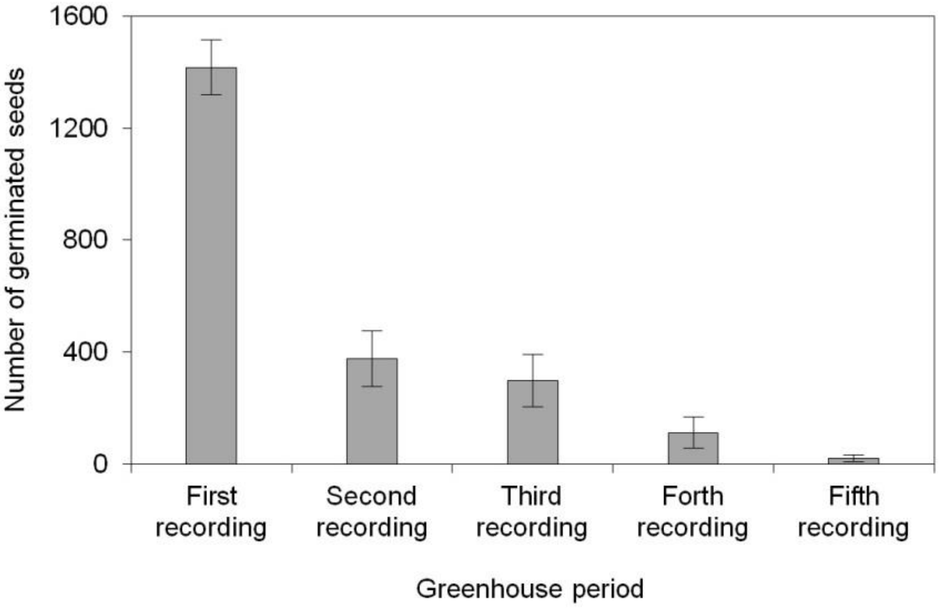
Mixture	Species	Seeding Rate %	Species Composition %
Mixture A	WWG	14	19
Mixture A	WPC	10	26
Mixture A	SOG	5	38
Mixture A	NOB	48	12
Mixture A	LBS	9	5
Mixture A	BWG	14	1
Mixture B	WWG	14	7
Mixture B	SOG	14	86
Mixture B	PPC	5	5
Mixture B	LBS	5	1
Mixture B	BWG	62	1
Mixture C	WWG	57	23
Mixture C	SOG	19	71
Mixture C	PPC	19	6
Mixture C	BWG	5	0
Mixture D	WWG	38	38
Mixture D	WPC	19	45
Mixture D	NOB	24	15
Mixture D	BWG	19	2
Mixture E	WWG	71	39
Mixture E	WPC	5	8
Mixture E	SOG	9	48
Mixture E	PPC	5	2
Mixture E	NOB	5	1
Mixture E	LBS	5	1
WWG+BBW	WWG	50	95
WWG+BBW	BWG	50	5
WWG+LBS	WWG	50	53
WWG+LBS	LBS	50	47
WWG+NOB	WWG	50	64
WWG+NOB	NOB	50	36
WWG+PPC	WWG	50	66
WWG+PPC	PPC	50	34
WWG+SOG	WWG	50	16
WWG+SOG	SOG	50	84
WWG+WPC	WWG	50	37
WWG+WPC	WPC	50	63



Appendix 9.7 Crude protein (N×6.25) in monocultures of WWG, SOG and LBS, and mixture plots in August of 2016 in Brandon, MB. The species composition of Mixtures A-E is provided in Table 4.2. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. Error bars represent one standard error around the mean; n = 4. Bars containing the same letter are not significantly different ($P \leq 0.05$) according to protected Fisher's LSD test.

Appendix 9.8 ADF, NDF and P concentrations (%) in a monoculture of WWG and mixture plots in August of 2016 in Brandon MB. The species composition of Mixtures A-E is provided in Table 4.2. Abbreviation: WWG: Western wheatgrass; BBW: Bluebunch wheatgrass; SOG: Side oats grama; LBS: Little blue stem; NOB: Nodding brome; PPC: Purple prairie clover; and WPC: White prairie clover. One standard error around the mean is shown after ±; *n* = 4.

Species	ADF (%)	NDF (%)	P
Mixture A	27.92±0.309	51.35±1.870	0.27±0.007
Mixture B	30.65±0.843	60.13±1.583	0.25±0.011
Mixture C	28.97±0.693	56.34±1.047	0.22±0.023
Mixture D	25.15±0.762	45.16±2.807	0.30±0.029
Mixture E	29.14±1.107	57.97±1.751	0.22±0.013
SOG+WWG	29.41±1.350	60.91±1.666	0.19±0.021
WWG	28.61±1.748	51.46±0.896	0.22±0.010
WWG+BWG	26.49±0.798	50.01±1.248	0.28±0.035
WWG+LBS	27.10±1.201	53.52±1.242	0.24±0.031
WWG+NOB	27.85±1.484	52.46±1.675	0.29±0.019
WWG+PPC	26.00±0.634	45.29±2.059	0.29±0.023
WWG+WPC	22.98±1.230	38.00±3.695	0.29±0.022



Appendix 9.9 Total number of germinated seeds during the course of greenhouse experiment. About 95% of germinated seeds was recorded from the first, second and third greenhouse period. Error bars represent one standard error around the mean; *n*=4.