# Perennial Forage Mixtures: An Integrated Approach to Investigating their Performance for Multifunctionality

by

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**Abstract**

Perennial forage mixtures are used in the feeding regimes of beef cattle producers in Alberta. This provides consistency in the supply of forage biomass and nutritive value to cover up lapses in annual forage supply, and adequately improve the performance of beef cattle. Additionally, perennial forage mixtures can potentially provide ecosystem services. However, there is skepticism regarding the correct combinations of grass and legume species to attain these benefits. Furthermore, beef cattle producers ask questions about the mixtures that can help improve their livestock-forage systems. To resolve this, a multiyear perennial forage field study was conducted at the Peace Country Beef and Forage Association, northwestern Alberta, Canada, to evaluate the performance of different forage mixtures in terms of productivity, water use efficiency, impact on soil quality, nitrogen fixation and transfer, performance of beef cattle categories on the mixtures, and to recommend some forage mixtures to producers for inclusion in their forage systems. Our findings showed that grass–legume mixtures consistently had a greater dry matter yield than monoculture grasses (by a 3.0 Mg ha$^{-1}$ difference). Total digestible nutrients (only in the first year of forage production) and crude protein were higher and neutral digestible fiber was lower in grass–legume mixtures. In contrast, grass monocultures had higher total digestible nutrients (in the second and third years of production) and 48 h neutral detergent fiber digestibility, and lower acid detergent fiber (in the second and third years of production). In terms of soil quality, complex grass–legume mixtures had greater biological activity (as $CO_2$ production) than monoculture grasses at 0–15 cm soil depth, whereas monoculture grasses had superior $CO_2$ production at 15–30 cm layer. Surface soil infiltration (0.79 cm h$^{-1}$) and compaction were improved under mixtures containing legumes. Overall, the apparent rate of soil carbon sequestration was higher under monoculture of Fleet meadow bromegrass (10 Mg C ha$^{-1}$ yr$^{-1}$) compared to grass–legume

mixtures. Furthermore, all legumes in the simple and complex grass–legume mixtures derived most of their nitrogen use from the atmosphere (>94%). However, alfalfa had a greater amount of fixed nitrogen (210.1 kg N $ha^{-1}$), followed by sainfoin (52.9 kg N $ha^{-1}$). Overall, more nitrogen was fixed by mixtures with more legume species and proportions, whereas a greater amount of nitrogen was transferred in mixtures with two grass species as compared to only one grass species. With regards to animal performance, grass–legume mixtures were adequate to support steers in obtaining an average daily gain (ADG) of 0.8 kg $day^{-1}$ whereas monoculture grasses, grass–legume and grass-only mixtures were adequate to support gestating and lactating beef cows to obtain average daily gain of 0.10 kg $day^{-1}$. In summary, perennial forage mixtures containing (i) AC Knowles hybrid bromegrass + spredor 5 alfalfa, (ii) AC Mountainview sainfoin + Veldt cicer milkvetch + spredor 5 alfalfa, (iii) AC Success hybrid bromegrass + AC Mountainview sainfoin + AC yellowhead alfalfa, (iv) AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + kirk crested wheatgrass + Italian ryegrass +AC yellowhead alfalfa + rugged alfalfa + Veldt cicer milkvetch + AC Mountainview sainfoin, and (v) AC Success hybrid bromegrass + AC yellowhead alfalfa + AC Mountain sainfoin have been identified as viable options for cultivation in Alberta based on multifunctionality analysis. However, trade-offs were noticed for these treatments. AC Knowles hybrid bromegrass + spredor 5 alfalfa and AC Mountainview sainfoin + Veldt cicer milkvetch + spredor 5 alfalfa had superior dry matter yield and nutritive value but poor at enhancing soil quality indicators. In addition, AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + kirk crested wheatgrass + Italian ryegrass +AC yellowhead alfalfa + rugged alfalfa + Veldt cicer milkvetch + AC Mountainview sainfoin, had high dry matter yield, nutritive value and also enhanced the soil compaction. These results may assist beef cattle producers to improve their forage livestock systems.

## Preface

A chapter in this thesis is a collaborative work with Agriculture and Agri-Food Canada, Government of Canada, Lethbridge, Alberta, under the support of Patrick Neuberger and Monika A. Gorzelak.

Chapter 2 of this thesis has been submitted as "Productivity and nutritive value of grass–legume forage mixtures in the Canadian Prairies: a synthesis" to the Agronomy Journal. The conceptualization, article search, formal analysis, methodology, visualization, writing – original draft, writing – review and editing was done by me. Akim Omokanye provided funding, methodology, project administration, resources, supervision, and writing – review and editing. Guillermo Hernandez-Ramirez assisted with conceptualization, data curation, formal analysis, funding acquisition, software supervision, writing – review and editing. Malinda S. Thilakarathna supported the conceptualization, methodology, visualization, writing – review and editing.

Chapter 3 is a manuscript titled "Does diversity of perennial forage species affect soil quality?" to be submitted to the Soil and Tillage Research Journal. The experimental plot design, field data collection, statistical data analysis, visualization, original draft writing, review and editing was done by me. Guillermo Hernandez-Ramirez helped with fund acquisition, conceptualization, data curation, data analysis, visualization, writing – editing and review. Akim Omokanye assisted with fund acquisition, project design and administration, data analysis, writing – editing and reviews. Malinda S. Thilakarathna assisted with data visualization, data analysis, writing – editing and review.

Chapter 4 is a manuscript titled "Productivity, nutritive value, and botanical composition of diverse forage species" has been submitted to Forage and Grass Science Journal. The field data collection, conceptualization, data analysis, original draft writing – editing and review was done by me. Akim Omokanye was responsible for experimental design, project administration, fund acquisition, data analysis, writing – editing and review. Guillermo Hernandez-Ramirez assisted with fund acquisition, data curation, data analysis, writing – editing and reviews. Malinda S. Thilakarathna supported the data visualization, data analysis, writing – editing and reviews.

Chapter 5 is a manuscript titled "Biomass and crude protein water-use efficiencies of perennial forage mixtures" to be submitted to Canadian Journal of Plant Science. The field data collection, conceptualization, data analysis, original draft writing – editing and review was done by me. Guillermo Hernandez-Ramirez assisted with data analysis, fund acquisition, project conceptualization, project administration, writing – editing and reviews. Akim Omokanye supported fund acquisition, project administration, data analysis, writing – editing and reviews. Malinda S. Thilakarathna assisted with data analysis, data visualization, writing – editing and reviews.

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Chapter 7 is manuscript titled "Predicting the performance of beef cattle on diverse perennial forage mixtures using the CowBytes ration-balancing software program" to be submitted to the Canadian Journal of Animal Science. The field data collection, conceptualization, statistical data analysis, original draft writing – editing and review was done by me. Akim Omokanye assisted with project funding, conceptualization, project management, data analysis, writing- editing and reviews. Guillermo Hernandez-Ramirez supported funding acquisition, conceptualization, data analysis, writing- editing and reviews. Malinda S. Thilakarathna assisted with conceptualization and writing-editing and reviews. Barry Yaremcio helped with model formulation, data analysis, data curation, software development, writing- editing and reviews.

Chapter 8 is a manuscript "Identifying top performers of temperate perennial forages based on cluster analysis" to be submitted to the Canadian Journal of Plant Science. The field data collection, conceptualization, statistical data analysis, original draft writing – editing and review was done by me. Akim Omokanye assisted with project funding, data analysis, data visualization, writing -editing and reviews. Guillermo Hernandez-Ramirez assisted with data visualization, fund acquisition, writing-editing and reviews. Malinda S. Thilakarathna was responsible for data visualization, writing-editing and reviews. Surendra Bhattarai assisted with data visualization, statistical data analysis, writing – editing and reviews, Blasius Azuhnwi assisted with data visualization, writing - editing and reviews.

## Dedication

I dedicate this work to my late father, Mr. Edmund Kofi Amoh for his unrelenting support and encouragement throughout my education. He was the pillar and solid rock behind me until his untimely demise in 2019. I also dedicate this to Mrs. Mary Martha Akosua-Boatemaa Peprah for her patience and commitment during this period. Finally, I dedicate this to all my friends and loved ones (in particular, Dr. Perejitei Bekewe) for their push and contribution to making this process successful. I say God richly bless you all.

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## Table of Contents

## List of Tables

**List of Figures**

# Chapter 1. General introduction

The global demand for meat is projected to increase by 200 million metric tonnes by 2050 (Hunter *et al*., 2017), owing to a projected rise in human growth and changes in dietary preferences around the globe (Alexandratos and Bruinsma, 2012). It is thus necessary for the meat industry to produce sufficient quantities to meet the demands of the rising human population predicted to also reach 9.7 billion by 2050 (FAO, 2017). A gradual expansion in the Canadian beef industry signifies a strong prospect of partially contributing to projected global meat demand. A recent report by Agriculture and Agri-Food Canada (AAFC, 2021) indicated that the Canadian beef industry is the $11^{th}$ largest producer and $7^{th}$ largest exporter of beef globally. In Canada, the Prairies Provinces (Alberta, Saskatchewan, and Manitoba) holds approximately 80% of the Canadian beef herd (Statistics Canada, 2019; Pogue et al., 2018). The province of Alberta currently accounts for 43.2% of beef cattle production in Canada (Statistics Canada, 2023). With the huge beef cattle production within the Canadian Prairies, it is imperative to produce sufficient forage to meet their nutritional demands.

Forage production is an important component of animal production systems, including the beef, dairy cattle, and small ruminant industries (Sheppard *et al*., 2015). In Canada, forage production is the largest by volume and the third most valuable crop product, only behind wheat and canola (Bonnefield Research, 2016; Statistics Canada, 2022). The province of Alberta accounts for approximately 43% of the forage production with approximately, 21.1 million hectares (Mha) of agricultural land supporting beef production (Canadian Roundtable for Sustainable Beef, 2016). These lands consist of the native grassland, tame grassland of commercial grass–legume mixtures, and hay lands comprising 12.9 Mha, 5 Mha, and 1.8 Mha respectively (Canadian Roundtable for Sustainable Beef, 2016). These forage lands are established for greenfeed from annual crops, cereal grain silage, and perennial forage to meet the feeding requirements of beef cattle (Fraser *et al*., 2004; Omokanye et al., 2019). Although annuals, either as monocultures or as mixtures, supply forage feed (Beef Cattle Research Council, 2016) and offer ecological services such as ameliorating water quality (Dabney *et al*., 2001) and soil quality (Wortman et al., 2012), these forage species are short-lived (McCartney and Fraser, 2010). (Wortman *et al*., 2012). However, their perennial counterparts have a greater impact on forage production and ecological services.

Perennial forages have higher longevity, with proper establishment and maintenance they can manifest persistence, productivity, and nutritive forage outputs (Aasen and Bjorge, 2009; Bonin and Tracy, 2012; Sanderson *et al*., 2013; Serajchi *et al*., 2017; Dhakal and Anowarul Islam, 2018), and provide feed that adequately meets the nutritional requirements of beef cattle (Kulathunga *et al*., 2016). Multispecies perennial forage mixtures can increase the livestock carrying capacity (Vasques *et al*., 2019) and improve animal performance and body condition scores (Coleman and Moore, 2003; Peprah *et al*., 2021). Compared to pure stands of monocultured grasses, mixed forages can improve palatability, intake, and digestibility (Naydenova and Vasileva, 2016). Perennial forage monocultures or mixtures can also be adopted as a long-term solution to curtail lapses in low forage productivity by annuals (Jefferson *et al*., 2005).

In addition to the enormous benefits of perennial mixtures to the cattle sector, mixed perennial forages are crucial for the proper functioning of the agroecosystem (Picasso *et al*., 2011) and the diversified landscapes that favor the stability of the environment and overall terrestrial ecosystems (Hooper *et al*., 2005). Perennial forage establishment benefits soils through nutrient cycling of nitrogen (N), carbon (C) and improves the overall functioning of microbial populations in the soil (Wedin and Russelle, 2020). Furthermore, perennial forage monocultures or mixtures are characterized by low soil movement or disturbances and stand persistence over several years, which are beneficial to the soil structure (Albayrak and Ekiz, 2005; Aasen and Bjorge, 2009). Soils under perennial forages can potentially increase the accumulation of soil organic matter (SOM) over time compared with monocultures. Increasing SOM supports water infiltration, aeration, soil structure, and water-holding capacity ( Franzluebbers, 2002; Grosbellet et al., 2011). In addition, yearly estimates suggest that legumes in mixtures supply nitrogen to grasses (Pirhofer-Walzl *et al*., 2012), eliminating the excessive use of synthetic fertilizers and mitigating the release of greenhouse gases into the atmosphere (Canadell *et al*., 2019).

The contribution of perennial forage mixes to forage productivity, quality, and ecosystem benefits cannot be overemphasized. Across Alberta, questions from beef producers focus on how to improve their pastures or hay land using combinations of grass and legume species to ensure stability in forage productivity requiring low maintenance, adaptable to various environmental conditions, and build soils through nutrient cycling. Previous studies have demonstrated the importance of annual forage mixtures within many parts of Alberta (Berkenkamp and Meeres, 1987; Jedel and Salmon, 1994; Omokanye et al., 2019), and a few other studies have focused on perennial forage mixtures within the Alberta ecoregions (Westerlund, 2018; MARA, 2018). However, these perennial forage trials were limited to fewer grass or legume species combinations in the same experimental plots. Therefore, there remains a knowledge gap in the field of multispecies perennial forage. This project intends to bridge the information gap by evaluating several highly diverse grass or legume species per stand in Northwestern Alberta, to understand their effects on improved forage biomass and quality compared to monoculture stands of grasses. This will provide producers with valuable information regarding the selection and combination of perennial forage species. The objectives of this study were;

I. To conduct a meta-analysis on the productivity and nutritive value of perennial grass–legume forage mixtures in the Canadian Prairies.

II. To evaluate the effects of diverse perennial forage mixtures on soil quality.

III. To determine the forage dry matter (DM) yield, nutritive value, and botanical composition of perennial forage mixtures.

IV. To examine the water-use efficiencies among the different perennial forage functional groups.

V. To quantify the biological nitrogen fixation (BNF) by legumes and the nitrogen transfer to grasses in simple and complex grass–legume mixtures.

VI. To predict the performance of beef cattle on diverse perennial forage species using the CowBytes® ration-balancing software program.

VII. To identify top performers of temperate perennial forages based on cluster analysis.

# Chapter 2. Productivity and nutritional value of perennial grass–legume forage mixtures in the Canadian Prairies: a synthesis

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**Study synopsis**

Growing grass–legume forages instead of monoculture grasses may improve livestock feed; however, a unified synthesis of these effects on the productivity and quality of forage within the Canadian Prairies is still unavailable. We compiled published data to examine the forage's dry matter yield (DMY), crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) of perennial grass–legume mixtures versus grass monocultures by calculating their relative effect sizes (TE), based on a natural logarithmic scale. Across the assembled experimental comparisons (up to 71) from the existing studies (eight), the results showed significant differences in DMY, CP, NDF, and ADF. Our meta-analysis revealed the positive effect sizes of the grass–legume mixtures for both DMY (overall TE = 0.04) and CP (0.05), and a neutral result for ADF (0.00), whereas NDF had a higher trend in grass monocultures (– 0.02). In addition, the overall improvements in both DMY and CP when combining grass with legumes were further influenced by the number of forage species, as well as by the type of legume species included in the mixtures. Mixtures with alfalfa (*Medicago sativa* L.) were higher in both DMY and CP than in mixtures including different legumes such as sainfoin (*Onobrychis viciifolia* Scop.) and cicer milkvetch (*Astragalus cicer* L.). This pronounced advantage resulted from the wide environmental adaptability and genetic traits of alfalfa stands. In certain rare cases, grass monocultures out-yielded or yielded similarly to grass–legume mixtures. Moreover, this phenomenon was only recorded over a short duration (≤1 year) and was attributed to the time of harvest and adequate rainfall. In summary, compared with grass monocultures, perennial grass–legume mixtures have the overall potential to enhance the DMY and nutritive value parameters evaluated in this meta-analysis, but the composition of plant species, the stage of maturity, edaphic-climatic factors, and management practices modulate these beneficial effects.

**Keywords:** perennial grass–legume mixtures, beef cattle performance, forage nutritional qualities, tannins, bloat, synthesis.

## 2.0 Introduction

Perennial forages are the bedrock for the sustainability of ruminant livestock production systems such as beef and dairy cattle, sheep, and goats. Forages account for about 80% of the feed requirements in beef cattle operations in the Canadian Prairies (Saskatchewan Forage Council, 2011; Sheppard et al., 2015). Common perennial forages that are grown in the Canadian Prairies include grasses such as timothy (*Phleum pratense* L.), bromegrasses (*Bromus* spp.), orchardgrass (*Dactylis glomerata* L.), ryegrass (*Lolium perenne* L.), and fescues (*Festuca* spp.), whereas the legume species include alfalfa (*Medicago sativa* L.), sainfoin (*Onobrychis viciifolia* Scop.), cicer milkvetch (*Astragalus cicer* L.), and red clover (*Trifolium pratense* L.). These can be established as monocultures or mixtures for silage, baled hay, haylage, or grazed pastures. Beef cattle producers can introduce a plethora of legumes in grass stands to obtain the desired forage yield and quality. For example, in the Canadian Prairies, alfalfa is predominantly used in perennial forage systems because of its adaptability to diverse environmental conditions, high yield, ability to ameliorate nutritive value, vigorous regrowth, and ability to fix atmospheric N (Bittman et al., 1991; Aasen and Bjorge, 2009). However, immature pure alfalfa stands can cause bloat in grazing cattle (Khatiwada et al., 2020) because of the rapid degradation of the protein content in the legumes' tissues by ruminal microbes (Majak et al., 2003). In contrast to alfalfa, both sainfoin and birdsfoot trefoil contain condensed tannins and are non-bloating legumes (Lees, 1992; Sheaffer et al., 1993). The condensed tannins bind to protein and inhibit the activities of ruminal microbes (Li et al., 2014). When mixed with alfalfa, sainfoin reduced the incidence of bloat. For instance, the presence of 35% sainfoin in an alfalfa–sainfoin mix was reported to reduce the incidence of bloat by 77% but did not eliminate bloat in grazing steers (Wang et al., 2006). On the other hand, cicer milkvetch is a non-bloating legume that does not contain condensed tannins (Berard et al., 2011); instead, its mesophyll cell walls are resistant to rupture, making it bloat-free by nature (Lees et al., 1982).

Perennial grass–legume mixtures have been demonstrated to improve forage yield and nutritive value parameters over their grass monoculture counterparts (Foster et al., 2014; Bélanger et al., 2014), and could be adopted to optimize forage–livestock systems across the Canadian Prairies (Khatiwada et al., 2020). Grazing cows on grass monocultures or grass–legume mixtures is a common practice in most parts of the Canadian Prairies during summer and can be extended to the fall and winter seasons (McGeough et al., 2017). Better cow performance (i.e., body weight and condition scores) has been found on perennial grass–legume mixtures than on grass monocultures (Alemu, 2016; Peprah, 2021; Durunna et al., 2015). This is because forage mixtures including legumes contain more crude protein (CP) (Bork et al., 2017) and offer lower concentrations of digestible fiber such as neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Bélanger et al., 2017) that enable increased intake. The higher consistency of protein concentrations in grass–legume mixtures is dependent upon the ability of the legumes to fix atmospheric N biologically (Snyder et al., 2016). Furthermore, various studies have reported the potential of grass–legume forages mixtures to provide improved ecosystem services by improving the soil's C storage (Arshad et al., 2004), soil structure (Arshad et al., 2011), and weed suppression (Serajchi et al., 2017).

Although several studies on perennial grass–legume mixtures across the Canadian Prairies (Alberta, Manitoba, and Saskatchewan) have focused extensively on the production and nutritive value of biomass (Jefferson et al., 2007; Foster et al., 2014, 2019; Kulathunga, et al., 2016), these studies were conducted in different locations, under different soil and climatic conditions, and management practices. Furthermore, the literature regarding the effect of perennial grass–legume mixtures on biomass or dry matter yield compared with monoculture grasses is inconsistent. For instance, the findings by Mischkolz et al. (2013) found that some grass monocultures outyielded grass–legume mixtures in Saskatchewan (Saskatoon and Swift Current) and various ecozones of Alberta, whereas other studies by Foster et al. (2014) in Melfort, Saskatchewan found no difference in yield between some grass monocultures and grass–legume mixtures. It also appears that the effect of these forage mixtures can be influenced by the number of plant species included in them (Foster et al., 2019; Quilichini et al., 2021). Therefore, these wide discrepancies and knowledge gaps remain to be addressed. In effect, reaching stronger conclusions about the value of growing legumes in forage production systems requires the assembly of an inclusive quantitative synthesis to disentangle these conflicting results in the literature.

A regional meta-analysis can provide a statistical quantitative framework for assembling and combining the information from existing reports (McAloon et al., 2016; McCarthy et al., 2010) to obtain clarity from the literature (Lean, et al., 2009). Therefore, we developed a meta-analysis of the available studies on the Canadian Prairies to determine the range and central tendency of the forage quality and productivity in comparisons of grass monocultures and grass–legume mixtures. We hypothesized that perennial grass–legume mixtures would provide more advantages in terms of biomass productivity and nutritive value (CP, NDF, and ADF).

## 2.1 Methodology

### 2.1.1 Literature search

A literature search was conducted using databases such as Google Scholar, Web of Science, and Science Direct to identify research papers for data extraction published between 1990 and 2021, with the last search conducted on 28 March 2023. The procedure included combining key words in different forms: "grass–legume mixture", "perennial forages", "livestock production", "Canadian Prairies", "forage nutritional qualities", and "forage biomass". Peer-reviewed literature was used as the primary reporting standard. The reference lists within the identified journal articles were also examined to search for any additional peer-reviewed publications.

### 2.1.2 Selection criteria

Research papers were initially screened by the title and abstract (Figure 2.1). Subsequently, they were included for further screening if (i) the paper was in English, (ii) the treatment of interest was grass–legume mixtures compared with grass monocultures, and (iii) the studies were conducted in the Canadian Prairies (i.e., Alberta, Manitoba, and Saskatchewan).

### 2.1.3 Inclusion and exclusion criteria

Studies were thoroughly assessed for inclusion on the basis of the experimental design, such as blocking, control, and replication. If these factors had not been clearly explained, an article was excluded. The publications that were used reported the mean effect values of grass monocultures and grass–legume mixtures. The mixtures were required to contain different forage-level combinations (i.e., mixtures containing two or more species of both grasses and legumes). The data were required to be original and not be reported in previous or subsequent studies. In addition, when available, precipitation data for the growing season was also included in the meta-analysis.

### 2.1.4 Data extraction

Eight journal articles met the selection criteria and were used to compile the final list of comparisons for evaluating dry matter yield [DMY; total observations ($n$) = 71] and crude protein ($n$ = 61). Furthermore, of the eight journal articles, four and five articles had data available for ADF ($n$ = 44) and NDF ($n$ = 42), respectively. The following data were extracted full reference, study location, soil classification, soil texture, precipitation, mean value of grass monocultures and grass–legume treatments, treatment levels, CP, ADF, and NDF were extracted. Furthermore, studies with reported $p$-values and sample sizes ($N$) were considered during the procedure to aid in calculating the standard errors. Subsequently, the effect sizes for DMY, CP, ADF, and NDF between the treatments (legume–grass mixtures) and the control groups (grass monocultures) were estimated by a natural logarithmic transformation of the mean values (Nasrollahi et al., 2015) as follows:

$$TE = \log M_{treat} - \log M_{cont} \qquad [1]$$

Where TE is the effect size, $M_{treat}$ is the mean of treatment, and $M_{\mathrm{cont}}$ is the mean of the controls.

### 2.1.5 Data analysis

An analysis of the extracted effect sizes (TE) was conducted. Before this, the standard errors were calculated from the effect sizes and the exact $p$-values provided in the studies using the **dmetar** package in R (version 4), as demonstrated by Altman and Bland (2011). The dmetar package provides functions that enable researchers to fit various models (i.e., metagen, metacont, or metabin) and produce forest plots for meta-analytic objects. A random effect model was conducted for the effect sizes, 95% confidence interval (CI), the statistical significance of the effect size, and the weights of each study using the metagen function (Nasrollahi et al., 2015).

A random effect model was used in this analysis because of the heterogeneity in the effect sizes of studies. The use of this model meant that the true effect size and all the different effect sizes were represented in the summary estimates, which eliminated the error of overlooking studies with smaller weights. In contrast to the random effect model, the fixed effect model assumes that the true effect size for all studies is identical. This can be misleading because smaller studies may be discounted when larger studies provide the researchers with relevant data about the same effect size (Imani et al., 2017). Forest plots were created to visualize the trend of the effect sizes compared with the true effect size. The $I^2$ statistic, which demonstrates the heterogeneity, variance, and *p*-value across studies was also extracted from the forest plots. Furthermore, publication bias, which exists when the probability of a study being published is affected by its results (Rothstein et al., 2005), was graphically represented with contour funnel plots and statistically tested using the Egger test (Egger et al., 1997).

## 2.2 Results

### 2.2.1 Forage DMY

In total, 71 experimental comparisons reported the DMY of perennial grass monocultures (baseline control) and grass–legume mixtures across the Canadian Prairies. These comparisons were extracted from the eight published studies (Figure 2.1). This sample size enabled us to extract the effect sizes (TE), SE, and *p*-values.

The forest plot visualized the frequent significant differences in the effect size estimations for the grass monoculture stands and grass–legume mixtures ($P$ <0.01; Figure 2.1). A pooled effect of 0.04 for DMY summarized the overall effect from the forest plot, demonstrating an overarching pattern in favor of the grass–legume mixtures (Table 2.1, Figure 2.2). In summary, among the 71 experimental comparisons, 12 showed a significant effect towards the control treatments (grass monocultures), 17 reported a significant effect towards grass–legume mixtures, and 42 reported a neutral effect. Out of these 29 comparisons, we found that five depicted less precision, with the weights ranging between 0.5% and 0.9%. All comparisons in the studies from Foster et al. (2021, 2019, and 2014) did not show any evidence of significance although they had more precise weights (1.1–1.6% in each comparison).

The grass–legume treatments increased DMY in the majority of the forage species combinations over the grass monocultures. For instance, an increase in the productivity of biomass from 6053 kg ha$^{-1}$ to 6721 kg ha$^{-1}$ was observed when a bromegrass monoculture was seeded with alfalfa in Lacombe and Eckville. Although the number of plant species included in a mixture was expected to change the DMY, our regional meta-analysis showed a counterintuitive, inverse effect. In fact, some studies with two-way grass–legume mixtures out-yielded some of the three-way or four-way grass–legume mixtures.

### 2.2.2 Nutritive Characteristics of Forage

According to the forest plots, grass–legume mixtures significantly improved the forage's nutritional attributes such as CP (for which more is better) and NDF (for which less is better) compared with the monoculture stands ($P < 0.01$; Table 2.1; Figure 2.4, and Figure 2.5). In detail, the results revealed that grass–legume mixtures had greater CP concentration overall than grass monocultures, with a size effect of 0.05 and a confidence interval ranging from 0.03 to 0.07. The legumes' contributions differed, depending on the species. For example, alfalfa contributed more to the CP concentrations of the mixture than sainfoin and cicer milkvetch did. Furthermore, the number of legume species present in the mixture also influenced the CP concentrations. A three-way treatment with two legumes, namely boreal sweet vetch (*Hedysarum boreale* Nutt.) and flexible milkvetch (*Astralgalus flexuosus* Douglas ex G. Don) or purple vetch (*Vicia americana* Muhl. Ex Willd.), was higher in CP than a five-way treatment with cicer milkvetch as the only legume in the mixture.

In terms of the detergent fiber content, the forest plot of NDF showed a pooled effect of –0.02 indicating that monoculture grasses had more NDF than grass–legume mixtures in the studies in the meta-analysis. The overall effect was also significant, with a 95% confidence interval of –0.03 and –0.01 (Figure 2.5), indicating that 95% of the true effects were between –0.03 and –0.01. In contrast, the ADF plot showed a non-significant pooled effect (0.00), with few of the individual experimental comparisons achieving significance (Figure 2.6).

### 2.2.3 Publication bias and representativeness of the data

We assessed publication bias across the assembled studies with a contour funnel plot (Figure 2.3) and Egger's test for plot symmetry and found no significant publication bias ($P$ = 0.0639). Moreover, high heterogeneity ($I^2$) among the outcomes of the individual studies for DMY, CP, NDF, and ADF, which ranged from 85 to 91% (Table 2.1), demonstrated the diversity and representativeness of the data within our meta-analysis.

## 2.3 Discussion

The presence of legumes in perennial forage mixtures often enhances the system's productivity overall by both improving the seasonal distribution of biomass and increasing the productivity of these mixtures later in the growing season (Sleugh et al., 2000). Across the

Canadian Prairies, forage yield increased when legumes were seeded with grasses (Foster et al., 2014; Bélanger et al., 2014; Khatiwada et al., 2020). Malhi et al. (2002) indicated that unfertilized bromegrass grown as a monoculture recorded the least DMY, but this doubled when the grass was grown in association with alfalfa. The increase in yield was attributed to the ability of the legume to supply N for uptake and utilization by the neighboring grasses (Thilakarathna et al., 2016; Munir et al., 2011; Whitbread et al., 2009; Temperton et al., 2007), as well as the existence of a well-defined complementarity effect between legumes and grasses (Picasso et al., 2011). Although grass–legume mixtures frequently had greater DMY than grass monocultures in our meta-analysis, a few comparisons of grass monocultures and grass–legume mixtures suggested otherwise (Mischkolz et al., 2013). It was noted that the reason for this was the time of forage harvest over the growing season (i.e., mid-harvest and late harvest). For example, blue bunch wheatgrass [*Pseudoregneria spicata* (Pursh.) Á Löve] and western wheatgrass [*Pascopyrum smithii* (Rydb.) Á Löve] yielded better than some grass–legume mixtures including these grasses when the harvest was carried out late in the growing season, showing increases of 10% and 25% respectively. Furthermore, edaphic-climatic factors may have also contributed to these divergent results. For instance, the sufficient rainfall recorded over the growing season (475 mm and 465 mm) at their two study sites (highest in May and June for Saskatoon, and evenly distributed throughout the season for Swift Current) may have further influenced the observed increase in grass biomass. This is supported by Kim et al. (2016), who found that yield increases in both short and tall grasses were only observed with adequate annual precipitation of 350–850 mm $yr^{-1}$. Additionally, the high natural fertility of the Chernozem soils in these study sites (Agricultural Region of Alberta Soil Inventory Database, Government of Alberta, 2020) enabled a substantial supply of nutrients, which were utilized efficiently by the grasses, leading to increased biomass production. However, although these soils may have a high N content, much of that N is immobilized and is not available for plants, so yields may still be lower than optimal (Thilakarathna et al., 2020).

Additionally, grass monocultures can yield as much as that of their mixtures with legumes, especially during their first year of production (Foster et. al., 2014). However, they reported a decline in productivity over subsequent years attributed to decreased soil N availability over time, as the production of grass directly depends on a sufficient N supply. This also agrees with Malhi et al. (2002), where an increase in DMY was observed in bromegrass monoculture stands receiving ammonium nitrate fertilization (2.8 Mg $ha^{-1}$ at 50 kg N $ha^{-1}$ and 9.5 Mg $ha^{-1}$ at 200 kg N $ha^{-1}$).

The growth habit, functional traits, resource availability, and resource utilization of legumes such as alfalfa over the growing season are driving factors of the overall production of forage biomass (Sheaffer et al., 2018). Our regional meta-analysis further confirmed this fact, in line with Biligetu et al. (2014), who found that grass–legume mixtures including alfalfa outyielded other grass–legume mixtures that included either sainfoin or cicer milkvetch, which was attributed to their distinct morphological and physiological traits. Aasen and Borje (2009) reported that alfalfa yields more, in either monocultures or mixtures, than other common perennial forage legume species.

The nutritive value of forage is an essential factor in feeding farm animals. The indicators such as CP, ADF, and NDF examined in this meta-analysis could serve as a base for determining the ability of forages to meet some of the nutritional requirements of a livestock enterprise. Our results clearly demonstrated that the CP concentration of grass–legume mixtures was consistently greater than that of grass monocultures. This significant difference in the CP of mixtures relative to grass monocultures was directly attributed to the presence of legumes in the stands (Sturludóttir et al., 2014; Bork et al., 2017). The effect of legume–grass mixtures on CP also varies among studies, depending on the legume species involved in the mixture. For instance, stands with alfalfa in the mixtures produced even greater CP concentrations than those with sainfoin and cicer milkvetch when harvested at similar growth stages (Malhi et al., 2002; Biligetu et al., 2014; Foster et al., 2019). Considering the variation across studies caused by the locations, our analysis did not conclusively resolve whether the legume species involved in the mixture significantly impacted the CP concentrations. In the comparisons across all the studies, a few experimental outcomes emerging from Saskatoon have suggested that grass can have greater CP concentrations than grass–legume mixtures in certain cases (Mischkolz et al., 2013). The CP concentrations in forage production systems may also depend on other factors such as genetics and management practices. In parallel, this general notion is consistent with Ren et al. (2021), who stated that the yield of alfalfa is a quantitative trait influenced by genetics and environmental factors.

Our meta-analyses of crude fibers showed two contrasting outcomes (i.e., NDF in Figure 2.5 vs ADF in Figure 2.6). Grass monocultures had greater NDF concentrations than grass–legume mixtures. This is contrary to the findings of Aponte et al. (2019), where higher NDF was reported in grasses with alfalfa than in monoculture grasses. In some instances, environmental factors such as the high ambient temperature influence concentrations of NDF in grasses by bringing about rapid rate of maturity and a rise in cell wall content (Thorvaldsson et al., 2007). Higher air temperatures can increase the rate of plant canopy development as well as reducing leaf-to stem ratio and digestibility (Buxton, 1996). Furthermore, drought stress could influence the physiological activities of plants and may cause forage quality indicators such as NDF to increase in concentrations (Küchenmeister et al., 2013).

The effect of later harvest of the forage crops to increase NDF is well established. During the early growing season, perennial plants contain less lignin and fewer fiber cells (Biligetu et al., 2014). As the season progresses, plants develop and accumulate more cellulose and lignified structures, increasing the NDF and ADF concentrations. For instance, studies by Foster et al. (2019) and Biligetu et al. (2014) reported higher NDF for monoculture grass harvested in late summer than in those harvested in early summer. Furthermore, Atis et al. (2012) also reported that NDF concentrations tended to increase significantly with delayed harvesting times. Collectively, our analysis showed that grass–legume mixtures tend to typically have less NDF than grass monocultures, which could favor their consumption and digestibility by farm animals.

Although we included all the available comparisons within our meta-analysis, the results for ADF revealed no consistent differences between grass monocultures and grass–legume mixtures. However, there was a slight tendency for lower ADF in the grass monocultures than grass–legume mixtures (Biligetu et al., 2014; Foster et al., 2019). Nevertheless, the experimental comparisons reported by Quilichini et al. (2021) showed the reverse, where grass monocultures actually had higher ADF than grass–legume combinations. The explanation for these apparent differences across studies could be the maturity stage and time of harvest. For instance, evidence in the literature suggests that harvesting grass in the afternoon rather than the morning results in forages with lower ADF concentrations due to dilution with total nonstructural carbohydrates produced by photosynthesis (Fisher et al., 1999; Huntington and Burns, 2007). This is also consistent with the results of Bertrand et al. (2008) which indicate a lower ADF in timothy grass harvested in the afternoon. Even when the harvests in most of the individual studies included in our meta-analysis were carried out at the same time of the season and day, there was probably considerable variation in the overall field methodologies across the compiled studies, likely leading to variations in ADF. This notion is further supported by the seminal work of Jung and

Vogel (1992), who argued that certain forage species have different processes of lignin formation, and the plant part analyzed is vital for predicting degradability.

## 2.4 Conclusion

This synthesis confirmed that perennial grass–legume mixtures enhance the overall productivity and nutritional qualities relative to grass monocultures within the Canadian Prairies. In effect, the inclusion of legumes boosts forage yield; though some grasses grown as monoculture were reported to match or even outyield the performance of grass–legume mixtures, this phenomenon only occurred rarely under favorable soil and weather conditions. Moreover, increasing the number of grasses and legume species in the mixtures tended to increase the total DMY. However, our meta-analysis demonstrated that this beneficial effect on DMY is not always true for the parameters of forage quality. The CP concentrations in perennial grass–legume mixtures were higher than those in grass monocultures, in which the CP was increased by the legume species present in the mixtures. Additionally, the detergent fiber contents showed that monoculture grasses had greater NDF, whereas there were no differences in ADF between grass–legume mixtures and grass monocultures. In summary, regardless of the study location across the Canadian Prairies, perennial grass–legume mixtures have the overall potential to enhance the DMY and nutritional qualities compared with monoculture grasses, but the plant composition of species, the stage of maturity, soil and climatic factors, and management practices modulate these beneficial effects.

## 2.5 Statement for data availability

The authors wish to declare their intent to release the data used for the research upon request.


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## 2.7 Conflict of interest

The authors declare no conflict of interest.

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## 2.9 References

Aasen, A., and Bjorge, M. (2009). Alberta Forage Manual. Alberta Agriculture and Rural Development Publication, Information Management Services. Agdex 120/20-1, 348

Alemu, A. W., Doce, R. R., Dick, A. C., Basarab, J. A., Kröbel, R., Haugen-Kozyra, K., and Baron, V. S. (2016). Effect of winter-feeding systems on farm greenhouse gas emissions. Agricultural Systems, 148, 28–37. https://doi.org/10.1016/j.agsy.2016.06.008

Altman, D. G., and Bland, J. M. (2011). Statistics notes: How to obtain the P value from a confidence interval. British Medical Journal (Online), 343(7825), 1–2. https://doi.org/10.1136/bmj.d2304

Aponte, A., Samarappuli, D., and Berti, M. T. (2019). Alfalfa–grass mixtures in comparison to grass and alfalfa monocultures. Agronomy Journal, 111(2), 628–638 https://doi.org/10.2134/agronj2017.12.0753

Arshad, M. A., Franzluebbers, A. J., and Azooz, R. H. (2004). Surface-soil structural properties under grass and cereal production on a Mollic Cyroboralf in Canada. Soil and Tillage Research, 77(1), 15–23. https://doi.org/10.1016/j.still.2003.10.004

Arshad, M. A., Soon, Y. K., and Ripmeester, J. A. (2011). Quality of soil organic matter and C storage as influenced by cropping systems in northwestern Alberta, Canada. Nutrient Cycling in Agroecosystems, 89(1), 71–79. https://doi.org/10.1007/s10705-010-9377-1

Atis, I., Konuskan, O., Duru, M., Gozubenli, H., and Yilmaz, S. (2012). Effect of harvesting time on yield, composition, and forage quality of some forage sorghum cultivars. International Journal of Agriculture and Biology, 14(6), 879–886

Bélanger, G., Castonguay, Y., and Lajeunesse, J. (2014). Benefits of mixing timothy with alfalfa for forage yield, nutritive value, and weed suppression in northern environments. Canadian Journal of Plant Science, 94(1), 51–60. https://doi.org/10.4141/CJPS2013-228

Bélanger, G., Tremblay, G. F., Papadopoulos, Y. A., Duynisveld, J., Lajeunesse, J., Lafrenière, C., and Fillmore, S. A. E. (2017). Yield and nutritive value of binary legume–grass mixtures under grazing or frequent cutting. Canadian Journal of Plant Science, 98(2), 395–407. https://doi.org/10.1139/CJPS-2017-0183

Berard, N. C., Wang, Y., Wittenberg, K. M., Krause, D. O., Coulman, B. E., McAllister, T. A., and Ominski, K. H. (2011). Condensed tannin concentrations found in vegetative and mature forage legumes grown in western Canada. Canadian Journal of Plant Science, 91(4), 669–675.

Bertrand, A., Tremblay, G. F., Pelletier, S., Castonguay, Y., and Bélanger, G. (2008). Yield and nutritive value of timothy as affected by temperature, photoperiod, and time of harvest. Grass and Forage Science, 63(4), 421–432. https://doi.org/10.1111/j.1365-2494.2008.00649.x

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. https://doi.org/10.4141/CJPS2013-269

Bittman, S., Waddington, J., and McCartney, D. H. (1991). Performance of alfalfa strains grown in mixture with smooth bromegrass as affected by management. Canadian Journal of Plant Science, 71(4), 1029–1037. https://doi.org/10.4141/cjps91-145

Bork, E. W., Gabruck, D. T., McLeod, E. M., and Hall, L. M. (2017). Five-year forage dynamics arising from four legume–grass seed mixes. Agronomy Journal, 109(6), 2789–2799. https://doi.org/10.2134/agronj2017.02.0069

Buxton, D. R. (1996). Quality related characteristics of forages as influenced by plant environment and agronomic factors. Animal Feed Science Technology, 59, 37–49. https://doi.org/10.1002/9781118860359.ch2

Durunna, O. N., Baron, V., Scott, S. L., Robins, C., Khakbazan, M., and Block, H. C. (2015). Effects of resting perennial pastures during the sensitive pre-dormancy period in western Manitoba: Pasture productivity and beef cattle performance. Canadian Journal of Animal

Science, 95(2), 129–141. https://doi.org/10.4141/CJAS-2014-046

Egger, M., Smith, G. D., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. British Medical Journal, 315, 629–634.

Fisher, D. S., Mayland, H. F., and Burns, J. C. (1999). Variation in ruminants' preference for tall fescue hays cut either at sundown or at sunup. Journal of Animal Science, 77, 762–768.

Foster, A., Biligetu, B., and Darambazar, E. (2019). Forage accumulation, nutritive value, and botanical composition of grass–cicer milkvetch mixtures under two harvest managements. Crop Science, 59(6), 2876–2885. https://doi.org/10.2135/cropsci2019.03.0198

Foster, A., Biligetu, B., Malhi, S. S., Gill, K. S., Mollison, B., and Leach, D. (2021). Harvest time and fertility effects on yield and quality of forage from alfalfa, hybrid bromegrass and their mixture. Agricultural Sciences, 12(04), 325–338. https://doi.org/10.4236/as.2021.124021

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. https://doi.org/10.4141/CJPS2013-095

Government of Alberta (2020). Agricultural region of Alberta soil inventory database. Accessed on 29 February 2024 at https://www.alberta.ca/agricultural-regions-of-alberta-soil-inventory-database

Huntington, G. B., and Burns, J. C. (2007). Afternoon harvest increases readily fermentable carbohydrate concentration and voluntary intake of gamagrass and switch grass baleage by beef steers. Journal of Animal Science, 85, 276–284.

Imani, M., Mirzaei, M., Baghbanzadeh-Nobari, B., and Ghaffari, M. H. (2017). Effects of forage provision to dairy calves on growth performance and rumen fermentation: A meta-analysis and meta-regression. Journal of Dairy Science, 100(2), 1136–1150. https://doi.org/10.3168/jds.2016-11561

Jefferson, P. G., Zentner, R. P., Selles, F. and Lemke, R. (2007). Performance of short-lived perennial grasses grown with and without alfalfa at a semiarid location in southern

Saskatchewan. *Canadian Journal of Plant Science*, 87(1), 59–65. https://doi.org/10.4141/P05-103

Jung, H. G., and Vogel, K. P. (1992). Lignification of switchgrass (Panicum virgatum) and big bluestem (Andropogon gerardii) plant parts during maturation and its effect on fibre degradability. Journal of the Science of Food and Agriculture, 59(2), 169–176. https://doi.org/10.1002/jsfa.2740590206

Khatiwada, B., Acharya, S. N., Larney, F. J., Lupwayi, N. Z., Smith, E. G., Islam, M. A., and Thomas, J. E. (2020). Benefits of mixed grass–legume pastures and pasture rejuvenation using bloat-free legumes in western Canada: A review. Canadian Journal of Plant Science, 100(5), 463–476. https://doi.org/10.1139/cjps-2019-0212

Kim, S., Williams, A., Kiniry, J. R., and Hawkes, C. V. (2016). Simulating diverse native C4 perennial grasses with varying rainfall. Journal of Arid Environments, 134, 97–103. https://doi.org/10.1016/j.jaridenv.2016.07.004

Küchenmeister, K., Küchenmeister, F., Kayser, M., Wrage-Mönnig, N., and Isselstein, J. (2013). Influence of drought stress on nutritive value of perennial forage legumes. International Journal of Plant Production 7(4), 693–710.

Kulathunga, D. G. R. S., Penner, G. B., Schoenau, J. J., Damiran, D., Larson, K., and Lardner, H. A. (2016). Effect of perennial forage system on forage characteristics, soil nutrients, cow performance, and system economics. Professional Animal Scientist, 32(6), 784–797. https://doi.org/10.15232/pas.2015-01490

Lean, I. J., Rabiee, A. R., Duffield, T. F., and Dohoo, I. R. (2009). Invited review: Use of meta-analysis in animal health and reproduction: Methods and applications. Journal of Dairy Science, 92, 3545–3565.

Lees, G. L. (1992). Condensed tannins in some forage legumes: Their role in the prevention of ruminant pasture bloat. In: Hemingway, R.W. and Laks, P.S. (Eds.), Plant polyphenols: Synthesis, properties, significance, Plenum Press, New York, NY (1992) pp.915–934.

Lees, G. L., Howarth, R.E. and Goplen, B. P. (1982). Morphological characteristics of leaves from some legume forages: Relation to digestion and mechanical strength. Canadian Journal of Botany, 60(10), 2126–2132.

Li, Y., Iwaasa, A. D., Wang, Y., Jin, L., Han, G., and Zhao, M. (2014). Condensed tannins concentration of selected prairie legume forages as affected by phenological stages during two consecutive growth seasons in western Canada. Canadian Journal of Plant Science, 94(5), 817–826. https://doi.org/10.4141/CJPS2013-234

Majak, W., McAllister, T. A., McCartney, D., Stanford, K., and Cheng, K.J. (2003). Bloat in cattle. Agriculture and Agri-Food Canada/Alberta Agriculture, Food and Rural Development, Edmonton, Alberta. AGDEX 420/60-1. 28 pp

Malhi, S. S., Zentner, R. P., and Heier, K. (2002). Effectiveness of alfalfa in reducing fertilizer N input for optimum forage yield, protein concentration, returns and energy performance of bromegrass–alfalfa mixtures. Nutrient Cycling in Agroecosystems, 62(3), 219–227. https://doi.org/10.1023/A:1021229824357

McAloon, C. G., Whyte, P., More, S. J., Green, M. J., O'Grady, L., Garcia, A. B., and Doherty, M. L. (2016). The effect of paratuberculosis on milk yield-A systematic review and meta-analysis. Journal of Dairy Science, 99(2), 1449–1460. https://doi.org/10.3168/jds.2015-10156

McCartney, D., and Fraser, J. (2010). The potential role of annual forage legumes in Canada: A review. Canadian Journal of Plant Science, 90(4), 403–420. https://doi.org/10.4141/CJPS07182

McGeough, E. J., Cattani, D. J., Koscielny, Z., Hewitt, B., and Ominski, K. H. (2017). Annual and perennial forages for fall/winter grazing in western Canada. Canadian Journal of Plant Science, 98(2), 247–254. https://doi.org/10.1139/cjps-2017-0228

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. https://doi.org/10.4141/CJPS2012-261

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. and PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Medicine 6, e1000097. https://doi.org/10.1371/journal.pmed.1000097

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. https://doi.org/10.1111/j.1365-2494.2011.00806.x

Nasrollahi, S.M., Imani M., and Zebeli, Q. A. (2015). Meta-analysis and meta-regression of the effect of forage particle size, level, source, and preservation method on feed intake, nutrient digestibility, and performance in dairy cows. Journal of Dairy Science. 98(12), 8926-8939. doi: 10.3168/jds.2015-9681

Peprah, S., Damiran, D., Biligetu, B., Iwaasa, A. D., Larson, K., and Lardner, H. A. (2021). Evaluation of cool-season binary mixtures as pasture: Herbage yield, nutritive value, and beef cattle performance. Livestock Science, 248(2021), 104501. https://doi.org/10.1016/j.livsci.2021.104501

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(4), 317–327. https://doi.org/10.1017/S1742170511000135

Quilichini, S. M., Schellenberg, M. P., and Biligetu, B. (2021). Introduction of four native legumes of the Canadian prairies in monoculture and in mixtures with meadow bromegrass (Bromus riparius). Canadian Journal of Plant Science, 101(5), 714–729. https://doi.org/10.1139/cjps-2020-0249

Ren, L., Bennett, J. A., Coulman, B., Liu, J., and Biligetu, B. (2021). Forage yield trend of alfalfa cultivars in the Canadian prairies and its relation to environmental factors and harvest management. Grass and Forage Science, 76(3), 390–399. https://doi.org/10.1111/gfs.12513

Rothstein, H. R., Sutton, J. A., and Borenstein, M. (2005). Publication bias in meta-Analysis: prevention, assessment, and adjustments. John Wiley & Sons.

Saskatchewan Forage Council (2011). An economic assessment of feed costs within the cow/calf sector. Western Canadian Feed Innovation Network, University of Saskatchewan, Saskatoon, SK.

Serajchi, M., Schellenberg, M. P., and Lamb, E. G. (2017). The potential of seven native north American forage species to suppress weeds through allelopathy. Canadian Journal of Plant Science, 97(5), 881–890. https://doi.org/10.1139/cjps-2016-0354

Sheaffer, C. C., Ehlke, N. J., Albrecht, K. A., Jungers, J. M., and Goplen, J. J. (2018). Forage legumes: clovers, birdsfoot trefoil, cicer milkvetch, crownvtech and alfalfa. Station Bulletin 608-2018. St. Paul, MN: Minnesota Agricultural Experiment Station.

Sheaffer, C. C., Mathison, R. D., Martin, N. P., Rabas, D. L., Ford, H. J., and Swanson, D. R. (1993). Forage legumes: clovers, birdsfoot trefoil, cicer milkvetch, crownvtech, sainfoin and alfalfa. Station Bulletin 597-1993. St. Paul, MN: Minnesota Agricultural Experiment Station, University of Minnesota

Sheppard, S. C., Bittman, S., Donohoe, G., Flaten, D., Wittenberg, K. M., Small, J. A., Berthiaume, R., McAllister, T. A., Beauchemin, K. A., McKinnon, J., Amiro, B. D., Macdonald, D., Mattos, F., and Ominski, K. H. (2015). Beef cattle husbandry practices across ecoregions of Canada in 2011. Canadian Journal of Animal Science, 95(2), 305–321. https://doi.org/10.4141/CJAS-2014-158

Sleugh, B., Moore, J. K., George, R. and Brummer, E. C. (2000). Binary legume mixtures improve forage yield, quality and seasonal distribution. Agronomy Journal. 92, 24–29.

Snyder, E. M., Karsten, H. D., Curran, W. S., Malcolm, G. M., and Hyde, J. A. (2016). Green manure comparison between winter wheat and corn: Weeds, yields, and economics. Agronomy Journal, 108(5), 2015–2025. https://doi.org/10.2134/agronj2016.02.0084

Sturludóttir, E., Brophy, C., Bélanger, G., Gustavsson, A. M., Jørgensen, M., Lunnan, T., and Helgadóttir, Á. (2014). Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. Grass and Forage Science, 69(2), 229–240. https://doi.org/10.1111/gfs.12037

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. https://doi.org/10.1007/s00442-006-0576-z

Thilakarathna, M. S., McElroy, M. S., Chapagain, T., Papadopoulos, Y. A., and Raizada, M. N. (2016). Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. Agronomy for Sustainable Development, 36(4), 1–16.

Thilakarathna, S.K., Hernandez-Ramirez, G., Puurveen, D., Kryzanowski, L., Lohstraeter, G., Powers, L., Quan, N., and Tenuta, M. (2020). Nitrous oxide emissions and nitrogen use efficiency in wheat: Nitrogen fertilization timing and formulation, soil nitrogen, and weather effects. Soil Science Society America Journal. 2020, 84, 1910–1927.

Thorvaldsson, G., Tremblay, G. F., and Kunelius, H. T. (2007). The effects of growth temperature on digestibility and fibre concentration of seven temperate grass species. Acta Agriculturae Scandinavica Section B: Soil and Plant Science, 57(4), 322–328. https://doi.org/10.1080/09064710600984221

Wang, Y., Berg, B. P., Barbieri, L. R., Veira, D. M., and McAllister, T. A. (2006). Comparison of alfalfa and mixed alfalfa–sainfoin pastures for grazing cattle: Effects on incidence of bloat, ruminal fermentation, and feed intake. Canadian Journal of Animal Science, 86(3), 383–392. https://doi.org/10.4141/A06-009

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, Australian Crop and Pasture Science, 60(12), 1147–1155. https://doi.org/10.1071/CP09058

## 2.10 Table

Table 2. 1 Summary of the outcome from the forest plots of dry matter yield (DMY), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF).

| **Outcome** | ***I*²‡ (%)** | **Effect size (TE)** | **95% CI†** | **Number of studies§** | **Number of comparisons§** | ***P*-value⸸** |
|---|---|---|---|---|---|---|
| DMY | 91 | 0.04 | –0.00; 0.08 | 8 | 71 | <0.01 |
| CP | 89 | 0.05 | 0.03; 0.07 | 8 | 61 | <0.01 |
| NDF | 90 | –0.02 | –0.03; –0.01 | 5 | 44 | <0.01 |
| ADF | 85 | 0.00 | –0.01; 0.00 | 4 | 42 | <0.01 |

‡ $I^2$ is the heterogeneity. † CI, confidence interval. §The number of studies and comparisons is for all outcomes extracted from the literature for the random effect analyses comparing perennial grass monocultures with grass–legume mixtures. ⸸The *p*-value indicates the significant differences between grass monocultures and grass–legume mixtures.

## 2.11 Figures

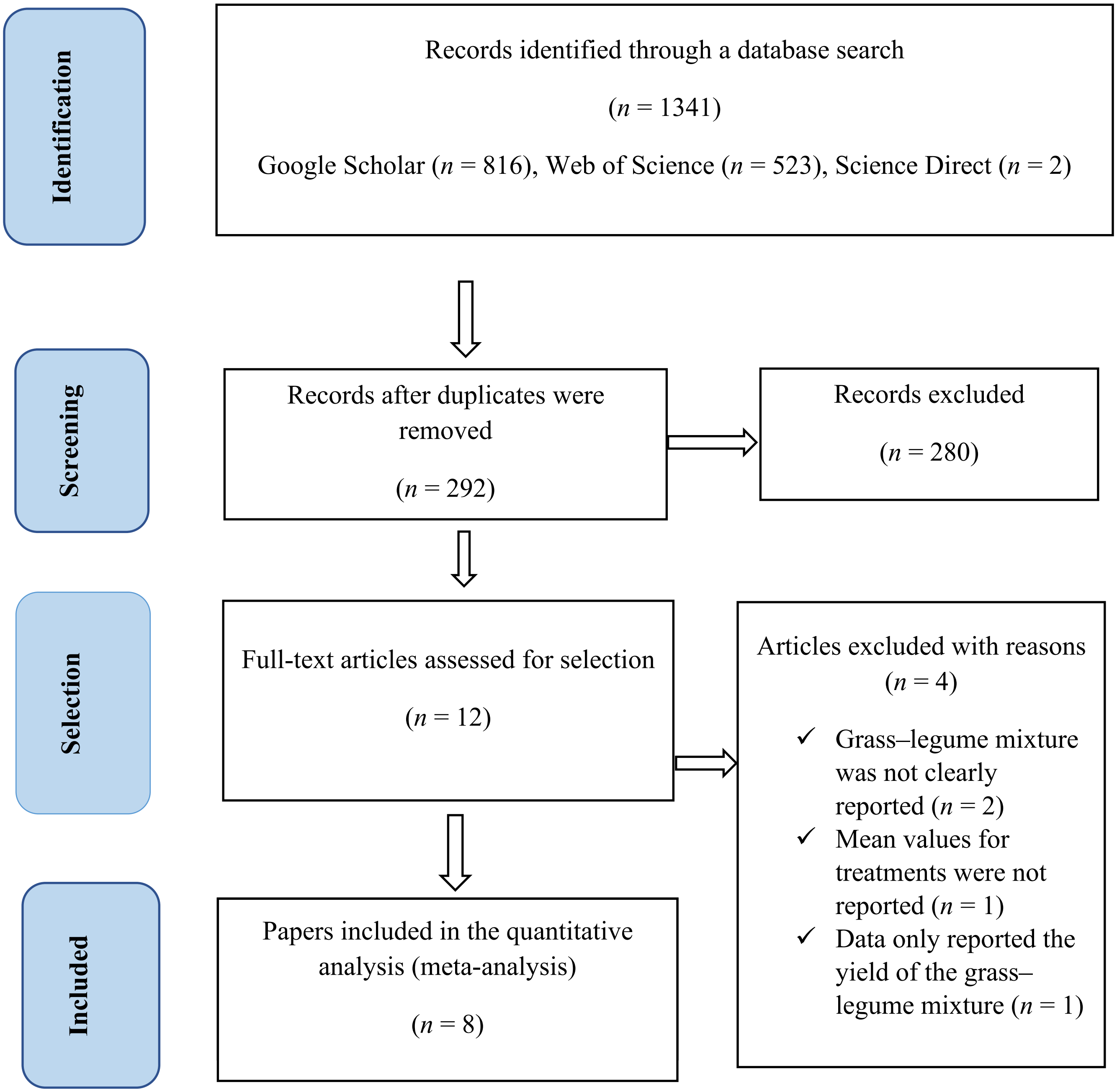


Figure 2. 1 A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart detailing the process of literature search and study collection for the meta-analysis. Adapted from Moher et al. (2009).

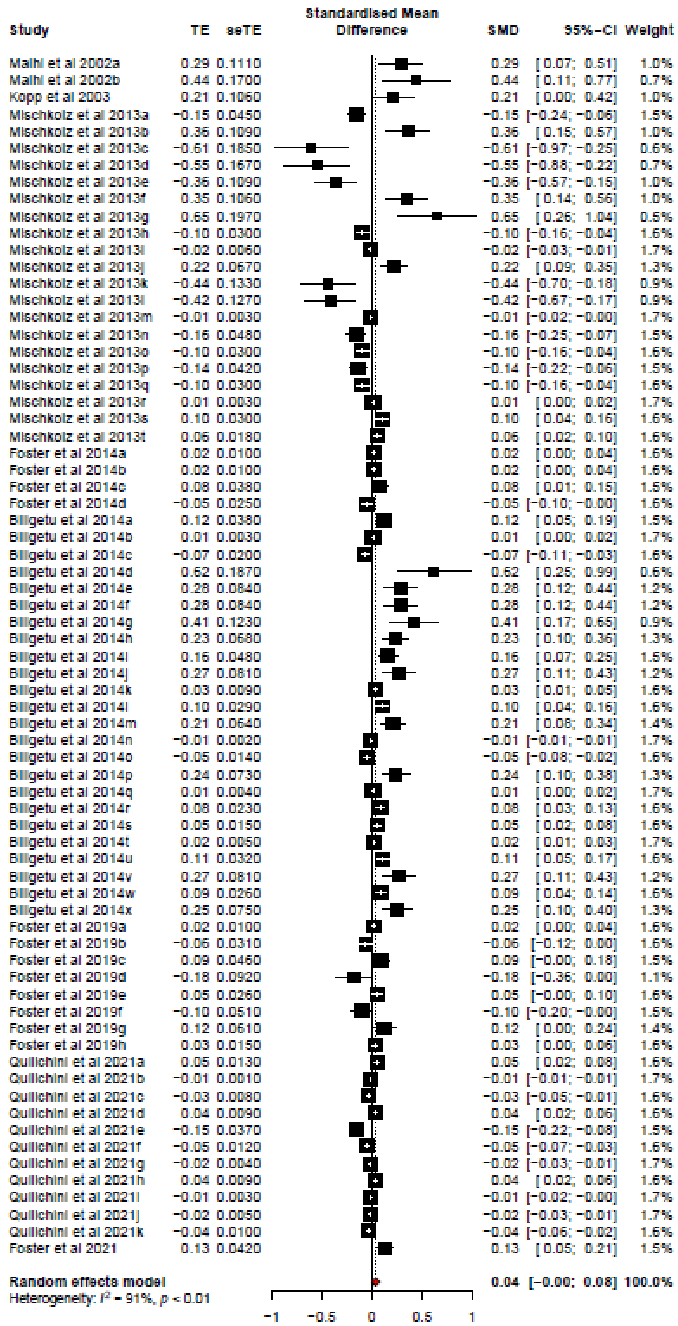


Figure 2. 2 A forest plot of the effect size (TE) and 95% confidence intervals (CI) for studies investigating the effect of perennial grass monocultures and grass–legume mixtures on dry matter yield. Point estimates and CI for each study are represented on each line. The weight of each study is displayed by a black box. Combined effect estimates (pooled effect) are presented at the bottom of each group with a red diamond symbol. The standard error (seTE) is the standard error of each study with its corresponding summarized mean difference (SMD).

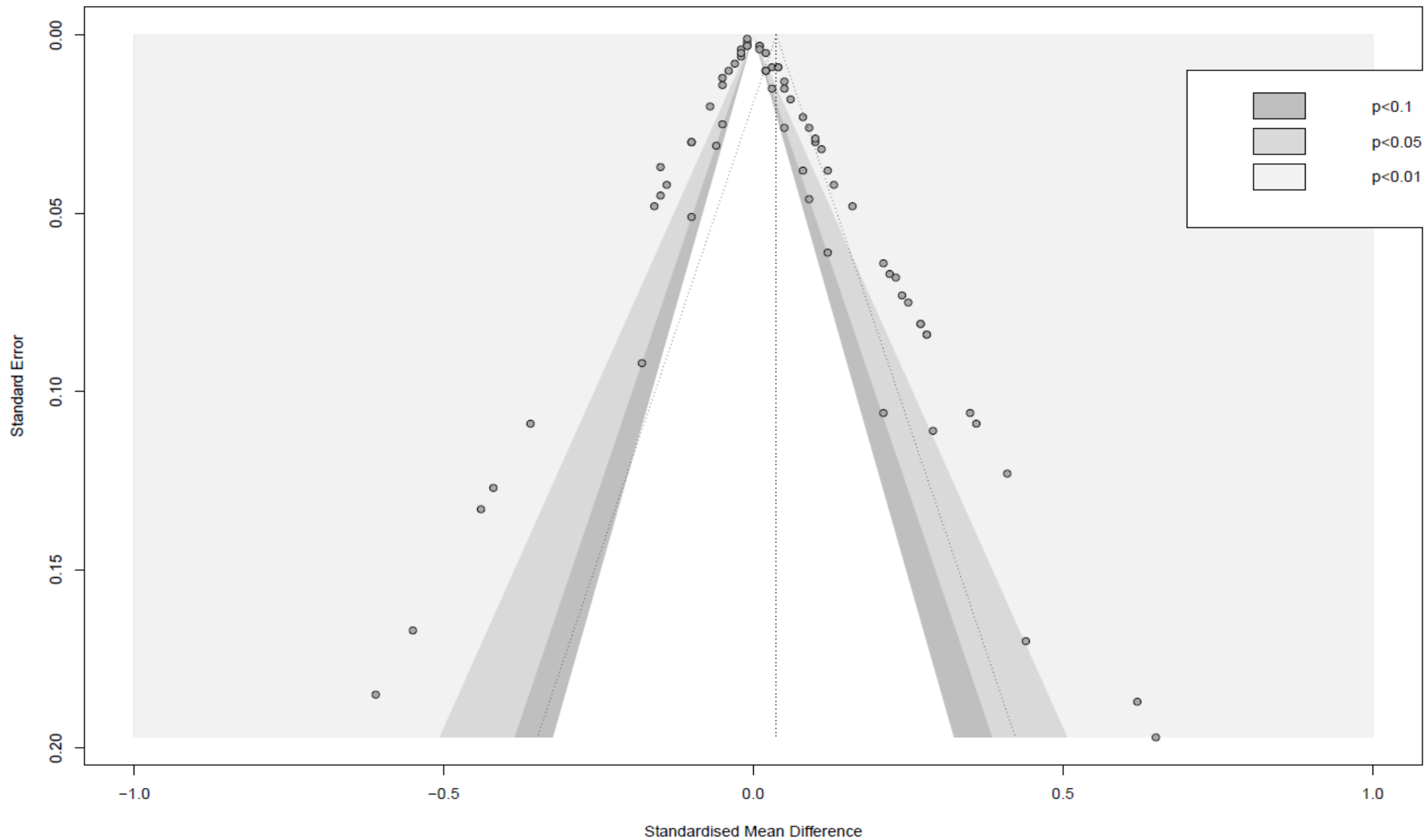


Figure 2. 3 Contour-enhanced funnel plot for the effect size of perennial grass monocultures and grass–legume mixtures on dry matter yield to test for publication bias. The contour lines correspond to statistical significance ($P = 0.01$, 0.05 or 0.1). Egger's test revealed no significant bias ( P = 0.0639).

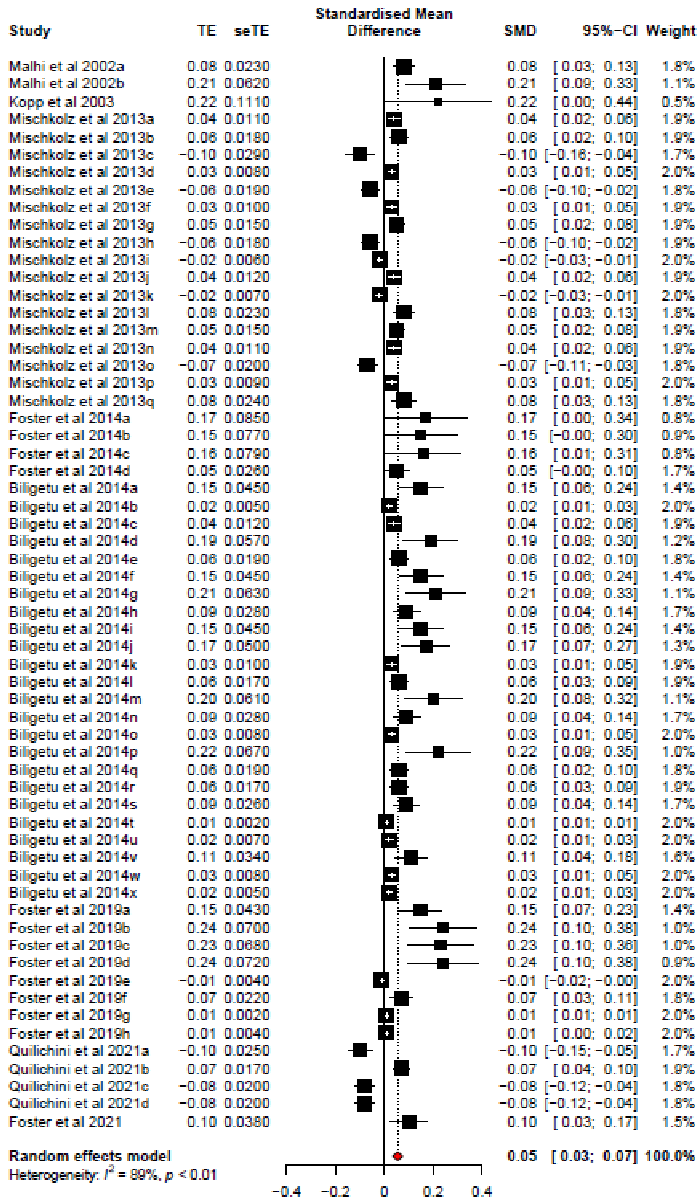


Figure 2. 4 A forest plot of the effect size (TE) and 95% confidence intervals (CI) for studies investigating the effect of perennial grass monocultures and grass–legume mixtures on crude protein. Point estimates and CI for each study are represented on each line. The weight of each study is displayed by a black box. Combined effect estimates (pooled effect) are presented at the bottom of each group with a red diamond symbol. The standard error (seTE) is the standard error of each study with its corresponding summarized mean difference (SMD).

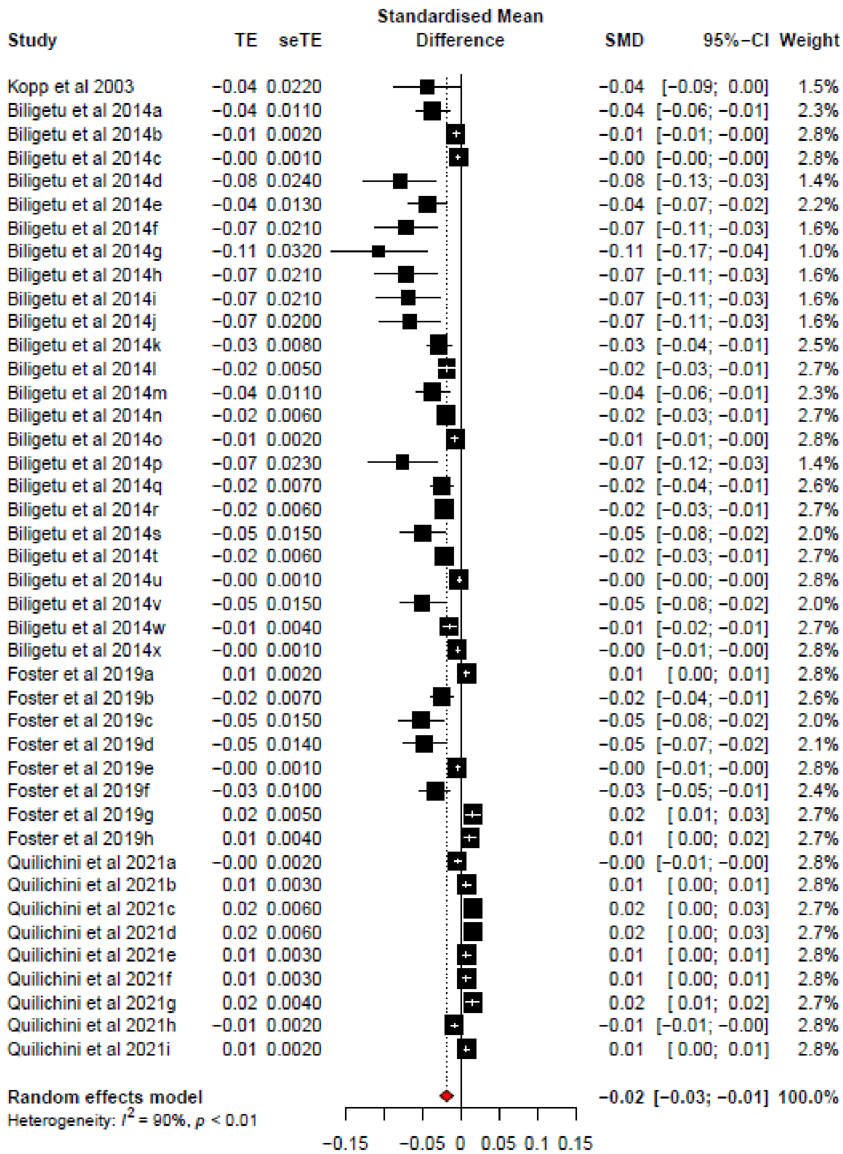


Figure 2. 5 A forest plot of the effect size (TE) and 95% confidence intervals (CI) for studies investigating the effect of perennial grass monocultures and grass–legume mixtures on neutral detergent fiber. Point estimates and CI for each study are represented on each line. The weight of each study is displayed by a black box. Combined effect estimates (pooled effect) are presented at the bottom of each group with a red diamond symbol. The standard error (seTE) is the standard error of each study with its corresponding summarized mean difference (SMD).

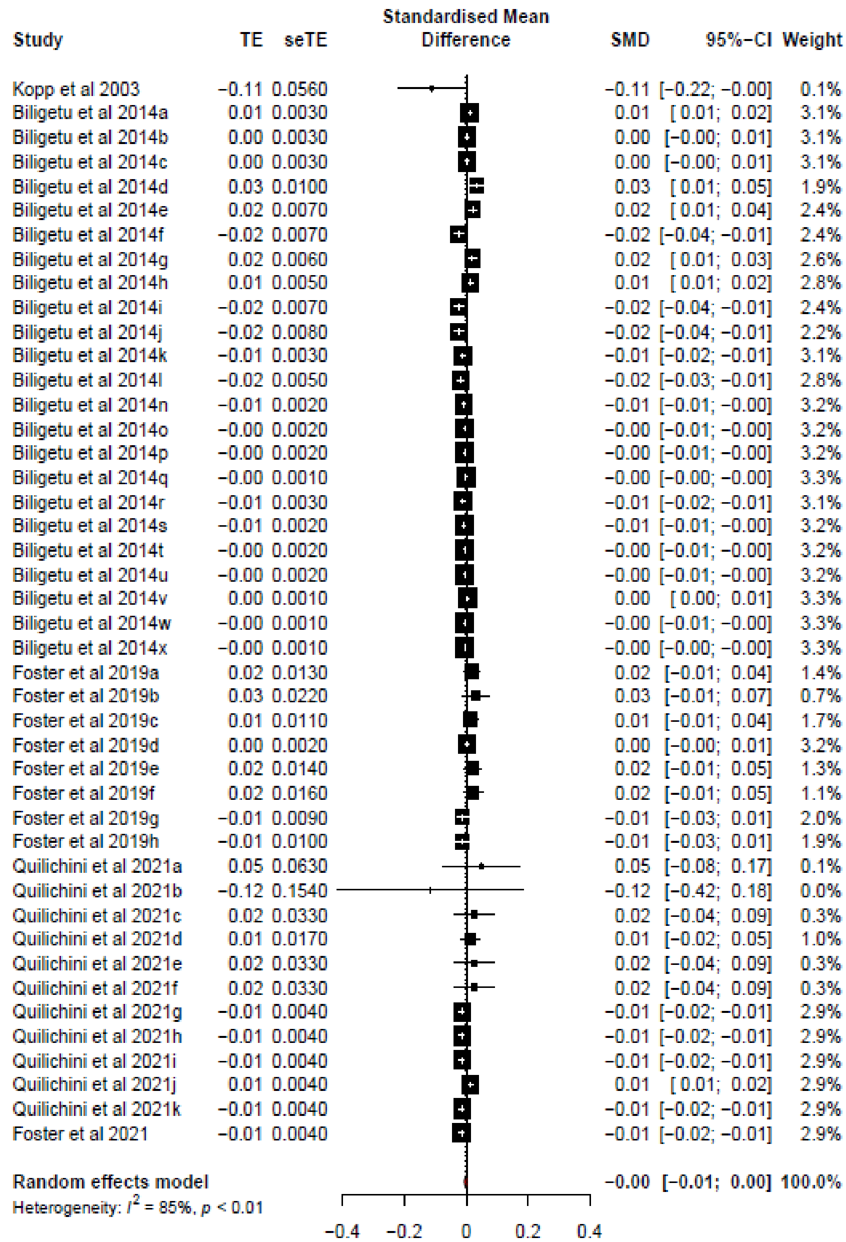


Figure 2. 6 A forest plot of the effect size (TE) and 95% confidence intervals (CI) for studies investigating the effect of perennial grass monocultures and grass–legume mixtures on acid detergent fiber. Point estimates and CI for each study are represented on each line. The weight of each study is displayed by a black box. Combined effect estimates (pooled effect) are presented at the bottom of each group with a red diamond symbol. The standard error (seTE) is the standard error of each study with its corresponding summarized mean difference (SMD).

# Chapter 3. Does the diversity of perennial forage species affect soil quality?

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## Highlights

- Perennial forage plant species improve the overall soil microbial $CO_2$ respiration.
- Grass–legume mixtures enhance surface soil infiltration rates and alleviate soil compaction.
- Soil C sequestration improves under perennial forage mixtures but grass monocultures have higher effects overall.
- Soil quality improves through cultivating perennial forage species.

## Study synopsis

Perennial forages grown either in mixtures or monocultures can improve the soil's quality. However, research documenting the impacts of perennial forages on soil quality in western Canada remains limited. Hence, a multiyear study (June 2020 to June 2023) was conducted in Fairview, Alberta, Canada, to evaluate the effects of different perennial forage species on the soil's physical (compaction, infiltration, and bulk density), biological ($CO_2$ respiration), and chemical [ammonium, nitrate, total N (TN), total C (TC), total organic C (TOC), C sequestration] properties. We established 15 forage treatments encompassing three monoculture grasses of wheatgrass (*Thinopyrum intermedium*; variety, greenleaf pubescent), meadow bromegrass (MB) (*Bromus commutatus*; variety, Fleet), hybrid bromegrass (HB) (AC Success, i.e., a cross between meadow bromegrass and smooth bromegrass (*Bromus inermis*), two simple mixtures (one grass and one legume), and 10 complex mixtures (i.e., one grass-only mixture, one legume-only mixture, and eight grass–legume mixtures). Baseline soil samples were collected before seeding forage at the beginning of the study. After harvest of the forage during the third year of production, soil samples were collected to evaluate changes over time as well as across the forage treatments. Our findings indicated that complex mixtures had greater biological activity (as $CO_2$ production) than monoculture grasses at the 0–15 cm soil depth ($P = 0.0386$), whereas the reverse effect was found at deeper layers (15–30 cm) (i.e., grasses > complex mixtures) ($P = 0.0027$). Although no difference in soil C storage was detected at the surface layer (0–15 cm), the monoculture grasses exhibited greater soil C gains at the subsurface layer (15–30 cm). When encompassing the entire 0–30 cm soil depth, cumulative soil C storage and the rate of C sequestration were highest for Fleet MB (71.0 Mg C $ha^{-1}$ and 10.0 Mg C $ha^{-1}$ $year^{-1}$, respectively) and lowest for the forage mixture of HB, yellowhead alfalfa (*Medicago sativa Subsp.falcata*), sainfoin (*Onobrychis viciifolia*), and cicer milkvetch (*Astragulus cicer*) (Mixture 10) (52.6 Mg C $ha^{-1}$ and 3.9 Mg C $ha^{-1}$ $year^{-1}$, respectively). In contrast, at depths of 0–30 cm, cumulative soil N storage was highest in the forage mixture of HB, greenleaf pubescent wheatgrass, Italian ryegrass (*Lolium multiflorum*), yellowhead alfalfa, and sainfoin (Mixture 19) (7.9 Mg N $ha^{-1}$). Comparatively, complex grass–legume mixtures reduced soil compaction, measured as penetration resistance. Likewise, the mixture of Fleet MB, yellowhead alfalfa, and sainfoin (Mixture 7) enhanced the water infiltration rate at the soil surface (i.e., 0.79 cm $h^{-1}$). Overall, diverse perennial forage was shown to be effective at improving soil quality by concomitantly alleviating compaction and increasing infiltration, whereas grasses were effective at improving the C storage, which can provide stability in the soil's structure.

**Keywords:** Perennial forage mixtures, soil compaction, bulk density, microbial activity, Soil quality, ammonium, nitrate

## 3.0 Introduction

Diversity in an agroecosystem enables the better use of a broad range of resource niches, providing consistency in productivity and enhancing sustainability. Additionally, the incorporation of perennial forage mixtures into cropping systems is a key factor in improving agricultural sustainability (Weißhuhn et al., 2017; Li et al., 2018). Although perennial forages are mostly used for providing balanced nutrition to beef and dairy cattle throughout the year, they also play a crucial role in the overall multifunctionality of agroecosystems (Entz et al., 1995). Furthermore, perennial legumes and grass forage improve the soil's health and quality by improving the soil organic matter (OM) and its physical properties (Gregory et al., 2005; Moore et al., 2000; Su, 2007). For example, perennial forages are known for improving the soil's structural quality and C sequestration while reducing nitrate ($NO_3$–N) leaching and erosion (Olmstead and Brummer, 2008; Kim et al., 2022). However, the right combinations of diverse perennial forage species that offer these ecosystem benefits still need more attention. This could be important in estimating or quantifying the influence of such forage species on soil quality.

Soil quality is the ability of a soil to supply sufficient nutrients and water sustainably while maintaining or increasing the resource niches and the environment, as well as plant, animal, and human health (Doran and Parkin, 1994). The essentiality of soil quality, therefore, revolves around the fulcrum of achieving land-use sustainability and management systems to regulate productivity and environmental protection (Doran and Zeiss, 2000). Although soil quality is complex, several soil indicators enable us to measure soil quality (Iheshiulo et al., 2023a), representing the physical, biological, and chemical attributes of the soil (Muñoz-Rojas, 2018) such as nutrient cycling, soil structure, bulk density (BD), microbial biomass, water infiltration rates, pH, and soil organic C (Doran and Parkin, 1996; Mocek and Owczarzak, 2011). These attributes directly or indirectly affect plants' performance in cropping systems (Iheshiulo et al., 2023b). However, the question of whether forage mixtures with many species perform better at improving the soil's attributes than fewer species established together still needs to be answered.

Previous research has demonstrated that perennial forage moderately improves the soil's physical quality through their diverse rooting patterns that could potentially influence the compaction and infiltration capacity of the soil ecosystem (Crotty et al., 2015; Luo, 2018; Daly et al., 2023). For instance, alfalfa (*Medicago sativa*) has deep and extending taproots which create channels to encourage water penetration. In addition, most perennial grasses have fibrous roots which help bind soil particles together and stabilize the soil's structure, as well as serve as N sinks by capturing free N in the soil (Clément et al., 2022; Elmy, 2020). Moreover, a simple grass–legume mixture showed significant soil aggregate stability and higher soil infiltration compared with pure grasses (Gijsman and Thomas, 1996). Furthermore, decreased soil microaggregates and increased macroaggregates as well as decreases in both penetration resistance and BD have been achieved through forage crop treatments such as alfalfa, purple crownvetch (*Securigera varia*), and bromegrass (*Bromus* spp.) (Gülser, 2006). However, it is unclear whether sufficient improvements will be achieved only within a short time (e.g., 3 years) after establishment of the forage.

Perennial mixtures including legume components can also positively impact the soil's biological properties. For instance, legumes within stands are critical in the enhancement of a conducive microenvironment between plants and soil for microbes to grow and thrive (Crotty et al., 2015; Luo et al., 2018). In addition, the presence and abundance of both macro- and microorganisms could be improved in grass–legume stands compared with pure grasses (Dhakal and Anowarul Islam, 2018; Bhandari et al., 2018). This biological activity also augments the production of $CO_2$ in the soil and can be used as a proxy to estimate the presence of living organisms (Murphy et al, 1998). However, the gap in knowledge regarding whether different legume cultivars seeded with grasses in mixtures provide a better overall biological benefit than a single legume species with grasses must be addressed. This can aid in understanding the intrinsic characteristics of individual forage plants and their contribution to the forage vegetation community.

Over the years, beef producers in the Canadian Prairies have been exploring methods of regenerative agriculture in their native grassland and cultivated stands of forage, with the core intention of improving soil health and minimizing overreliance on synthetic resources. Regenerative agriculture is a "farming strategy that uses natural processes to increase biological activity, enhance soil health, improve nutrient cycling, restore landscape function, and produce food and fiber, while preserving or increasing farm profitability" (Khangura et al., 2023). Both annual and perennial forages can be used to achieve the objectives of regenerative agriculture through improved soil quality and nutrient recycling. However, perennials provide the most benefits because of their ability to grow and thrive over many years (Daly et al., 2023). Nevertheless, there is little information on the combinations of perennial forages that can significantly achieve this goal. It is therefore important to explore the effect of a broad range of perennial forage mixtures on soil quality indicators such as the physical properties (BD, compaction as penetration and surface infiltration), chemical nutrients [TC, $NO_3$–N, ammonium ($NH_4$–N), TOC, C and N sequestration), and biological activities (microbial $CO_2$ respiration) of the heavy-textured clay soil in northwestern Alberta. The results will deepen our understanding of forage mixtures and their roles in enhancing soil quality. We hypothesized that complex, highly diverse forage mixtures would significantly improve the physical, chemical, and biological properties of the soil compared with either simple mixtures or monoculture grasses. Therefore, the objective of the study was to quantify the effect of contrasting perennial forage mixtures on certain soil quality attributes representing the soil's physical, chemical, and biological qualities.

## 3.1 Materials and methods

### 3.1.1 Experimental site and weather

A perennial forage field experiment was established at the Peace Country Beef & Forage Association research farm in Fairview, northwestern Alberta, Canada (56°04′53″N, 118°26′05″W, 670 m above sea level) in June 2020 and was evaluated for three consecutive production years (2021, 2022, and 2023). The selected field had stubble from a previous crop, namely canola (*Brassica napus*), and had a history of several years of wheat (*Triticum aestivum*)–canola rotations for grain production. The soil classification was Eluviated Black Chernozem according to the Canadian System of Soil Classification (Agricultural Region of Alberta Soil Inventory Database, 2020). The weather data during the growing seasons of the field experiment and their long-term averages for the site (Table 3.1) were obtained from the Alberta Climate Information Service (2020) (Fairview AGDM).

### 3.1.2 Experimental design and treatments

The experimental design for the trial was a randomized complete block design with four replications ($n$ = 4). The treatments consisted of two simple mixtures (one grass and one legume) and 10 complex diverse perennial forage species (i.e., three or more grass–legume) mixtures, consisting of one grass-only mixture, one legume-only mixture, and eight grass–legume mixtures (Table 3.2). In addition, three pure stands of perennial grasses were used for comparison. The grass species used in the mixtures or monocultures were wheatgrass (*Thinopyrum intermedium*; greenleaf pubescent), meadow bromegrass (MB) (*Bromus commutatus*; variety, Fleet), and hybrid bromegrass (HB) (AC Success grass, i.e., a cross between meadow bromegrass and smooth bromegrass (*Bromus inermis*). A complete list of treatments is available in Appendix 1.

### 3.1.3 Pre-establishment soil measurements (baseline)

Eight initial random composite baseline soil samples (0–15 cm) were collected from each block replicate ($n$ = 4) before seeding. The soil samples were submitted to the A&L Laboratory (London, Ontario, Canada) for chemical analyses (e.g., $NO_3$–N, P, K, and pH). Another set of soil samples was taken at 0–7.5 cm, 7.5–15 cm, 0–15 cm, and 15–30 cm for tests of the soil's wet aggregation (Kemper and Rosenau, 1986), and biological assessments (TN, TC, TOC, and $CO_2$ production). Soil compaction (0–7.5 cm, 7.5–15 cm, 0–15 cm, and 0–30 cm) was measured as penetration resistance using a SpotOn digital probe (Spectrum Technologies, WA, USA). Surface infiltration was measured using single infiltration rings (7.5 cm and 15 cm in diameter). BD (0–7.5 cm, 7.5–5 cm, 0–15 cm, and 15–30 cm) was also measured with a BD sampling probe (AMS Inc., ID, USA) using the core method (Kelley et al., 1947; USDA, 2001).

### 3.1.4 Establishment of perennial mixtures and grass monoculture stands

The site received 95.3 kg N $ha^{-1}$ of anhydrous ammonia in May 2020, 1 month before seeding. Seeding rates for the treatments were calculated on the basis of the monoculture seeding rate recommendations for perennial forages by Hutton et al. (2005) (Appendix 1). Each experimental plot was 2 m wide by 8 m in length. Adequate alley space (2–3 m) was left between block replicates. Before seeding, alfalfa seeds were inoculated with Nitragin Gold Alfalfa inoculants from Northstar Seeds, Edmonton, Canada, at a rate of 1.5 g per 250 g of seeds, whereas birdsfoot trefoil (*Lotus corniculatus*), cicer milkvetch (*Astralagus cicer*), and sainfoin *(Onobrychis viciifolia*) received no inoculants because of the lack of commercially available inoculants in the region. All grasses and legumes were seeded at a depth of 1.3 cm on 26 June 2020 with a six-row

Fabro plot drill (Fabro Ltd Swift Current, SK, Canada) equipped with disc-type openers at a 23-cm row spacing. In the establishment year (2020) and the first year of production (2021), the plots were sprayed with Basagran Forte at 4.05 mL $ha^{-1}$ for in-crop weed control. Additionally, dandelions (*Taraxacum officinale*), volunteer wheat (*Triticum aestivum*), and canola (*Brassica napus* L.) were occasionally handpicked. Subsequently, in the growing seasons of 2022 and 2023, no further weed control measures (chemical spraying or manual) were carried out.

### 3.1.5 Within-season compaction measurements

As a proxy of soil compaction, penetration resistance at depths of 0–7.5 cm, 0–15 cm, and 0–23 cm was measured weekly from mid-May to late June in 2021, 2022, and 2023 in each experimental plot with a SpotOn digital soil compaction meter (Innoquest Inc., IL, USA). These soil data provide an indication of the vertical pressure and resistance encountered by penetrating roots. The soil penetrometer monitoring was coupled with reading the volumetric water content (time domain reflectometry, TDR-350) for every measurement date, soil depth, and experimental plot.

## 3.2 Postharvest measurements

### 3.2.1 Soil compaction and water infiltration

After harvest of the forage in June 2023, the single ring (7.5 cm and 15 cm in diameter) method was used to determine the rate of water infiltration. The measurement was taken within the inner rows to prevent outer row effects. Two infiltration readings were taken and timed according to the method of the Natural Resources Conservation Service (USDA, 2001) within the same day to avoid bias and ensure continuity. The measurement was stopped after 25 minutes if the first or the second water reading did not drain, the height of any remaining water was measured. Subsequently, the infiltration rate ($IR$) was calculated as $IR = H/t$ (USDA, 2001), where $H$ is the height of infiltrated water and $t$ is the time taken for the water to infiltrate. Final soil penetration resistance readings at 0–7.5 cm, 0–15 cm, and 0–30 cm were also measured after the harvest on 23 June 2023 for each experimental plot with a SpotOn digital soil compaction meter.

### 3.2.2 Bulk density

Soil samples for measurements of BD were manually taken on 23 June 2023 soon after harvest by taking soil cores at depths of 0–15 cm and 15–30 cm, each 5 cm in diameter. The soil cores were dried at 105ºC to a constant weight. The BD was subsequently determined using the dried soil mass (g) divided by the volume of the metal ring ($cm^3$) according to the protocol of the USDA (2001).

### 3.2.3 Carbon dioxide respiration, TN, TC, TOC, C and N sequestration, $NO_3$–N, and $NH_4$–N

Soil samples at depths of 0–15 cm and 15–30 cm for both biological and chemical analyses were collected on 23 June 2023 from each individual plot after harvest and submitted to the Chinook Applied Research Association and Natural Resources Analytical Laboratory for analysis. The TOC, TC, and TN were analyzed using the dry combustion method (ThermoScientific, FlashSmart 2000 Organic Elemental Analyzer) (Matejovic, 1997). In addition, the Comprehensive Assessment of Soil Health (CASH) protocol modified method known as 'sealed chamber alkali trap respirometry' was used to analyze $CO_2$ respiration (Zibilske, 1994). Chemical analyses were determined for soil $NO_3$–N and $NH_4$–N via the 2M KCl extraction method with a Thermo Gallery Plus Beermaster Autoanalyzer (Thermo Fisher Scientific, Vantaa, Finland) (Bower and Holm-Hansen, 1980). The soil C sequestration (Mg C $ha^{-1}$) was calculated from the TOC values via the formula postulated by Poeplau and Don (2015) as $SOC_{\text{stock}} = TOC_{\text{conc}} \times BD \times D$, where $SOC_{\text{conc}}$ is the TOC concentration (%), $BD$ is the BD of individual depth increments (g $cm^{-3}$), and $D$ is the thickness of the individual depth increment (cm). Similarly, soil N sequestration (Mg N $ha^{-1}$) was estimated from the TN values as $SON_{\text{stock}} = STN_{\text{conc}} \times BD \times D$, where $STN_{\text{conc}}$ is the soil nitrogen concentration (%), $BD$ is the BD of individual depth increments (g $cm^{-3}$), and $D$ is the thickness of the individual depth increments (cm).

### 3.3 Statistical analysis

Data were subjected to analysis of variance (ANOVA) as a two-way factor with a randomized complete block design using the NLME package (Pinheiro et al., 2020) in the R statistical program (Version 4.0) (R Core Team 2020). The forage treatments and depths were used as fixed effects in the analysis, and the random effect consisted of the block (replicates). The baseline values were used as covariates in the model. Due to the significance of treatments x depths, results were presented on a year-to-year basis. A 95% confidence interval was used to test the significance of the baseline values from post-harvest values at soil depths. Before ANOVA, the normality of the residuals was tested using the Shapiro–Wilk statistic, and the homogeneity of variance was tested using Levene's test. No data transformations were required, as the residuals of all data were homogeneous and normally distributed. Following ANOVA, Tukey's honestly significant difference test was used to separate treatment means when significant ($P < 0.05$).

## 3.4 Results

### 3.4.1 The soil's physical, chemical, and biological properties before establishment.

The baseline physical and chemical properties are listed in Table 3.3 and Appendix 2b, respectively. The baseline results showed a low soil compaction of 217.2 kPa at 0–7.5 cm which increased steadily downwards through the soil profile (Table 3.3). At 7.5–15 cm, the soil compaction was 333.7 kPa. In addition, between 0–15 cm and 0–30 cm, the soil compaction increased by 20.7 kPa. The soil's surface infiltration was 0.58 cm $h^{-1}$ prior to establishment of the perennial forage treatments.

The total $CO_2$ respiration prior to establishment of the perennial forage in June 2020 indicated higher $CO_2$ concentrations of 0.45 mg $CO_2$ $g^{-1}$ soil in the first 0–7.5 cm of the soil than 7.5–15 cm, which had 0.26 mg $CO_2$ $g^{-1}$ soil. The analysis of soil taken directly from the depth of 0–15 cm found 0.39 mg $CO_2$ $g^{-1}$ soil, equivalent to the average $CO_2$ concentration of 0.35 mg $CO_2$ $g^{-1}$ soil obtained at 0–7.5 cm and 7.5–15 cm (Table 3.3). The results further demonstrated that with an increase in soil depth, the amount of $CO_2$ produced declined. For instance, a $CO_2$ concentration of 0.25 mg $CO_2$ $g^{-1}$ soil was observed at a depth of 15–30 cm. This indicated a decrease of 0.25 and 0.01 mg $CO_2$ $g^{-1}$ soil for depths of 0–7.5 cm and 7.5–15 cm, respectively.

Higher concentrations of TOC (30 g $kg^{-1}$) and TC (31 g $kg^{-1}$) were also found at the depth of 0–7.5 cm than at 7.5–15 cm (23 and 24 mg $kg^{-1}$, respectively), and 15–30 cm (12 and 12 mg $kg^{-1}$, respectively) (Table 3.3).

### 3.5 Soil properties after the harvest

#### 3.5.1 Carbon dioxide respiration

The $CO_2$ produced at depths of 0–15 cm was significantly different among forage treatments (*P* = 0.0386). This varied between 0.29 mg $CO_2$ $g^{-1}$ soil for AC Success hybrid bromegrass (SHBG) to 0.66 mg $CO_2$ $g^{-1}$ soil for the grass-only mixture (Table 3.4). Except for Fleet MB (0.46 mg $CO_2$ $g^{-1}$ soil), all simple and complex mixtures produced more $CO_2$ than monoculture grasses of SHBG (0.29 mg $CO_2$ $g^{-1}$ soil) and greenleaf pubescent wheatgrass (GPWG), 0.39 mg $CO_2$ $g^{-1}$ soil. Interestingly, Fleet MB also produced more $CO_2$ than complex grass–legume mixture M10 and M20. Furthermore, simple mixtures (M1 and M5) showed similar results to those of complex mixtures such as M14, M18, M19, M22, and M23 (Table 3.4). Mixtures with one grass and two legumes, such as M7 (0.65 mg $CO_2$ $g^{-1}$ soil) and M21 (0.63 mg $CO_2$ $g^{-1}$ soil), also had similar results. However, if we consider $CO_2$ production prior to establishment of the perennial forage (0.39 mg $CO_2$ $g^{-1}$ soil), it was apparent that in the first 0–15 cm of the soil, all forage treatments had higher $CO_2$ concentrations, except for SHBG and GPWG (Tables 3.3 and 3.4).

At a soil depth of 15–30 cm, the $CO_2$ produced under the forage treatments varied significantly (*P* = 0.0027). The results showed that Fleet MB produced the most $CO_2$ (0.83 mg $CO_2$ $g^{-1}$ soil), followed by SHBG (0.62 mg $CO_2$ $g^{-1}$ soil). Apart from M23 (0.38 mg $CO_2$ $g^{-1}$ soil), there was no difference in $CO_2$ production between simple and complex mixtures. It appears that monoculture grasses produced more $CO_2$ than all the other treatments in this study. In addition, except for M5, M10, and M19, all forage treatments produced more $CO_2$ than the baseline values (0.25 mg $CO_2$ $g^{-1}$ soil) prior to establishment of the forage (Table 3.4). A general observation of our results between the two different soil depths showed that $CO_2$ production was higher at 0–15 cm but dropped to approximately 50% at 15–30 cm, except under monoculture grasses (Table 3.4). For instance, Fleet MB had 0.46 mg $CO_2$ $g^{-1}$ soil within the first 0–15 cm but increased to 0.83 mg $CO_2$ $g^{-1}$ soil at a depth of 15–30 cm. Similarly, SHBG had 0.29 mg $CO_2$ $g^{-1}$ soil but increased to

0.62 mg $CO_2$ $g^{-1}$ soil at 15–30 cm. Alternatively, M0 recorded 0.66 mg $CO_2$ $g^{-1}$ soil within the first 0–15 cm but decreased to 0.32 mg $CO_2$ $g^{-1}$ soil at 15–30 cm, whereas M7 had 0.65 mg $CO_2$ $g^{-1}$ soil (0–15 cm) and also decreased to 0.36 mg $CO_2$ $g^{-1}$ soil (15–30 cm) (Table 3.4).

### 3.5.2 Water infiltration rates at the soil's surface

Infiltration among treatments was significantly different after harvest ($P$ = 0.024). The lowest infiltration rate was recorded for M18 and GPWG (0.33 cm $h^{-1}$), whereas the highest was found for M7 (0.79 cm $h^{-1}$) (Table 3.4). Fleet MB (0.51 cm $h^{-1}$) was similar to M5, M10, M19, M20, and M23. In addition, the monoculture grasses (SHBG and GPWG) had similar soil infiltration rates to the grass-only mixture (M0) and some complex grass–legume mixtures (M14, M18, M21, and M22). Evidently, the number of species within each treatment did not influence the soil infiltration rate. For instance, there was no difference in the soil infiltration rate between the eight-way (M23), six-way (M22), and three-way mixtures (M14 and M21) (Table 3.4).

By comparing the infiltration rate (0.58 cm $h^{-1}$) prior to establishment of the forage with after harvest, we found that only M7 (0.79 cm $h^{-1}$) improved the soil infiltration rate considerably. This indicated that the soil infiltration rate improved by approximately 0.21 cm $h^{-1}$. It was also noted that the soil infiltration rates decreased substantially in most treatments. For example, the infiltration rates were reduced by more than 0.20 cm $h^{-1}$ in M14 (0.28 cm $h^{-1}$), M18 (0.33 cm $h^{-1}$), M22 (0.36 cm $h^{-1}$), M21 (0.38 cm $h^{-1}$), and GPWG (0.33 cm $h^{-1}$) (Table 3.4).

### 3.5.3 Bulk density

BD at depths of 0–15 and 15–30 cm was not significantly different among treatments ($P$ = 0.770 and 0.443, respectively). The treatment M5 had 1.1 g $cm^{-3}$ within the first 0–15 cm of the soil but increased to 1.4 g $cm^{-3}$ at 15–30 cm. In addition, Fleet MB recorded a BD of 1.2 g $cm^{-3}$ at 0–15 cm but increased to 1.5 g $cm^{-3}$ at 15–30 cm. Only M23 (1.0 g $cm^{-3}$) showed a slight reduction in BD at 15–30 cm soil depth. Prior to establishment of the forage, the BD was 1.09 g $cm^{-3}$ at 0–15 cm and 1.3 g $cm^{-3}$ at 15–30 cm. These results showed that none of the forage treatments changed BD at a soil depth of 0–15 cm. Similarly, forage treatments did not change BD at depths of 15–30 cm. Instead, the BD under some treatments such as M0 (1.4 g $cm^{-3}$), M5 (1.4 g $cm^{-3}$), and Fleet MB (1.5 g $cm^{-3}$) actually increased at 15–30 cm (Table 3.4).

### 3.5.4 Soil compaction

After establishment of the forage, the results showed that there were considerable improvements in soil compaction at 0–7.5 cm ($P$ = 0.034). In particular, complex grass–legume mixtures such as the four-way (M18), five-way (M19), six-way (M20 and M22), and eight-way (M23) mixtures were more effective in reducing soil compaction, considering the levels (217.2 kPa) prior to establishment of the forage (Tables 3.3 and 3.5). Alternatively, the values of soil compaction obtained at the start of the study were surpassed by monoculture grasses (Fleet MB, SHBG, and GPWG), and simple mixtures of M1 and M5. Additionally, soil compaction levels increased substantially among the treatments at a depth of 0–15 cm ($P$ = 0.042) compared with 0–7.5 cm. A comparison among the treatments revealed that M23 (248 kPa), M20 (256.1 kPa), M18 (277.2 kPa), and M22 (264.3 kPa) had the lowest soil compaction levels. Our results also indicated that monoculture GPWG (381.4 kPa), the complex grass–legume mixtures of M10 (380.5 kPa) and M7 (391.3 kPa), and the simple mixture of M1 (384.2 kPa) had higher compaction values (374.8 kPa) than those recorded before establishment of the forage (Tables 3.3 and 3.5).

The results at a depth of 0–30 cm showed that Fleet MB (225.8 kPa), M23 (243.6 kPa), M22 (248.7 kPa), M20 (277.8 kPa), M19 (268.9 kPa), and M18 (263.4 kPa) had a significant impact on soil compaction ($P$ = 0.006). Compared with the soil compaction prior to establishment of the forage (395.5 kPa), all forage treatments produced a reduction in their soil compaction levels, with the exception of M5 (421.6 kPa) and M21 (455.0 kPa) (Table 3.5). Furthermore, the four-way, five-way, six-way, and eight-way treatments produced noticeable changes in soil compaction compared with the three-way mixtures, the simple mixtures, and monoculture SHBG and GPWG.

### 3.5.5 Soil ammonium and nitrate

The $NH_4$–N concentrations at 0–15 cm differed among the treatments ($P$ = 0.042). The monoculture Fleet MB had the highest $NH_4$–N concentration (10.5 mg $kg^{-1}$), whereas M19 had the lowest (3.9 mg $kg^{-1}$), although it was not significantly different from GWPG, M1, M0, and M10 (Table 3.6). The monocultures of SHBG and GPWG did not vary from the simple mixtures (M1 and M5) and the grass-only mixture (M0). Among the complex grass–legume mixtures, the eight-species treatment (M23) had the highest $NH_4$–N concentration (7.7 mg $kg^{-1}$) but was similar to the three-way (M14 and M21), four-way (M18), and six-way (M22 and M20) mixtures. At a soil depth of 15–30 cm, the results indicated that the $NH_4$–N concentrations were significantly different among the treatments ($P$ = 0.037). The $NH_4$–N concentrations produced under all treatments ranged from 2.6 mg $kg^{-1}$ for M23 to 0.87 mg $kg^{-1}$ for SHBG. Additionally, $NH_4$–N concentrations decreased substantially under all treatments compared with a soil depth of 0–15 cm. For example, Fleet MB decreased by 8.8 mg $kg^{-1}$, whereas M23 decreased by 4.1 mg $kg^{-1}$. Notably, except for M23, M0, and SHBG, all treatments were statistically similar (Table 3.6).

The $NO_3$–N concentrations at 0–15 cm were 5.4–15.2 mg $kg^{-1}$ for monoculture grasses, 5.6–6.8 mg $kg^{-1}$ for simple mixtures, 6.3–13.8 mg $kg^{-1}$ for complex grass–legume mixtures, and 4.3 mg $kg^{-1}$ for the grass-only mixture of M0 (Table 3.6). Among the complex grass–legume mixtures, the $NO_3$–N concentrations were greater under the eight- and six-species mixtures compared with the three-, four-, and five-species mixtures, with the exception of M14. Generally, Fleet MB, M1, M0, and M19 had the lowest $NO_3$–N concentrations. If we compare the $NO_3$–N concentrations prior to establishment of the forage (33 mg $kg^{-1}$), the results showed that the soils under all treatments were low in $NO_3$–N at the end of the study (Appendix 2b). At a depth of 15–30 cm, the $NO_3$–N concentrations decreased compared with that at 0–15 cm. However, the $NO_3$–N concentrations at 15–30 cm differed significantly among treatments ($P$ = 0.035). Both GPWG and M5 had the highest $NO_3$–N concentrations, whereas M0 had the lowest (Table 3.6). Except for GPWG, SHBG, M1, M0, and M21, all treatments were statistically similar, albeit with slight differences in concentrations.

### 3.5.6 Soil TN, TC, and TOC

The results for soil TN concentrations sampled at 0–15 cm were statistically different among the treatments ($P$ = 0.044). M19 had the highest TN (3.0 g $kg^{-1}$), whereas GPWG and M20 had the lowest (1.7 g $kg^{-1}$ each). Apart from GPWG, M5, M20, and M19, there were no differences among the treatments in this study (Table 3.6). The soil TN concentration (3.0 g $kg^{-1}$) prior to establishment of the forage also further demonstrated that M19 was the only treatment that maintained the soil TN concentration over the entire study period. At a depth of 15–30 cm, TN concentrations decreased further, with the exception of Fleet MB, GPWG, and M20. For instance, Fleet MB increased by 1.0 g N $kg^{-1}$, whereas GPWG and M20 increased by 0.3 g N $kg^{-1}$. Although there was a decrease in TN concentrations, no treatments depleted the soil below the concentration (1.0 g N $kg^{-1}$) recorded prior to establishment of the forage (Table 3.3). Instead, Fleet MB, GPWG, and M20 slightly increased the soil TN concentration.

The soil TC and TOC concentrations showed no significant difference (*P* = 0.754 and 0.891, respectively) among the treatments at a depth of 0–15 cm. However, TC ranged from 18 g $kg^{-1}$ for GPWG to 24 g $kg^{-1}$ for M0, whereas TOC varied between 15 g $kg^{-1}$ for M5 and 22 g $kg^{-1}$ for M0 (Table 3.6). The soil C slightly decreased among treatments over the entire study (2–6 g $kg^{-1}$ for TC and 2–9 g $kg^{-1}$ for TOC) compared with the baseline TC (26 g $kg^{-1}$) and TOC (24 g $kg^{-1}$) concentrations (Table 3.3). In contrast to the depth of 0–15 cm, significant differences in TC and TOC (*P* = 0.0281 and 0.038, respectively) were observed among the forage treatments at 15–30 cm (Table 3.6). Fleet MB and GPWG had the highest TC, whereas M10, M14, M18, and M21 had the lowest. Furthermore, M5 had the highest TOC (16 g $kg^{-1}$), whereas M10, M18, and M21 recorded the lowest of 10 g $kg^{-1}$ each. By comparing the results after the entire study with the baseline results for TC and TOC (12 g $kg^{-1}$ each), it was found that monoculture grasses, simple mixtures, and complex grass–legume mixtures had higher TC concentrations at 15–30 cm. Nevertheless, monocultured grasses had the highest TC (14–18 g $kg^{-1}$) among the treatments (Tables 3.3 and 3.6). Similarly, TOC was higher in soils of Fleet MB and GPWG monocultured grasses, and of the simple mixture M5. Here, the complex grass–legume mixtures did not increase the TOC concentrations of the soil.

### 3.5.7 Soil C and N sequestration

The amount of soil C accumulated at 0–15 cm was not statistically different among the forage treatments (*P* = 0.932), although M0 (42 Mg C $ha^{-1}$) and M1 (40.4 Mg C $ha^{-1}$) were slightly better than the rest of the forage treatments (Table 3.7). At a depth of 15–30 cm, the C sequestered by the soil differed significantly among the treatments (*P* = 0.005). The M5 treatment recorded the highest (34.6 Mg C $ha^{-1}$), followed by Fleet MB (31.7 Mg C $ha^{-1}$) and GWPG (31.0 Mg C $ha^{-1}$). With the exception of SHBG, monoculture grasses sequestered significantly more C than both the grass-only mixture and the grass–legume mixtures (Table 3.7). For instance, Fleet MB had 7.7 and 4.9 Mg C $ha^{-1}$ more than M7 and M0, respectively. The cumulative amount of C stored in 0–30 cm of the soil also showed significant differences among the forage treatments (*P* = 0.041). The highest level of C sequestration was found for Fleet MB (71.0 Mg C $ha^{-1}$), whereas the lowest was for M10 (52.6 Mg C $ha^{-1}$). The rest of the forage treatments did not differ statistically, although there were slight differences, ranging between 55.9 Mg C $ha^{-1}$ for M18 to 69.8 Mg C $ha^{-1}$ for M0 (Table 3.7).

The baseline results prior to establishment of the forage showed 20 Mg C $ha^{-1}$ of C was stored at 0–15 cm, whereas 21 Mg C $ha^{-1}$ was found at 15–30 cm (Table 3.3). In addition, 41 Mg C $ha^{-1}$ of C was stored across the whole soil depth (0–30 cm). Comparatively, the forage treatments substantially improved the C stock at both soil depths and in the whole soil layer 3 years after establishment of the forage. For instance, the forage treatment (M5) with the lowest level of C sequestration (32.6 Mg C $ha^{-1}$) at 0–15 cm added approximately 12.6 Mg C $ha^{-1}$ of C to the soil, whereas M0, with the highest (42.9 Mg C $ha^{-1}$), added 22.9 Mg C $ha^{-1}$ to the soil. Cumulatively, at 0–30 cm, Fleet MB which had the highest level of C sequestration (71.0 Mg C $ha^{-1}$), added 30 Mg C $ha^{-1}$ to the soil, whereas M10, which had the lowest (52.6 Mg C $ha^{-1}$), added 11.6 Mg C $ha^{-1}$ (Table 3.7). The annual soil C sequestration rate also showed that Fleet MB had the highest C sequestration rate (10.0 Mg C $ha^{-1}$ $y^{-1}$), followed by M0 (9.6 Mg C $ha^{-1}$ $y^{-1}$), whereas M10 had the lowest (3.9 Mg C $ha^{-1}$ $y^{-1}$).

Prior to establishment of the forage, the soil N content was 6.9 Mg $ha^{-1}$ (Table 3.3). Following 3 years of the forage treatments, the N sequestration throughout the soil (0–30 cm) showed significant differences among the forage treatments ($P$ = 0.042, Table 3.7). The N sequestration ranged between 5.2 Mg N $ha^{-1}$ for M18 to 7.9 Mg N $ha^{-1}$ for M19. The soil under M19 was the highest among all treatments.

## 3.6 Discussion

### 3.6.1 Forage mixtures improved soil microbial activity

High $CO_2$ production in the first 0–15 cm of soil under the mixtures compared with the monoculture grasses, except for Fleet MB, suggested that more microbial activity occurred in soils with diverse plant species. This observation was made for all mixtures: grass-only, legume-only, and grass–legume mixtures. For example, the grass–legume mixture M7 had greater $CO_2$ production, indicating significant microbial activity compared with monoculture grasses. Our findings are in accordance with those of Yan et al. (2022) and Lange et al. (2017), who found that grass–legume mixtures alter the rhizosphere and composition of microbial communities, providing favorable conditions for microbial interactions. In addition, having diverse plant rooting systems could release a large amount of root exudates into the soil environment. These act as an organic C source for the growth of rhizospheric microorganisms (Alkorta and Garbisu, 2001). As a result, the densities of rhizospheric microorganisms can be increased in mixtures compared with monoculture grasses. Although grass–legume mixtures seem to be conducive to microbial activity, our results also revealed that this phenomenon is not always true. This is because we found that Fleet MB performed better than the three-way and six-way grass–legume mixtures. According to our findings, one could conclude that the number of species within a treatment did not influence the ability of microbes to populate the soil. Instead, this is linked to the type of plant species and their functional traits, as we found simple mixtures produced similar results to very complex mixtures. Wieland et al. (2001) also described the variation in the microbial communities in the rhizosphere of crop species, where they found differences in the responses of alfalfa and clover (*Trifolium* spp.) to soil microbes. Furthermore, the general improvement in soil $CO_2$ production at the end of the study compared with the microbial activity at baseline prior to establishment of the forage can be linked to the previous crops cultivated in the soil. The study site had been under canola–wheat rotations for over 5 years prior to establishment of the forage. As found by Hansen et al. (2019), the microbial community associated with canola–wheat rotations contained significantly less fungi, less abundant mycorrhizae, and lower total microbial biomass.

A decline in soil $CO_2$ production at 15–30 cm was expected for all treatments because of a decrease in microbial activity at deeper soil depths. This result is supported by Taylor et al. (2002), who posited that microbial numbers decreased with depth, as indicated by viable counts and calculations based on biomass C and extracted DNA. However, observations of monoculture grasses were counterintuitive, as $CO_2$ production increased at deeper soil depths under these treatments. This can be explained by the rooting systems of these grasses. Grasses are monocotyledonous. In general, the fibrous root system of monocotyledons has a larger surface area than the root system of dicotyledons, which could explain the higher microbe–root surface contact, likely enabling this increased biological activity. Root profiling also showed that monoculture grasses, particularly Fleet MB, grew vigorously and spread to about 15–25 cm compared with the mixed treatments with legume components (Appendix 2a). Furthermore, we observed that more organic material (C) was found within the 15–30 cm zone of the soil (Table 3.6). This provided microbes with more substrate to decompose within this layer, resulting in greater $CO_2$ production.

### 3.6.2 Soil infiltration rates and compaction improved under mixtures whereas no change was observed in soil BD

The marginal changes in soil infiltration rates produced by the forage treatments indicated that different combinations of plant species can influence the soil differently. Although the infiltration rates differed among treatments, most values were within the range of the standard infiltration rate for clay soils. As reported by Hillel (1982), the infiltration rates in most clayey soils ranges from 0.10 to 0.51 cm $h^{-1}$. In line with Iheshiulo et al. (2023a), our results also indicated that the majority of forage treatments either maintained or even decreased their infiltration rates. This was partly caused by the compaction of clayey soils. Silva et al. (2008) postulated that in compacted soils such as clays, the total porosity reduces, mainly affecting macropores, which strongly prevents water from infiltrating. Noticeably, the only treatment (M7) that substantially improved the soil infiltration rate was among the treatments that had lower soil compaction. This improvement in the infiltration rate met the criteria of loam soils, which range from 0.51 to 1.0 cm $h^{-1}$ (Hillel, 1982).

Contrary to our expectations, the forage treatments did not change the BD of such heavily clay-textured soils. In this study, it was expected that in the presence of varying degrees of roots from both grasses and legumes, soil particles would loosen, alter their aggregations, and decrease BD. However, this was not the case, as we recorded soil BD values that were similar to those prior to establishment of the forage. This occurred partly because the field for the study had not been plowed for a long time (approximately 5 years). As described by Alamooti and Navabzadeh (2007), plowing generally decreases BD and increases pore space, which is beneficial. Our results also agree with those of Hurisso et al. (2013) and Iheshiulo et al. (2023b), who reported that soil BD did not differ at their study sites and sampling depths under a long-term no-till management system. Additionally, the increase in soil BD at deeper soil depths, as revealed by our findings, could be linked to consolidation as well as the reduced amount of OM within this layer of soil. Generally, soils enriched with OM are lighter than mineral soils (Kim et al., 2022; Daly et al., 2023). Hence, soils with higher OM would typically have a lower BD and vice versa.

Although soil compaction can influence BD, our results indicated that compaction was reduced across soil depths compared with the values prior to establishment of the forage. However, there were more changes in the first 0–7.5 cm than at depths of 0–15 and 0–30 cm. These changes were more evident under complex grass–legume mixtures than under monoculture grasses or simple mixtures. A similar pattern was also observed during the growing seasons (Figure 3.1). Here, the root growth patterns of complex grass–legume mixtures were partly responsible for the noticeable changes observed in the first 0–7.5 cm of the soil (Appendix 2a). This result is supported by the findings of Hamza and Anderson (2005), and Cochrane and Aylmore (1994), who reported that although soil compaction restricts root growth, when they grow different plant roots are effective in creating and stabilizing the soil's structural features. Moreover, the soil moisture content can also influence measurements of soil compaction. Thus, with higher soil moisture, soils become easier to penetrate compared with dry soils. However, our results showed that the moisture content at a depth of 0–15 cm was generally higher than at 0–7.5 cm. Hence, soil moisture may not have been the reason for the lower soil compaction at 0–7.5 cm. Furthermore, increased soil compaction at deeper depths was expected because the topsoil is loosened to subsoil. Nevertheless, soil compaction generally improved at both 0–15 and 0–30 cm compared with the values prior to establishment of the forage. This effect was ultimately attributed to the growth and expansion of the roots. With the increased root biomass of perennials, soil compaction can be alleviated over time (Daly et al., 2023).

### 3.6.3 Monoculture grasses are superior at improving C sequestration whereas N decreases under forage treatments

The initial objective here was to identify suitable treatments that could improve the nutrient pools (TN, TC, $NH_4$–N, and $NO_3$–N). Our findings suggest that soil $NH_4$–N and $NO_3$–N concentrations were higher under monoculture grass (Fleet MB) and complex grass–legume mixtures (M14 and M18) within the first 0–15 cm. However, the concentrations were lower than those reported prior to the start of the study, indicating the depletion of both nutrients. This is partly because N is one of the most important plant nutrients and is required in large quantities for the vegetative growth of crop plants, including for the synthesis of chlorophyll and protein, which affect crop yield (Kumar et al., 2021). However, because there were no external N fertilizer inputs, the plants only depended on the N fixed by legumes or via mineralization during the entire study period. Lower concentrations of $NH_4$–N and $NO_3$–N are also linked to the removal of crop biomass through harvesting. During each production year, the forage was cut and carried as hay. These recurrent harvests took away the nutrient resources within the plants' canopy tissues. Hence, the continuous removal of crops without the addition of fertilizer can lead to nutrient depletion (Li et al., 2018). This agrees with Kimura et al. (2015), who reported that approximately 237 kg of N $ha^{-1}$ is removed annually by harvesting perennial switchgrass (*Panicum virgatum*). Although both nutrients were lower in the soil, it appeared that higher concentrations of $NO_3$–N than $NH_4$–N were observed. These observations are supported by the characteristics of both $NH_4$–N and $NO_3$–N. Generally, $NH_4$–N is positively charged and has a higher propensity for attachment to soil particles, which are negatively charged. Again, most $NH_4$–N is absorbed by plants before the soil conditions allow its conversion into nitrites or nitrates. In contrast, $NO_3$–N is negatively charged and readily available in soil solutions for plant uptake (Keeney, 1970; Prakasa Rao and Puttanna, 2000). Additionally, with contrasting types of plant species (legumes and grasses), we expected higher concentrations of $NH_4$–N and $NO_3$–N owing to the functional traits of the individual plant species (Fornara and Tilman, 2008). For instance, plant root exudates and the decomposition of individual plant parts contribute differently to nutrient cycling (Bürgmann et al., 2005; Meier et al., 2017). However, our results were contradictory, as a high number of plant species did not necessarily improve the $NH_4$–N and $NO_3$–N concentrations. The decrease in both nutrients at a soil depth of 15–30 cm was probably caused by the lower OM (C) substrate within that zone of the rhizosphere (Table 3.6). In contrast, with more OM at that depth, increased microbial activity would have occurred, leading to effective mineralization (Lange et al., 2017), which could improve the nutrient pool. Our findings also indicated that monoculture grasses and simple mixtures had a higher pool of nitrates than complex mixtures. Here, we speculate that this occurred because there was greater competition and utilization of nutrients under complex mixtures than under monoculture grass (one species) and simple mixtures (two species). This is consistent with Wang et al. (2020), who reported that interspecific plant competition decreased the soil's TN content and mineralized C.

Interestingly, according to the estimated soil N storage at 0–30 cm, the treatments depleted the TN content of the soil, with the exception of M19 (Table 3.7). This was parallel to our expectations, because of the presence of different legumes in the mixtures. Based on the ability of legumes to fix atmospheric N (Synder et al., 2016), we had initially expected an improvement in soil TN under the treatments dominated by legumes. However, this did not occur. One could conclude that forage plants were effectively utilizing the N in their canopies. Alternatively, it is possible that nutrients were present at their lowest concentrations at the time of sampling, as the soils were sampled at the end of the last growing season in this study.

The concentrations of TC and TOC were found to be slightly low at 0–15 cm, with no statistical difference across treatments. However, there was a substantial increase at 15–30 cm compared with the baseline value prior to establishment of the forage. Although soil TC and TOC improved across treatments, those of monoculture grasses were slightly higher than those of simple and complex mixtures. This is partly attributed to grasses having a greater C content than legumes in their tissues. This leads to a slower microbial decomposition rate under grasses, thereby increasing the OM content over time. The results by Kim et al. (2022) showed how aboveground plant attributes impact the accrual of stable C pools in the soil. This was further supported by Conant et al. (2017), who reported that more C was stored as OM, particularly in grass-based management systems.

Similarly, C sequestration by all forage treatments improved after multiple years of forage cultivation in line with findings of Amiro et al. (2017) which reported high C in a perennial phase (507g C $m^{-2}$) than annual cropping system (155g C $m^{-2}$) over a four-year period. However, monoculture grasses had the highest accrual of stored soil C. This can be attributed to the amount of organic C accumulated by these grass treatments over time. As found by Franzluebbers (2012), soils rooted with perennial grasses have a higher OM content and thus can contribute to higher soil C sequestration, which can also help to mitigate climate change. In further detail, the grass components have extensive and deep root systems, which are essential for hiding recently fixed C and enables an active surface for biological activities. This was demonstrated by our field observations (Appendix 2a), where the large mass and surface area of grass roots were revealed.

### 3.7 Conclusion

This study has shown that highly diverse forage mixtures considerably increased the microbial activity in the topsoil layer (0–15 cm) compared with monoculture grasses. However, high microbial activity was also found in soils under monoculture grasses at 15–30 cm, which was attributed to the higher soil organic C within this subsurface in the grass rhizosphere. In addition, having a higher number of legumes within the mixtures did not commensurate with the increased $CO_2$ production. An increase in microbial activity coupled with an adequate C:N ratios (e.g., 20:1) of the organic material ensures effective mineralization, leading to an increase in the nutrient pools, which improves soil fertility. Complex grass–legume mixtures with higher number of plant species compared with either simple mixtures or monoculture grasses greatly reduced soil compaction. This change in soil compaction can be caused by the varying root growth patterns of the mixtures. The reduction in soil compaction improves the soil's quality through enhancing greater movement of solutions, which ensures a better redistribution of nutrients within the soil. Furthermore, the infiltration rate at the soil surface was also improved under complex grass–legume mixtures compared with simple mixtures and monoculture grasses. Conversely, monoculture grasses increased the TC and TOC of the soil compared with simple grass-legume and complex grass–legume mixtures. All complex mixtures, simple mixtures, and monoculture grasses sequestered soil C over the study period. Soil C influences the soil's quality by improving the source of nutrients through mineralization. In summary, both grass–legume mixtures and monoculture grasses can be used to improve the soil's biological properties, whereas complex grass–legume mixtures can improve soil compaction and infiltration rates substantially. Monoculture grasses also improved the soil's rate of C sequestration. We expect that additional changes in soil properties could be observed beyond the 3-yr period of the forage stands.

**Conflict of interest declaration**

The authors declare no conflict of interest.

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## 3.8 References


Alamooti, M. Y. and Navabzadeh, M. (2007). Investigation of plowing depth effect on some soil physical properties. Pakistan Journal of Biological Sciences 10 (24): 4510–4514 https://doi.org/10.3923/pjbs.2007.4510.4514

Alberta Climate Information Service (2020). Current and historical Alberta weather 384 station data viewer. Data provided by Alberta Agriculture and Forestry. Retrieved on 21 May 2021, from https://acis.alberta.ca/acis/weather-data-viewer.jsp

Alkorta I, Garbisu C. (2001). Phytoremediation of organic contaminants in soils. Bioresource Technology, 79, 273–276.

Amiro, B. D., Tenuta, M., Gervais, M., Glenn, A. J., and Gao, X. (2017). A decade of carbon flux measurements with annual and perennial crop rotations on the Canadian Prairies. Agricultural and Forest Meteorology, 247, 491–502.

Bhandari, K. B., West, C. P., Acosta-Martinez, V., Cotton, J., and Cano, A. (2018). Soil health indicators as affected by diverse forage species and mixtures in semi-arid pastures. Applied Soil Ecology, 132, 179–186. https://doi.org/10.1016/j.apsoil.2018.09.002

Bower, C. E. and Holm-Hansen, T. (1980). A salicylate–hypochlorite method for determining ammonium in seawater. Canadian Journal of Fisheries and Aquatic Sciences, 37, 794–798.

Bürgmann, H., Meier, S., Bunge, M., Widmer, F., and Zeyer, J. (2005). Effects of model root exudates on structure and activity of a soil diazotroph community. Environmental Microbiology, 7, 1711–1724.

Clément, C., Sleiderink, J., Fiil, S., George, A., Efstathios, S., Bodin, D., Kristian, D., and Kristensen, T. (2022). Comparing the deep root growth and water uptake of intermediate wheatgrass (Kernza®) to alfalfa. Plant and Soil 472, 369–390. https://doi.org/10.1007/s11104-021-05248-6

Cochrane, H. R., and Aylmore, L.A.G. (1994). The effects of plant roots on soil structure. In: Clarke, C. J., George, R. J., Bell, R. W., Beetson, G. R., Mauger, G. W., and Hobbs. R. J., Eds., Proceedings of the Third Triennial Conference “Soils 94”. (pp 207–212). Australian Society of Soil Science.

Conant, R. T., Cerri, C. E. P., Osborne, B. B., and Paustian, K. (2017). Grassland management impacts on soil carbon stocks: a new synthesis. Applied Ecology, 27, 662–668. https://doi.org/10.1002/eap.1473

Crotty, F. V., Fychan, R., Scullion, J., Sanderson, R., and Marley, C. L. (2015). Assessing the impact of agricultural forage crops on soil biodiversity and abundance. Soil Biology and

Biochemistry, 91, 119–126. https://doi.org/10.1016/j.soilbio.2015.08.036

Daly, E.J., K. Kim, Hernandez-Ramirez, G., and K. Klimchuk (2023). The response of soil physical quality parameters to a perennial grain crop. Agriculture, Ecosystems and Environment, 343, 108265. https://doi.org/10.1016/j.agee.2022.108265

Dhakal, D., and Anowarul Islam, M. (2018). Grass–legume mixtures for improved soil health in cultivated agroecosystem. Sustainability, 10, 2718. https://doi.org/10.3390/su10082718

Doran, J. W., and Zeiss, M. R. (2000). Parent material and soil physical properties. Encyclopedia of Earth Sciences Series, 15, 3–9. https://doi.org/10.1007/978-90-481-3585-1_107

Doran, J.W. and Parkin, T.B. (1994). Defining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F. and Stewart, B.A., Eds., Defining Soil Quality for a Sustainable Environment. (pp. 3–21). Soil Science Society of America. http://dx.doi.org/10.2136/sssaspecpub35.c1

Doran, J.W. and Parkin, T.B. (1996). Qualitative indicators of soil quality: a minimum data set. In: Doran, J.W. and Jones, A. J., eds., Methods for Assessing Soil Quality. (pp. 25–37). Soil Science Society of America. https://doi.org/10.2136/sssaspecpub49.c2

Elmy, K. R. (2020). Cover Cropping in Western Canada, 5$^{th}$ ed. Friesen Press.

Entz, M. H., Bullied, W. J., and Katepa-Mupondwa, F. (1995). Rotational benefits of forages in Canadian Prairies cropping systems. Journal of Production Agriculture, 4,521–528. https://doi.org/10.2134/jpa1995.0521

Fornara, D. A. and Tilman, D. (2008). Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology, 96, 314–322. https://doi.org/10.1111/j.1365-2745.2007.01345.x

Franzluebbers, A. J. (2012). Grass roots of soil carbon sequestration. Carbon Management, 3, 9–11. https://doi.org/10.4155/cmt.11.73

Gijsman, A. J., and Thomas, R. J. (1996). Evaluation of some physical properties of an oxisol after conversion of native sannana into legume-based or pure grass pastures. Tropical Grassland, 30, 237–248.

Government of Alberta (2020). Agricultural region of Alberta soil inventory database. Accessed on 29 February 2024 at https://www.alberta.ca/agricultural-regions-of-alberta-soil-inventory-database

Gregory, M. M., Shea, K. L., and Bakko, E. B. (2005). Comparing agroecosystems: effects of cropping and tillage patterns on soil, water, energy use and productivity. Renewable

Agriculture and Food Systems, 20, 81–90.

Gülser, C. (2006). Effect of forage cropping treatments on soil structure and relationships with fractal dimensions. Geoderma, 131, 33–44. https://doi.org/10.1016/j.geoderma.2005.03.004

Hamza, M. A., and Anderson, W. K. (2005). Soil compaction in cropping systems: a review of the nature, causes and possible solutions. Soil and Tillage Research 82, 121–145. https://doi.org/10.1016/j.still.2004.08.009

Hansen, J. C., Schillinger, W. F., Sullivan, T. S., and Paulitz, T. C. (2019). Soil microbial biomass and fungi reduced with canola introduced into long-term monoculture wheat rotations. Frontiers in Microbiology, 19, 1045–1059. https://doi.org/10.3389/fmicb.2019.01488

Hillel, D. (1982). Introduction to soil physics. Academic Press.

Hurisso, T. T., Norton, J. B., and Norton, U. (2013). Soil profile carbon and nitrogen in prairie, perennial grass–legume mixture and wheat–fallow production in the central High Plains, USA. Agriculture, Ecosystems and Environment, 181,179–187. https://doi.org/10.1016/j.agee.2013.10.008

Hutton, G., Berg, B., Najda, H., Johns, M., Cole, D., and Yoder, C. (2005). Perennial forage establishment in Alberta. Government of Alberta. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9682/$file/120_22-3.pdf?OpenElement

Iheshiulo, E. M. A., Larney, F. J., Hernandez-Ramirez, G., Luce, M. St., Liu, K., and Chau, H.W. (2023a). Crop rotations influence soil hydraulic and physical quality under no-till on the Canadian Prairies. Agriculture, Ecosystem, and Environment, 361, 108820. https://doi.org/10.1016/j.agee.2023.108820

Iheshiulo, E. M. A., Larney, F. J., Hernandez-Ramirez, G., Luce, M. St., Liu, K., and Chau, H.W. (2023b). Do diversified crop rotations influence soil physical health? A meta-analysis. Soil and Tillage Research 233, 105781 https://doi.org/10.1016/j.still.2023.105781

Keeney, D. R. (1970). Nitrates in plants and waters. Journal of Milk and Food Technology, (10), 425–432.

Kelley, O. J., Hardman, J. A. and Jennings, D. S. (1947). A soil-sampling machine for obtaining two, three, and four inches cores of undisturbed soil to a depth of six feet. Soil Science, American Proceedings 12, 85–87.

Kemper, W.D., Rosenau, R.C. (1986). Aggregate stability and size distribution. In: Klute, A., (ed.) Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. Agronomy Monographs No. 9. (pp. 425–442). Society of Agronomy and Soil Science Society of

America.

Khangura, R., Ferris, D., Wagg, C., and Bowyer, J. (2023). Regenerative agriculture—a literature review on the practices and mechanisms used to improve soil health. Sustainability, 15, 1–41. https://doi.org/10.3390/su15032338

Kim, K., Daly, E. J., Gorzelak, M., Hernandez-Ramirez, G. (2022). Soil organic matter pools response to perennial grain cropping and nitrogen fertilizer. Soil and Tillage Research, 220, 105376. https://doi.org/10.1016/j.still.2022.105376

Kimura, E., Collins, H. P., and Fransen, S. (2015). Biomass production and nutrient removal by switchgrass under irrigation. Agronomy Journal, 107, 204–210. https://doi.org/10.2134/agronj14.0259

Kumar, S., Kumar, S., and Mohapatra, T. (2021). Interaction between macro- and micro-nutrients in plants. Frontiers in Plant Science, 12, 665583. https://doi.org/10.3389/fpls.2021.665583

Lange, M., Eisenhauer, N., Sierra, C. et al. (2017). Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications, 6, 6707. https://doi.org/10.1038/ncomms7707

Li, J. C., Hernandez-Ramirez, G., Kiani, M., Quideau, S., Smith, E., Janzen, H., Larney, F., Puurveen, D. (2018). Soil organic matter dynamics in long-term temperate agroecosystems: rotation and nutrient addition effects. Canadian Journal of Soil Science, 98, 232–245.

Luo, C., Deng, Y., Inubushi, K., Liang, J., Zhu, S., Wei, Z., et al. (2018). Sludge biochar amendment and alfalfa revegetation improve soil physicochemical properties and increase diversity of soil microbes in soils from a rare earth element mining wasteland. International Journal of Environmental Research and Public Health 15, 965–986. https://doi.org/10.3390/ijerph15050965

Matejovic, I. (1997). Determination of carbon and nitrogen in samples of various soils by the dry combustion. Communications in Soil Science and Plant Analysis, 28, 1499–1511. https://doi.org/10.1080/00103629709369892

Meier, I. C., Finzi, A. C., and Phillips, R. P. (2017). Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. Soil Biology and Biochemistry, 106, 119–128.

Mocek, A., and Owczarzak, W. (2011). Parent material and soil physical properties. In Gliński, J., Horabik, J., Lipiec, J. (eds), Encyclopedia of Earth Sciences Series (pp. 543–547). https://doi.org/10.1007/978-90-481-3585-1_107.

Moore, J. M., Klose, S., and Tabatabai, M. A. (2000). Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biology and Fertility of Soils, 31, 200–210.

Muñoz-Rojas, M. (2018). Soil quality indicators: critical tools in ecosystem restoration. Current Opinion in Environmental Science and Health, 5, 47–52. https://doi.org/10.1016/j.coesh.2018.04.007

Murphy, D. V., Sparling, G. P., Fillery, I. R . P. (1998). Stratification of microbial biomass C and N and gross N mineralization with soil depth in two contrasting Western Australian agricultural soils. Australian Journal of Soil Research, 36, 45–55.

Olmstead, J., and Brummer, E. C. (2008). Benefits and barriers to perennial forage crops in Iowa corn and soybean rotations. Renewable Agriculture and Food Systems, 2*3*(2), 97–107. https://doi.org/10.1017/S1742170507001937

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. (2020). Nlme: linear and nonlinear mixed effects models. R package version 3.1-149.URL: https://CRAN.R-project.org/package=nlme.

Poeplau, C., and Don, A. (2015). Carbon sequestration in agricultural soils via cultivation of cover crops – a meta-analysis. Agriculture, Ecosystems and Environment, 200, 33–41. https://doi.org/10.1016/j.agee.2014.10.024

Prakasa Rao, E. V. S. and Puttanna, K. (2000). Nitrates, agriculture and environment. Current Science, 79,1163–1168.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Silva, S., Barros, N., Costa, L., and Leite, F. (2008). Soil compaction and eucalyptus growth in response to forwarder traffic intensity and load. Revista Brasileira Ciencia do Solo 32, 921–932.

Snyder, E. M., Karsten, H. D., Curran, W. S., Malcolm, G. M., and Hyde, J. A. (2016). Green manure comparison between winter wheat and corn: weeds, yields, and economics. Agronomy Journal, 108(5), 2015–2025. https://doi.org/10.2134/agronj2016.02.0084

Su, Y. Z. (2007). Soil carbon and nitrogen sequestration following the conversion of cropland to alfalfa forage land in northwest China. Soil and Tillage Research, 92, 181–189.

Taylor, J. P., Wilson, B., Mills, M. S., and Burns, R. G. (2002). Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biology and Biochemistry, 34, 387–401. https://doi.org/10.1016/S0038-0717(01)00199-7

USDA (2001). Soil infiltration: soil health – Guide for Educators.

https://www.nrcs.usda.gov/sites/default/files/2022-10/Soil%20Infiltration.pdf (accessed 20 March 2021).

Wang, M., Yang, J., Gao, H., Xu, W., Dong, M., Shen, G., Xu, J., Xu, X., Xue, J., Xu, C. Y., and Zhou, X. (2020). Interspecific plant competition increases soil labile organic carbon and nitrogen contents. Forest Ecology and Management, 462, 117991. https://doi.org/10.1016/j.foreco.2020.11799

Weißhuhn, P., Reckling, M., Stachow, U., and Wiggering, H. (2017). Supporting agricultural ecosystem services through the integration of perennial polycultures into crop rotations. Sustainability, 9, 2267. https://doi.org/10.3390/su9122267

Wieland, G., Neumann, R., and Backhaus, H. (2001). Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. Applied and Environmental Microbiology, 67, 5849–5854. doi: 10.1128/AEM.67.12.5849-5854.2001.

Yan, H., Gu, S., Li, S., Shen, W., Zhou, X., Yu, H., Ma, K., Zhao, Y., Wang, Y., Zheng, H., Deng, Y., and Lu, G. (2022). Grass–legume mixtures enhance forage production via the bacterial community. Agriculture, Ecosystems and Environment, 338, 108087. https://doi.org/10.1016/j.agee.2022.108087

Zibilske, L. M. (1994). Carbon mineralization. In Weaver R. W., Angle S., Bottomley P., Bezdicek D., Smith S., Tabatabai A., Wollum A., and Mickelson S. H., (eds), Methods in Soil Analysis, Part 2. Microbiological and Biochemical Properties. (pp. 835–863) Soil Science Society of America.

## 3.9 Tables

Table 3. 1 Rainfall, and maximum and minimum temperatures with their long-term average during the growing seasons of 2020, 2021, 2022, and 2023 at Fairview, Alberta, Canada.

| Month | 2020 | 2021 | 2022 | 2023 | Long-term average |
|---|---|---|---|---|---|
| Rainfall (mm month$^{-1}$) | | | | | |
| May | 19.1 | 13.3 | 53.4 | 12.7 | 35.1 |
| June | 67.2 | 43.3 | 60.9 | 60.9 | 94.5 |
| July | 89.8 | 23.5 | 18.6 | 3.5 | 161.4 |
| August | 53.9 | 40.5 | 31.4 | 1.7 | 203.1 |
| Cumulative | 230.0 | 120.6 | 164.3 | 40.8 | 494.1 |
| Maximum air temperature (°C) | | | | | |
| May | 24.8 | 16.6 | 13.8 | 23.2 | 14.8 |
| June | 26.8 | 20.2 | 21.0 | 22.9 | 14.0 |
| July | 29.2 | 22.2 | 23.9 | 23.4 | 15.9 |
| August | 29.2 | 21.3 | 25.5 | 22.5 | 14.6 |
| Minimum air temperature (°C) | | | | | |
| May | –2.3 | 3.1 | 2.6 | 6.4 | 10.3 |
| June | 2.6 | 7.5 | 8.9 | 9.0 | 9.4 |
| July | 7.0 | 9.2 | 10.3 | 10.7 | 11.1 |
| August | 0.3 | 7.7 | 10.8 | 9.7 | 13.7 |

Table 3. 2 A complete list of forage grass monocultures and mixtures grown at Fairview, Alberta, Canada.

| | |
|---|---|
| Monoculture grasses | GPWG |
| | Fleet MB |
| | SHBG |
| Simple and complex mixtures | (M0) MB (Fleet) + HB (AC Success) + HB (AC Knowles) + GPWG + CWG |
| | (M1) MB (Fleet) + AL(s5) |
| | (M5) HB (AC Success) + AL(s5) |
| | (M7) MB (Fleet) + AL(s5) |
| | (M10) HB (AC Success) + AL(y) + SF + CMV |
| | (M14) SF + CMV + AL(s5) |
| | (M18) HB (AC Success) + GPWG + AL(Y) + SF |
| | (M19) HB (AC Success) + GPWG + IR + AL (y) + SF |
| | (M20) HB (AC Success) + GPWG + AL(R) + AL(y) + CMV + SF |
| | (M21) HB (AC Success) + SF + AL(y) |
| | (M22) HB (AC Success) + GPWG + AL(y) + CMV + SF + BT |
| | (M23) HB (AC Success) + GPWG + CWG + IR + AL(y) + AL(R) + CMV + SF |

M, mixture; GPWG, greenleaf pubescent wheatgrass; HB, hybrid bromegrass; CWG, crested wheatgrass; SHBG, Success hybrid bromegrass; MB, meadow bromegrass; AL(s5), Spredor 5 alfalfa; AL(y), yellowhead alfalfa; AL(R), rugged alfalfa; SF, sainfoin; CMV, cicer milkvetch; BT, birdsfoot trefoil; IR, Italian ryegrass.

Table 3. 3 Baseline data of soil nitrogen, carbon, and physical properties measured prior to seeding in June 2020 at Fairview, Alberta, Canada.

| Soil depth | 0–7.5 cm | | 7.5–15 cm | | 0–15 cm | | 15–30 cm | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| N and C properties | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | | |
| Total $CO_2$ (mg $CO_2$ $g^{-1}$ soil) | 0.45 | 0.05 | 0.26 | 0.01 | 0.39 | 0.01 | 0.25 | 0.01 | | |
| C:N ratio | 11.2 | 0.66 | 10.4 | 0.20 | 10.0 | 0.09 | 8.7 | 0.54 | | |
| TC (g $kg^{-1}$) | 31.0 | 13.0 | 24.0 | 3.50 | 26.0 | 2.40 | 12.0 | 2.10 | | |
| TOC (g $kg^{-1}$) | 30.0 | 12.0 | 23.0 | 3.90 | 24.0 | 1.90 | 12.0 | 1.50 | | |
| TN (g $kg^{-1}$) | 3.0 | 1.10 | 2.0 | 0.30 | 3.0 | 1.10 | 1.0 | 0.30 | | |
| C storage (Mg C $ha^{-1}$) | | | | | 20.0 | - | 21.0 | - | | |
| N storage (Mg N $ha^{-1}$) | | | | | 4.90 | - | 2.2 | - | | |
| Physical properties | | | | | | | | | | |
| Soil depth | 0–7.5 cm | 7.5–15 cm | 0–15 cm | 15–30 cm | 0–15 cm | 15–30 cm | 0–30 cm | | 0–30 cm | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Compaction (kPa) | 217.2 | 110.2 | 333.7 | 141.9 | 374.8 | 142.4 | 0 | 0 | 395.5 | 65.7 |
| Bulk density (g $cm^{-3}$) | 1.43 | 0.06 | 1.69 | 0.03 | 1.09 | 0 | 1.3 | 0.04 | | |
| Soil wet aggregate (%) | 47.2 | 4.8 | 42.7 | 5.6 | 53.5 | 5.1 | 74.7 | 5.6 | | |
| Infiltration (cm $h^{-1}$) | | | 0.58 | | | | | | | |
| SEM | | | 0.08 | | | | | | | |

SEM, standard error of means; TOC, total organic C; TN, total N; TC, total C.

Table 3. 4 Carbon dioxide production, soil bulk density, and surface infiltration rates under perennial forage at the end of the study in June 2023 at Fairview, Alberta, Canada.

| Treatments | Carbon dioxide production 0–15 cm | 95% CI | 15–30 cm | 95% CI | Treatments | Bulk density ($g\ cm^{-3}$) 0–15cm | 15–30cm | Infiltration ($cm\ h^{-1}$) | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| | $mg\ CO_2\ g^{-1}$ soil | | | | | | | | |
| Fleet MB | 0.46 bcd | 0.44–0.48 | 0.83 a | 0.02–1.68 | Fleet MB | 1.2 a | 1.5 a | 0.51 ab | 0.47–0.55 |
| SHBG | 0.29 d | 0.27–0.31 | 0.62 ab | 0.16–1.08 | SHBG | 1.1 a | 1.3 a | 0.41 b | 0.31–0.36 |
| GPWG | 0.39 c | 0.36–0.42 | 0.46 b | 0.44–0.48 | GPWG | 1.2 a | 1.3 a | 0.33 ab | 0.31–0.35 |
| M1 | 0.51 abc | 0.21–0.81 | 0.34 c | 0.32–0.37 | M1 | 1.2 a | 1.3 a | 0.41 b | 0.39–0.44 |
| M5 | 0.54 abc | 0.50–0.58 | 0.28 c | 0.27–0.29 | M5 | 1.1 a | 1.4 a | 0.48 ab | 0.35–0.47 |
| M7 | 0.65 a | 0.63–0.68 | 0.36 c | 0.33–0.39 | M7 | 1.2 a | 1.2 a | 0.79 a | 0.75–0.83 |
| M0 | 0.66 a | 0.63–0.69 | 0.32 c | 0.29–0.35 | M0 | 1.2 a | 1.4 a | 0.41 b | 0.39–0.44 |
| M10 | 0.42 cd | 0.41–0.43 | 0.28 c | 0.27–0.29 | M10 | 1.1 a | 1.2 a | 0.51 ab | 0.47–0.55 |
| M14 | 0.56 abc | 0.54–0.58 | 0.30 c | 0.28–0.32 | M14 | 1.2 a | 1.3 a | 0.28 ab | 0.27–0.29 |
| M18 | 0.58 abc | 0.56–0.60 | 0.30 c | 0.28–0.32 | M18 | 1.1 a | 1.3 a | 0.33 b | 0.31–0.35 |
| M19 | 0.53 abc | 0.52–0.54 | 0.29 c | 0.27–0.31 | M19 | 1.3 a | 1.3 a | 0.51 ab | 0.21–0.81 |
| M20 | 0.43 bcd | 0.21–0.65 | 0.31 c | 0.29–0.34 | M20 | 1.1 a | 1.2 a | 0.53 ab | 0.52–0.54 |
| M21 | 0.60 b | 0.56–0.64 | 0.35 c | 0.32–0.38 | M21 | 1.2 a | 1.3 a | 0.38 b | 0.35–0.41 |
| M22 | 0.51 abc | 0.47–0.55 | 0.35 c | 0.32–0.38 | M22 | 1.2 a | 1.2 a | 0.36 b | 0.33–0.39 |
| M23 | 0.53 abc | 0.52–0.54 | 0.38 bc | 0.34–0.42 | M23 | 1.2 a | 1.1 a | 0.51 ab | 0.47–0.55 |
| *P*-value | 0.0386 | – | 0.0027 | – | | 0.77 | 0.443 | 0.024 | – |

95% CI, 95% confidence interval; Fleet MB, Fleet meadow bromegrass; SHBG, Success hybrid bromegrass; GPWG, greenleaf pubescent wheatgrass. M1 and M5 are simple grass–legume mixtures, M0 is a grass-only mixture, M14 is a legume-only mixture, and M7, M10, M18, M19, M20, M21, M22, and M23 are complex grass–legume mixtures. Values with the same letters within each column are not significantly different ($P > 0.05$). *P* values for treatments x depth effects for $CO_2$ production and BD are <0.001 and 0.015, respectively.

Table 3. 5 Soil compaction and volumetric moisture readings after harvest of the perennial forage in June 2023 at Fairview, Alberta, Canada.

| Treatments | Compaction (kPa) | Moisture (%) | Compaction (kPa) | Moisture (%) | Compaction (kPa) | Moisture (%) |
|---|---|---|---|---|---|---|
| Soil depth | 0–7.5 cm | | 0–15 cm | | 0–30 cm | |
| M1 | 246.1 c | 9.4 ab | 384.2 ab | 9.2 abc | 390.4 b | 6.7 d |
| M5 | 268.8 bc | 8.7 b | 388.4 ab | 7.1 d | 421.6 ab | 5.2 e |
| M7 | 220.5 d | 9.7 ab | 391.3 a | 11.1 a | 364.5 c | 8.7 c |
| M0 | 257.3 c | 10.1 a | 301.3 d | 10.6 a | 370.4 c | 6.8 d |
| M10 | 279.3 ab | 8.2 bc | 380.5 ab | 9.1 abc | 368.3 c | 10.1 a |
| M14 | 280.2 ab | 7.9 bc | 364.5 abc | 8.3 c | 300.5 d | 8.3 c |
| M18 | 213.7 de | 7.5 c | 277.2 de | 5.6 e | 263.4 e | 7.1 cd |
| M19 | 210.6 de | 10.4 a | 250.5 e | 9.7 ab | 268.9 e | 10.3 a |
| M20 | 200.2 de | 6.6 d | 256.1 e | 7.9 d | 277.8 e | 9.7 ab |
| M21 | 294.3 a | 8.6 b | 300.5 d | 9.9 ab | 455.0 a | 5.2 e |
| M22 | 196.4 e | 7.8 c | 264.3 de | 10.3 ab | 248.7 e | 8.8 c |
| M23 | 190.5 e | 8.2 bc | 248.8 e | 8.9 c | 243.6 e | 7.8 cd |
| Fleet MB | 266.1 bc | 10.6 a | 366.6 abc | 6.8 e | 225.8 f | 10.6 a |
| SHBG | 273.2 abc | 5.9 d | 394.8 a | 6.6 e | 346.2 c | 7.7 cd |
| GPWG | 255.7 c | 7.2 c | 381.4 ab | 8.9 c | 385.8 b | 5.6 e |
| *P*-value | 0.034 | 0.045 | 0.042 | 0.014 | 0.006 | 0.044 |

Fleet MB, Fleet meadow bromegrass; SHBG, Success hybrid bromegrass; GPWG, greenleaf pubescent wheatgrass. M1 and M5 are simple grass–legume mixtures, M0 is a grass-only mixture, M14 is a legume-only mixture, and M7, M10, M18, M19, M20, M21, M22, and M23 are complex grass–legume mixtures. Values with the same letters within a column are not significantly different ($P > 0.05$). The $P$ value for treatments x depths effects is $<0.0001$.

Table 3. 6 Ammonium nitrogen ($NH_4$–N), nitrate nitrogen ($NO_3$–N), total carbon (TC), total organic carbon (TOC), total nitrogen (TN), and C:N ratios of soils sampled at 0–15 cm and 15–30 cm at the end of the experiment in June 2023 at Fairview, Alberta, Canada.

| Treatments | Soil depth increment | | | |
|---|---|---|---|---|
| | 0–15 cm | | 15–30cm | |
| | $NH_4$–N | $NO_3$–N | $NH_4$–N | $NO_3$–N |
| | mg kg$^{-1}$ | | mg kg$^{-1}$ | |
| Fleet MB | 10.5 a | 5.4 c | 1.7 ab | 2.6 abc |
| SHBG | 6.5 ab | 15.2 a | 0.87 b | 4.8 ab |
| GPWG | 2.2 b | 7.7 bc | 1.7 ab | 5.4 a |
| M1 | 3.4 b | 5.6 c | 1.3 ab | 2.5 abc |
| M5 | 5.6 ab | 6.8 bc | 2.3 ab | 5.5 a |
| M7 | 4.9 ab | 7.9 bc | 1.5 ab | 2.8 abc |
| M0 | 3.1 b | 4.3 c | 1.1 b | 1.2 c |
| M10 | 4.2 b | 8.2 abc | 1.7 ab | 3.4 abc |
| M14 | 6.3 ab | 13.8 ab | 1.4 ab | 3.5 abc |
| M18 | 5.2 ab | 10.6 abc | 1.2 ab | 3.8 abc |
| M19 | 3.9 b | 6.3 c | 1.5 ab | 4.2 abc |
| M20 | 4.8 ab | 6.8 bc | 1.8 ab | 3.1 abc |
| M21 | 5.4 ab | 7.4 bc | 1.6 ab | 2.1 bc |
| M22 | 6.9 ab | 8.5 abc | 1.5 ab | 2.6 abc |
| M23 | 7.7 ab | 8.5 abc | 2.6 a | 3.4 abc |
| *P*-value | 0.05 | 0.02 | 0.701 | 0.358 |

| Treatments | Soil depth increment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0–15 cm | | | | 15–30 cm | | | |
| | TC | TOC | TN | C:N ratio | TC | TOC | TN | C:N ratio |
| | g kg$^{-1}$ | | | | | | | |
| Fleet MB | 23 a | 20 a | 2.0 ab | 10.0 ab | 18 ab | 14 abc | 3.0 ab | 4.7 e |
| SHBG | 23 a | 19 a | 2.0 ab | 9.5 b | 14 abc | 12 abc | 1.0 b | 12.0 b |
| GPWG | 19 a | 17 a | 1.7 b | 10.0 ab | 18 a | 15 ab | 2.0 a | 7.5 d |
| M1 | 22 a | 20 a | 2.0 ab | 10.0 ab | 13 abc | 11 bc | 1.0 b | 11.0 bc |
| M5 | 18 a | 15 a | 1.7b | 8.8 bc | 16 abc | 16 a | 1.0 ab | 16.0 a |
| M7 | 22 a | 20 a | 2.0 ab | 10.0 ab | 13 abc | 13 abc | 1.0 b | 13.0 ab |
| M0 | 24 a | 22 a | 2.0 ab | 11.0 a | 14 abc | 13 abc | 1.0 b | 13.0 ab |
| M10 | 19 a | 16 a | 2.0 ab | 8.0 c | 11 c | 10 c | 1.0 b | 10.0 c |
| M14 | 21 a | 20 a | 2.0 ab | 10.0 ab | 11 c | 11 bc | 1.0 b | 11.0 bc |
| M18 | 22 a | 21 a | 2.0 ab | 11.0 a | 12 c | 10 c | 1.0 b | 10.0 c |
| M19 | 21 a | 20 a | 3.0 a | 6.7 d | 12 abc | 11 abc | 1.0 b | 11.0 bc |
| M20 | 19 a | 16 a | 1.7 b | 9.4 b | 16 abc | 13 abc | 2.0 b | 6.5 d |
| M21 | 21 a | 20 a | 2.0 ab | 10.0 ab | 11 c | 10 c | 1.0 b | 10.0 c |
| M22 | 22 a | 21 a | 2.0 ab | 11.0 a | 14 abc | 12 abc | 1.0 b | 12.0 b |
| M23 | 21 a | 19 a | 2.0 ab | 9.5 b | 14 abc | 11 abc | 1.0 ab | 11.0 bc |
| | 0.75 | 0.89 | 0.91 | 0.041 | 0.0281 | 0.0383 | 0.062 | 0.022 |

Fleet MB, Fleet meadow bromegrass; SHBG, Success hybrid bromegrass; GPWG, greenleaf pubescent wheatgrass. M1 and M5 are simple grass–legume mixtures, M0 is a grass-only mixture, M14 is a legume-only mixture, and M7, M10, M18, M19, M20, M21, M22, and M23 are complex grass–legume mixtures. Values with same letters within a column are not significantly different ($P > 0.05$). The *P* values for treatments x depth effects of NH4–N, NO3–N, TC, TOC, and TN are 0.005, 0.001, 0.004, 0.014, <0.0001, respectively.

Table 3. 7 Cumulative and rate of soil C and N sequestration by perennial forage at two soil depths after the forage experiment in June 2023 at Fairview, Alberta, Canada.

| Treatments | Soil C sequestration 0–15 cm | 15–30 cm | Cumulative (0–30cm) | | Soil carbon sequestration rate (Mg C ha$^{-1}$ year$^{-1}$) | 95% CI | Treatments | Soil N sequestration 0–15 cm | 15–30 cm | Cumulative (0–30 cm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mg C ha$^{-1}$ | | | SEM | | | | Mg N ha$^{-1}$ | | | SEM |
| Fleet MB | 39.3a | 31.7ab | 71.0a | 5.01 | 10.0a | 9.1–10.9 | Fleet MB | 3.4b | 3.1ab | 6.4ab | 1.3 |
| SHBG | 38.6a | 23.2abcd | 61.8ab | 2.14 | 7.0d | 6.6–7.4 | SHBG | 3.4b | 1.9b | 5.4b | 1.9 |
| GPWG | 36.2a | 31.0abc | 67.2ab | 2.36 | 8.8b | 8.3–9.3 | GPWG | 3.1b | 3.4a | 6.5ab | 1.2 |
| M1 | 40.4a | 24.3abcd | 64.7ab | 1.22 | 7.9c | 7.3–8.5 | M1 | 3.7b | 1.9b | 5.6b | 1.1 |
| M5 | 32.6a | 34.6a | 67.2ab | 2.36 | 8.8b | 8.3–9.3 | M5 | 2.9b | 2.9ab | 5.8ab | 2.5 |
| M7 | 38.5a | 24.0 abcd | 62.5ab | 1.99 | 7.2d | 6.6–7.8 | M7 | 3.5b | 1.9b | 5.4b | 1.9 |
| M0 | 42.9a | 26.8abcd | 69.8ab | 2.00 | 9.6ab | 8.2–11.0 | M0 | 3.6b | 2.0ab | 5.6b | 1.1 |
| M10 | 33.4a | 19.1cd | 52.6b | 4.17 | 3.9g | 3.6–4.2 | M10 | 3.4b | 1.9b | 5.2b | 1.8 |
| M14 | 35.5a | 23.1abcd | 58.6ab | 3.38 | 5.9e | 5.4–6.4 | M14 | 3.4b | 2.0b | 5.5b | 1.1 |
| M18 | 35.0a | 20.9bcd | 55.9ab | 1.34 | 5.0f | 4.2–5.8 | M18 | 3.2b | 2.0b | 5.2b | 1.8 |
| M19 | 39.8a | 19.9bcd | 59.7ab | 1.70 | 6.3e | 5.7–6.9 | M19 | 6.1a | 1.8b | 7.9a | 3.6 |
| M20 | 36.7a | 23.7abcd | 60.5ab | 2.56 | 6.5e | 5.8–7.2 | M20 | 3.2b | 3.0ab | 6.1ab | 2.2 |
| M21 | 39.4a | 19.6bcd | 59.1ab | 1.68 | 6.1e | 5.5–6.8 | M21 | 3.7b | 2.0b | 5.6ab | 1.1 |
| M22 | 38.9a | 22.7abcd | 61.7ab | 1.50 | 6.9d | 6.6–7.2 | M22 | 3.6b | 1.7b | 5.3b | 1.7 |
| M23 | 39.6a | 17.5d | 57.1ab | 2.26 | 5.4f | 4.8–6.0 | M23 | 3.7b | 2.0ab | 5.7ab | 2.7 |
| *P*-value | 0.932 | 0.005 | 0.041 | – | 0.0012 | – | | 0.03 | 0.025 | 0.0423 | – |

95% CI, 95% confidence interval; SEM, standard error of the mean; FMB, Fleet meadow bromegrass; SHBG, Success hybrid bromegrass; GPWG, greenleaf pubescent wheatgrass. M1 and M5 are simple grass–legume mixtures, M0 is a grass-only mixture, M14 is a legume-only mixture, and M7, M10, M18, M19, M20, M21, M22, and M23 are complex grass–legume mixtures. Values with the same letters within a column are not significantly different ($P > 0.05$). Prior to seeding, the soil carbon sequestered at 0–15 cm was 20 Mg C ha$^{-1}$, at 15–30 cm was 21 Mg C ha$^{-1}$, whereas at 0–30 cm was 41 Mg C ha$^{-1}$. The rate of soil C sequestration was estimated by subtracting the baseline C from the final amount of C sequestration, divided by the two sample collection dates (26 June 2020 to 23 June 2023). Prior to seeding, the soil N storage at 0–15 cm was 4.9 Mg N ha$^{-1}$, at 15–30 cm was 2.2 Mg N ha$^{-1}$, and at 0–30 cm was 6.9 Mg N ha$^{-1}$. The $P$ values for treatments x depth effects for soil C and N sequestration were <0.0001 and 0.0012, respectively.

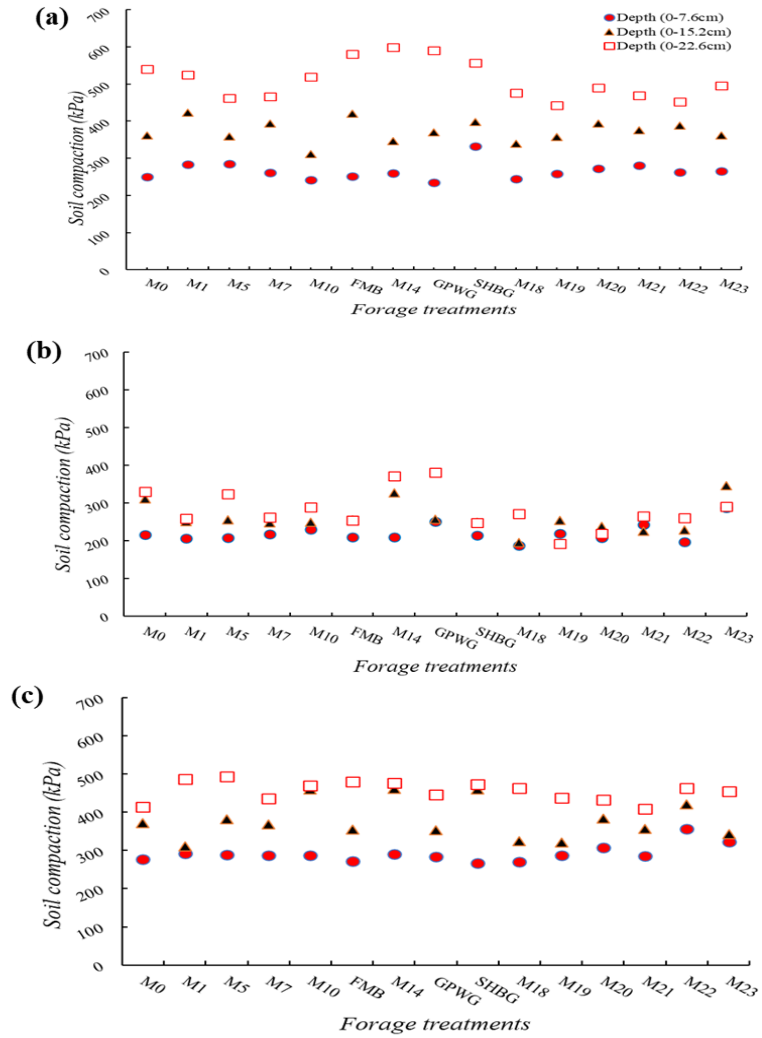


Figure 3. 1 Soil compaction measured over 5 weeks during the growing seasons of (a) 2021 during the first year of production, (b) 2022 during the second year of production, and (c) 2023 during the third year of production. M0 is grass-only mixture, M14 is legume-only mixture, M1 and M5 are simple grass-legume mixtures, M7, M10, M18, M19, M20, M21, M22, and M23 are complex grass-legume mixtures, FMB is Fleet meadow bromegrass, SHBG is Success hybrid bromegrass, GPWG is greenleaf pubescent wheatgrass.

## Chapter 4. Forage dry matter yield, nutritive value, and botanical composition of diverse perennial forage species mixtures

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## Study synopsis

This field study examined the dry matter yield, nutritive value, and botanical composition of perennial forage mixtures and monoculture grasses for three consecutive production years. Forage treatments consisting of six simple mixtures (i.e., one grass and one legume), 18 complex mixtures (i.e., three or more grasses and legumes), and five monoculture grasses as a control were evaluated. The forage dry matter (DM) yield of grass–legume mixtures was superior during the first (2.4 Mg $ha^{-1}$) and third (4.9 Mg $ha^{-1}$) production years (2021 and 2023), with monoculture meadow bromegrass (*Bromus comunatus*) producing higher DM yield (4.8 Mg $ha^{-1}$) in the second year of production (2022). Crude protein was higher and neutral detergent fiber (NDF) was lower in forage from grass–legume mixtures compared with monoculture grasses. Furthermore, acid detergent fiber (ADF) was lower in grass–legume mixtures during 2021, whereas monoculture grasses were lower in ADF during 2022 and 2023. The total digestible nutrients (TDN) were also highest in grass–legume mixtures in 2021, whereas monoculture grasses had the highest TDN in 2022 and 2023. The NDF digestibility over 48h of monoculture grasses was higher than that of grass–legume mixtures. Grass–legume mixtures demonstrated greater calcium and phosphorus content of 1.03% and 0.02%, respectively, over monoculture grasses, whereas potassium was higher in monoculture grasses (0.87% vs. 0.45% for legume–grass mixtures). The botanical composition of alfalfa (*Medicago sativa*) was dominant compared with sainfoin (*Onobrychis viciifolia*), cicer milkvetch (*Astragulus cicer*), and birdsfoot trefoil (*Lotus corniculatus*).

Keywords: grass–legume mixtures, dry matter yield, crude fiber, nutritive value, weed suppression, botanical composition

## 4.0 Introduction

In Canada, forage production uses more arable land than any other single crop species, accounting for about 21.2 million hectares, including both natural grasslands and cultivated forages (Bonnefield Research, 2016; Beef Cattle Research Council, 2023a). In addition, one-third of all agricultural lands is dedicated to beef production (Canadian Roundtable for Sustainable Beef, 2016). These lands consist of the native grassland, tame grassland with commercial grass–legume mixtures, and hay lands, comprising 12.9 Mha, 5 Mha, and 1.8 Mha, respectively (Canadian Roundtable for Sustainable Beef, 2016). Within Canada, Alberta holds approximately 43% of the forage production land and also leads the country in beef cattle herds (43.2%) (Statistics Canada, 2022). Forage crops, particularly perennials, are vital for sustaining winter feeding. For instance, many perennials are fed during the winter periods as bale grazing, stockpiling, or swath grazing when annuals are not available. This allows the beef producer to extend the grazing season and offset the costs associated with mechanical harvesting of biomass and managing the manure produced by confined animals (McGeough et al., 2017). In some cases, baled and stored hay may retain much of their nutrients better than those forage plants left standing on the field for grazing during the winter periods. According to the Beef Cattle Research Council (2023a), about 80% of a beef animal's diet during its lifetime depends on forage, entailing both annuals and perennials. However, annuals only complement perennials by producing forage for the beef cattle when the perennials are dormant or growing slowly (Ball et al., 2008).

In Western Canada, alfalfa (*Medicago sativa*) is the most successful predominant forage legume grown in mixtures with perennial grasses because of its adaptability to diverse environmental conditions, high yield, improved nutritive value, vigorous regrowth, and ability to fix atmospheric N (Bittman et al., 1991; Aasen and Bjorge, 2009). Although binary alfalfa–grass mixtures have been documented to significantly boost dry matter (DM) yield compared with either pure grasses or alfalfa monocultures (Bélanger et al., 2017; Foster et al., 2014), combinations of perennial grasses with other legumes such as sainfoin (*Onobrychis viciifolia*), cicer milkvetch (*Astragulus cicer*), and birdsfoot trefoil (*Lotus corniculatus*) in both simple and complex mixtures have not been fully documented. Hence, an exploration of their relative contributions within mixtures to yield, persistence and nutritive value will lead to a more comprehensive knowledge of forage production systems as well as their ability to serve as an alternative feed source for beef cattle operations.

Previous research has shown that seeding grasses in association with legumes provides a more sustainable source of forage yields than monoculture grasses (Cecen et al, 2005; Malhi et al., 2002; Bélanger et al., 2017). This is occasioned by the ability of legumes to provide N for use by grasses and the complementary relationships that exist within the agroecosystem (Muir et al., 2011; Whitbread et al., 2009; Temperton et al., 2007). In addition, the inclusion of both legumes and nonlegumes in forage cropping systems provides contrasting morphologies of canopies and root systems, which allow better utilization of spatial and nutrient niches (Helgadóttir et al., 2018; Khatiwada et al., 2020). This indicates that a plethora of different forage species can impact the sustainability of established perennial stands in different ways, as well as the productivity of herbage and other system benefits (Tilman et al., 1996). It is thus crucial to understand the agronomic characteristics, synergy, competition, and survival rate of the species combinations used in forage production systems (Sanderson et al., 2005). Moreover, in a forage mixture, the compatibility of forage species is important for better establishment, productivity, and persistence of the stand (Hutton et al., 2005; Aasen and Bjorge, 2009).

Perennial forage mixtures provide balanced diverse rations that can help to curtail the negative effects that arise from the one-way (one single ingredient) feeding of animals and enhance better animal productivity and performance. In terms of feed nutritional qualities, legume–grass forage mixtures can produce the right proportions of roughage in terms of crude protein (CP) and carbohydrates. Yüksel and Balabanlı (2021) reported that in general, alfalfa–grass mixtures have significantly better CP concentrations, CP yield, neutral detergent fiber (NDF), and acid detergent fiber (ADF). In further details, higher CP was recorded for both alfalfa + perennial ryegrass (*Lolium perenne*) and alfalfa + meadow fescue (*Festuca pratensis*) mixtures (14.93% and 14.80%, respectively). Furthermore, Schellenberg et al. (2012) studied seeded native cool-season mixtures and found higher CP concentrations in a 14-species mixture in comparison with a seven-species mixture, although ADF and NDF were similar between the two mixtures over the 3 years of the study. However, there is skepticism surrounding whether the number of species within forage mixtures alters the nutritive quality or if this is solely dependent on the traits of the individual forage species.

Consequently, a better understanding of the drivers of the yield, persistence, and quality of forage will contribute to the current knowledge base; at the same time, this new knowledge can be applied as a vital tool for the successful operation of the livestock sector (Judy et al., 2015). It is therefore important to provide adequate information to beef and dairy producers on how the biomass of perennial forage mixtures can potentially supply better nutrition to animals and deliver excellent performance. In this study, we hypothesized that (i) the DM yield and nutritive value of simple and complex grass–legume mixtures would be superior to those of monoculture grasses and grass-only mixtures; (ii) following the stand's establishment, the proportion of legumes within forage mixtures would diminish rapidly over time compared with grasses; and (iii) the reduction in invasive weed species would be dependent upon the complexity of the forage mixtures. The primary objective of our study was to examine the botanical composition, DM yield, and nutritive value across a broad range of perennial forage mixtures and grass monoculture options over three continuous years of production.

## 4.1 Materials and methods

### 4.1.1 Experimental site and weather

A perennial forage field experiment was established at the research farm of the Peace Country Beef and Forage Association in Fairview, northwestern Alberta, Canada (56°04′53″N, 118°26′05″W, 670 m above sea level) in June 2020 and was evaluated for three forage production years (2021, 2022, and 2023). The selected field had stubble from a previous canola (*Brassica napus*) crop. The site had a history of several years of wheat (*Triticum aestivum*)–canola rotations for grain production, with canola being the last crop before the experiment started. The soil classification was Eluviated Black Chernozem according to the Canadian System of Soil Classification (Government of Alberta, 2020). The weather information during the growing seasons and their long-term averages for the site (Table 4.1) were obtained from the Alberta Climate Information Service.

### 4.1.2 Experimental design and forage mixtures

The experimental design for the trial was a randomized complete block design with four replications ($n$ = 4). The treatments consisted of six simple grass–legume mixtures (one grass and one legume) and 18 complex diverse perennial forage species mixtures (i.e., three or more grasses and/or legumes) mixtures, including three grass-only mixtures, two legume-only mixtures and 13 grass–legume mixtures. In addition, there were five pure stands of perennial grasses which were used for comparisons. The grass species used in the mixtures and monocultures were wheatgrass (*Thinopyrum intermedium*; varieties: Kirk crested and greenleaf pubescent), orchardgrass (OG) (*Dactylis glomerata*), timothygrass (TG) (*Phleum pratense*), meadow bromegrasses (*Bromus commutatus*; variety: Fleet), and hybrid bromegrass [AC Knowles and AC Success, i.e., a cross between meadow bromegrass and smooth bromegrass (*Bromus inermis*)] (Table 4.2).

### 4.1.3 Establishment of perennial mixtures and grass monoculture stands

The seeding rates for the treatments were calculated from the seeding rates for monocultures of perennial forages in Alberta recommended by Hutton et al. (2005) (Appendix 1). Each experimental plot size was 2 m wide by 8 m in length. Adequate alley space (2–3 m) was left between block replicates. Before seeding, alfalfa seeds were inoculated with Nitragin Gold alfalfa inoculants from Northstar Seeds, Edmonton, Canada, at a rate of 1.5 g per 250 g of seeds, whereas birdsfoot trefoil, cicer milkvetch and sainfoin received no inoculants because of the lack of commercially available inoculants in the region. All grasses and legumes were seeded at a depth of 1.3 cm on 26 June 2020 with a six-row custom-made Fabro plot drill equipped with disc-type openers with a 23-cm row spacing. In the establishment year (2020) and the first production year (2021), the plots were sprayed with Basagran Forte at 4.05 mL $ha^{-1}$ for in-crop weed control. Additionally, dandelions (*Taraxacum officinale*), volunteer wheat, and canola were occasionally handpicked out of the plots as an additional weed control measure. Subsequently, during the production years of 2022 and 2023, no further weed control measures (chemical spraying or manual) were carried out.

#### 4.1.4 Normalized difference vegetation index measurements

A Green Seeker handheld crop sensor (Trimble Agriculture, Westminster, CO, USA) was used to measure the weekly forage plant coverage and greenness of a targeted area within the inner four rows of the plots to avoid border effects (from early spring to harvest in 2021–2023). The crop sensor works with infrared lights and measures the amount of each type of light that is reflected back from the plant. The sensor displays the measured value in terms of a normalized difference vegetation index (NDVI) reading ranging from 0.00 to 0.99 on its liquid crystal display screen. The higher the reading (i.e., closer to 0.99), the healthier the plant and vice versa. To use the equipment, the sensor was set to zero and subsequently passed horizontally above the experimental plot at a consistent height of 31 cm above the ground over the end of the targeted area and back to the starting point. The final value was recorded when movement stopped at the point where the reading started. Two readings were taken and averaged per plot.

#### 4.1.5 Botanical composition estimation

Shortly prior to harvest time on 2 July 2021, 29 June 2022, and 21 June 2023, forage plants were sampled from a defined area (excluding the two outside rows) in the experimental plots for estimating the botanical composition. A quadrat of 0.5 m by 0.5 m was randomly placed on each of the plots, and forage plants within this area were clipped to about 7.5 cm above the ground. Grasses, legumes, and weeds (including forbs and sedges) were subsequently hand-separated, weighed fresh, oven-dried at 105°C until completely dried and reweighed to determine the botanical composition on a dry weight basis as posited by Mercier et al. (2020) as follows:

$$\frac{\text{Individual component DM (g)}}{\text{total sample DM (g)}} \text{ x } 100 \qquad (1)$$

#### 4.1.6 Forage harvest and quality analysis

On 3 July 2021, 30 June 2022, and 22 June 2023, an area of 3 m encompassing the four inner rows of the plots was harvested at the stage of 10–15% of the alfalfa legume blossom (Bonin and Tomlin, 1968). The aboveground biomass was harvested at a stubble height of 7.5 cm using a custom-made self-propelled small-plot forage harvester (Swift Machine and Welding Ltd., Swift Current-Saskatchewan, Canada). The harvested biomass was weighed fresh, and subsamples (up to 1000 g) were air-dried to determine the forage's DM content, which was used to calculate the forage yield on a DM basis (Mg $ha^{-1}$). The dried forage subsamples were analyzed in a commercial laboratory (A&L Canada Laboratory, London, Ontario, Canada) for feed quality parameters, including CP, acid detergent fiber (ADF) and neutral detergent fiber (NDF) content. The total N concentration was determined using the micro-Kjeldahl method (AOAC, 1984), and the total N was multiplied by 6.25 to determine the CP content (Schroeder, 1994). The NDF and ADF were determined using an ANKOM2000 fiber analyzer (Model 2000, ANKOM, Fairport, NY, USA). Total digestible nutrient (TDN) levels were calculated via equations provided by Weiss and Hall (2020), whereas forage minerals (calcium, phosphorus, potassium, and magnesium) were determined by near-infrared spectroscopy (Amari & Abe, 1997). The in vitro neutral detergent fiber digestibility (NDFD) was determined by using a 48-h incubation in buffered rumen fluid (Goering and Van Soest, 1970).

### 4.1.7 Statistical analysis

Research data were subjected to analysis of variance (ANOVA) in a randomized complete block design using the NLME package with mixed effect model (Pinheiro et al., 2020) in the R statistical program (Version 4.0) (R Core Team 2020). In the model, forage treatments x year interaction were considered as fixed effects while block replications were set as random effects. Due to the significant forage treatments x year effects, year was treated as fixed effect to understand the year-to-year trend of forage dry matter yield. Botanical composition and forage nutritive value data were analyzed using the mixed effect model. In botanical composition, forage treatments were treated as fixed effects when block replication were set as random effects. In nutritive value analysis, forage treatments x year interaction was fixed effects. The significant forage treatments x year interaction for nutritive values (CP, NDF, ADF, TDN, NDFD-48h) was treated as fixed effect to know the year-to-year trends of forage nutritive values. Before the ANOVA, the normality of the residuals was tested using the Shapiro–Wilk statistic, and the homogeneity of variance was tested using Levene's test. No data transformations were required, as the residuals of all data were homogeneous and normally distributed. Following the ANOVA, Tukey's honestly significant difference was used to separate the treatment means when significant.

## 4.2 Results

### 4.2.1 Dry matter yield

Based on the significance of the forage treatments x year interactions ($P = 0.014$), results were presented on a year-to-year basis to understand the trend of forage DM yield. The forage DM yield varied significantly among all treatments in the first (2021, $p < 0.0001$), second (2022, $p < 0.0001$), and third (2023, $p < 0.0001$) production years. In 2021, the forage DM yield generally varied from 0.77 to 2.40 Mg ha$^{-1}$ (Figure 4.1), with two of the complex mixtures that had legumes (M14 and M17) recording the highest forage DM yield of 2.40 Mg ha$^{-1}$ each. The three grass-only mixtures [i.e., Mixture (M) M0, M13, and M15] yielded between 1.22 and 1.95 Mg ha$^{-1}$, whereas for the five monoculture grasses, forage DM yield varied from 0.77 Mg ha$^{-1}$ for Fleet meadow bromegrass (FMB) to 1.27 Mg ha$^{-1}$ for TG. In general, except for TG and M8, all mixtures outyielded the monoculture grass stands.

In 2022, forage DM yield ranged from 1.62 to 4.77 Mg ha$^{-1}$ ($p < 0.0001$, Figure 4.1). FMB had the highest yield of 4.77 Mg ha$^{-1}$, followed by M4 with 4.07 Mg ha$^{-1}$, whereas two of the grass-only mixtures (M13 and M15) had a significantly lower forage DM yield (1.85–1.62 Mg ha$^{-1}$). Generally, three-way mixtures (M14 and M21) had higher DM yields than mixtures with four, five, six, and eight species in complex stands (Figure 4.1).

In 2023, M6 had the highest DM yield of 4.93 Mg ha$^{-1}$, whereas TG significantly produced lower yield (0.53 Mg ha$^{-1}$) than most treatments (Figure 4.1). Overall, simple mixtures, particularly M6 (4.93 Mg ha$^{-1}$), M4 (4.3 Mg ha$^{-1}$), and M2 (4.08 Mg ha$^{-1}$), and complex mixtures such as M14 (4.7 Mg ha$^{-1}$), M21 (4.2 Mg ha$^{-1}$), and M23 (4.4 Mg ha$^{-1}$) had greater yields ($P < 0.0001$). In general, the DM yield for all grass–legume mixtures increased, whereas there was a decrease in monoculture grasses and grass-only mixtures. For instance, the DM yield for M0 decreased from 3.07 to 1.95 Mg ha$^{-1}$, whereas that of FMB decreased from 4.77 to 1.88 Mg ha$^{-1}$.

Cumulatively, over the three production years, M14 (10.9 Mg ha$^{-1}$), M4 (10.4 Mg ha$^{-1}$), M21, and M23 (10.2 Mg ha$^{-1}$ each) had the highest DM yield ($P < 0.0001$). Overall, monoculture grasses or grass-only mixtures had the lowest cumulative DM yield, ranging from 4.1 Mg ha$^{-1}$ for TG to 7.4 Mg ha$^{-1}$ for FMB (Appendix 3g).

### 4.2.2 Nutritive value of forage

The forage quality indicators reported here, particularly CP, NDF, ADF, TDN, and 48-h NDFD differed significantly ($P < 0.05$) among the forage treatments. Additionally, the forage treatments x year interactions for nutritive values was significant, hence treated as fixed effects to comprehend how they varied during each forage production year (Figures 4.2– 4.6). In every forage production year, the forage calcium was significantly impacted, but not phosphorus or potassium.

### 4.2.3 Crude protein

In 2021, all forage treatments had CP contents above 10%, varying from 10.4% for greenleaf pubescent wheatgrass (GPWG) to 15.2% for M21. Higher and similar CP contents (14.5–15.2, Figure 4.2) were observed for M12 (33.3% legumes), M16 (100% legumes), M21 (70% legumes), and M22 (90% legumes) ($P = 0.0004$). Monoculture TG, FMB, and Kirk crested wheatgrass (KCG) had ~1.7% higher CP content than all grass-only mixtures. Simple mixtures had higher CP contents than all the monoculture grasses (except TG) (Figure 4.2). Furthermore, grass–legume mixtures consisting of five or six species (M19 and M20) had lower CP contents (a net difference of 1.2%) than all simple grass–legume mixtures and complex eight-species stands.

In 2022, the CP content in forage samples varied from 10.7% to 17.0% ($P < 0.0001$). The treatments M1 (50% legumes), M12 (33.3% legumes), M14 (100% legumes), and M22 (90% legumes) had the highest CP contents (17.02% each). Except for M0 (12.30%), all grass-only mixtures had the same CP content as monoculture grasses (Figure 4.2). Complex grass–legume mixtures (four, five, six, and eight species) had 2.3% CP content more than monoculture grass stands.

The CP content in the forages ranged between 18% for M3 to 11.7% for M0 in the third year of forage production (2023) ($P < 0.0001$). There was a substantial increase in the CP content for all complex grass–legume mixtures and monoculture grasses. Alternatively, grass-only mixtures and some simple mixtures decreased in CP, except for M3 and M6 (Figure 4.2).

### 4.2.4 Neutral detergent fiber and acid detergent fiber

Significant differences in NDF and ADF were observed across the forage treatments in 2021 ($P < 0.0001$ and $P = 0.0266$, respectively) (Figures 4.3 and 4.4). The forage treatments M16, M21, and M22 had the lowest NDF of 45.7%, 46.6%, and 46.7%, respectively, whereas M12, M21, and M22 had the lowest ADF of 33.9%. These results indicate that in terms of NDF and ADF, the abovementioned forage treatments had the lowest fiber contents and hence were the best treatments during the year. Overall, the NDF for all forage treatments ranged from 45.7% to 58.9%, whereas ADF ranged from 33.9% to 43.7%. Notably, all grass-only mixtures had higher ADF than simple and complex grass–legume mixtures, but this was not the case for monoculture grasses. Additionally, all grass-only mixtures (M0, M13, and M15) had higher NDF contents (54.0%, 59.2%, and 55.5%, respectively) than the simple and complex grass–legume mixtures as well as TG and KCG monoculture grasses (Figure 4.3). This indicates that forage treatments consisting of only grass components were higher in fiber, which could affect forage intake and utilization.

In 2022, a significant difference was observed in both forage NDF ($P < 0.0001$) and ADF ($P = 0.0001$). Across all treatments, NDF ranged from 44.2% to 57.6%, whereas ADF changed from 34.2% to 37.1%. M14 and M16 had the lowest NDF (44.2% and 44.5%, respectively), whereas M13 had the lowest for ADF (34.2%) compared with all the other forage treatments. This makes M13, M14, and M16 the treatments with the lowest NDF and ADF and hence, the best performers in terms of the crude fiber content.

In 2023, the NDF and ADF of the forage varied significantly across treatments ($P < 0.0001$ and $P = 0.0162$, respectively). M3 had the lowest NDF of 45.6%, whereas KCG recorded the lowest ADF of 31.3% (Figures 4.3 and 4.4). Apart from M3, the NDF in all simple and complex grass–legume mixtures was higher. However, within the monoculture grasses, there was an increase in NDF for GPWG (60%), KCG (53.1%), and TG (54.9%), which is likely to affect forage intake.

### 4.2.5 Mineral concentrations

In 2021, the results for the mineral concentrations in the forage samples showed that only Ca was significantly different ($P < 0.0001$) across forage treatments, whereas both P and K were not significantly different ($P = 0.935$ and $P = 0.2579$, respectively). The Ca content ranged from 0.57% to 1.13%, with M22 recording the highest and OG the lowest. In general, the best performing treatments were legume-dominated, whereas the poorest treatments were either monoculture grasses or grass-only mixtures (Table 4.3). Even though there was no statistical difference in P and K contents across the forage treatments, P content ranged between 0.14% and 0.19%, whereas K ranged widely between 0.42% and 1.93%.

In 2022, the content of Ca, P, and K were significantly different across forage treatments ($P < 0.0001$, $P = 0.001$, and $P = 0.0004$, respectively). If we compare the highest-producing mixtures in 2021 versus 2022, the content of Ca in the forage samples increased from 1.13% to 1.66% (a net difference of 0.53%) (Table 4.3). M14 was the best performer (1.66%), whereas M0 recorded the lowest content of 0.90%. Furthermore, there was an overall increase in the amount of Ca in the forage samples in 2022. Complex legume-dominated mixtures such as M14, M16, and M22 were better performers than either simple mixtures, grass-only mixtures, or monoculture grasses. In addition, the content of P in the forage samples ranged from 0.22% to 0.27%. M1 recorded the highest content of 0.27%, and FMB had the lowest (0.22%). Apart from M0, the P content followed the same trend as Ca, where legume-dominated mixtures were better than their grass counterparts (Table 4.3). However, the P content in the forage samples increased in 2022. On the other hand, the K content in the forage samples ranged from 1.48% to 1.76%. M0 had the highest K content of 1.76%, and M22 had the lowest (1.48%). Here, it appeared that monoculture grasses or grass-only mixtures had a higher K content than most simple and complex grass–legume treatments. Nevertheless, the K content decreased during 2022 compared with 2021.

In 2023, the Ca and P content were significantly different among the forage treatments ($P < 0.0001$), whereas K was not significantly different ($P = 0.705$). Ca content ranged between 0.73% for FMB and 1.78% for M14, whereas P varied from 1.69% for M8 to 2.73% for OG. In addition, P content was generally 0.23–0.27%. In comparison with 2022, there was a decrease in Ca for both monoculture grasses and grass-only mixtures. For instance, M15 decreased by 0.18%, whereas FMB also declined by 0.70%. Alternatively, the Ca content increased in all complex grass–legume and simple mixtures during the year (Table 4.3). The P content in the forages was similar to that in 2022, with the exception of a few monoculture grasses, which had a slight increase. Notable among these were GPWG (0.27%) and FMB (0.26%). Furthermore, except for

KCG (1.86%), all monoculture grasses were higher in K than all the other treatments tested in the study. For example, OG, which had the highest K content, had approximately 0.59% and 0.78% more than the simple and complex grass–legume mixtures with the highest K content (M4 and M11, respectively). Overall, an increasing trend was observed in Ca and K concentrations over the 3 years, whereas P only increased in the second year of production and remained constant in the third year of production (Table 4.3).

**4.2.6 Total digestible nutrients**

The TDN of the forage treatments varied between 53.9% and 64.9% in 2021 ($P < 0.0001$), 61.3% and 67.6% in 2022 ($P < 0.0001$), and 61.2% and 68.1% in 2023 ($P < 0.0001$). In the first year (2021), monoculture grasses recorded TDN of 55.5–57.3%, all grass-only mixtures recorded 58.7–63.0%, simple mixtures had 59.4–63.0%, and complex grass–legume mixtures had 58.3–64.9% (Figure 4.5). The results showed that forage from complex grass–legume mixtures had higher energy availability than monoculture grasses and grass-only or simple grass–legume mixtures. These results also demonstrated that six-species mixtures including three grasses and three legumes had higher TDN (64.1%) than those with two grasses and four legumes (59.1%), as evident in M20 and M22, and was superior to an eight-species mixture of four grasses and four legumes (62.8%) (Figure 4.5). Compared with 2021, the overall TDN values of the forage samples increased to 61.3–67.6%, with the exception of M21 and M22 in 2022. Apart from TG and OG, all grass-only mixtures (i.e., M0, M13, and M15) recorded better TDN (1.3% more) than all monoculture grasses (Figure 4.5). Highly diversified grass–legume mixtures such as M23 (eight species) also produced lower TDN than moderately diversified (four-, five-, and six-species) mixtures. In 2023, TDN varied between 61.2% for M6 to 68.1% for KCG. Generally, there was a decline in TDN for all forage treatments compared with 2022. However, monoculture grasses and grass-only mixtures had a higher TDN than both simple and complex mixtures (Figure 4.5 In addition, complex grass–legume mixtures, particularly M12 (64.7%), M17 (64.6%), and M18 (64.1%), had higher TDN than all simple mixtures tested in the study.

### 4.2.7 Neutral detergent fiber digestibility

In 2021, 48-h NDFD was significantly different among the treatments ($P$ = 0.0001), revealing the effects of different forage combinations on NDFD. Likewise, NDFD differed significantly across the treatments in 2022 and 2023 as well ($P$ < 0.0001). The results from 2021 showed that the NDFD values for GPWG and all simple mixtures except M1 were similar (averaging 49.9%). However, the NDFD for grass-only mixtures was better than that of all monoculture grasses and grass–legume mixtures (Figure 4.6). Among the grass treatments, the highest NDFD was recorded in M0 (57.0%), whereas the lowest was observed in monoculture OG (46.5%). Furthermore, it was evident that the eight-species mixture had lower NDFD than either the five- or six-species treatments. It also appeared that forage treatments with more grasses had higher NDFD than mixtures that were heavily dominated by legumes. During 2022, NDFD increased substantially between 5.1% and 16.1% among all the forage treatments (Figure 4.6). Grass monocultures and all grass-only mixtures recorded the highest NDFD (61.5% on average) compared with the grass–legume mixtures. Interestingly, simple mixtures such as M4 and M5 as well as the eight-species mixture (M23) produced similar NDFD values. Similarly, in 2023, monoculture grasses such as FMB and KCG (65.7% each), GWPG (66.5%), and grass-only mixtures, particularly M0 (63.4%), M13 (62.5%), and M15 (61.3%), had the highest NDFD. In addition, highly diverse treatments such as M23 (54.4%) and M18 (54.3%) had higher NDFD than all simple mixtures (Figure 4.6).

### 4.2.8 Normalized difference vegetation index

The results for NDVI taken during each production year showed that the growth patterns of forage treatments were significantly different ($P$ < 0.05) (Figure 4.7). In 2021, the vegetation growth patterns of all treatments peaked in the third week of vegetation greenness in the spring and remained so until harvest time. Generally, monoculture grasses and grass-only mixtures had poorer canopy cover, whereas grass–legume mixtures showed greater vegetation cover. Treatments with high proportions of legumes had greater canopy cover (0.51–0.61) compared with those with few legumes. For instance, M14 (100% legumes), M16 (100% legumes), M22 (90% legumes), and M21 (70% legumes) were the top performers over the 5-week period.

In 2022, the NDVI for all treatments in the spring was above 0.40. The vegetation cover for treatments substantially increased until harvest of the forage. Generally, the lowest vegetation cover during the spring was observed in all monoculture grasses and grass-only mixtures, similar to the results in 2021. However, by the end of Week 5, monocultures of FMB and GPWG had the highest vegetation cover (0.50) among all the monoculture grasses (Figure 4.7). Legume-dominated complex mixtures and simple mixtures had greater vegetation cover from the third week (above 0.50) until Week 5. For instance, the vegetation cover increased in M14 (100% legumes), M22 (90% legumes), M6 (50%), M4 (50% legumes), and M21 (70% legumes).

In 2023, the greenness of monoculture grasses and grass-only mixtures was poor from the start of the season (0.35–0.50) and slowly increased to 0.65 in Week 5 for M15. By contrast, grass–legume mixtures had high vegetation cover in the spring, ranging from 0.50 for M9 to 0.64 for M14. This steadily increased throughout the weeks leading up to harvest of the forage. By Week 5, the NDVI for grass–legume mixtures ranged from 0.70 for M9 to 0.80 for M6 and M14. Across all the three production years, M14 was the standout treatment that showed the greatest vegetation cover from early spring to harvest. Overall, the NDVI of all treatments in 2021 was lower than in 2022 and 2023 (Figure 4.7).

#### 4.2.9 Botanical composition of forage combinations

The botanical composition of species based on their proportion of DM during the first production year (2021) demonstrated that legumes, namely Spredor 5 and AC yellowhead alfalfa, were greater contributors to biomass than their grass counterparts. In simple mixtures, the alfalfa cultivars recorded more than 50% of the total DM weight (Appendix 3h), with the highest proportion of alfalfa recorded in M3 and M6 (73.6% and 75%, respectively). The dry biomass of the weed population ranged between 15.3% and 20.3%. It was noted that stands of Spredor 5 alfalfa with either AC Success or AC Knowles hybrid bromegrasses had a lower proportion of weed dry matter (10.6% and 10.1%, respectively) (Appendix 3h). Among the grass species combined with alfalfa cultivars in simple mixtures, AC Success hybrid bromegrass was observed to compete better in mixtures compared with either FMB or AC Knowles hybrid bromegrass. In the second production year (2022), the proportion of DM from alfalfa in the mixed stands decreased drastically by approximately 45%, whereas grasses and weeds increased by about 20% and 40%, respectively. Grass species recorded 30.4–44.0% of DM, and weeds had 32.2–39.9%. Increased growth of grasses and weeds was evident during 2022, and this was reflected in the DM results (Appendix 3i). The DM of grasses, legumes, and weeds in the third year of production (2023) followed similar trends to 2022. The proportions of grasses and weeds were higher than those of legumes. For instance, weeds also dominated in 2023 with an increase of 2–5% (Figure 4.8). Evidently, the proportions of weeds were lower in mixtures with Spredor 5 alfalfa (M3 and M4) compared with those of AC yellowhead alfalfa (M1 and M2).

Similar to the simple mixtures, the composition of species within the three-species mixtures revealed that alfalfa cultivars dominated the stands in terms of dry biomass in 2021. The results for alfalfa ranged between 27.1% and 61.7 %, followed by sainfoin (16.0 to 31.9%) and cicer milkvetch (5.2%) (Appendix 3h). For grasses, the AC Success hybrid bromegrass in M8 recorded the highest contribution (29.8%) across all the three-species mixtures (Appendix 3h). Furthermore, the proportions of weed biomass ranged from 6.1% in M14 to 38.6% in M12. This demonstrated that a lower proportion of weeds was witnessed in three-species mixtures compared with simple mixtures. In 2022, the DM of alfalfa species within three-species stands decreased to 16.3–25.8%. A decrease was also recorded for sainfoin in certain stands. For instance, sainfoin decreased by 9.7% in M17 and by 3.5% in M21 (Appendix 3i). Conversely, there was an increase in sainfoin's contribution in some stands. For example, sainfoin increased by 13.2% in M7 and by 4.8% in M14. Grasses in these three-way mixtures, particularly AC Success hybrid bromegrass and FMB, also increased in M12, M17, and M21 (4.3–10.2%). Fewer weed were observed in stands with a single legume seeded with two grasses compared with two species of legumes seeded with one grass ( Appendix 3i). In 2023, the proportions of alfalfa, sainfoin (3–10%), and cicer milkvetch (4.4%) increased slightly compared with 2022. Grasses generally increased but the proportions of weeds were lower (5.3–28.5%). Surprisingly, one grass–two legume stands were better at suppressing weeds (Figure 4.8).

In 2021, the DM composition of FMB and GPWG in all grass-only mixtures with four or more species was the lowest (11.8% each) in M0 (Appendix 3h). In the second year of forage production, the proportion of both grasses increased significantly in the grass-only mixtures by approximately 2.7% and 19.4%, respectively. The proportion of weeds in M15 (3.0%) and M13 (18.4%) was lower during the first year of production compared with the second year (21.9% and 26.6%, respectively). The proportion of DM from grasses and legumes in mixtures with four or more species demonstrated the clearly superior contribution of alfalfa cultivars. The other legumes (i.e., sainfoin, birdsfoot trefoil, and cicer milkvetch) were proportionally low (Appendix 3i). For instance, within M22, birdsfoot trefoil was absent (0%), whereas cicer milkvetch recorded only 2.3% in M23. On average, AC Success hybrid bromegrass recorded more DM compared with the other grasses present in these mixtures. Mixtures with three grass species seeded with two legumes showed better competitiveness compared with those seeded with more than two legumes. The DM of weeds was also lower in mixtures with a higher number of grass and legume species. This effect was evident in M23, which was composed of eight species (11.2%), compared with M22 (19.2%), which was a six-species mixture. However, about 19.2 % of weeds were suppressed in 2022. In 2023, the DM proportion of alfalfa generally decreased for AC yellowhead but increased for rugged alfalfa. There was an improvement in the proportions of sainfoin in M9, M16, M19, M20, and M22 (Figure 4.8). Cicer milkvetch was lower (3.1–18.6%), whereas birdsfoot trefoil also decreased by 11.3% in M22. Among the grasses, AC Knowles hybrid bromegrass decreased in M0 (9.2%), whereas AC Success hybrid bromegrass increased in M0 (24.5%), M10 (23.3%), M20 (16.8%), and M22 (10.5%). The DM of weeds was generally higher than in 2022. However, M20 (17.3%) and M22 (17%), which are six-species mixtures, were best in terms of weed suppression (Figure 4.8). Approximately 13.3% of weeds were suppressed by complex mixtures during the growing season of 2023 relative to 2022.

## 4.3 Discussion

### 4.3.1 Productivity of grass–legume versus grass-only mixtures or monoculture grasses

Comparatively, grass–legume mixtures had greater yields than monoculture grasses and grass-only mixtures in the dry growing season of 2021. This is attributed to the presence of legumes within the mixtures and their supportive functions. For instance, alfalfa, which has extending taproots, can extract soil moisture from deeper depths, enabling it to grow well under harsh weather conditions. The ability of legumes to fix atmospheric N and transfer this to grasses suggests that grasses can still be productive in harsh periods. In contrast, monoculture grasses did not obtain this support, resulting in lower DM yields. In addition, the growth and expansion of legumes' structures such as leaves can provide soil cover. This reduces evaporation from the soil's surface, allowing neighboring grasses to make use of the limited water resources in the soil. This was observed in the vegetation cover of highly legume-dominated mixtures (Figure 4.7), where a high NDVI was reported. Our findings are supported by those of Malézieux et al. (2009), Roscher et al. (2016), and Helgadóttir et al. (2018), who found that complementary effects in grass–legume mixtures resulted in high DM yields. Weggler and Elsäßer (2023) also reported that grass–legume mixtures had greater biomass yields in a drought-affected year. Although complexity of mixtures may considerably influence the DM yield (Tillman et al., 2001; Picasso et al., 2011; Mischkolz et al., 2016; Jing et al., 2017), our present study revealed that this phenomenon (i.e., higher productivity with increasing plant diversity) is not always true. This is because we observed three-way mixtures outyielding a six-species stand (M20 and M22) and an eight-species stand (M23). This finding partly attributed to the low botanical composition of individual species within the mixtures. For instance, in M22, birdsfoot trefoil was completely absent in 2021, whereas the proportion of cicer milkvetch was very low. This indicates that when plant species within mixtures fail during establishment, it considerably affects the biomass yield regardless of the number of species present in the mixture. Nelson and Moser (1994) also posited that the intrinsic characteristics of individual plant species are crucial to the overall productivity of forage.

Generally, forage DM yield increased across treatments in 2022, with grass–legume mixtures having consistently greater yields. However, FMB had the highest DM yield. This is attributed to the stout rhizomatous growth habit of meadow bromegrasses and their capacity to recover quickly during moist periods to produce a higher DM yield (Agriculture Canada, 1993). We also speculate that the existence of less competition among cultivars of the same species for bioavailable nutrients in the soil may have further influenced these results, consistent with Dyer and Rice (1999), who stated that intraspecific competition could significantly influence plant growth in the absence of interspecific competition. The increase in all forage yield is probably linked to the adequate amount of precipitation that was recorded during the growing season of 2022 (73.6 mm). This is consistent with Serajchi et al. (2017), who reported forage yield advantages in wet periods of their study in Swift Current, Saskatchewan, where moisture limitations drove primary productivity. In addition, our findings can be attributed to the well-established rooting systems of forage plants, which enabled them to take up resources for better formation and expansion of the canopy. Bolinder et al. (2002) found that perennial forage plants develop better root distribution and biomass during the second year of production.

Grass–legume mixtures were still superior in DM yields in 2023. However, monoculture grasses and grass-only mixtures decreased substantially. This can be explained by a probable decline in nitrogen in soils under grasses resulting in a lower DM yield. Our present study also demonstrated that there could be deviations in DM yield between grass–legume mixtures and monoculture grasses from one year to the other, depending on the environmental conditions. However, cumulatively, grass–legume mixtures had superior DM yields.

### 4.3.2 Legume-dominated mixtures have better nutritive value

Our overall finding of the higher nutritional qualities of grass–legume mixtures over monoculture grasses and grass-only mixtures reinforces the importance of including legumes in our forage production systems. CP content was high in treatments dominated by legume species and increased steadily across the forage production years. These were usually treatments with high proportions of legumes. For instance, across the production years, M1 and M3 (50% legumes), M14 (100% legumes), M16 (100% legumes), M22 (90% legumes), and M21 (70% legumes) had a better CP content. This is because legumes are self-reliant for acquiring nitrogen, which is an important component of protein. Our results are consistent with Sheaffer et al. (1990) and Zemenchik et al. (2002), who reported that variations in CP were strongly related to the proportions of legumes within grass–legume mixtures. Furthermore, the increase in CP content as the study progressed can be attributed to the botanical composition of the stands. In 2021, legumes such as birdsfoot trefoil and cicer milkvetch were poorly established, whereas in both 2022 and 2023, their contributions to the forage community were substantial. This indicates that CP content is not always dependent on the diversity of forage stand but instead on the functional traits and roles of the individual forage species and their collective contributions to the stands (Spehn et al., 2002). We also speculate that N uptake and utilization by forage plants was limited in the drier growing season of 2021, which resulted in several morphological and biochemical alterations within plants. During dry periods, the propensity of plant roots to take up water and nutrients generally declines, resulting in a reduction in key plant processes, including protein synthesis and photosynthesis (Alam, 1994). Conclusively, the CP contents in all forage treatments over the three years were adequate or even above adequate to meet the protein needs of cattle at different development stages (i.e., gestation, lactation, and calving). For instance, the model of the National Research Council

(2000) predicts a requirement of 7–8% CP in the diet for dry cows in early gestation for maintenance, which increases to about 11–13% in young growing and lactating cows.

The forage's fiber contents, defined by NDF and ADF, showed that legume-dominated mixtures had the overall advantage of a lower fiber content. This was evident across the three forage production years, where treatments with high proportions of legumes such as M14 (100% legumes), M21 (70%), and M22 (90% legumes) had lower NDF and ADF. This is because legumes have a lower hemicellulose content (Crowder, 1985), whereas grasses have hollow stems, fibrous tissues, and a degree of lignification of the cell wall (Arzani et al., 2003). This indicates that ADF content is higher in grasses than in legumes, consistent with Tuna et al. (2004), who reported high ADF contents in grasses. In parallel, Sleugh et al. (2000) mentioned that NDF content decreased by 30% in a Kura clover (*Trifolium ambiguum* Bieb.)–intermediate wheatgrass mixture. A general decrease in NDF content in 2022 and an increase in 2023, as well as the reduction in ADF during both years (2022 and 2023) is linked to the influence of harvest times and the plants' maturity stage. In 2022 and 2023, forage was harvested 3 and 7 days earlier, respectively, compared with the previous year (2021), which could have influenced the differences in the observed results. This is because forage plants were at different development stage during 2021 compared with 2022 and 2023. For example, grasses were at the flowering stage in 2021 but in the late boot stage in 2022 and 2023. Our results are also consistent with several studies (Waldie et al., 1983; Elizalde et al.,1994; Ball et al., 2001; Yu et al., 2003), which reported an increase in crude fiber concentrations with increasing plant maturity and vice versa. In addition, high crude fiber in forage plants during 2021 were caused by the extreme moisture stress during the growing season. As reported by Leen and Martin (2004), plants grown under moisture stress are usually lower in digestibility (high in fiber) than those grown under normal moisture conditions. For feeding forage to cattle, studies have identified the importance of moderately lower NDF and ADF contents for better feed intake and digestibility (Belyea et al., 1993; Beauchemin,1996; Arelovich et al., 2008). Our findings on crude fiber indicated that most of the forage treatments could potentially allow the optimum forage consumption and digestibility for beef cattle. This is because our ADF values are within the range suggested for both legumes (20–35%) and grasses (30–45%), whereas NDF values above 70% in both grasses and legumes will restrict forage intake (Beef Cattle Research Council, 2023b).

The ability of forage to provide animals with an adequate supply of minerals is dependent on the mineral concentration and also the bioavailability of the mineral (O'Dell, 1984). As expected, forage treatments including legume components were higher in Ca compared with grasses, consistent with the study by Kunelius et al. (2006), who found that TG–alfalfa mixtures were enriched in Ca relative to pure grasses. This also agrees with Beef Cattle Research Council (2023a), which stated that grasses are often lower in both Ca and P, whereas alfalfa and other legumes are higher in Ca contents but are seldom higher in P. In legumes, phosphorus has been noted as one of the most limiting soil nutrients during the growing season. This is partly because P is central to the production of adenosine triphosphate, which is a key source of energy for legumes, particularly during biological N fixation and energy transfer (Berg, 2009; Rotaru and Sinclair, 2009).

The higher contents of K in both monoculture grasses and grass-only mixtures compared with legume-dominated mixtures is consistent with Kelling et al. (2014), who posited that grasses are more efficient than legumes in their capacity to extract K from the soil. In the feeding of beef cattle, Ca, K, and P are important for proper body functioning and performance. For instance, Ca, K, and P are responsible for reproduction, milk production, bone formation, and the nervous system's function (Beef Cattle Research Council , 2023a). According to the results from our study, the contents of Ca and K were within the acceptable ranges for beef cattle, whereas P met the requirements for lactating and dry cows but not entirely for growing calves (Table 4.3). As posited by the National Research Council  (2000), the Ca requirements in the diet of lactating cows is 0.31% and that for dry cows is 0.18%, whereas growing calves require a high 0.58%. In parallel, the P requirement of lactating cows is 0.21%, that of dry cows is 0.16%, and that of growing calves is 0.26%; however, only M0, M1, M2, and M5 in our study were able to meet the requirements for growing calves in 2022 (Table 4.3), whereas M1, M3, M6, M12, M21, M23, OG, GPWG, and KCG met these requirements in 2023 (Table 4.3). Furthermore, the K contents were adequate in all forage combinations for both lactating and dry cows (0.60%), although growing calves require 0.70%.

The results for energy content across all forage treatments indicated that complex grass–legume mixtures contained more TDN than monoculture grasses and grass-only mixtures in 2021. This was expected because of the legumes' contributions to the mixtures. Generally, legumes have less structural material and are higher in energy than grasses (Rayburn, 2002), as observed in complex legume-dominated mixtures such as M21 (70% legumes), M22 (90% legumes), and M16 (100% legumes). This was consistent with Foster et al. (2021), who reported lower TDN in bromegrass than in either alfalfa monocultures or alfalfa–bromegrass mixtures. The overall improvement in TDN for all treatments in 2022 and 2023 further emphasizes the greater accumulation of biomass during the year. However, monoculture grasses and grass-only mixtures had higher TDN compared with grass–legume mixtures. We believe that because the ADF of forage monoculture grasses in 2022 and 2023 was lower, it allowed better digestibility, resulting in greater energy, which influenced the TDN content. The TDN values during our 3-year study met the acceptable energy requirements for beef cattle. For instance, the National Research Council (2000) stated that the TDN requirements for cattle are 54–64%. The requirements for dry and pregnant cows are 54% and 55%, respectively, compared with 59% for young cattle and 62% for lactating cattle. High levels of TDN in the forage is important for cattle during the very cold winter seasons for maintaining body temperature and function.

The 48-h NDFD for monoculture grasses and grass-only mixtures was higher than that of mixtures containing legumes across the three production years. This is parallel to the findings on legumes because of their protein content and less fibrous characteristics, making it easier for ruminal microbes to degrade them (Brown and Pitman, 1991; Buxton and Redfearn, 1997). This occurred because although grasses contain high amounts of fiber, they have greater overall digestibility. In addition, grass silage or hay have a very wide range of NDFD because grass species are so diverse and are utilized at extreme ranges in maturity (e.g., grazing on vegetative grass versus feeding on straw). The substantial increase in the NDFD of monoculture grasses and grass-only mixtures in 2022 and 2023 compared with 2021 commensurate with the NDF. However, research has shown that a decrease in NDF increases NDFD and subsequently allows cows to eat more to support their performance (beef or milk) (Oba and Alan, 1999). Although the maturity of plants can play a role in these results, our study rules out this effect because all forages were harvested simultaneously in an identical manner.

### 4.3.3 Botanical composition of forage species

The botanical composition of individual grass and legume species in forage mixtures could provide a guide to producers on species' competition and survival during the production years.

The botanical composition of legumes in simple mixtures was higher in 2021 compared with both grasses and weeds during 2022 and 2023. In our study, the high proportion of legumes, particularly alfalfa, during the first year was partly caused by the ability of the legume to thrive well within mixtures under extreme weather conditions. This is because alfalfa is a competitive legume which has either tap, branch, rhizomatous, or creeping rooting systems, all penetrating deep into the soil to acquire nutrients and water (Agriculture Canada, 1987; Aasen and Bjorge, 2009). The low proportions of weeds in 2021 may have been caused by the hand-weeding measures undertaken at the beginning of the growing season in the first year of forage production. However, the general abundance of weeds in 2022 and 2023 suggests that simple mixtures were relatively ineffective at weed suppression.

Alfalfa species competed well relative to sainfoin and cicer milkvetch. In addition, the grasses also competed well within mixtures containing sainfoin and cicer milkvetch in 2021. Our results are consistent with Biligetu et al. (2014), who reported that in drier regions of Western Canada, cool-season grasses were highly competitive with cicer milkvetch. This competition effect was evident in the extreme dry conditions of 2021 in our study. We further stress, as per our observations, that grasses, particularly AC Success hybrid bromegrasses, were also competitive with the other legume species (sainfoin, birdsfoot trefoil, and alfalfa). In addition, although the proportion of alfalfa was high in 2021 but declined in 2022, it was slightly better in 2023 and was generally very persistent in all production years, unlike sainfoin, birdsfoot trefoil, and cicer milkvetch. Birdsfoot trefoil was almost absent during the first year in highly diverse mixtures. This was probably a result of their slow establishment, hardiness, competitiveness, and persistence (Aasen and Bjorge, 2009). The proportions of weeds in these complex mixtures decreased drastically compared with simple mixtures and three-species mixtures. This is consistent with Nyfeler et al. (2009), Drenovsky and James (2010), and Bonin and Tracy (2012), who demonstrated that species-rich forage mixtures can actually suppress weeds.

### 4.4 Conclusion

Establishing forage stands that include legume components significantly increased the DM yield of forage. Each of the forage mixtures including legumes yielded consistently more DM than grass-only mixtures or monoculture grass stands, particularly in the first production year. However, an exception to this overarching conclusion was insightful, as within the second production year, when there was adequate moisture, the monoculture of FMB demonstrated superior DM yield, indicating that under favorable environmental conditions, some pure grasses can actually outyield grass–legume mixtures. Nevertheless, monoculture grasses and grass-only mixtures decreased substantially in the third year of production. A cumulative DM yield over the 3-year study period showed that mixtures with legumes were superior. The complexity of mixtures did not always provide a yield advantage in this study, as certain simple mixtures outyielded complex mixtures. Similar to the findings on DM yield, nutritional value indicators such as CP and TDN (only in the first production year) were higher, whereas ADF (only in the first year of production) and NDF were lower in legume-dominated stands. In addition, ADF was lower in grass-only mixtures and monoculture grasses, but TDN was higher (in both 2022 and 2023) and 48-h NDFD was higher (in 2021, 2022, and 2023). Forage combinations including legumes also showed a higher Ca and P content, although K was higher in grasses. Even given these differences, all forage mixtures in the study met the nutritional requirements of beef cattle. The proportions of alfalfa cultivars in mixtures were higher than those of both grasses and weeds during the first production year (2021), but this phenomenon changed in 2022 and 2023, when a sharp decrease was observed in the proportion of alfalfa and an increase in both grasses and weeds. AC Success hybrid bromegrass competed well in mixtures compared with the other main grass species tested in the study, whereas the legume sainfoin was a weak competitor in the final production year. In addition, cicer milkvetch and birdsfoot trefoil were slow to establish but achieved a significant proportion of DM by the third production year. It is underscored that the highly diverse six- and eight-species mixtures suppressed weeds better than the two-way, three-way, and four-way forage mixtures. Approximately, 19.2% and 13.3% of weeds were suppressed by the more complex mixtures in 2022 and 2023, respectively.

This study can impact beef cattle production by providing producers with valuable information on high-yielding and nutritious mixtures, how legume and grass species persist over time, and the appropriate period for rejuvenating forage stands.

**Data availability statement**

The authors declare their intent to release the data used for the research upon request.

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**Conflict of interest**

The authors declare no conflict of interest.

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### 4.5 References


Aasen, A., and Bjorge, M. (2009). Forage. Alberta Agriculture and Rural Development, Information Management Services. https://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/agdex16/$FILE/120_20-1_2009.pdf (accessed 20 March 2021).

Agriculture Canada. (1987). Growing and managing alfalfa in Canada. Agriculture Canada.

Agriculture Canada. (1993). Meadow bromegrass. Research Program Service. Agriculture Canada.

Alam, S. M. (1994). Nutrient by plant under stress condition. In: Pessarakli M. (ed.): Handbook of Plant and Crop Stress (pp. 227–246). Marcel Dekker, New York.

Alberta Climate Information Service (2020). Current and historical Alberta weather station data viewer. Data provided by Alberta Agriculture and Forestry. Retrieved on 21 May 2021, from https://acis.alberta.ca/acis/weather-data-viewer.jsp

Amari, M., and Abe, A. (1997). Application of near infrared reflectance spectroscopy to forage analysis and prediction of TDN contents. *Japan Agricultural Research Quarterly*, *31*(1), 55–63.

AOAC. (1984). Official Methods of Analysis, 14th Edition. AOAC.

Arelovich, H. M., Abney, C. S., Vizcarra J. A., and Galyean, M. L. (2008). Effects of dietary neutral detergent fiber on intakes of dry matter and net energy by dairy and beef cattle: analysis of published data. *Professional Animal Scientist*, *24*, 375–383.

Arzani, H., Khatibi, F., Basiri, M., and Fish, E. (2003). Comparison of forage quality of 22 Zagros Mountain rangeland species. In: Kirilov, A., Todorov, N., and Katerov, I., eds., Proceedings of the 12th Symposium of the European Grassland Federation Pleven, Bulgaria, 26–28 May (pp. 222–225). British Grassland Society.

Ball, D., Collins, M., Lacefield, G., Martin, N., Mertens, D., Olson, K., Putnam, D., Undersander, D., and Wolf, M. (2001). Understanding forage quality. American Farm Bureau Federation Publication.

Ball, D., Ballard, E.N., Kennedy, M., Lacefield, G.D., and Undersander, D. (2008). Extending Grazing and Reducing Stored Feed Needs. Publication 8-01. Grazing Lands Conservation

Initiative.

Beauchemin, K. A. (1996). Using ADF and NDF in dairy cattle diet formulation – A western Canadian perspective. *Animal Feed Science and Technology, 58*, 101–111. https://doi.org/10.1016/0377-8401(95)00877-2

Beef Cattle Research Council. (2023a). Nutrition in beef Cattle. Accessed on 10 May 2023 at https://www.beefresearch.ca/topics/nutrition-in beef cattle/

Beef Cattle Research Council. (2023b). Forage species. Accessed on 7 May 2023, at https://www.beefresearch.ca/topics/forage-species/

Bélanger, G., Castonguay, Y., and Lajeunesse, J. (2014). Benefits of mixing timothy with alfalfa for forage yield, nutritive value, and weed suppression in northern environments. *Canadian Journal of Plant Science, 94,* 51–60.

Bélanger, G., Tremblay, G. F., Papadopoulos, Y. A., Duynisveld, J., Lajeunesse, J., Lafrenière, C., and Fillmore, S. A. E. (2017). Yield and nutritive value of binary legume–grass mixtures under grazing or frequent cutting. *Canadian Journal of Plant Science, 98*(2), 395–407. https://doi.org/10.1139/CJPS-2017-0183

Belyea, R. L., Steevens, B. J., Garner, G. B., Whittier, J. C., and Sewell, H. B. (1993). Using NDF and ADF to Balance Diets. Extension Publications. http://hdl.handle.net/10355/3631

Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology, 84*(1),11–8. doi: 10.1007/s00253-009-2092-7

Biligetu, B., Jefferson, P., Muri, R., and Schellenberg, M. (2014). Forage yield, nutritive value and persistence in a binary mixture of warm- and cool- grasses with legume species. *Canadian Journal of Plant Science, 94*, 1139–1148.

Bittman, S., Waddington, J., and McCartney, D. H. (1991). Performance of alfalfa strains grown in mixture with smooth bromegrass as affected by management. *Canadian Journal of Plant Science*, *71*(4), 1029–1037. https://doi.org/10.4141/cjps91-145.

Bolinder, M. A., Angers, D. A., Bélanger, G., Michaud, R., and Laverdière, M. R. (2022). Root biomass and shoot to root ratios of perennial forage crops in eastern Canada. *Canadian Journal of Plant Science, 82*, 731–737.

Bonin, C. L., and Tracy, B. F. (2012). Diversity influences forage yield and stability in perennial prairie plant mixtures. *Agriculture Ecosystem Environment, 162*, 1–7. doi:https://doi-org

Bonin, S. G., and Tomlin, D. C. (1968). Effects of nitrogen on herbage yields of timothy harvested at various stages. *Canadian Journal of Plant Science, 48*, 501–509. https://doi.org/10.4141/cjps68-097

Bonnefield Research. (2016). Canada's forage crop: The Overlooked Cornerstone of Canadian Agriculture. Accessed on 7 May 2023 at https://bonnefield.com/wp-content/uploads/2020/04/Forage_review.pdf

Brown, W. F, Lai, Z. Q., and Pitman, W. D. (1991). In vitro fibre digestion: Associative effects in tropical grass–legume mixtures. *Tropical Grasslands, 25,* 297–304.

Buxton, D. R. and Redfearn, D. D. (1997). Plant limitations to fiber digestion and utilization. *Journal of Nutrition, 127*, 814–818.

Canadian Roundtable for Sustainable Beef. (2016). National Beef Assessment and Strategy Summary Report the Go-To Forum on Sustainable. Accessed on 17 October 2022 at https://crsb.ca/wp-content/uploads/2021/12/Assessment-and-Strategy_summary_report_2016.pdf

Canan, T., Levent, C., and Koc, F. (2004). Determination of nutritional value of some legume and grasses. *Pakistan Journal of Biological Science, 7*(10), 1750–1753.

Cecen, S., Erdurmus, C., and Oten, M. (2005). Determination of suitable forage plants that can be used in artificial pasture areas in Antalya conditions. In: Kokten K, Celiktas N, Atis I, Hatipoglu R, Tukel T, eds., VI Turkey Field Crop Congress, 5–9 September 2005. Antalya, pp. 386–390.

Crowder, L.V. (1985). Pasture management for optimum ruminant production. In: McDowell, L.R. (ed). Nutrition of Grazing Ruminants in Warm Climates (pp. 103–128). Academic

Press.

Drenovsky, R. E., and James, J. J. (2010). Designing invasion-resistant plant communities. The role of plant functional traits. *Rangelands, 32,* 32–37.

Dyer, A. R., and Rice, K. J. (1999). Effects of competition on resource availability and growth of a California bunchgrass. Ecology *80*(8), 2697–2710.

Elizalde, J. C., Merchen, N. R., and Faulkner, D. B. (1994). Fractionation of fiber and crude protein in fresh forages during the spring growth. *Journal of Animal Science, 77*, 476–484.

Foster, A., Biligetu, B., Malhi, S. S., Gill, K. S., Mollison, B., and Leach, D. (2021). Harvest time and fertility effects on yield and quality of forage from alfalfa, hybrid bromegrass and their mixture. *Agricultural Sciences, 12*(04), 325–338. https://doi.org/10.4236/as.2021.124021

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.Volume 94. pp. 41-50. https://doi.org/10.4141/CJPS2013-095

Goering, H. K., and Van, P. J. (1970). Forage fiber analyses. *U.S.* Agricultural Research Service, 379, 387–598.

Government of Alberta (2020). Agricultural region of Alberta soil inventory database. Accessed on 29 February 2024 at https://www.alberta.ca/agricultural-regions-of-alberta-soil-inventory-database

Helgadóttir, Á., Suter, M., Gylfadóttir, T., Kristjánsdóttir, T. A., and Lüscher, A. (2018). Grass–legume mixtures sustain strong yield advantage over monocultures under cool maritime growing conditions over a period of 5 years. *Annals of Botany, 122*(2), 337–345. https://doi.org/10.1093/aob/mcy074

Hutton, G., Berg, B., Najda, H., Johns, M., Cole, D., and Yoder, C. (2005). Perennial forage establishment in Alberta. Government of Alberta. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9682/$file/120_22-3.pdf?OpenElement

Jing, J., Søegaard, K., Cong, W. F., and Eriksen, J. (2017). Species diversity effects on productivity, persistence, and quality of multispecies swards in a four-year experiment. *PLoS ONE*, *12*(1) https://doi.org/10.1371/journal.pone.0169208

Judy, J. V., Jenkins, K. H., Klopfenstein, T. J., Stalker, L. A., and Volesky, J. D. (2015). Effects of stocking rate on forage nutrient composition of Nebraska sandhills upland range when grazed in early summer. *Journal of Animal Science*, *93*(9), 4343–4349. https://doi.org/10.2527/jas.2015-9185

Kelley, O. J., Hardman, J. A., and Jennings, D. S. (1947). A soil-sampling machine for obtaining two, three, and four inches cores of undisturbed soil to a depth of six feet. Soil Science, American Proceedings 12:85–87.

Kelling, K., Peters, J., Rankin, M., and Undersander, D. (2014). Potassium in forages. *Focus on Forage, 4*, 1–2.

Khatiwada, B., Acharya, S. N., Larney, F. J., Lupwayi, N. Z., Smith, E. G., Islam, M. A., and Thomas, J. E. (2020). Benefits of mixed grass–legume pastures and pasture rejuvenation using bloat-free legumes in western Canada: A review. *Canadian Journal of Plant Science, 100*(5), 463–476. https://doi.org/10.1139/cjps-2019-0212

Kunelius, H. T., and Durr, G. H., McRae, K. B., and Fillmore, A. E. (2006). Performance of timothy-based grass / legume mixtures in cold winter region. *Journal of Agronomy and Crop Science, 192*, 159–167.

Leen J., and Martin N. P. (2004). Forage quality tests and interpretation. Agriculture, Food and Environment Sciences. 6, 67–80.

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*, 43–62

Malhi, S. S., Zentner, R. P., and Heier, K. (2002). Effectiveness of alfalfa in reducing fertilizer N input for optimum forage yield, protein concentration, returns and energy performance of bromegrass–alfalfa mixtures. *Nutrient Cycling in Agroecosystems, 62*(3), 219–227. https://doi.org/10.1023/A:1021229824357

McGeough, E. J., Cattani, D. J., Koscielny, Z., Hewitt, B., and Ominski, K. H. (2017). Annual and perennial forages for fall/winter grazing in western Canada. *Canadian Journal of Plant Science, 98*(2), 247–254. https://doi.org/10.1139/cjps-2017-0228

Mercier, K. M., Teutsch, C. D., Fike, J. H., Munsell, J. F., Tracy, B. F., and Strahm, B. D. (2020). Impact of increasing shade levels on the dry-matter yield and botanical composition of multispecies forage stands. *Grass and Forage Science, 75*, 291–302. https://doi.org/10.1111/gfs.12489

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.

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. https://doi.org/10.1111/j.1365-2494.2011.00806.x

National Research Council (2000). Nutrient Requirements of Beef Cattle, 7th Revised Edition. The National Academies Press.

Nelson, C.J. and L.E. Moser. 1994. Plant factors affecting forage quality. p. 115–154. In: G. C. Fahey, Jr. (ed.) Forage Quality, Evaluation, and Utilization. ASA, CSSA, SSSA, Madison, Wisc.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., and Lüscher, A. (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology, 46,* 683–691.

O’Dell, B. L. (1984). Bioavailability of trace elements. *Nutrition Reviews, 42*, 301–308.

Oba, M., and Allen, M. S. (1999). Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows.

*Journal of Dairy Science, 82*, 589–591.

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 Agricultural Food Systems, 26*, 317–327.

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*, 331–342.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. (2020). nlme: linear and nonlinear mixed effects models. R package version 4-149. Accessed 29 February 2024 at https://CRAN.R-project.org/package=nlme

Rayburn, E. B. (2002) Forage management. West Virginia University Extension Service, 5817–5820.R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Roscher, C., Schmid, B., Kolle, O., and Schulze, E. D. (2016). Complementarity among four highly productive grassland species depends on resource availability. *Oecologia, 181*(2), 571–582. https://doi.org/10.1007/s00442-016-3587-4

Rotaru, V., and Sinclair, T. R. (2009). Interactive influence of phosphorus and iron on nitrogen fixation by soybean. *Environmental and Experimental Botany, 66*(1), 94–99

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*(5), 1465–1471. https://doi.org/10.2134/agronj2005.0032

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. https://doi.org/10.4141/CJPS2011-273

Schroeder, J. W. (1994). Interpreting forage analysis. Extension Dairy Specialist, North Dakota State University. Accessed on 6 June 2023 at: https://library.ndsu.edu/ir/bitstream/handle/10365/9133/AS-1080

1994.pdf?sequence=2&isAllowed=y

Serajchi, M., Schellenberg, M. P., Mischkolz, J. M., and Lamb, E. G. (2017). Mixtures of native perennial forage species produce higher yields than monocultures in a long-term study. *Canadian Journal of Plant Science*, *98*(3), 633–647. https://doi.org/10.1139/cjps-2017-0087

Sheaffer, C. C., Miller, D.W. and Marten, G. C. (1990). Grass dominance and mixture yield quality in perennial grass–alfalfa mixtures. *Journal of Production Agriculture, 3*, 480–485.

Sleugh, B., Moore, J. K., George, R., and Brummer, E. C. (2000). Binary legume mixtures improve forage yield, quality, and seasonal distribution. *Agronomy Journal, 92,* 24–29.

Spehn, E. M., Schmid, B., Hector, A., Caldeira, M. C., Dimitrakopoulos, P. G., Finn, J. A., Jumpponen, A., Donnovan, G. O., Pereira, J. S., Schulze, E., Troumbis, A. Y., and Ko, C. (2002). The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. Oikos, 98, 205–218.

Statistics Canada. (2022). Production of principal field crops, November 2022. Government of Canada. Accessed on 29 December 2023 at: https://www150.statcan.gc.ca/n1/daily-quotidien/221202/dq221202b-eng.htm

Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B., and Buchmann, N. (2007). Positive interactions between nitrogen-fixing legumes and four different neighboring species in a biodiversity experiment. *Oecologia, 151*(2), 190–205. https://doi.org/10.1007/s00442-006-0576-z

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*, 843–845.

Tilman, D., Wedin, D., and Knops, J. (1996). Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379, 718–720.

Tuna, C., Tuna, L. C., and Koc, F. (2004). Determination of nutritional value of some legumes and grasses. *Pakistan Journal of Biological Science, 7*, 1750–1753.

Waldie, O., Wright, S. B., and Cohen, R. D. H. (1983). The effects of advancing maturity on crude protein and digestibility of meadow foxtail (*Alopecurus pratensis*) and timothy (*Phleum pratense*). *Canadian Journal of Plant Science, 63*, 1083–1085. https://doi.org/10.4141/cjps83-139

Weggler, K., and Elsäßer, M. (2023). The influence of nitrogen applications and low rainfall conditions on yield of mixed grass–legume grassland for 2 years. *Nitrogen* 2023, *4*, 194-208. https://doi.org/10.3390/nitrogen4020013

Weiss, W. P., and Hall, M. B. (2020). Laboratory methods for evaluating forage quality. *Forages: Science Grassland Agriculture, 2*, 659–672. doi: 10.1002/9781119436669.ch36

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. *Australian Crop and Pasture Science, 60*(12), 1147–1155. https://doi.org/10.1071/CP09058

Yu, P., Christensen, D. A., McKinnon, J. J., and Market, J. D. (2003). Effect of variety and maturity on chemical composition, carbohydrate and protein subfractions, in vitro rumen degradability and energy values of timothy and alfalfa. *Canadian Journal of Animal Science, 83*, 279–290. https://doi.org/10.4141/A02-053

Yüksel, O., and Balabanlı, C. (2021). Yield and some quality properties of binary alfalfa–grass mixtures in different mixture ratios. *Turkish Journal of Agriculture – Food Science and Technology, 9*(6), 1020–1029. https://doi.org/10.24925/turjaf.v9i6.1020-1029.40

Zemenchik, R.A., Albrecht, K.A., and Shaver, R.D. (2002). Improved nutritive value of kura clover and birdsfoot trefoil with grass monocultures. *Agrononmy Journal, 94*, 1131–1138.

## 4.6 Tables

Table 4. 1 Cumulative monthly rainfall, maximum and minimum temperatures, and their long-term average during the growing seasons of 2021–2023 at Fairview, Alberta, Canada.

| Month | 2020 | 2021 | 2022 | 2023 | Long-term average |
|---|---|---|---|---|---|
| Rainfall (mm month$^{-1}$) | | | | | |
| May | 19.1 | 13.3 | 53.4 | 12.7 | 35.1 |
| June | 67.2 | 43.3 | 60.9 | 60.9 | 94.5 |
| July | 89.8 | 23.5 | 18.6 | 3.5 | 161.4 |
| August | 53.9 | 40.5 | 31.4 | 1.7 | 203.1 |
| Cumulative | 230.0 | 120.6 | 164.3 | 40.8 | 494.1 |
| Maximum air temperature (°C) | | | | | |
| May | 24.8 | 16.6 | 13.8 | 23.2 | 14.8 |
| June | 26.8 | 20.2 | 21.0 | 22.9 | 14.0 |
| July | 29.2 | 22.2 | 23.9 | 23.4 | 15.9 |
| August | 29.2 | 21.3 | 25.5 | 22.5 | 14.6 |
| Minimum air temperature (°C) | | | | | |
| May | –2.3 | 3.1 | 2.6 | 6.4 | 10.3 |
| June | 2.6 | 7.5 | 8.9 | 9.0 | 9.4 |
| July | 7.0 | 9.2 | 10.3 | 10.7 | 11.1 |
| August | 0.3 | 7.7 | 10.8 | 9.7 | 13.7 |

Table 4. 2 A complete treatment list of forage monocultures and mixtures grown at the study site in Fairview, Alberta.

| Monoculture grasses and simple mixtures | Complex mixtures: 3-species | Complex mixtures: 4 or more species |
|---|---|---|
| *OG | M7: MB (Fleet) + AL (y) + SF | M0: MB (Fleet) + HB (AC Success) + HB (AC Knowles) + GPWG + CWG |
| *GPWG | M8: HB (AC Success) + AL (y) + SF | M9: MB (Fleet) + AL (y) + SF + CMV |
| *MB | M11: MB (Fleet) + GPWG + AL (y) | M10: HB (AC Success) + AL(y) + SF + CMV |
| *CWG | M12: HB (AC Success) + GPWG + AL (y) | M15: HB (AC Success) + GPWG + CWG + IR + SB (Manchar) |
| *TG | M14: SF + CMV + AL (s5) | M16: AL(y) + AL (R) + CMV + SF +BT |
| M1: MB (Fleet) + AL(s5) | M17: HB (AC Success) + AL (y) + SF | M18: HB (AC Success) + GPWG + AL (y) + SF |
| M2: HB (AC Success) + AL(y) | M13: TF + SW + STL | M19: HB (AC Success) + GPWG + IR + AL (y) + SF |
| M3: HB (AC Success) + AL (s5) | M21: HB (AC Success) + SF + AL(y) | M20: HB (AC Success) + GPWG + AL(R) + AL (y) + CMV + SF |
| M4: MB (Fleet) + AL (s5) | | M22: HB (AC Success) + GPWG + AL (y) + CMV + SF + BT |
| M5: HB (AC Success) + AL(s5) | | M23: HB (AC Success) + GPWG + CWG + IR + AL (y) + AL (R) + CMV + SF |
| M6: HB (AC Knowles) + AL(s5) | | |

Asterisks (*) indicate monocultures. M, mixture; OG, orchardgrass; GPWG, greenleaf pubescent wheatgrass; HB, hybrid bromegrass; CWG, crested wheatgrass; TG, timothygrass; MB, meadow bromegrass; AL(s5), Spredor 5 alfalfa; AL(y), yellowhead alfalfa; AL(R), rugged alfalfa; SF, sainfoin; CMV, cicer milkvetch; BT, birdsfoot trefoil; IR, Italian ryegrass; SB, smooth bromegrass; TF, tall fescue; SW, slender wheatgrass; STL, AC Saltlander wheatgrass.

Table 4. 3 Analysis of Variance (ANOVA) *p*-values and mean grouping for mineral concentration in perennial forage mixtures and monoculture grasses for three production years (2021–2023). See Appendix 1 for a detailed list of the experiment treatments in the study.

| Treatment | Monoculture grasses | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Calcium (%) | | | Phosphorus (%) | | | Potassium (%) | | |
| | 2021 | 2022 | 2023 | 2021 | 2022 | 2023 | 2021 | 2022 | 2023 |
| TG | 0.90bcde | 1.12ghi | 1.02h | 0.17ab | 0.23ef | 0.25abc | 1.51a | 1.49i | 2.40ab |
| FMB | 0.69efg | 1.43abcd | 0.73j | 0.14ab | 0.22g | 0.24abc | 1.76a | 1.67abcd | 2.49a |
| OG | 0.57g | 1.29efgh | 1.30g | 0.15ab | 0.25abc | 0.26abc | 1.75a | 1.75abc | 2.73a |
| GPWG | 0.79def | 1.43abc | 0.78ij | 0.14ab | 0.24cde | 0.27a | 1.42a | 1.53fghi | 2.51de |
| KCG | 1.07abc | 1.41bcd | 1.09h | 0.14ab | 0.25abc | 0.26ab | 1.42a | 1.73abcd | 1.86cde |
| Treatment | Simple mixtures | | | | | | | | |
| | Calcium (%) | | | Phosphorus (%) | | | Potassium (%) | | |
| M1 | 0.90bcd | 1.45abc | 1.50def | 0.16a | 0.27a | 0.27a | 1.72a | 1.76a | 2.04cd |
| M2 | 0.95abc | 1.36bcd | 1.53cde | 0.15a | 0.27ab | 0.25abc | 1.67a | 1.71abcd | 1.89cde |
| M3 | 0.96 abc | 1.55abc | 1.71abc | 0.19a | 0.25abcd | 0.26a | 1.69a | 1.67abcd | 1.90cde |
| M4 | 0.88bcd | 1.41bcd | 1.50def | 0.15ab | 0.25abcd | 0.23c | 1.75a | 1.68abcd | 2.14bc |
| M5 | 0.86cde | 1.41bcd | 1.66abcd | 0.16ab | 0.26abc | 0.25abc | 1.79a | 1.64abcd | 1.84cde |
| M6 | 0.97abc | 1.60ab | 1.71abc | 0.15ab | 0.24cdef | 0.26ab | 1.70a | 1.61cdef | 1.80de |
| Treatment | Complex mixtures | | | | | | | | |
| | Calcium (%) | | | Phosphorus (%) | | | Potassium (%) | | |
| M0 | 0.59fg | 0.90i | 0.82ij | 0.16a | 0.26abc | 0.24abc | 1.68a | 1.76a | 2.03cde |
| M7 | 0.98abc | 1.21fgh | 1.33fg | 0.16ab | 0.25abc | 0.24abc | 1.63a | 1.70abcd | 1.89cde |
| M8 | 1.05abc | 1.38bcdef | 1.61abcd | 0.17ab | 0.24cde | 0.25abc | 1.68a | 1.61bcde | 1.69e |
| M9 | 0.90bcde | 1.26efgh | 1.47defg | 0.16ab | 0.25abc | 0.25abc | 1.61a | 1.70abcd | 1.88cde |
| M10 | 0.96abc | 1.34cdefgh | 1.49def | 0.16ab | 0.25abc | 0.24abc | 1.70a | 1.65abcd | 1.78de |
| M11 | 0.88bcd | 1.38bcdef | 1.62abcd | 0.17ab | 0.25abc | 0.25abc | 1.74a | 1.70abcd | 1.95cde |
| M12 | 0.97abc | 1.48abcde | 1.58bcd | 0.18ab | 0.25abc | 0.26abc | 1.83a | 1.70abcd | 1.83cde |
| M13 | 0.62fg | 1.06hi | 0.91hi | 0.16ab | 0.25abc | 0.25abc | 1.63a | 1.72abc | 1.84cde |
| M14 | 1.10ab | 1.66a | 1.78a | 0.17ab | 0.23def | 0.25abc | 1.92a | 1.50hi | 1.77de |
| M15 | 0.82def | 1.13ghi | 0.95hi | 0.17ab | 0.25abc | 0.25abc | 1.63a | 1.69abcd | 1.83cde |
| M16 | 1.11ab | 1.53abc | 1.76ab | 0.16ab | 0.24cde | 0.25abc | 1.69a | 1.60defg | 1.82cde |
| M17 | 1.08abc | 1.40bcd | 1.51def | 0.16ab | 0.24cde | 0.25abc | 1.62a | 1.59defg | 1.79de |
| M18 | 0.93abc | 1.43abcd | 1.51def | 0.16ab | 0.25abc | 0.25abc | 1.82a | 1.65abcd | 1.78de |
| M19 | 0.96 abc | 1.30def | 1.36efg | 0.16ab | 0.24cde | 0.25abc | 1.71a | 1.62abcd | 1.97cde |
| M20 | 0.99abc | 1.34cde | 1.53cde | 0.15ab | 0.23fg | 0.23bc | 1.57a | 1.50ghi | 1.87cde |
| M21 | 1.09abc | 1.49abc | 1.57cd | 0.19ab | 0.25abc | 0.26ab | 1.93a | 1.73abcd | 1.92cde |
| M22 | 1.13 a | 1.56abc | 1.64abcd | 0.17ab | 0.24cde | 0.25abc | 1.68a | 1.48i | 1.74de |
| M23 | 0.92abcd | 1.44abc | 1.59bcd | 0.17ab | 0.23efg | 0.27a | 1.82a | 1.56efg | 1.72de |
| *P*-value | <0.001 | <0.0001 | <0.0001 | 0.935 | 0.001 | 0.705 | 0.2504 | 0.0004 | <0.0001 |

Treatments with the same letters within a column indicate no significant difference, according to Tukey's honestly significant difference.TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures.

## 4.7 Figures

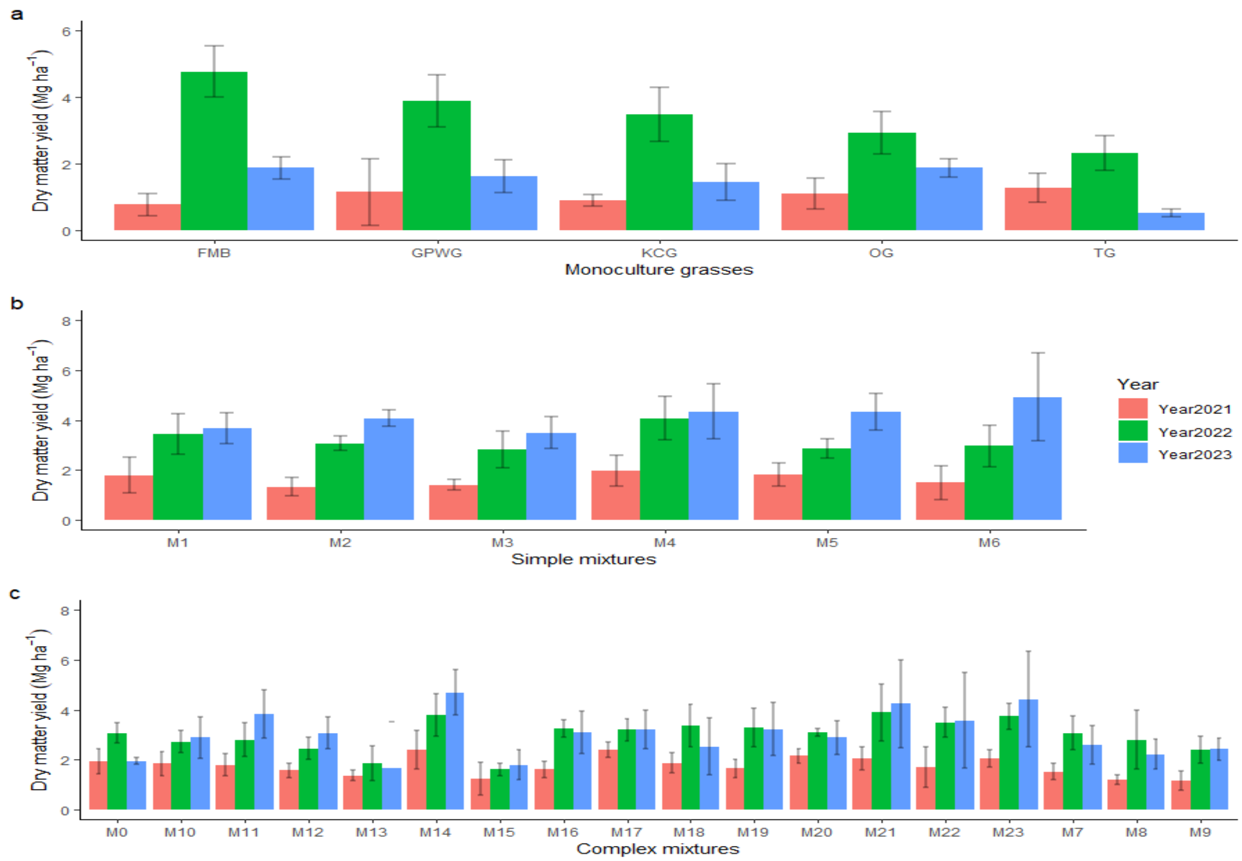


Figure 4. 1 Annual dry matter (DM) yield of (a) monoculture grasses, (b) simple and (c) complex mixtures for three production years (2021–2023). TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures. *P*-value of forage treatments x year for dry matter yield was 0.014, whereas *P*-values for 2021, 2022, and 2023 were $P < 0.0001$. DM yields for monoculture grasses were higher in 2022 than in 2021 and 2023. DM yields for simple mixtures in 2023 were higher than in 2022 and 2023, and DM yields for complex mixtures were comparatively higher in both 2022 and 2023 than in 2021.

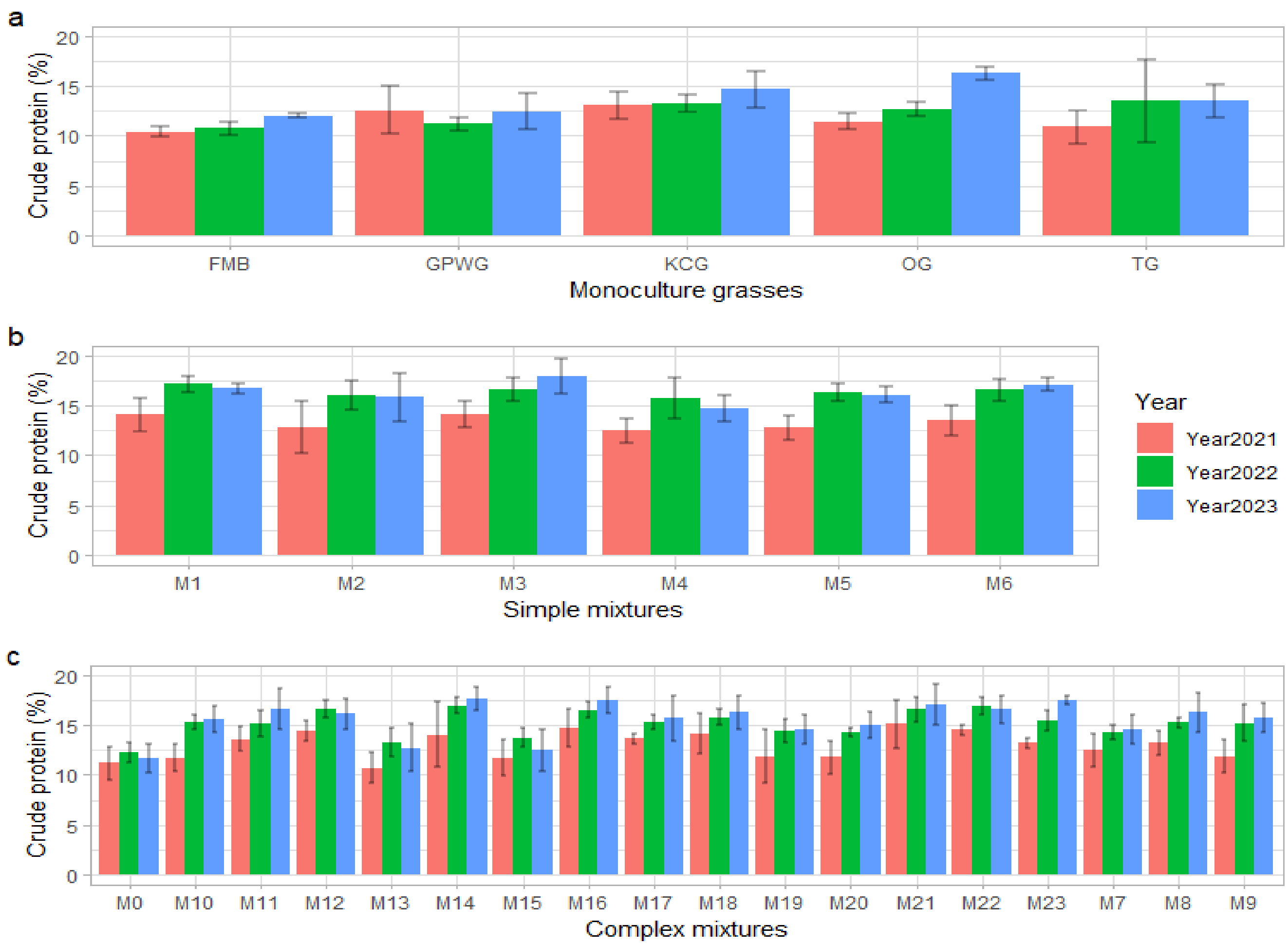


Figure 4. 2 Crude protein (CP) of (a) monoculture grasses, (b) simple and (c) complex mixtures (2021–2023). *P*-values for 2021, 2022, and 2023 were 0.0004, <0.0001, and <0.0001, respectively, whereas *P*-value for forage treatments x year for CP was 0.001. TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures. The CP content for monoculture grasses was higher in 2023 than in 2021 and 2022. The CP content was greater for both simple and complex mixtures in 2022 and 2023 than in 2021. See Table 4.2 for a list of the species combinations for each treatment.

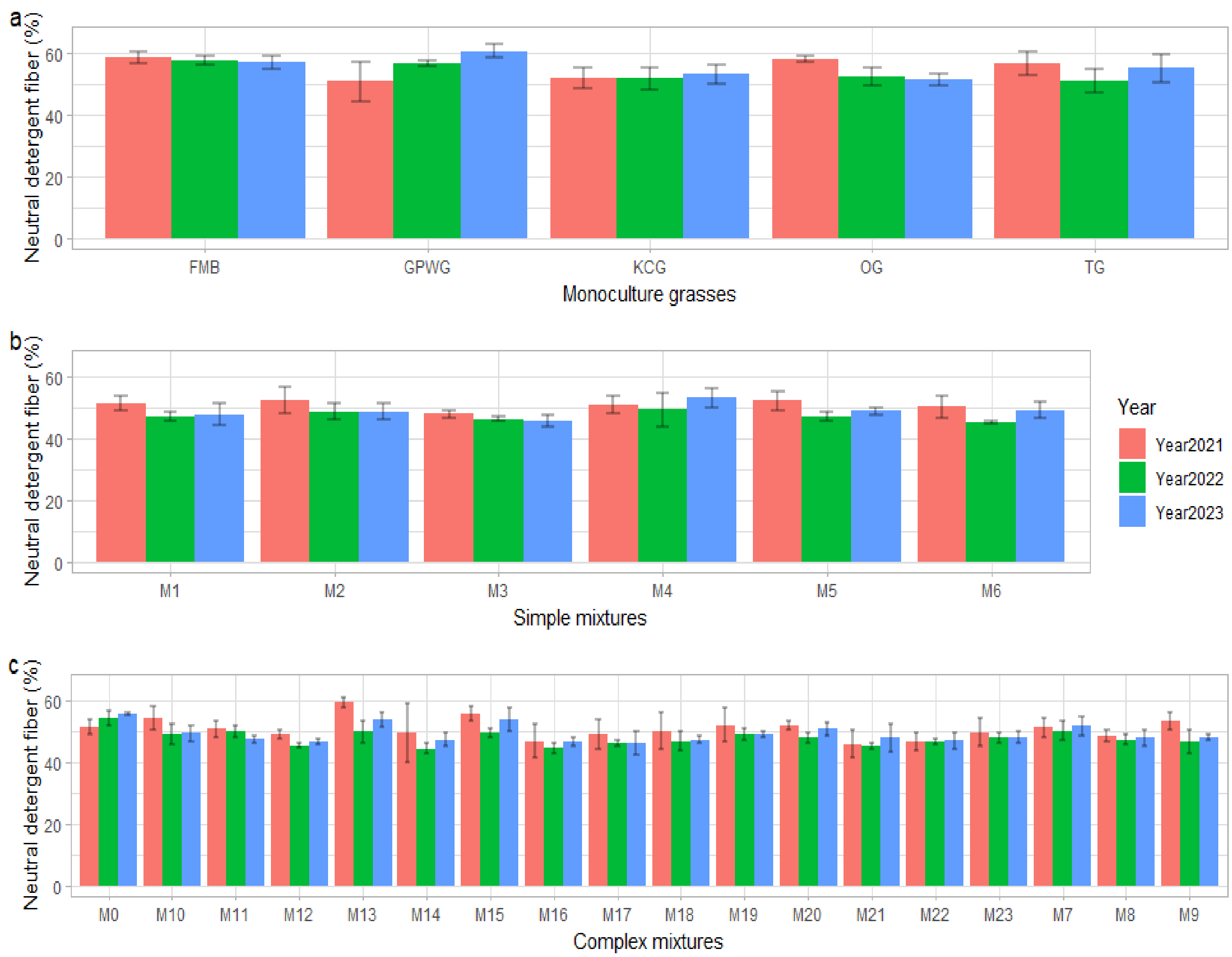


Figure 4. 3 Neutral detergent fiber of (a) monoculture grasses, (b) simple and (c) complex mixtures (2021–2023). *P*-values for 2021, 2022 and 2023 were $p < 0.0001$, respectively, whereas *P*-value of forage treatments x year for NDF was 0.013. TG (timothy grass), OG (orchardgrass), FMB (Fleet meadow brome grass), KCG (kirk crested wheatgrass), and GPWG (greenleaf pubescent wheatgrass). M0, M13, and M15 are grass-only mixtures while M1 to M6 are simple grass–legume mixtures. The NDF content for forages were better (lower) in 2022 compared to both 2021 and 2023. See Table 4.2 for list of detailed species combination for each treatment.

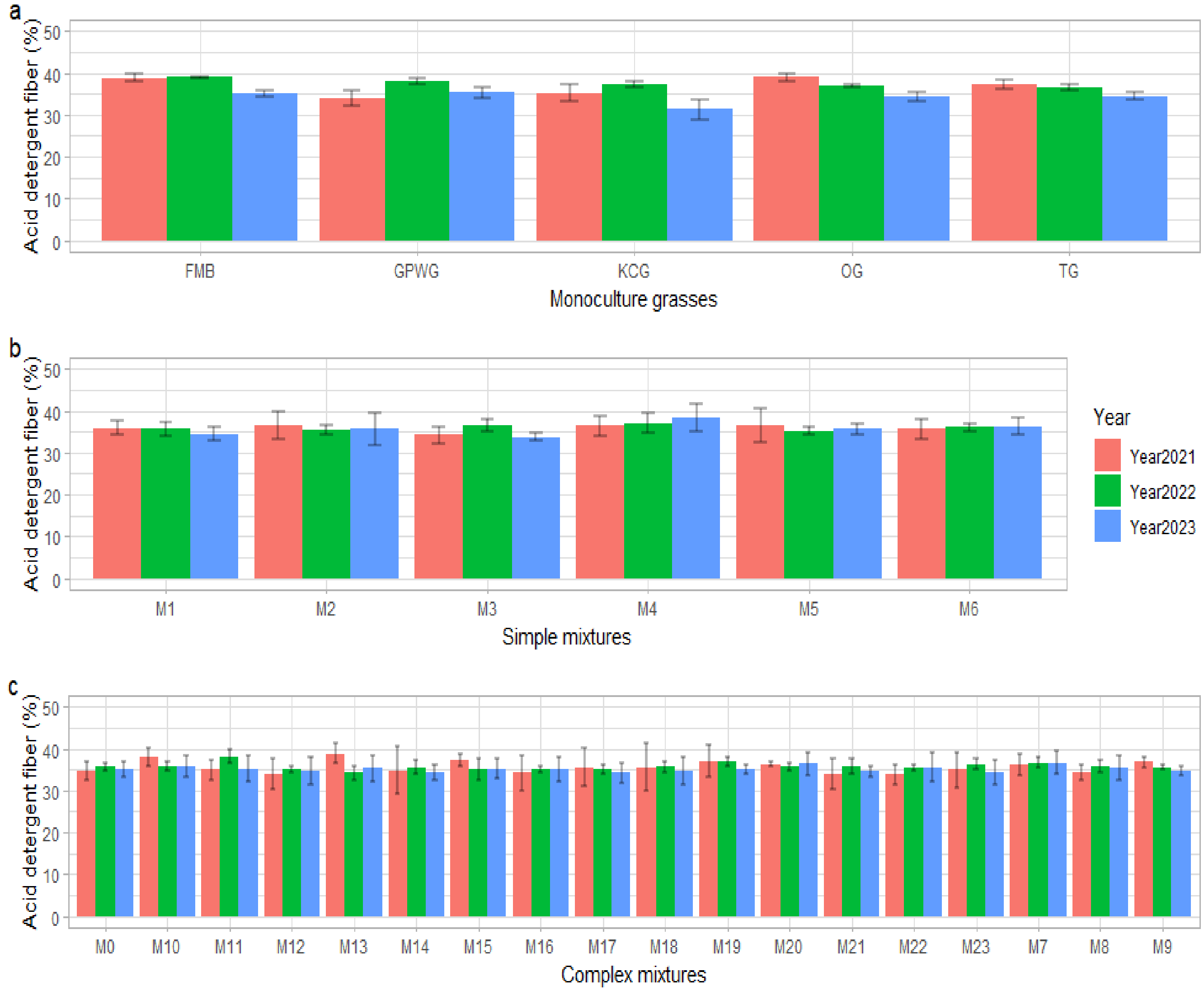


Figure 4. 4 Acid detergent fiber of (a) monoculture grasses, (b) simple and (c) complex mixtures (2021–2023). *P*-values for 2021 2022, and 2023 were 0.026, <0.0001, and 0.0162, respectively, whereas *P*-value of forage treatments x year for ADF was 0.002. TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures. See Table 4.2 for a list of the species combinations for each treatment.

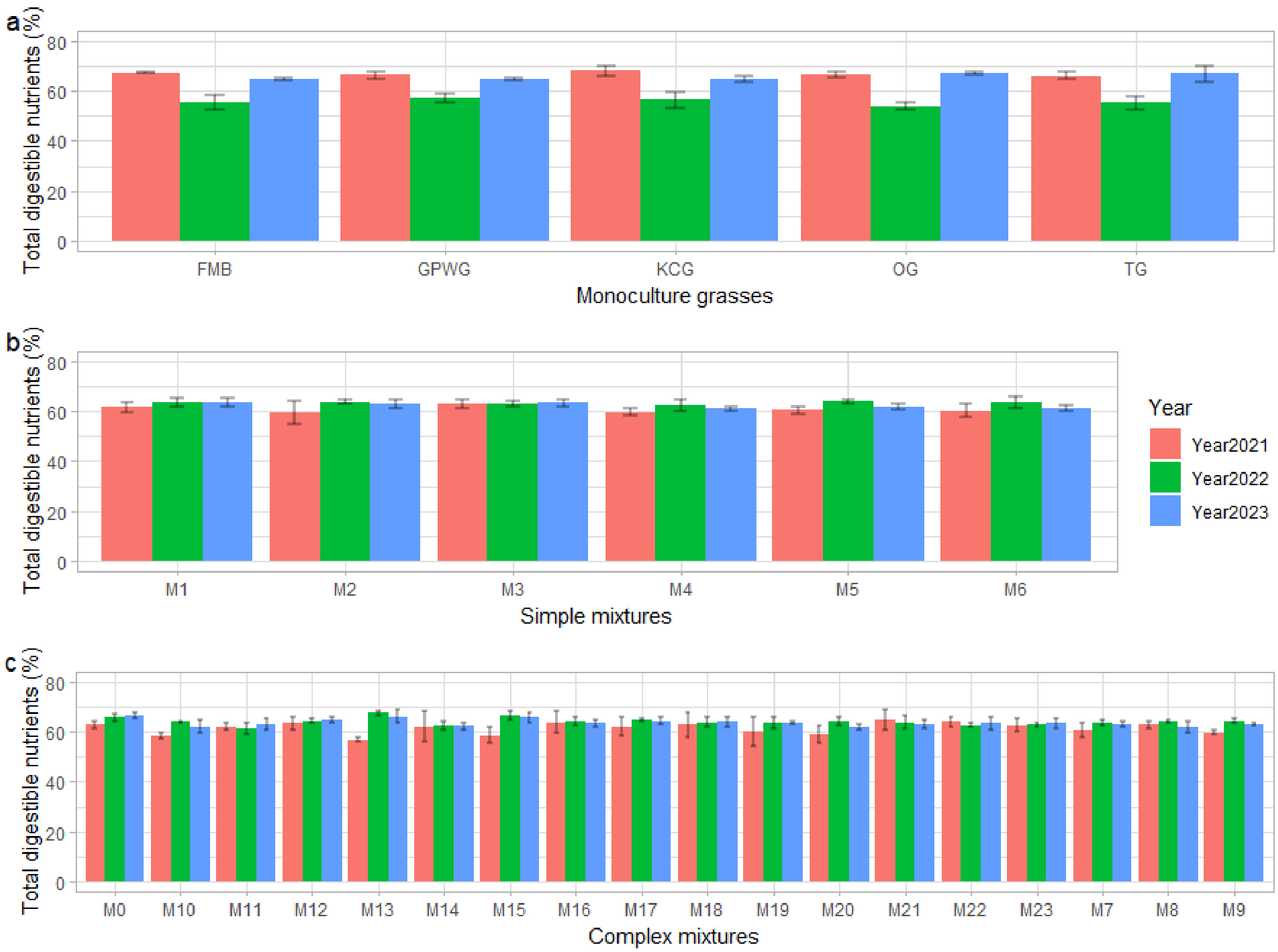


Figure 4. 5 Total digestible nutrients (TDN) of (a) monoculture grasses, (b) simple and (c) complex mixtures (2021–2023). *P*-values for 2021, 2022, and 2023 were <0.0001 whereas the *P*-value of forage treatments x year for TDN was <0.001. TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures. The TDN for monocultures grasses were higher in 2021 and 2022 than in 2023; simple mixtures had higher TDN in both 2022 and 2023 compared with 2021. Complex mixtures were comparatively similar in TDN across the 3 years. See Table 1 for a list of the species combinations for each treatment.

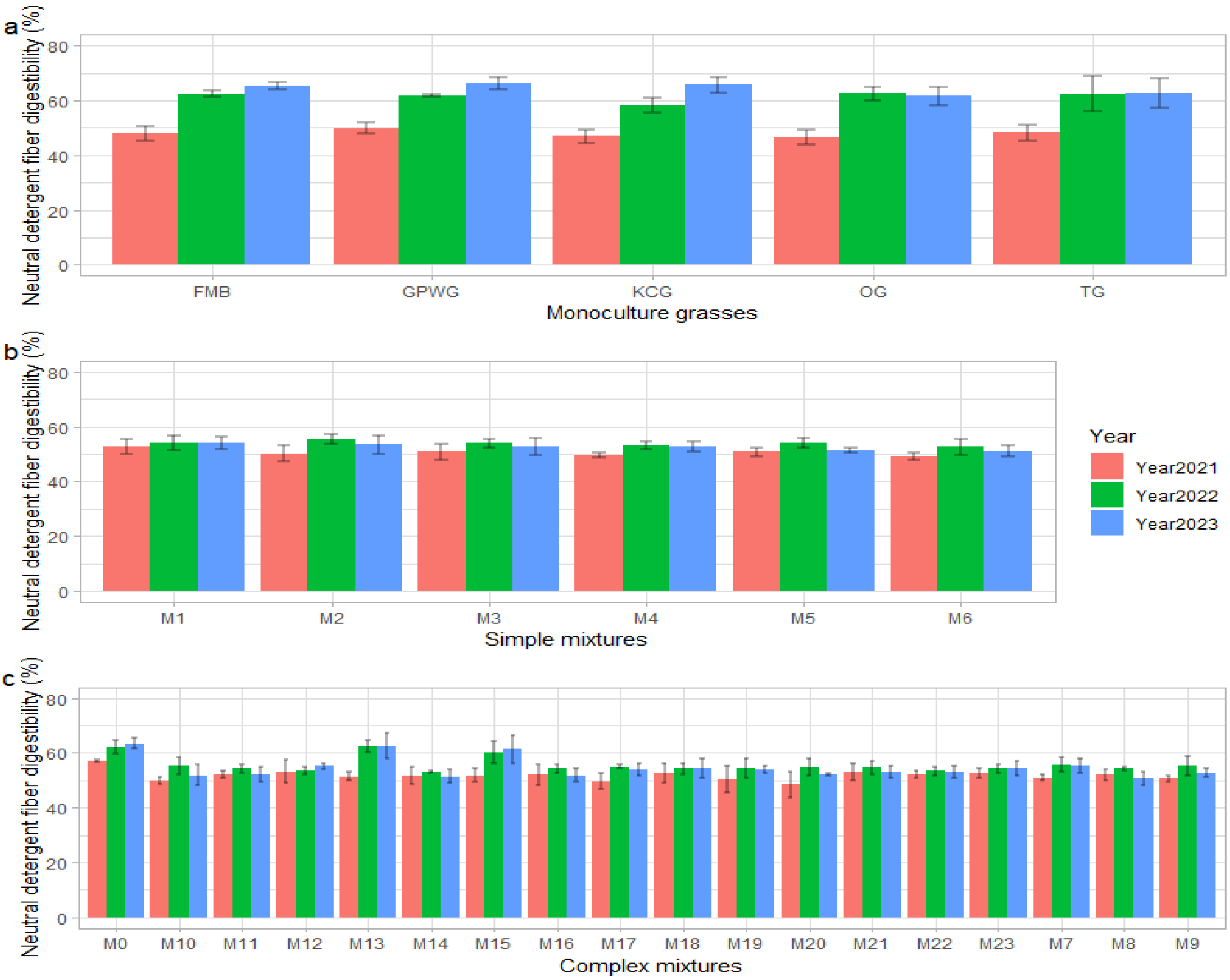


Figure 4. 6 Neutral detergent fiber digestibility (NDFD) of (a) monoculture grasses, (b) simple and (c) complex mixtures (2021–2023). *P*-values for 2021, 2022, and 2023 were 0.0001, <0.0001, and <0.0001, respectively, whereas the *P*-value of forage treatments x year for NDFD was 0.046. TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures. NDFD for monoculture grasses and simple mixtures was superior in 2022 and 2023 compared with 2021. See Table 4.2 for a list of the species combinations for each treatment.

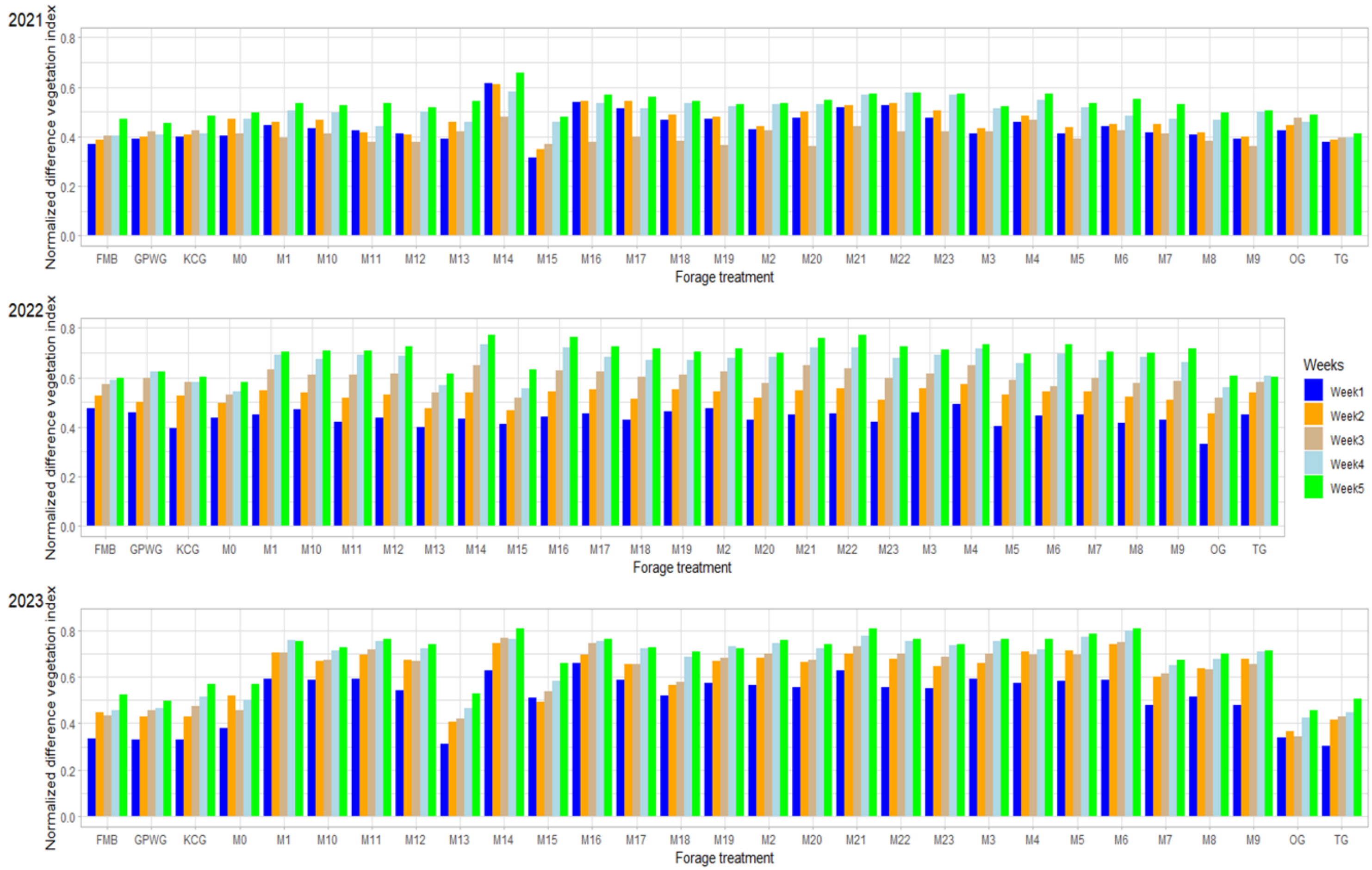


Figure 4. 7 Normalized difference vegetation index (NDVI) of perennial forage mixtures and monoculture grasses for 2021–2022. This shows the growth pattern of forage treatments in terms of their greenness and is a proxy of biomass accumulation over 5 weeks during the growing season. The NDVI ranges between 0 and 0.9. The higher the value, the better growth of the plant. TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures. See Appendix 1 for a list of the treatments in the study.

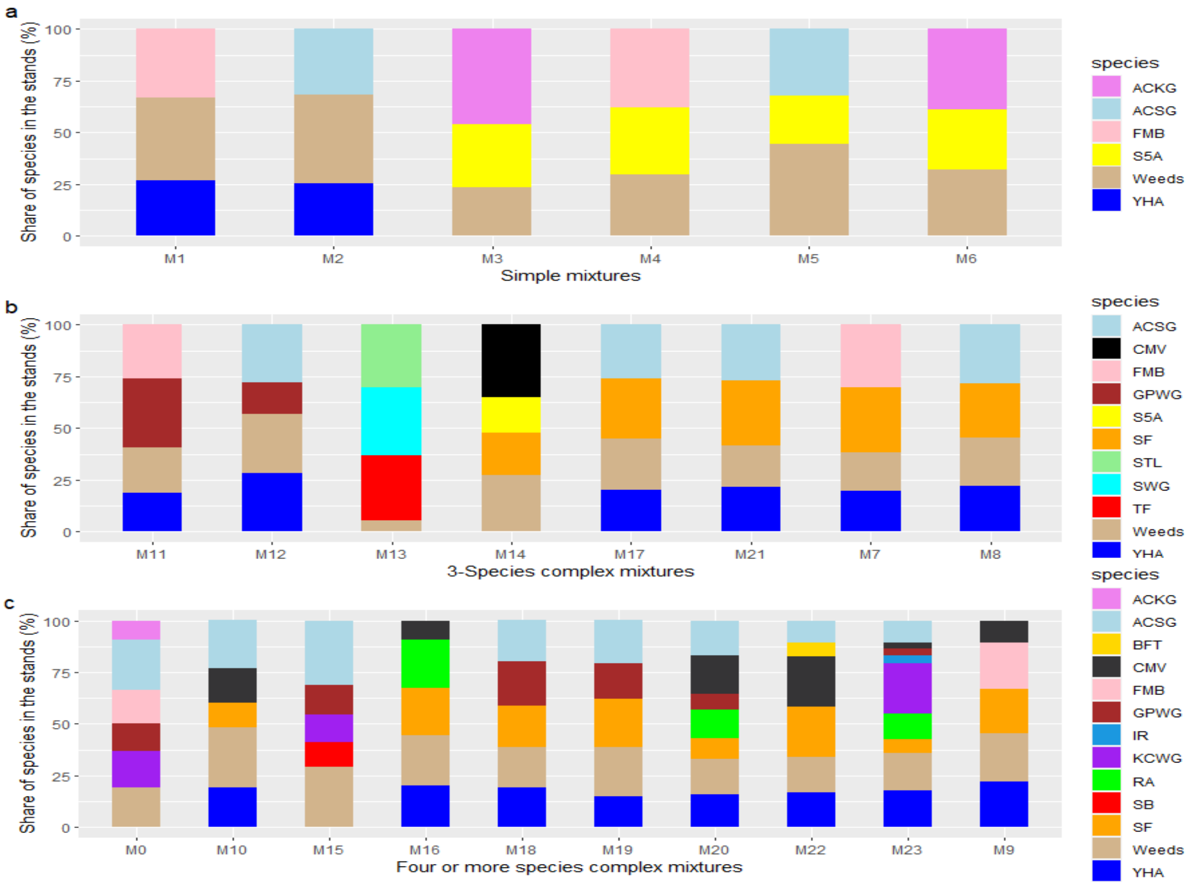


Figure 4. 8 Botanical composition of individual species of grasses, legumes, and weeds expressed a percentage of dry matter weight during the third year of production (2023). CMV, cicer milkvetch; FMB, Fleet meadow bromegrass, BFT; birdsfoot trefoil; IR, Italian rye grass; KCWG, Kirk crested wheatgrass; SB, smooth bromegrass; ACSG, AC Success hybrid bromegrass; ACKG, AC Knowles hybrid bromegrass; GPWG, greenleaf pubescent wheatgrass; RA, rugged alfalfa; SF, sainfoin; YHA, yellowhead alfalfa; SWG, slender wheatgrass; STL, AC Saltander wheatgrass; TF, tall fescue; s5A, Spredor 5 alfalfa. The average proportion of weeds as a percentage of dry matter was 25.2%. See Table 1 for a list of the species combinations for each treatment.

**Chapter 5. Biomass and crude protein water-use efficiencies of perennial forage mixtures**

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## Study synopsis

This 3-yr field study examined the effects of six simple mixtures (one grass and one legume species), 18 complex mixtures (thus three or more species, comprising two legume-only mixtures, three grass-only mixtures, and 13 grass–legume mixtures), and five monoculture grasses on water use efficiency (WUE) and how these forage species mixtures can result in the production of more protein per unit of water use. We continuously measured changes in topsoil water content from early spring to harvest within each production year (2021, 2022, and 2023) as well as crop biomass and crude protein (CP) productivities (i.e., CP yield). In 2021, where seasonal precipitation was low (21.9 mm), both simple and complex grass–legume mixtures significantly increased biomass WUE ($WUE_{BM}$) as well as WUE of CP ($WUE_{CP}$) compared to monoculture grasses or grass-only mixtures. During the second year of production in 2022 when seasonal precipitation was more abundant (73.6 mm), these differential margins between forage treatments receded. Monoculture grasses particularly timothy, often showed better $WUE_{BM}$ (38.2 kg DM ha$^{-1}$ mm$^{-1}$) than simple (23.5–27.3 kg DM ha$^{-1}$ mm$^{-1}$) and complex forage mixtures (13.3 to 31.9 kg DM ha$^{-1}$ mm$^{-1}$). In the third year (2023, seasonal precipitation of 56.7 mm) all monoculture grasses and grass-only mixtures recorded the overall lowest $WUE_{BM}$. Conversely, significant improvements in $WUE_{CP}$ were associated with forage treatments, which include legumes. For instance, in 2021, a 3-legume mixture (treatment M14) had the highest $WUE_{CP}$ (1560.7 kg CP ha$^{-1}$ mm$^{-1}$), while Fleet meadow bromegrass had the lowest $WUE_{CP}$ (367.3 kg CP ha$^{-1}$ mm$^{-1}$), showing a substantial differential margin of 1178.4 kg CP ha$^{-1}$ mm$^{-1}$. This was similar to 2022 when even a simple grass–legume mixture (M4, 548.8 kg CP ha$^{-1}$ mm$^{-1}$) showed higher $WUE_{CP}$ than a grass-only mixture (M15) by a difference of 367.3 kg CP ha$^{-1}$ mm$^{-1}$. This study further showed that grass–legume mixtures substantially improved $WUE_{BM}$ and $WUE_{CP}$ in a relatively drier first production season (2021) due to the moderated water consumption by productive plants but decreased both $WUE_{BM}$ and $WUE_{CP}$ in the moist years (2022 and 2023). An increase in water availability can support nutrient bioavailability and uptake by forages, translating into better biomass or CP yield. Further research can focus on quantifying the impact of individual species within mixtures and their contributions to overall WUE.

**Keywords:** water use, biomass, crude protein, grass–legume mixtures, monoculture grass, yield

## 5.0 Introduction

Forage productivity can be altered by erratic precipitation as well as increasing and extremely-fluctuating ambient temperatures as exacerbated by escalating climate change (Moore and Ghahramani, 2013; IPCC, 2014). Even at the present, these collective effects already generate scarcity of water for growing forages, detrimentally impacting the feed supply to the livestock sector globally (Kulshreshtha and Wheaton, 2013; Attia-Ismail, 2019). These concerns substantiate the need for understanding how to improve the efficiency of water use in forage production systems.

Water-use efficiency (WUE) is described as to how plants make adequate use of water to optimize photosynthesis while minimizing its loss (Hatfield and Dold, 2019; Gelley et al., 2020). Furthermore, focusing on crop yield, WUE is quantified as the relationship of aboveground biomass or economic yield to the water use or evapotranspiration (Bramley et al., 2013). Soil water availability and usage by grasses and legumes have been reported as the most limiting factor that affects the growth and development of forages in various parts of the world (Holecheck et al., 1998; Havlin et.al., 1999; Naqvi et al., 2015). Interestingly, forages that are mostly cultivated in drier areas with limited availability of water have higher WUE in comparison to plants adapted to environments with adequate water availability (Suhartanto et al., 2019). In other words, when experiencing growing conditions without any excess or even optimum of water availability, key plant traits such as canopy architecture and physiology begin interplaying to drive biomass accrual and yield performance (Kim et al., 2022).

In general, different plant species can intrinsically diverge in their WUE; for instance, grasses versus legumes (Siddique et al., 2001). In certain circumstances, legume forages have been reported to use water more efficiently than grass forages (Jefferson and Cutforth, 2005). Furthermore, research found that a simple alfalfa-grass mixture increased WUE by 25% over a pure stand of grass (Dhakal *et al*., 2020), indicating that water could possibly be utilized more efficiently within mixed cropping systems than monoculture systems. Likewise, according to Lindenmayer et al (2008), warm-season forage crops are more efficient in water use than cool-season crops, while annual forages often use more water than perennial forages.

While previous research has explored WUE, these studies focused either on monoculture perennials and simple grass–legume mixtures (Jefferson and Cutforth, 2005; Attram, 2015; Dhakal *et al*., 2020) or on annuals (Campbell et al., 1987; Biederbeck and Bourman, 1994; Cutforth et al., 2013; Lee et al., 2022). However, to date, little is known about WUE of diverse perennial forage mixtures. Similarly, we are unaware of any studies which have experimentally examined WUE of diverse perennial forage mixtures in cold northern regions such as the Peace Region of Western Canada. Therefore, filling this knowledge gap could be crucial to producers whose forage agriculture usually relies upon seasonal precipitation, without supplementary irrigation (Omokanye et al., 2021).

The interaction between nitrogen (N) and water availabilities is key in forage biomass production. Thus, at limiting soil water availability, N absorption and utilization by forage plants could be hindered, resulting in decreased biomass yield and crude protein (CP) content (Araus, et al., 2020). However, forage legumes in mixtures that are predominantly high in CP could ensure a more stable nitrogen use within systems as well as water use due to its ability to fix atmospheric N and access soil moisture at various soil depths (Bittman et al., 1991; Aasen and Bjorge, 2009). Furthermore, since CP is an important evaluation indicator for forage quality and animal performance (Coleman and Moore, 2003), it is therefore relevant to explore the response of crude protein production to water use by forage systems. This can be undertaken by estimating water use efficiency ($WUE_{CP}$) as a metric to aid in selecting suitable combinations of perennial forage species in providing high-quality forage feed for livestock production in Western Canada.

Within Alberta, Canada, forage lands account for up to 43% of the total farmland producing approximately 80% of the forage requirements of beef cattle (Statistics Canada, 2019). Moreover, since Alberta accounts for about 41% of the national cattle herd (Alberta Beef Producers Annual Report, 2020), there is a growing need for the cultivation of more sustainable perennial forages to ensure a consistent forage supply and quality to the sector. It is therefore pertinent to understand and identify the combinations of forage species that use water more efficiently in particular considering the ongoing effects of climate change on forage productivity. We hypothesize that perennial grass–legume mixtures would use water more efficiently than monoculture grasses. As there is a paucity of comparative information on the WUE of perennial forage mixtures in northwestern Alberta, this study sought (i) to quantify biomass water-use efficiency ($WUE_{BM}$) and crude protein water-use efficiency ($WUE_{CP}$) of perennial forage mixtures relative to monocultures and (ii) to estimate seasonal water availability uptake from topsoil by forages.

## 5.1 Materials and methods

### 5.1.1 Site description and experimental design

A perennial forage field experiment was established at the research farm of the Peace Country Beef & Forage Association, located at 670 m above sea level in Fairview, northwestern Alberta, Canada (lat. 56°04′53′N, long. 118°26′05′W) in June 2020 and was evaluated for three consecutive production years (2021, 2022, and 2023). The selected field was a previous canola (*Brassica napus* L.) crop stubble. The field also had a history of several years of wheat (*Triticum aestivum*) canola -rotation for grain production. The soil at the experimental site was classified as Eluviated Black Chernozem according to the Canadian System of Soil Classification (Agricultural Region of Alberta Soil Inventory Database AGRASID; Government of Alberta, 2020). Further soil characteristics at the experimental site have been provided earlier by Gyamfi et al. (unpublished data). The weather information during the growing seasons and their long-term averages for the site (Table 5.1) was obtained from the Alberta Climate Information Service (ACIS).

A randomized complete block design (RCBD) with four replications ($n$ = 4) was used in this trial. Forage treatments comprised six grass–legume (one grass and one legume) and 18 complex diverse perennial forage species (i.e., three or more grass–legume) mixtures. Within the complex mixtures were three grass-only mixtures, two legume-only mixtures and 13 grass–legume mixtures. In addition, there were five pure stands of perennial grasses which were used for comparisons. The grass species used in mixtures and monocultures were wheatgrass (*Thinopyrum intermedium*; varieties- Kirk crested wheatgrass and greenleaf pubescent), orchardgrass (*Dactylis glomerata*), timothy grass (*Phleum pratense*), meadow bromegrass (*Bromus commutatus*; variety- Fleet), and hybrid bromegrasses (AC Knowles and AC Success, i.e., a cross between meadow bromegrass and smooth bromegrass) (Table 5.2).

On 26 June 2020, all grass and legume seeds were seeded at 1.3 cm depth with a six-row Fabro plot drill equipped with disc-type openers at 23 cm row spacing on experimental plots, measuring 2 m wide by 8 m in length with 2 m alleys between replicates. The seeding rates were estimated based on the monoculture seeding rate recommendations of perennial forage by Hutton et al. (2005) (Appendix 1). Before seeding, alfalfa seeds were inoculated with Nitragin Gold alfalfa inoculants from Northstar Seeds, Edmonton, Canada at a rate of 1.5 g per 250 g of seeds, whereas birdsfoot trefoil (*Lotus corniculatus*), cicer milkvetch (*Astralagus cicer*) and sainfoin *(Onobrychis viciifolia*) were not inoculated due to the lack of commercially available inoculants. In the establishment year (2020) and the first year of production (2021), the plots were sprayed with Basagran Forte at 4.05 mL $ha^{-1}$ for in-crop weed control to ensure a good forage establishment. Additionally, dandelions (*Taraxacum officinale*), volunteer wheat and canola were occasionally handpicked. Subsequently, from 2022 to 2023, no further weed control measures (chemical spraying or manual) were carried out.

### 5.1.2 Plant sample collection and measurement

On 3 July 2021, 30 June 2022, and 22 June 2023, an area of 3 m length encompassing 4 inner rows of the plots was harvested when 10–15% of alfalfa legumes in the stands had blossomed (Bonin and Tomlin, 1968). A stubble height of 7.5 cm of grasses and legumes above the ground was implemented during harvest using a custom-made self-propelled forage harvester (Swift Machine and Welding Ltd., Swift Current-Saskatchewan, Canada). The harvested biomass was subsequently weighed for every experimental plot and a fresh sub-sample of (not more than 1000 g) was oven-dried at 105°C to constant weight to determine forage dry matter content which was used to calculate yield on dry matter basis in (kg $ha^{-1}$). Dried forage samples from the three production years were submitted to A&L Canada Laboratory (London, Ontario) for assessing crude protein (CP) content. Total N concentration was determined using micro-Kjeldahl method (AOAC, 1984) and the total %N multiplied by 6.25 to determine CP content (Schroeder, 1994).

### 5.1.3 Water-use efficiency determination

Water use efficiency was calculated using the formula postulated by Gao et al. (2009).

$$\text{WUE} = \frac{Y(Kg\ \text{ha}-1\ )}{ET(mm)}$$

Evapotranspiration (ET) is expressed as:

$$ET = \Delta S + P + I + U - R - D_w$$

where **ΔS** denotes the seasonal changes in water content in the soil determined by using volumetric water content (VWC) measurement. The volumetric water content was measured weekly from vegetative stage of forage plants to early or mid -blossom stage prior to harvest (mid-May to early July 2021). This was done using the Time Domain Reflectometry (TDR- 350) sensor with a rod length of 0 to 15.2 cm soil depth increment. The changes in the VWC were converted from percentages (%) value into the water column (mm) by multiplying the measured VWC by the soil depth of 15.2 cm thickness. In both 2022 and 2023, the VWC reading was done using a rod length of 0 to 25.4 cm soil depth increment due to an anticipated deeper development in root systems of the forage treatments. Although perennials can extract water beyond the depths explained above, this study was limited in this regard.

The **P** represents the total rainfall in (mm) during the entire growing season in each study year. For instance, the rainfall for each growing season was accounted for from early May to the last week of June or early week of July when the forage biomass was harvested. Data was obtained from the AICS weather station. **I** connotes irrigation and since the plots were not irrigated, **I** was not considered in the calculation. Run off (**R**) was considered negligible in the equation due to the flat topography of the experimental site at the Fairview research farm. The upward capillary (**U**) flow to the root and downward (**D***w*) drainage was also assumed negligible based on Darcy's law (De Medeiros et al., 2005; Kar et al., 2007).

Y represents the biomass yield or CP yield. This comprises the forage dry matter measured in kg $ha^{-1}$ ($WUE_{BM}$), and the CP yield obtained by multiplying the DM yield and CP concentration of the forage as the Y for 'CP WUE ($WUE_{CP}$)'.

### 5.1.4 Statistical analysis

Research data were subjected to two-way analysis of variance (ANOVA) in randomized complete block design (RCBD) using the NLME program with mixed effect model (Pinheiro et al., 2020) in the R statistical program (Version 4.0)(R Core Team 2020). In the mixed effect model, forage treatments x year interaction was considered fixed effects while block replications were set as random effect in the model. Due to the significant forage treatments x year effects, year was treated as fixed effect to understand the year-to-year trend of both biomass water use efficiency ($WUE_{BM}$) and crude protein water use efficiency ($WUE_{CP}$). Before ANOVA, normality of residuals was tested using the Shapiro-Wilk statistic and homogeneity of variances was tested using Levene's test. No data transformations were required, as residuals of all data were homogeneous and normally distributed. Following significant ANOVAs, Tukey's honestly significant difference (HSD) was used to separate treatment means when proven significant ($P < 0.05$).

## 5.2 Results

### 5.2.1 Biomass water-use efficiency ($WUE_{BM}$)

Focusing on biomass production, water-use efficiencies of perennial mixtures and monoculture grasses during the first year of production (2021) were significantly different across treatments ($P = 0.0046$). Treatment M14 recorded the highest $WUE_{BM}$ (116.8 kg dry matter (DM) ha$^{-1}$ mm$^{-1}$, while orchard grass (OG), Fleet meadow bromegrass (FMB), and Kirk crested wheatgrass (KCG) had the lowest $WUE_{BM}$ with only 37.0 kg DM ha$^{-1}$ mm$^{-1}$. In addition, $WUE_{BM}$ of simple mixtures were between 62.3 and 81.8 kg DM ha$^{-1}$ mm$^{-1}$, while those of complex mixtures varied wider between 56.1 and 116.8 kg DM ha$^{-1}$ mm$^{-1}$ (Figure 5.1). Apart from M8, M9, and M15, all complex mixtures had greater $WUE_{BM}$ than all monoculture grasses and simple mixtures. Highly diversified forage combinations such as mixtures with six and eight species were also more water-use efficient compared to most 3-way or 4-way species mixtures, as evident in M20 and M23 (Figure 5.1).

In 2022, $WUE_{BM}$ across forage treatments varied significantly between 13.3 and 38.4 kg DM ha$^{-1}$ mm$^{-1}$ ($P < 0.0001$). Timothy grass had the highest $WUE_{BM}$ of 38.4 kg DM ha$^{-1}$ mm$^{-1}$ while M15 recorded the lowest (13.3 kg DM ha$^{-1}$ mm$^{-1}$). All monoculture grasses, except for OG were higher in $WUE_{BM}$ than all simple mixtures (with the exception of M4), grass-only, or grass–legume complex mixtures (Figure 5.1). In addition, complex grass–legume mixtures of eight-species had consistently higher $WUE_{BM}$ compared to either four-, five-, or six- species mixtures.

During the third year of production (2023), $WUE_{BM}$ was significantly different among forage treatments ($P < 0.0001$). The $WUE_{BM}$ ranged from 8.5 kg DM $ha^{-1}$ $mm^{-1}$ for timothy grass to 75.2 kg DM $ha^{-1}$ $mm^{-1}$ for M6. Here, WUE for simple grass–legume mixtures were higher than monoculture grasses, grass-only mixtures, and some complex grass–legume mixtures such as M7, M8, M9, M10, M16, M17, M18, and M20 (Figure 5.1). Our results further showed that complex mixtures with many forage species (M19, M20, M22, and M23) also had greater WUE compared to those mixtures with fewer plant species such as M7, M8, M9, and M10. In general, water-use efficiency in forage treatments was higher during 2021 than in both 2022 and 2023.

### 5.2.2 Crude protein water use-efficiency ($WUE_{CP}$)

Over the three years of forage production (2021, 2022, and 2023), $WUE_{CP}$ was significantly different across forage treatments (2021 with $p = 0.0008$; 2022 with $p < 0.0001$; 2023 with $p < 0.0001$) (Figure 5.2). In 2021, M14 had the highest $WUE_{CP}$ (1560.7 kg CP $ha^{-1}$ $mm^{-1}$), and M14 was also among the second best in both 2022 (537.2 kg CP $ha^{-1}$ $mm^{-1}$) and 2023 (1161.0 kg CP $ha^{-1}$ $mm^{-1}$). Generally, the poorest $WUE_{CP}$ across the three study years was recorded in either all grass-only mixtures or grass monocultures, whereas all legume-dominated mixtures were better than their grass counterparts. Conversely, complex grass–legume mixtures performed better than simple grass–legume mixtures (Figure 5.2). In 2022, M4 had the highest $WUE_{CP}$ with 548.8 kg CP $ha^{-1}$ $mm^{-1}$, but it was only among the 4th highest performers in 2021. Alternatively, M6 had the highest $WUE_{CP}$ of 1283.0 kg CP $ha^{-1}$ $mm^{-1}$ in 2023, but it was the 6th best performer in both 2021 (957.1 kg CP $ha^{-1}$ $mm^{-1}$) and 2022 (395.6 kg CP $ha^{-1}$ $mm^{-1}$). Furthermore, simple mixtures had relatively similar $WUE_{CP}$ to complex grass–legume mixtures in both 2022 and 2023, a result that was absent in 2021.

## 5.3 Discussion

During the first year (2021) of production of forage stands, monoculture grasses and grass-only mixtures had the lowest water-use efficiency based on $WUE_{BM}$ results. Conversely, legume-dominated mixtures can provide the highest $WUE_{BM}$. This is consistent with Jefferson and Cutforth (2005) who reported that legumes are more water use efficient than grasses. In our study, these findings are in part attributable to the inadequate precipitation recorded during the growing season of 2021 (21.9 mm). This effect was also evident from the soil moisture observed beneath grass-only stands, where lower topsoil moisture was also recorded under these grass-only mixtures (Figures 5.3, 5.4, and 5.5). For example, grass-legume mixtures accessed water at different soil depths. The grasses with shallow rooting systems can utilize moisture at topsoil whereas the legumes with taproots accessed water at deeper depth. This creates a balance in the use of water across the soil profile resulting in an efficient use of resources. The relatively dry soil and weather conditions influenced the biomass of grasses and their $WUE_{BM}$. As previously reported by Wright et al. (2021), monoculture grasses grew 25% less during dry years, but 30% greater during wet years. This showcases the sensitivity of WUE metrics while integrating how biomass accumulation respond across contrasting growing conditions (Hendrickson et al., 2013; Kim et al., 2022).

The diversity within grass–legume mixtures can improve ecosystem functions through their complementarity, hence improving yield and WUE (Tillman and Downing, 1994; Isbell et al., 2015). The synergetic manifestation of complementarity effects among plant species within a given forage mixture can account for a better water uptake and utilization, potentially translating into biomass accumulation, stand persistence and nutritive value. Within this framework, specific traits of plant species such as the root architecture and leaf morphology of alfalfa can enable substantial increases in overall $WUE_{BM}$. This is supported by Moot et al. (2008), who reported that alfalfa extracted 328 mm of water at deeper soil layers than perennial ryegrass which only extracted 243 mm of water. In other words, alfalfa enabled a high recovery of rainwater stored at depth in the soil profile, leading to a WUE for alfalfa of 40 kg DM $ha^{-1}$ $mm^{-1}$ while perennial ryegrass registered less than half, with only 18 kg DM $ha^{-1}$ $mm^{-1}$. Additionally, as posited by Jefferson and Cutforth, (2005), alfalfa has higher water leaf potential. This generates a greater osmotic capacity, which helps alfalfa plants to adjust during water stress periods and maintain higher turgor. In our study, findings from the first year of production (2021), reinforce the important role of polycultures such as grass–legume mixtures over grass monocultures in cropping systems in semi-arid areas. By focusing on $WUE_{BM}$ across the three years of production in our study (2021, 2022, and 2023), it became self-evident that water-use efficiency was higher in 2021 while the opposite was observed in both 2022 and 2023. This observation agrees with Hussain et al. (2022) who indicated that higher water-use efficiency during drier season can be linked to disproportionately reduced water consumption by plants. Moreover, our results of seasonal water uptake across the three years also revealed how variation in soil moisture is a major driving factor for crop yield (Table 5.3). For example, forage yields were lower in 2021 when soil moisture was low compared to both 2022 and 2023 when soil moisture was relatively high. This is because water is essential for plant cell development and expansion (McElrone et al., 2013) Our findings of better $WUE_{BM}$ for both simple and complex mixtures that include legumes relative to either grass-only mixtures or monoculture grasses over the 3-yr study adds to the knowledge base. This is the first report in the literature that documents how simple or complex forage polyculture systems can result in better WUE than grass monocrop systems over three years of production that encompassed major seasonal fluctuations in moisture availability.

During the first year of production (i.e., drier 2021), $WUE_{CP}$ trended low for both monoculture grasses and grass-only mixtures whereas legume-dominated mixtures were slightly higher, reflecting patterns of crude protein accumulation across forage mixtures. The CP concentrations per unit of biomass for grass–legume mixtures highlights the efficiency of legumes at converting water uptake efficiently into CP production. This assertion resulted in contrasting grass monocropping. The diminished $WUE_{CP}$ in monoculture grasses emerges in part from their inability of grasses to fix atmospheric N. This is supported by Carlsson and Huss-Danell (2014) who stated that as grasses are non-fixers of N, they demand greater N supply than fixers such as legumes and other N fixers. However, within polycultures, legumes can make available N to non-legumes such as grasses via: (i) decomposition of legume roots and nodules, (ii) roots exudates, and (iii) mycorrhiza mediation (Thilakarathna et al., 2016). Furthermore, in our study, the prevalent dry soil conditions in the first year of production could overall have hindered N utilization. For instance, N uptake by roots could be affected due to constrained mass flow and diffusion as driven by limitations in soil water (Dunham and Nye, 1973; Lambers et al., 2008).

During a season with drought stress, our observations of low $WUE_{BM}$ and $WUE_{CP}$ in monoculture grasses or grass-only mixtures could be elucidated by a probable use of energy by the plants towards the acquisition of light, water, and nutrients instead of producing biomass and subsequently crude protein accrual. This observation agrees with Farooq et al. (2009) and Jaleel et al. (2009) who indicated that limited water availability affects most physiological and morphological responses, including reduced absorption of photosynthetic active radiation which translates into decreased crop yield.

Crude protein concentrations increased significantly in both monoculture grasses and grass–legume mixtures in the second and third years of forage production (2022 and 2023), but generally, $WUE_{CP}$ remained higher in grass–legume mixtures. These growing seasons received fair amounts of precipitation (73.6 mm in 2022 and 56.7 mm in 2023) compared to the previous season of 2021 (21.9 mm). This was also evident in higher soil moisture content during the second and third years of forage production (Figures 5.3, 5.4, and 5.5). In the case of monoculture grasses or grass-only mixtures, a slight increase in CP concentrations during 2022 and 2023 is attributable to a probable increase in water and nitrogen uptake, as well as better utilization; hence, better development of plant structures resulted in improved biomass and protein content. Other speculation is that plants expended their energies to amassing aboveground biomass instead of scavenging the soil for water and nutrients.

Complex grass–legume mixtures were better in terms of $WUE_{CP}$ than simple grass–legume mixtures in both 2021 and 2023. This could be explained by the intrinsic traits of multiple plant species combined within a cropping system. In our study, under complex grass–legume mixtures, one could conceptualize that nutrient pools were accessible for uptake, utilization, and recycling within these forage systems with several plant species which can have slightly different fertility requirements. This becomes contrasting to simple mixtures that included only two plant species, which had to probably pose intense intraspecific competition for limiting resources. This implies that under limiting supply, complex grass–legume mixtures can use water and other resources more efficiently to produce forage biomass resulting also in better CP concentrations and $WUE_{CP}$ over simple mixtures.

## 5.4 Conclusion

This study showed the $WUE_{BM}$ capacity of simple grass–legume mixtures, complex mixtures, and monoculture grasses over three years of continual forage production with contrasting precipitations (dry in 2021 while moist in both 2022 and 2023). During the establishment phases, monoculture grasses or grass-only mixtures were deficient at using water for producing biomass during the dry year 2021, while either simple or complex legume-dominated stands were better at this process. This translated into a better DM yield and crude protein accrual by legumes, and subsequently increasing $WUE_{CP}$. This phenomenon was also attributable to the materialization of complementarity effects that existed within the grass–legume systems. Even though there was a general improvement of $WUE_{BM}$ and $WUE_{CP}$ in the second and third years of production across all forage systems probably in connection to a better nutrient bioavailability, uptake, and utilization, legume-dominated mixtures were still superior to their monoculture counterparts. A critical observation of $WUE_{BM}$ over the entire 3- yr study was that in the drier year 2021, water was more efficiently used by forage mixtures compared to moist years 2022 and 2023. This was elucidated by the divergent abilities of different forage species to use soil water at a reduced availability during the periods of scarcity. Conclusively, this study has reinforced the important contributions of polyculture over monoculture within cropping systems as per their advantage for more efficient use of scarce water. This further supports the view of cultivating grass–legume mixtures instead of monoculture grasses in forage-livestock production systems.

## 5.5 References


Aasen, A., and Bjorge, M. (2009). Forage. Alberta Agriculture and Rural Development, Information Management Services. https://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/agdex16/$FILE/120_20-1_2009.pdf (accessed 20 March 2021).

Alberta Beef Producers Annual Report (2020). https://www.albertabeef.org/files/annual-reports/d5aN2ubsRRwNZvwA6PaAc2rSDn5mZvwdnbAZNvLu.pdf (accessed 6 October, 2021).

Alberta Climate Information Service-ACIS (2020). Current and historical Alberta weather station data viewer. Data provided by Alberta Agriculture and Forestry. Retrieved on May 21 2021, from https://acis.alberta.ca/acis/weather-data-viewer.jsp

AOAC. (1984). Official Methods of Analysis, 14th Edition. AOAC.

Araus, V., Swift, J., Alvarez, J. M., Henry, A., and Coruzzi, G. M. (2020). A balancing act: How plants integrate nitrogen and water signals. Journal of Experimental Botany, 71(15), 4442–4451. https://doi.org/10.1093/jxb/eraa054

Attia-Ismail, S. (2019). Impacts of climate change on forages production, world wide efforts for mitigation and adaptation and the concept of smart-climate agriculture ARIDLANDS: Biodiversity, Management and Conservation, Nova Science Publisher, Inc, New York, USA, 2019, pp.207–233.

Attram, J. (2015). Determination of water use efficiency for forage legume in Southern Alberta with emphasize on dryland and irrigated alfalfa cultivars. University of Lethbridge, Canada.(Publication No. 1585934)[Masters dissertation].

Biederbeck, V.O., and Bourman, O. T. (1994). Water use by annual green manure legumes in dryland cropping systems. Agronomy Journal, 86, 543–549.

Bittman, S., Waddington, J., and McCartney, D. H. (1991). Performance of alfalfa strains grown in mixture with smooth bromegrass as affected by management. Canadian Journal of Plant Science, 71(4), 1029–1037. https://doi.org/10.4141/cjps91-145

Bonin, S.G., and Tomlin, D. C. (1968). Effects of nitrogen on herbage yields of timothy harvested at various stages. Canadian Journal of Plant Science , 48, 501–509. https://doi.org/10.4141/cjps68-097

Bramley, H., Turner, N., and Siddique, K. (2013). Water use efficiency. In Genomics and Breeding for Climate-resilient Crops. (Vol. 2, pp. 225–268). https://doi.org/10.1007/978-3-642-37048-9_6

Campbell, C. A., Zentner, R. P., and Steppuhn, H. (1987). Effect of crop rotations and fertilizers on moisture conserved and moisture use by spring wheat in southwestern Saskatchewan. Canadian Journal of Soil Science, 67, 457–472.

Carlsson, G., and Huss-Danell K. (2014). Does nitrogen transfer between plants confound $^{15}$N-based quantifications of $N_2$ fixation? Plant Soil, 374, 345–358. doi:10.1007/s11104-013-1802-1

Coleman, S.W., and Moore, J. E. (2003). Feed quality and animal performance. Field Crops Research. 84, 17– 29. doi:https://doi.org/10.1016/S0378-4290(03)00138-2

Cutforth, H. W., Jefferson, P. G., Campbell, C. A., and Ljunggren, R. H. (2013). Yield, water use, and protein content of spring wheat grown after six years of alfalfa, crested wheatgrass, or spring wheat in semiarid southwestern Saskatchewan. Canadian Journal of Plant Science, 93(5), 983. https://doi.org/10.4141/CJPS2013-503

De Medeiros, G. A., Arruda, F. B., and Sakai, E. (2005). Crop coefficient for irrigation beans derived using three reference evaporation methods. Agriculture and Forest Meterorology. 115, 135–143.

Dhakal, D., and Anowarul Islam, M. (2018). Grass–legume mixtures for improved soil health in cultivated agroecosystem. Sustainability, 10, 2718. https://doi.org/10.3390/su10082718

Dunham, R. J., and Nye, P. H. (1973). Influence of soil-water content on uptake of ions by roots. Soil-water content gradients near a plane of onion roots. *Journal of applied ecology* 10, 585–598.

Durack, P.J., Wijffels, S.E., and Matear, R. J. (2014). Ocean salinities reveal strong global water cycle intensification during 1950 to 2000. Science 2012, 336, 455–458.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., and Basra, S. M. A (2009). Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development, 29, 185–212.

Gao, Y, Duan, A., Sun, J., Li, F., Liu, Z., Liu, H., and Liu, Z. (2009). Crop coefficient and water-use efficiency of winter wheat/spring maize strip intercropping. Field Crops Research 111, 65–73.

Gelley, C., Ashworth, A., Keyser, P., Nave, R., and Rhinehart, J. (2020). Water- use efficiency of forage crops in the Southeastern United States, Agronomy, 10, 1377. https://doi.org/10.3390/agronomy10091377

Government of Alberta (2020). Agricultural region of Alberta soil inventory database. Accessed on 29 February 2024 at https://www.alberta.ca/agricultural-regions-of-alberta-soil-inventory-database

Gyamfi, H., Hernandez Ramirez, G., Omokanye, A., and Thilakarathna, M. S. (2024). Does diversity of perennial forage species affect soil quality?. [Unpublished manuscript]. University of Alberta, Edmonton, AB.

Hatfield, J. L., and Dold, C. (2019). Water-use efficiency: Advances and challenges in a changing climate. *Frontiers in Plant Science*, *10*, 1–14. https://doi.org/10.3389/fpls.2019.00103

Havlin, J. L., Beaton, J. D., Tisdale, S. L., and Nelson, W. L. (1999). Soil fertility and fertilizers: an introduction to nutrient management. 6th ed. Prentice Hall Inc., Upper Saddle River, NJ. 499 pp.

Hendrickson, J. R., Schmer, M. R., and Sanderson, M. A. (2013). Water use efficiency by switchgrass compared to a native grass or a native grass alfalfa mixture. Bioenergy Research, 6(2), 746–754. https://doi.org/10.1007/s12155-012-9290-3

Holechek, J. L., Pieper, R. D., and Herbel, C. H. (1998). Range management: principles and practices. 3rd ed. Prentice Hall Inc., Upper Saddle River, NJ. 542 pp.

Hussain, T., Hussain, N., Tahir, M., Raina, A., Ikram, S., Maqbool, S., Fraz Ali, M., and Duangpan, S. (2022). Impacts of drought stress on water use efficiency and grain productivity of rice and utilization of genotypic variability to combat climate change. *Agronomy*, 12(10), 2518. https://doi.org/10.3390/agronomy12102518

Hutton, G., Berg, B., Najda, H., Johns, M., Cole, D., and Yoder, C. (2005). Perennial forage establishment in Alberta. Government of Alberta. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9682/$file/120_22-3.pdf?OpenElement

IPCC. (2014). Climate Change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change / R. Pachauri and L. Meyer (editors), Geneva, Switzerland, IPCC, 151p., ISBN: 978-92-9169-143-2.

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid. B., et al. (2015). Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature, 526, 574–77.

Jaleel, C. A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H. J., Somasundaram, R., and Panneerselvam, R. (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. International Journal of Agriculture & Biology, 11, 100–105.

Jefferson, P. G., and Cutforth, H. W. (2005). Comparative forage yield, water use, and water use efficiency of alfalfa, crested wheatgrass and spring wheat in a semiarid climate in southern Saskatchewan. *Canadian Journal of Plant Science*, *85*(4), 877–888. https://doi.org/10.4141/P04-115

Kar, G., Kumar, A., and Martha, M. (2007). Water use efficiency and crop coefficients of dry season oilseed crops. Agricultural Water Management. 87, 73–82.

Kim, K., Daly, E.J, Flesch, T.K., Coates, T.W., and Hernandez-Ramirez, G. (2022). Carbon and water dynamics of a perennial versus an annual grain crop in temperate agroecosystems. Agriculture and Forest Meteorology 314, 108805 https://doi.org/10.1016/j.agrformet.2021.108805

Kulshreshtha, S., and Wheaton, E. (2013). Climate change adaptation and food production in Canada: Some research challenges. In WIT Transactions on Ecology and the Environment (Vol. 170). https://doi.org/10.2495/FENV130101

Lambers, H., Chapin, F., and Pons, T. (2008). Plant physiological ecology. Springer, New York, p 540.

Lee, A., Omokanye, A., and Hernandez-Ramirez, G. (2022). Oat- pea and pea-canola intercropping with and without nitrogen fertilization alter biomass and crude protein water-use efficiencies [Masters dissertation, University of Alberta] Accessed on May 28, 2023, at https://era.library.ualberta.ca

Lenth, R. (2020). Emmeans: Estimated marginal means, aka Least-Squares Means. R package version 1.5.2-1. https://CRAN.R-project.org/package=emmeans

Lindenmayer, B., Hansen, N., Crookston, M., Brummer, J., and Jha, A. (2008). Strategies for reducing alfalfa consumptive water use. p. 52–61. In: Proc. America Geophysical Union Hydrology Days. Colorado State University.

McElrone, A. J., Choat, B., Gambetta, G. A., and Brodersen, C. R. (2013). Water Uptake and Transport in Vascular Plants. Nature Education Knowledge 4(5):6

Moore, A. D., and Ghahramani, A. (2013). Climate change and broadacre livestock production across southern Australia. Impacts of climate change on pasture and livestock productivity, and on sustainable levels of profitability. Global Change Biology, 19, 1440–1455.

Moot, D. J., Brown, H. E., Pollock, K., and Mills, A. (2008). Yield and water use of temperate pastures in summer dry environments. Proceedings of the New Zealand Grassland Association, 70, 51–57. https://doi.org/10.33584/jnzg.2008.70.2724

Naqvi, M., Kumar, D., De, K., and Sejian, V. (2015). Climate change and water availability for livestock: Impact of both quality and quantity. In Climate Change impact on livestock: Adaptation and Mitigation; Sejian, V., Gaughan, J., Baumgard, L., Prasad, C.S., Eds.; Springer-Verlag GMbH Publisher: New Delhi, India, 2015; pp. 81–96. https://doi.org/10.1007/978-81-322-2265-1_6

Omokanye, A., Hernandez-Ramirez, G., Lardner, H. A., Al-Maqtari, B., Gill, K. S., and Lee, A. (2021). Alternative forage feeds for beef cattle in Northwestern Alberta, Canada: Forage yield and nutritive value of forage brassicas and forbs. Journal of Applied Animal Research. DOI: 10.1080/09712119.2021.1933990

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. (2020). Nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-149, URL: https://CRAN.R-project.org/package=nlme

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Schroeder, J. W. (1994). Interpreting forage analysis. Extension Dairy Specialist (NDSU), AS-1080, North Dakota State University. Available online: https://library.ndsu.edu/ir/bitstream/handle/10365/9133/AS-1080-1994.pdf?sequence=2&isAllowed=y (accessed on 6 June 2023).

Siddique K. H. M., Regan, K. L, Tennant, D., and Thomson B. D. (2001). Water use and water use efficiency of cool season grain legumes in low rainfall Mediterranean-type environments. European Journal of Agronomy, 15, 267–280.

Statistics Canada. (2019). Livestock estimates, July 1, 2019. Government of Canada, July 2019, 1–5.

Suhartanto, B., Widodo, S., Kustantinah, and Lestari, E. S. (2019). Potential of forage production on dry land agriculture with mixed cropping pattern. IOP Conference Series: Earth and Environmental Science, 387, 012061. https://doi.org/10.1088/1755-1315/387/1012061

Thilakarathna, M. S., Mcelroy, M. S., Chapagain, T., Papadopoulos, Y. A., and Raizada, M. N. (2016). Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems . A review. Agronomy for Sustainable Development. https://doi.org/10.1007/s13593-016-0396-4

Tilman, D., and J. A. Downing. (1994). Biodiversity and stability in grasslands. *Nature,* 367, 363–365.

Vermeulen, S. J., Campbell, B. M., and Ingram, J. S. I. (2012). Climate change and food systems. Annual Review of Environment and Resources, 37, 195–222. https://doi.org/10.1146/annurev-environ-020411-130608

Volesky, D. J. and Berger, A. (2010). Forage production with limited irrigation. NebGuide, Range and Forage Pasture Management, 4–7. http://extensionpublications.unl.edu./asssest/pdf/g2012.pdf

Wright, A. J., Mommer, L., Barry, K., and Van Ruijven, J. (2021). Stress gradients and biodiversity: monoculture vulnerability drives stronger biodiversity effects during drought years. *Ecology* 102(1). doi: 10.1002/ecy.3193

## 5.6 Tables

Table 5. 1 Cumulative monthly rainfall, maximum and minimum temperatures with their long-time average during the growing season (2021, 2022, and 2023) at Fairview, Alberta, Canada.

| Month | 2020 | 2021 | 2022 | 2023 | Long-term average |
|---|---|---|---|---|---|
| Rainfall (mm month$^{-1}$) | | | | | |
| May | 19.1 | 13.3 | 53.4 | 12.7 | 35.1 |
| June | 67.2 | 43.3 | 60.9 | 60.9 | 94.5 |
| July | 89.8 | 23.5 | 18.6 | 3.5 | 161.4 |
| August | 53.9 | 40.5 | 31.4 | 1.7 | 203.1 |
| Cumulative | 230.0 | 120.6 | 164.3 | 40.8 | 494.1 |
| Maximum air temperature (°C) | | | | | |
| May | 24.8 | 16.6 | 13.8 | 23.2 | 14.8 |
| June | 26.8 | 20.2 | 21.0 | 22.9 | 14.0 |
| July | 29.2 | 22.2 | 23.9 | 23.4 | 15.9 |
| August | 29.2 | 21.3 | 25.5 | 22.5 | 14.6 |
| Minimum air temperature (°C) | | | | | |
| May | –2.3 | 3.1 | 2.6 | 6.4 | 10.3 |
| June | 2.6 | 7.5 | 8.9 | 9.0 | 9.4 |
| July | 7.0 | 9.2 | 10.3 | 10.7 | 11.1 |
| August | 0.3 | 7.7 | 10.8 | 9.7 | 13.7 |

Table 5. 2 A complete treatment list of forage monocultures and mixtures grown at Fairview, Alberta, Canada.

| Monoculture grasses and simple mixtures | Complex mixtures 3-species | 4 or more species |
|---|---|---|
| OG | M7: MB (Fleet) + AL (y) + SF | M0: MB (Fleet) + HB (AC Success) + HB (AC Knowles) + GPWG + CWG |
| GPWG | M8: HB (AC Success) + AL (y) + SF | |
| MB | M11: MB (Fleet) + GPWG + AL (y) | M9: MB (Fleet) + AL (y) + SF + CMV |
| CWG | M12: HB (AC Success) + GPWG + AL (y) | M10: HB (AC Success) + AL(y) + SF + CMV |
| TG | M14: SF + CMV + AL (s5) | M15: HB (AC Success) + GPWG + CWG + IR + SB (Manchar) |
| M1: MB (Fleet) + AL(s5) | M17: HB (AC Success) + AL (y) + SF | |
| M2: HB (AC Success) + AL(y) | M13: TF + SW + STL | M16: AL(y) + AL (R) + CMV + SF +BT |
| M3: HB (AC Success) + AL (s5) | M21: HB (AC Success) + SF + AL(y) | M18: HB (AC Success) + GPWG + AL (y) + SF |
| M4: MB (Fleet) + AL (s5) | | M19: HB (AC Success) + GPWG + IR + AL (y) + SF |
| M5: HB (AC Success) + AL(s5) | | M20: HB (AC Success) + GPWG + AL(R) + AL (y) + CMV + SF |
| M6: HB (AC Knowles) + AL(s5) | | M22: HB (AC Success) + GPWG + AL (y) + CMV + SF + BT |
| | | M23: HB (AC Success) + GPWG + CWG + IR + AL (y) + AL (R) + CMV + SF |

M, mixture; OG, orchardgrass; GPWG, greenleaf pubescent wheatgrass; HB, hybrid bromegrass; CWG, crested wheatgrass; TG, timothy grass; MB, meadow bromegrass; AL(s5), Spredor 5 alfalfa; AL(y), yellowhead alfalfa; AL(R), Rugged alfalfa; SF, sainfoin; CMV, cicer milkvetch; BT, birdsfoot trefoil; IR, Italian ryegrass; SB, smooth bromegrass; TF, tall fescue; SW, slender wheatgrass; STL, Saltander grass.

Table 5. 3 ANOVA *p*-values of seasonal water uptake by perennial forage mixtures and monoculture grasses (2021–2023). See Table 1 for a detailed list of the experimental treatments in the study.

| Treatments | Seasonal water uptake (mm) | | |
|---|---|---|---|
| | **2021** | **2022** | **2023** |
| | **0 - 15.2 cm topsoil layer** | **0 - 25.4 cm topsoil layer** | **0 - 25.4 cm topsoil layer** |
| M0 | 5.8 bcd | 55.0 abc | 13.9 abcd |
| M1 | 6.6 abcd | 54.4 abc | 10.2 bcdefg |
| M2 | 7.5 abcd | 54.6 abc | 12.7 abcde |
| M3 | 9.1 abcd | 46.6 bc | 18.3 a |
| M4 | 9.0 abcd | 44.1 c | 8.5 bcdefg |
| M5 | 8.5 abcd | 50.8 bc | 7.3 cdefg |
| M6 | 7.5 abcd | 53.3 abc | 9.0 bcdefg |
| M7 | 11.6 ab | 52.7 abc | 19.2 a |
| M8 | 6.7 abcd | 53.1 abc | 14.9 abc |
| M9 | 5.5 bcd | 53.2 abc | 7.8 cdefg |
| M10 | 7.9 abcd | 47.2 bc | 9.1 bcdefg |
| M11 | 11.0 ab | 48.9 bc | 9.7 bcdefg |
| M12 | 6.9 abcd | 48.2 bc | 6.3 defg |
| M13 | 10.0 abc | 53.5 abc | 9.4 bcdefg |
| M14 | 9.5 abcd | 49.6 bc | 14.9 abc |
| M15 | 7.6 abcd | 45.8 bc | 6.1 efg |
| M16 | 12.7 a | 47.9 bc | 14.6 abc |
| M17 | 8.1 abcd | 51.3 bc | 15.8 ab |
| M18 | 8.5 abcd | 50.5 bc | 7.8 cdefg |
| M19 | 6.8 abcd | 48.3 bc | 6.7 defg |
| M20 | 9.7 abcd | 46.6 bc | 9.4 bcdefg |
| M21 | 5.6 bcd | 48.4 bc | 13.4 abcde |
| M22 | 8.3 abcd | 46.7 bc | 12.6 abcde |
| M23 | 8.9 abcd | 44.0 c | 15.6 ab |
| TG | 7.8 abcd | 50.8 bc | 3.1 g |
| OG | 3.2 d | 56.7 ab | 13.1 abcde |
| FMB | 7.9 abcd | 64.4 a | 11.6 abcdef |
| GPWG | 3.8 cd | 53.9 abc | 8.7 bcdefg |
| KCG | 6.4 abcd | 51.0 bc | 4.1 fg |
| Mean | 7.9 | 50.7 | 10.8 |
| *P*-value | 0.021 | 0.025 | 0.012 |

Treatments with same letters in the columns indicate no significant difference based on Tukey HSD test. TG denotes timothy grass, OG is orchard grass, FMB connotes Fleet meadow bromegrass, KCG is Kirk crested wheatgrass and GPWG represents greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures while M1 to M6 are simple grass-legume mixtures. Seasonal water uptake by forage treatments was highest in 2022 but lowest in both 2021 and 2023.

## 5.7 Figures

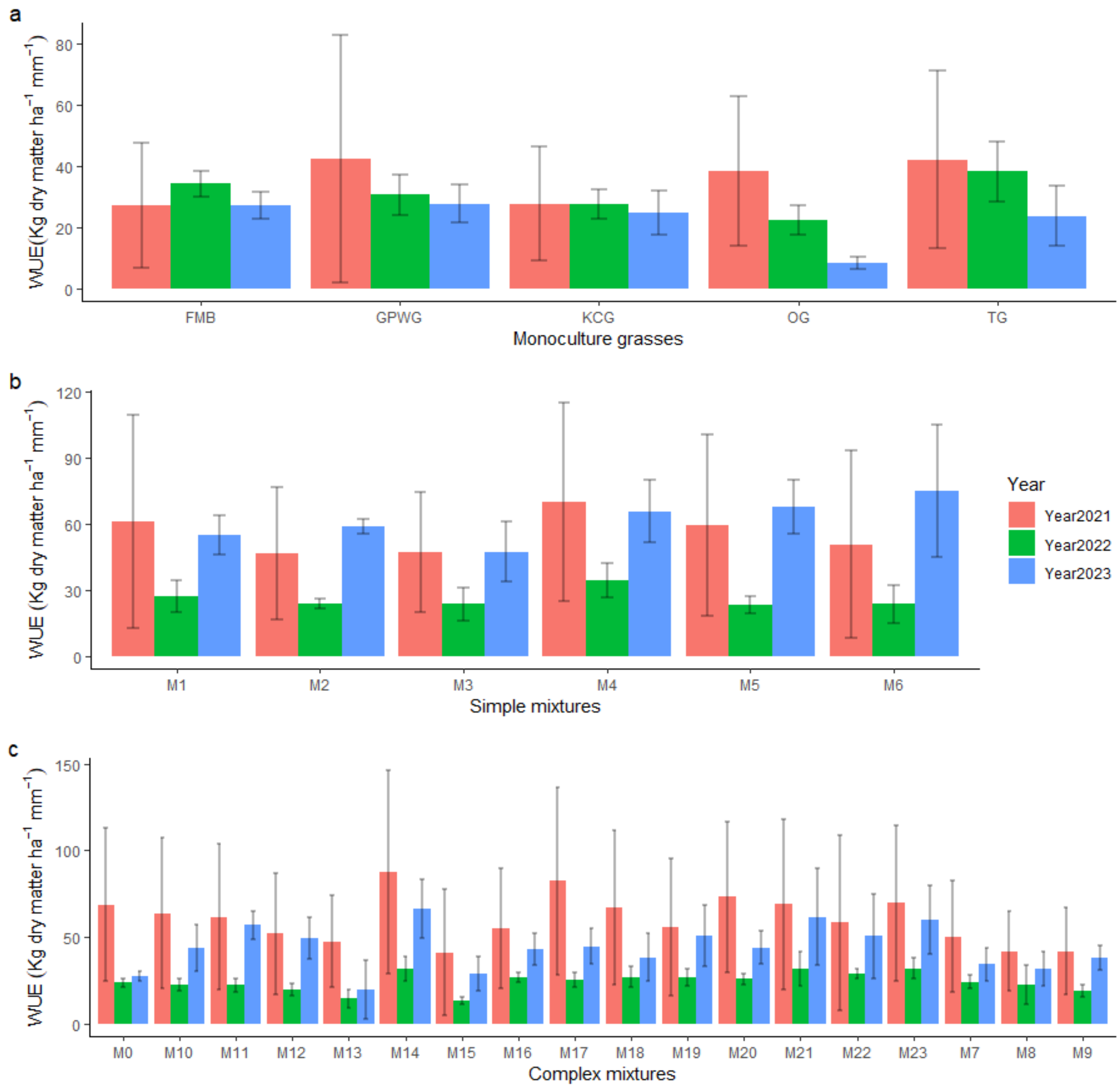


Figure 5. 1 Yearly biomass water-use efficiency ($WUE_{BM}$) of (a) Fleet meadow bromegrass (FMB), greenleaf pubescent wheatgrass (GPWG), Kirk crested grass (KCG), orchardgrass (OG), timothy grass (TG), (b) simple mixtures consisting of one grass and one legume, and (c) complex forage mixtures comprising 3 or more grasses or legumes grown in 2021–2023 at Fairview, Alberta, Canada. The *P*-values were 0.0046, < 0.0001, and < 0.0001 for 2021, 2022, and 2023 respectively, whereas *P*-value of forage treatments x year effects of $WUE_{BM}$ was 0.016. A complete list of simple and complex (M0 to M23) forage mixtures is organized in Table 5.2.

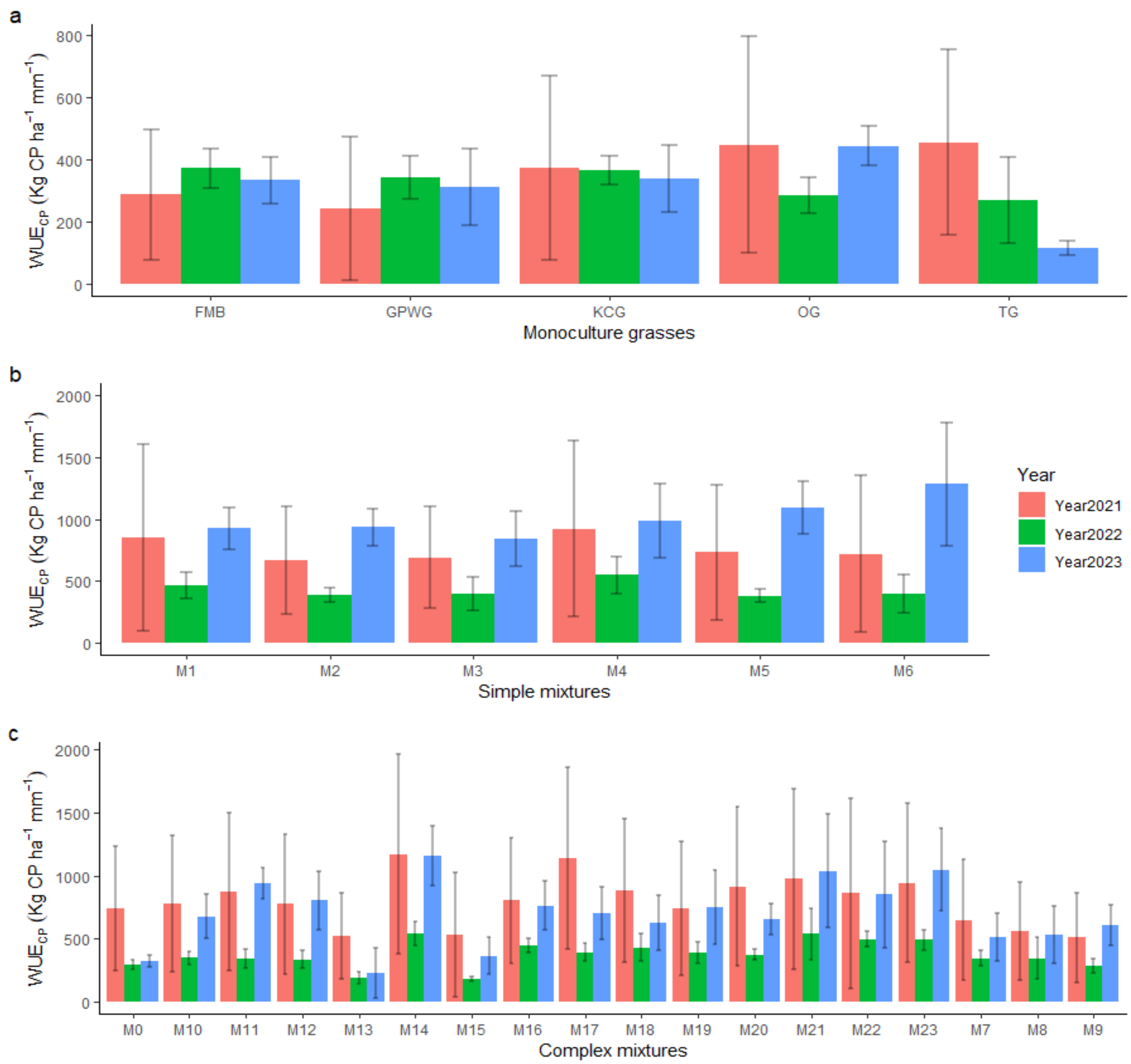


Figure 5. 2 Yearly crude protein water-use efficiency (WUEcp) of (a) Fleet meadow bromegrass (FMB), greenleaf pubescent wheatgrass (GPWG), Kirk crested grass (KCG), orchardgrass (OG), timothy grass (TG), (b) simple mixtures consisting of one grass and one legume, and (c) complex forage mixtures comprising 3 or more grasses or legumes grown in 2021, 2022, and 2023 at Fairview, Alberta, Canada. The *P*-values were 0.0008, < 0.0001, and < 0.0001 for 2021, 2022, and 2023, respectively, whereas *P*-value for forage treatments x year effects of $WUE_{(CP)}$ was <0.001. A complete list of simple and complex (M0 to M23 ) forage mixtures is organized in Table 5.2.

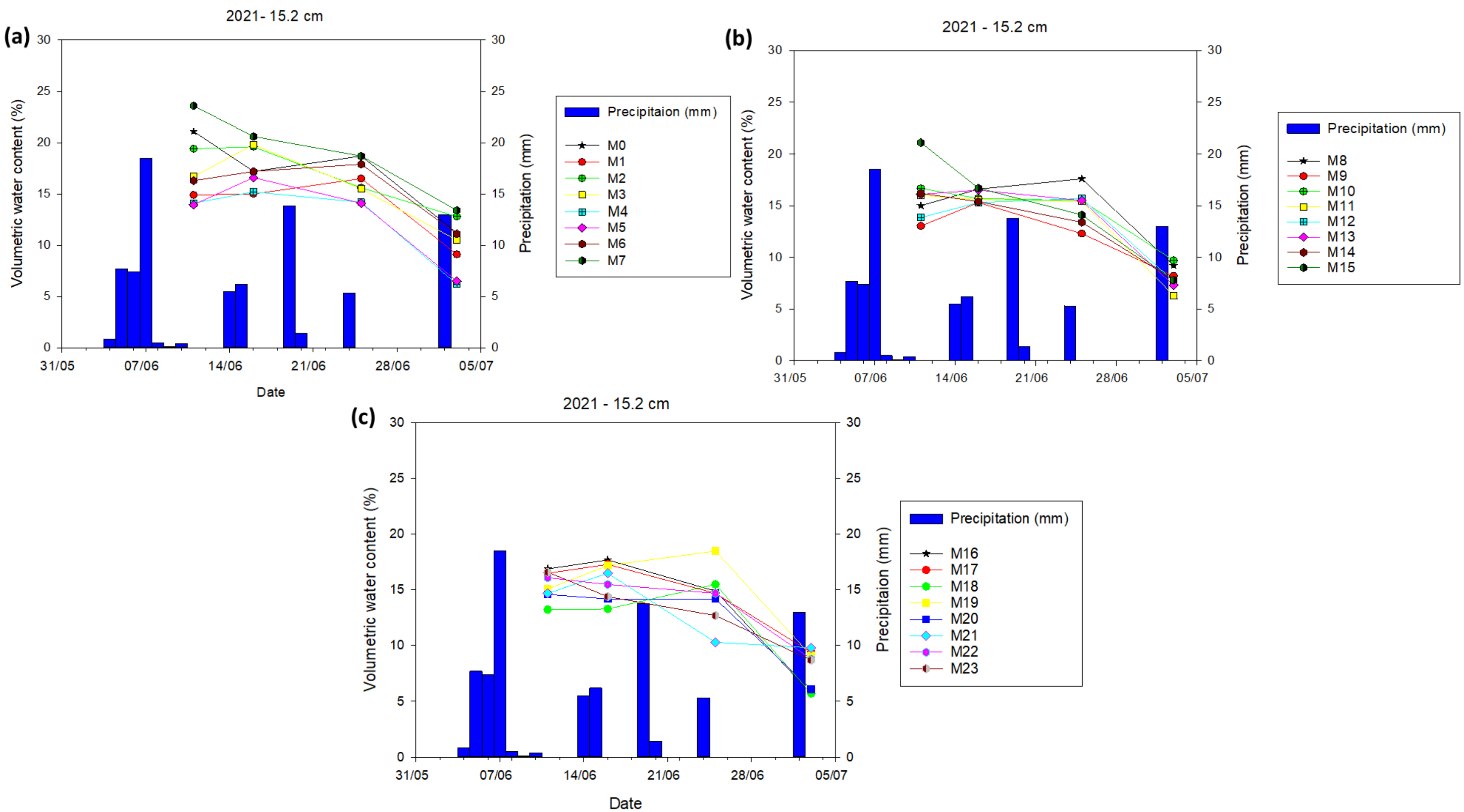


Figure 5. 3 Volumetric water content (%) taken at 0–15.2 cm soil depth for the first growing season (2021) along with daily rainfall (mm $day^{-1}$). The cumulative seasonal rainfall was 120.6 mm. The rainfall patterns showed that soil moisture readings under forage treatments increased after a major rainfall. Grass-only mixtures (M0, M13, and M15) were generally low in soil moisture during the growing season. Forage treatment M1 to M6 are simple grass–legume mixtures whereas M7 to 23 are complex mixtures.

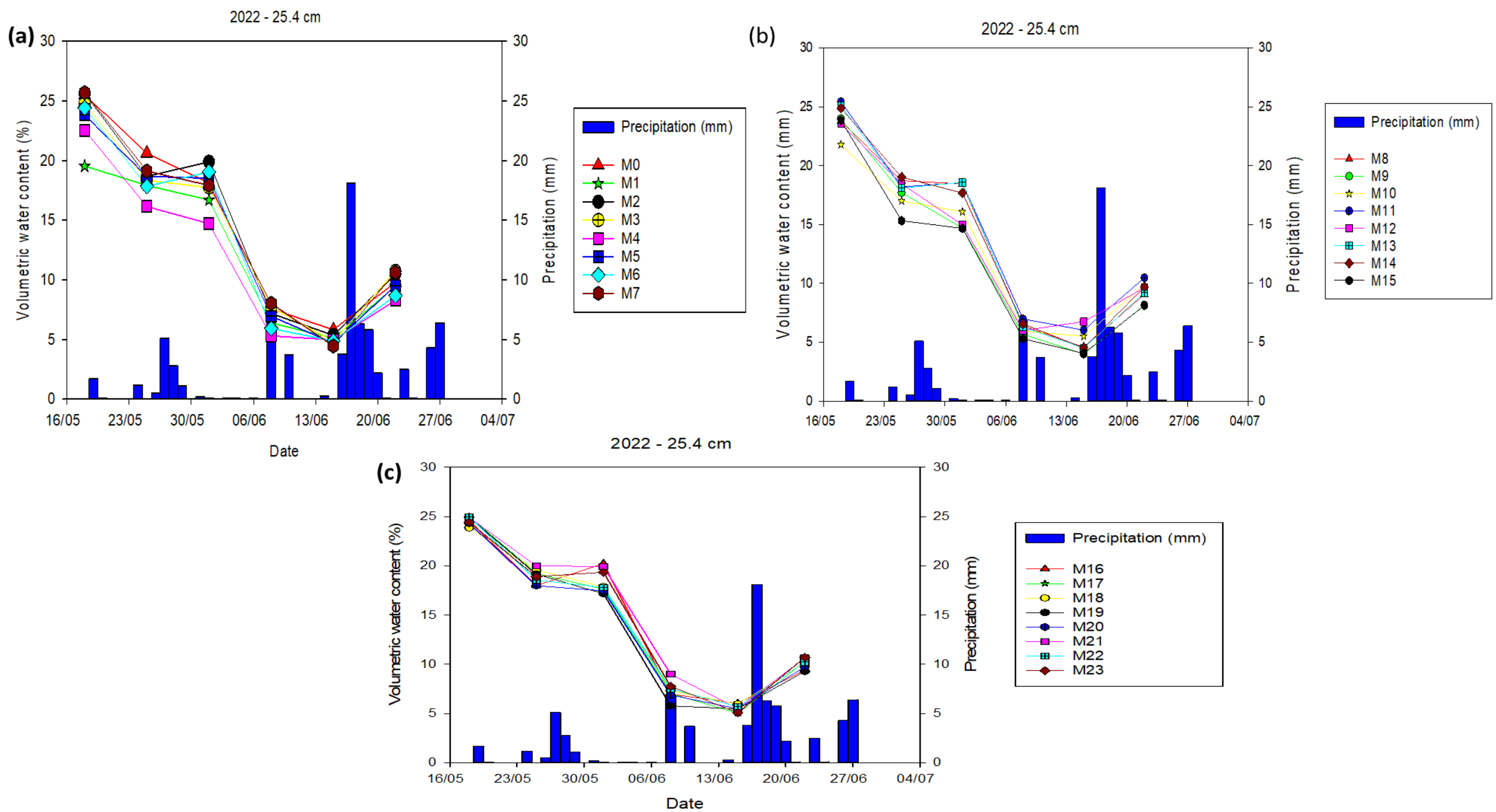


Figure 5. 4 Volumetric water content (%) taken at 0–25.4 cm soil depth for the second growing season (2022) along with daily rainfall (mm day$^{-1}$). The cumulative seasonal rainfall was 164.3 mm. The moisture readings were highest in the early season, but declined gradually mid-season. A critical look at the moisture readings under forage treatments indicated that soil moisture was particularly higher under highly legume-dominated mixtures to compared grass-only mixtures (M0, M13, and M15). Forage treatment M1 to M6 are simple grass–legume mixtures whereas M7 to 23 are complex mixtures.

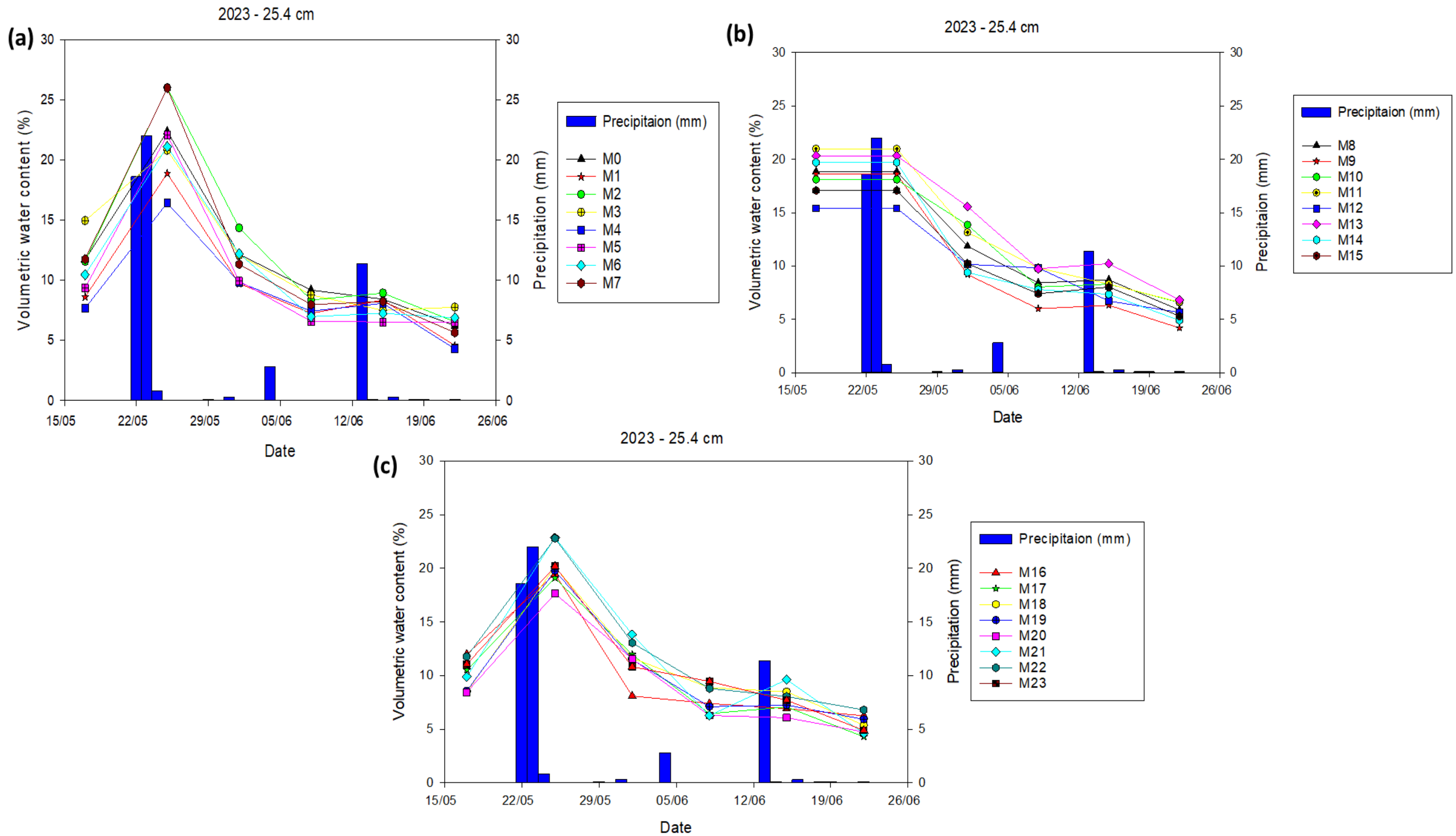


Figure 5. 5 Volumetric water content (%) taken at 0–25.4 cm soil depth for the third growing season (2023) along with daily rainfall (mm day$^{-1}$). The cumulative seasonal rainfall was 40.8 mm. The moisture readings were highest early season due to a possible snow melt but declined gradually mid-season. A critical look at the moisture readings under forage treatments indicated that soil moisture was particularly higher under highly legume-dominated mixtures compared with grass-only mixtures (M0, M13, and M15). Forage treatment M1 to M6 are simple grass–legume mixtures whereas M7 to 23 are complex mixtures.

# Chapter 6. Nitrogen fixation and transfer under simple and complex perennial forage mixtures


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**Study synopsis**

Nitrogen is one of the most important elements in crop production systems and is the most limiting nutrient in agroecosystems. Within grass–legume systems, legumes can fix atmospheric N, which can partially support the N requirements of grasses while reducing the dependency on synthetic sources of N. Nevertheless, skepticism surrounding whether highly diversified grass–legume mixtures enhance the fixation and transfer of N requires attention. To understand this, the study sought to evaluate the fixation and transfer of N in simple and complex grass–legume mixtures under field conditions using a $^{15}N$ dilution technique. This technique involved the application of $^{15}N$ enriched fertilizer to the soil in which both legumes and grasses are growing. Both plants uptake the same ratio of the enriched fertilizer but the legumes reduces its ratio when it incorporates atmospheric nitrogen into the soil. The results showed that all legume species derived more than 95% of their N via biological fixation. The overall ranking of N fixation by legume species across the treatments was alfalfa (*Medicago sativa*) > sainfoin (*Onobrychis viciifolia*) > cicer milkvetch (*Astragalus cicer*). Significant differences were observed among distinct alfalfa and sainfoin cultivars in forage mixtures in the amount of N fixed (ranging from 60.1 to 210.1 kg N $ha^{-1}$ and 37.0 to 52.9 kg N $ha^{-1}$, respectively). Significant differences across mixes of legume species were observed for their N transfers to AC Success hybrid bromegrass (*Bromus riparius* × *Bromus inermis*) and greenleaf pubescent wheatgrass (*Thinopyrum intermedium*), corresponding to N transfers ranging from 19.6 to 36.2 kg N $ha^{-1}$. A complex forage mixture including bromegrass, wheatgrass, alfalfa, and sainfoin (M18) showed the highest N transfer of 36.2 kg N $ha^{-1}$. The results suggested that the fixation and transfer of N are influenced by increasing the species richness as well as the proportion of legumes to grass within a given forage mixture.

**Keywords**: nitrogen fixation, nitrogen transfer, grass–legume mixtures, symbiosis, *Rhizobium,* $^{15}N$ dilution method

## 6.0 Introduction

About 78% of Earth's atmosphere is nitrogen gas ($N_2$), making it a dominant element in nature (Stein and Klotz, 2016). Nitrogen is an essential plant nutrient for agricultural production. However, N is the most limiting crop production factor globally (Fageria, 2014). Synthetic N and other forms of fertilizers have been utilized in agricultural systems to augment crop yields in many countries since the invention of the Haber Bosch N fixation process; however, the excessive use of N inputs has resulted in devastating environmental problems such as eutrophication of water, groundwater contamination, global warming, loss of biodiversity, and stratospheric ozone depletion (Lee and Jones-Lee, 2005; Rütting et al., 2018; Chai et al., 2019).

With sustainable agriculture gaining more attention over the years, N fixation by legumes via a symbiotic relationship with *Rhizobia* bacteria (Carranca, 2013; Walker et al., 2015) instead of synthetic fertilizers has become a reliable alternative (Garg and Geetanjali, 2007; Fustec et al., 2010). Furthermore, the ability of pure stands of legumes or grass–legume combinations to fix atmospheric $N_2$ has been explored in agricultural systems, particularly for forage production (Campillo et al., 2005; Nyfeler et al., 2011). For instance, Heichel et al. (1981) reported an average of 148 kg $ha^{-1}$ of N fixed through symbiosis by two populations of alfalfa (*Medicago sativa*) during the growing season. Sainfoin (*Onobrychis viciifolia*) also has the ability to fix biological N, even though some authors found lower amounts of fixed N compared with alfalfa and clover (*Trifolium repens*) (Provorov and Tikhonovich, 2003; Hardarson and Atkins, 2003; Prosser et al., 2006). In addition, cicer milkvetch (*Astragalus cicer*) has been reported to fix substantial amounts of $N_2$ in pure and mixed stands (Papastylianou, 1988; Papastylianou and Danso, 1989). For example, Papastylianou and Danso (1990) found that vetch biologically fixed approximately 95 kg N $ha^{-1}$ at 106 days after emergence in a vetch–oat (*Avena sativa*) mixtures, whereas between 43.3 and 59.3 kg N $ha^{-1}$ was reported in vetch–switchgrass (*Panicum virgatum*) mixtures (Ashworth et al., 2017). In addition, other studies have highlighted the transfer of N to grasses within grass–legume mixtures (Ta and Faris, 1987; Høgh-Jensen and Schjoerring, 1997, 2000; Rasmussen et al., 2007; Thilakarathna et al., 2012, 2016a). For instance, Høgh-Jensen and Schjoerring (2000) reported that the average amount of N transferred from clover to ryegrass (*Lolium* spp.) were 1.7 and 3.6 g $m^{-2}$ in the first and second production years, respectively. However, these studies focused on the fixation and transfer of N within simple grass–legume mixtures. Therefore, knowledge gaps still exist in quantifying biological N fixation and the transfer of N within multiple legume and grass species grown together in diversified forage cropping systems under field conditions.

Within grass–legume systems, the fixed N benefits the legumes, their companions, or subsequent crops (Bullied et al., 2002) through N transfer, as demonstrated in many grass–legume mixtures (Pirhofer-Walzl et al., 2012; Thilakarathna et al., 2016a; Islam and Adjesiwor, 2018). Nitrogen transfer is the movement of N from one living plant (the donor) to another (the receiver) in a community of diversified plant populations usually from a legume to non-legumes (Høgh-Jensen and Schjoerring, 2000; Moyer-Henry et al., 2006). According to Yong et al. (2015), N transfer occurs in both directions; however, the majority of N tends to flow from relatively high N fixers to non-fixers. Nitrogen transfer also promotes more efficient utilization of N, reduces N losses, and maintains good biomass production (Thilakarathna et al., 2016a). It has been reported that the transfer of N from legumes constitutes up to 50% of the N in non-legumes (Soussana and Hartwig, 1996). Furthermore, N-transfer routes in forage systems can be categorized as belowground and aboveground (Høgh-Jensen and Schjoerring, 2000; Watt et al., 2003; Peoples et al., 2015; Thilakarathna et al., 2016a). By contrast, aboveground N transfer takes place through the decomposition of leaf and shoot litter and animal excreta through grazing (Ledgard, 2001; Thilakarathna et al., 2016a). In the context of grass–legume forage systems, belowground N transfer can involve: (1) decomposition of the root tissues of legumes and the subsequent uptake of the released N by neighboring plants (Munroe and Isaac, 2014), (2) root exudation of soluble N compounds by legumes and their uptake by receivers (Paynel et al., 2008), and (3) the transfer of N mediated by plant-associated mycorrhizae (Thilakarathna et al., 2016a).

A plethora of studies have described methods for quantifying N transfer within grass–legume mixtures in the field, namely, the natural abundance of $^{15}N$, $^{15}N$ isotope dilution, and total N difference (Unkovich and Pate, 2000; Carlsson and Huss-Danell, 2003; Thilakarathna et al., 2016b). The $^{15}N$ natural abundance method relies on the natural $^{15}N$ enrichment of plant-available soil N relative to atmospheric $N_2$ (Schipanski and Drinkwater, 2012). It also depends on significant differences in the $^{15}N$: $^{14}N$ ratio between atmospheric $N_2$ and the soil N pool which the legumes and non-legumes are using (Unkovich et al., 1993). The $^{15}N$ dilution method relies on the introduction of external $^{15}N$-enriched fertilizer into the soil where both the fixing plant (legume) and the non-fixing plant (grass) are cultivated (Danso, 1986). The drawback is that the availability of N in the soil and its uptake may differ between the test and reference crops. In contrast the total N difference technique is based on comparisons between the total N yield in a N-fixing crop and a non-fixing reference crop. However, the uptake of soil N may be affected when the root architecture of the reference crop is N-deficient (Mårtensson and Ljunggren, 1984).

A thorough understanding of the mechanisms that govern the fixation and transfer of N is pertinent for assessing its losses to the environment and its benefits in agricultural forage systems. We hypothesized that the fixation and transfer of $N_2$ by legumes would increase with increasing species richness. Hence, this project intended to generate knowledge about the fixation and transfer of N among mixtures with single legume versus two or more legumes and grasses in the field during the forage production year after establishment of the stand. The objectives of this study were to (i) quantify the biological $N_2$ fixation under simple and complex grass–legume mixtures, and (ii) determine the $N_2$ transferred to neighboring grasses within simple and complex grass–legume mixtures and (iii)

## 6.1 Material and methods

### 6.1.1 Experimental site and weather

A perennial forage field experiment was established at the research farm of the Peace Country Beef & Forage Association in Fairview, northwestern Alberta, Canada (56°0453″ N, 118° 26′05″W, 670 m above sea level) in June 2020 and was evaluated for three production years (2021, 2022, and 2023). The selected field had a canola (*Brassica napus*) crop stubble. The field had a history of several years of a wheat (*Triticum aestivum*)–canola rotation for grain production. The soil's pH and organic matter content were 5.2 and 3.1%, respectively. The soil's classification is Eluviated Black Chernozem according to the Canadian System of Soil Classification (Government of Alberta, 2020). The weather information during the growing seasons and their long-term averages for the site (Table 6.1) were obtained from the Alberta Climate Information Service (2020).

### 6.1.2 Experimental design and forage treatments

The experimental design for the trial was a randomized complete block design with four replications ($n = 4$). The treatments consisted of one simple grass–legume mixture (one grass and one legume) and four complex diverse perennial forage species mixtures (i.e., three or more grasses and legumes) (Table 6.2). In addition, two pure stands of perennial grass were used as the reference plants. The grass species used in this study were wheatgrass (*Thinopyrum intermedium*; varieties- greenleaf pubescent) and hybrid bromegrass (*Bromus riparius x B. inermis*; variety- AC Success, Table 6.2). In this study, we used AC Success hybrid bromegrass and greenleaf pubescent wheatgrass (*Thinopyrum intermedium*) as the reference plants, and the legume species AC Yellowhead alfalfa (*Medicago sativa* ssp. *falcata*), AC Mountainview sainfoin, and Veldt cicer milkvetch.

### 6.1.3 Establishment of perennial forage stands

The seeding rates for the treatments were based on those recommended for monocultures of perennial forages given by Hutton et al. (2005) (Table 6.2). Each experimental seeding plot was 2 m wide and 8 m long. An adequate alley space (2–3 m) was left between the block replicates. Before seeding, the alfalfa seeds were inoculated with Nitragin Gold alfalfa inoculants from Northstar Seeds, Edmonton, Canada, at a rate of 1.5 g per 250 g of seeds, whereas cicer milkvetch, and sainfoin received no inoculants because of the lack of commercially available inoculants. All grasses and legumes were seeded at a depth of 1.3 cm on 26 June 2020 with a six-row Fabro plot drill equipped with disc-type openers at a 23 cm row spacing. In the establishment year (2020) and the first production year (2021), the plots were sprayed with Basagran Forte at 4.05 mL $ha^{-1}$ for in-crop weed control. Additionally, dandelions (*Taraxacum officinale*), volunteer wheat, and canola were occasionally handpicked. Subsequently, in 2022–2023, no further weed control measures (chemical spraying or manual) were carried out.

### 6.1.4 The $^{15}N$ dilution method

Using the simplified mass balance of the two end members along with $^{15}N$ isotopic trace labeling (Thilakarathna and Hernandez-Ramirez, 2021), the fixation and transfer of atmospheric N was quantified. The $N_2$ fixation was estimated using the $^{15}N$ dilution method (Danso, 1986). This method relies on the differential dilution of $^{15}N$-labeled fertilizer in the soil and fixed N in plants (Fried and Middelboe, 1977). In this method, both fixing and non-fixing plants are established in soils to which the same amount of $^{15}N$ enriched fertilizer is applied. The assumption here is that both plants uptake the same ratio of $^{15}N/^{14}N$, and hence will contain the same amount of $^{15}N/^{14}N$. However, the fixing plant has a lower ratio of $^{15}N/^{14}N$ because of the N incorporated from the air (dinitrogen; $N_2$) into the system (Harderson and Danso, 1993).

### 6.1.5 Using $^{15}N$ labelling to determine the fixation and transfer of $N_2$

At the beginning of the third year of forage production (2023), plots for $^{15}N$ labeling were selected out of the 29 treatments seeded in 2020. These included the following experimental treatments: M2 (containing alfalfa and hybrid bromegrass), M8 (containing alfalfa, sainfoin, and hybrid bromegrass), M10 (containing alfalfa, sainfoin, cicer milkvetch, and hybrid bromegrass), M18 (containing alfalfa, sainfoin, hybrid bromegrass, and pubescent wheatgrass), and M21 (containing alfalfa, sainfoin, and hybrid bromegrass), and pure stands of AC Success hybrid bromegrass and greenleaf pubescent wheatgrass. Each consisted of four replicates. Subsequently, a 46 cm × 30 cm quadrat microplot was inserted into the soil (2 cm depth) to isolate the legumes from their grass counterparts within the mixtures for $^{15}N$ labelling. Similarly, pure stands of AC Success hybrid bromegrass and greenleaf pubescent wheatgrass were isolated with similar quadrat microplots. Both the legumes in the mixtures and the pure grasses inside the quadrat were uniformly labeled with 10 atom% $^{15}N$ potassium nitrate (Sigma-Aldrich, Oakville, ON, Canada) on 8 May 2023, at 0.73 kg N $ha^{-1}$, according to the $^{15}N$ dilution procedure (Mallarino et al., 1990). The water-based $^{15}N$ solution (10 L $m^{-2}$) was applied to the marked area inside the quadrat and the same volume of water (10 L $m^{-2}$) was carefully used to water it down.

### 6.1.6 Forage harvest and analysis

Forage plants within the 46 × 30 cm (0.138 $m^2$) quadrats were harvested when 50% of the legumes in the mixtures had blossomed. Grasses and legumes were clipped with scissors to a stubble height of 4 cm from the ground. The herbage from the mixture was separated by species. Subsequently, the harvested material was dried separately at 65°C in a forced air oven to determine the dry matter (DM) yield. Dried species were individually ground using a Wiley mill (standard model 3, Arthur H. Thomas Co., Philadelphia, USA) to pass through a 1-mm sieve, followed by a bead mill (Retsch, Germany). The ground samples were encapsulated in 5-mg batches using 8 mm × 5 mm micro elemental capsules. The samples were then analyzed for $\delta^{15}N$ and total N in the aboveground DM using a mass spectrometer (Costech ECS4010 Elemental Analyzer coupled to a Delta V mass spectrometer) (Thilakarathna et al., 2016b). The proportion of N derived from the atmosphere by the legume species in the mixtures (%Ndfa) was calculated according to the $^{15}N$ dilution method (Hardarson and Danso, 1993).

$$\%\ \mathrm{Ndfa} = \left(1 - \frac{atom\%\ ^{15}N\ excess_{(legume)}}{atom\%\ ^{15}N\ excess_{(pure\ grass)}}\right) \mathrm{x}\ 100 \qquad \text{Eq. [1]}$$

where *%Ndfa* is the percentage of nitrogen derived from the atmosphere, *atom%* $^{15}N$ *excess* $_{(legume)}$ is the atom% of $^{15}N$ of legumes in mixtures, and *atom%* $^{15}N$ *excess* $_{(pure\ grass)}$ is the atom% of $^{15}N$ in pure grasses.

An estimate of the fixed N transferred from legumes to grass in mixed plots was determined as follows (Thilakarathna et al., 2016b):

$$\%\ \mathrm{N\ transfer} = \left(1 - \frac{atom\%\ ^{15}N\ excess_{(mix\ grass)}}{atom\%\ ^{15}N\ excess_{(pure\ grass)}}\right) \mathrm{x}\ 100 \qquad \text{Eq. [2]}$$

where *atom%* $^{15}N$ *excess* $_{(mix\ grass)}$ is the atom% of $^{15}N$ of grasses in the mixtures and *atom%* $^{15}N$ *excess* $_{(pure\ grass)}$ is the atom% of $^{15}N$ in pure grasses.

Once the plant N had been partitioned into fixed fractions based on %Ndfa, the amount of $N_2$ fixed on a mass basis (g N $m^{-2}$) was calculated as follows (Peoples et al., 2002):

$$N\ Fixed = \frac{(\%Ndfa\ x\ plant\ N)}{100} \qquad \text{Eq. [3]}$$

where the amount of plant N is derived from the DM in DM $m^{-2}$ multiplied by the percentage of tissue N content. In addition, the amount of N transferred to grasses on a mass basis (g N $m^{-2}$) was:

$$N\ transfer = \frac{(\%\ N\ transfer\ x\ total\ grass\ N)}{100} \qquad \text{Eq. [4]}$$

Subsequently, N fixed and N transferred (g N $m^{-2}$) were converted to units kg N $ha^{-1}$.

### 6.1.7 Statistical analysis

The data on N fixation by legumes and N transfer during the 2023 growing season were analyzed with the NLME program using a mixed effect model with analysis of variance (Pinheiro et al. 2020), in R statistical software (version 4.2.3). Legume and grass species were considered to be fixed effects, whereas block replicates were considered to be random effects. Before the analysis of variance, the normality of the residuals was tested using the Shapiro–Wilk statistic, and the homogeneity of variance was tested using Levene's test. No data transformations were required, as the residuals of all the data were homogeneous and normally distributed. Following the analysis of variance, Tukey's honestly significant difference test was used to separate the treatment means when they were significant ($P < 0.05$).

## 6.2 Results

### 6.2.1 Dry matter and N concentration in plant tissues

The DM ($P = 0.041$) and tissue N concentration ($P = 0.006$) were different in alfalfa among the forage treatments. The N concentration in plant tissues ranged from 1.7 to 2.9%, whereas DM ranged from 31.8 to 103.3 g DM $m^{-2}$ (Table 6.3). Similarly, there were differences in the DM and N concentration in sainfoin among the forage treatments ($P = 0.011$ and $P = 0.017$, respectively). The DM ranged from 23.3 to 35.0 g DM $m^{-2}$, whereas the N concentration ranged from 1.5 to 2.3%. Cicer milkvetch recorded the least DM (14.3 g DM $m^{-2}$). Generally, alfalfa had more DM and higher N concentrations than sainfoin and cicer milkvetch. For example, alfalfa had 68 and 89 g DM $m^{-2}$ more than sainfoin and cicer milkvetch, respectively in M18 (Table 6.3).

### 6.2.2 The proportions of N derived from the atmosphere (% Ndfa) and fixed N

All legume species within the treatments, namely AC Yellowhead alfalfa, AC Mountainview sainfoin, and Veldt cicer milkvetch, had a high N fixation capacity (%Ndfa) of more than 94% (Table 6.4). The %Ndfa of AC Yellowhead alfalfa was not significantly different ($P = 0.143$) among the different forage mixture treatments. This value ranged from 95.74% for M2 (containing alfalfa and hybrid bromegrass) to 99.02% for M10 (containing alfalfa, sainfoin, cicer milkvetch, and hybrid bromegrass). However, the amount of N fixed (kg N $ha^{-1}$) by AC Yellowhead alfalfa was significantly different ($P = 0.024$) among the treatments. Similar to

%Ndfa, M10 had the highest amount of fixed N (210.1 kg N ha$^{-1}$), followed by M21 (containing alfalfa, sainfoin, and hybrid bromegrass) (176.1 kg N ha$^{-2}$) (Table 6.4). There was no significant difference in %Ndfa for AC Mountainview sainfoin ($P$ = 0.228) among the treatments, ranging from 97.1% for M18 (containing alfalfa, sainfoin, hybrid bromegrass, and pubescent wheatgrass) to 99.48% for M10 (Table 6.4). However, the amount of fixed N (kg N ha$^{-1}$) was significantly different, with that in M10 being slightly higher (52.9 kg N ha$^{-1}$), followed by both M8 (containing alfalfa, sainfoin, and hybrid bromegrass) and M21 (50.0 kg N ha$^{-1}$ each), and M18 (37.0 kg N ha$^{-1}$) (Table 6.4). The %Ndfa for Veldt cicer milkvetch was 98.3%, whereas the amount of fixed N was 23.9 kg N ha$^{-1}$. Generally, our results showed that AC Yellowhead alfalfa fixed a greater amount of N than AC Mountainview sainfoin and Veldt cicer milkvetch. For instance, alfalfa fixed 43.5, 157.2, 23.1, and 126.1 kg N ha$^{-1}$ more than sainfoin in M8, M10, M18, and M21, respectively. Similarly, alfalfa fixed 186.2 kg N ha$^{-1}$ more than cicer milkvetch within M10 (Table 6.4).

The total amount of fixed N (kg N ha$^{-1}$) was significantly different ($P$ = 0.018) among the legume species within the forage treatments (Table 6.4). M10, consisting of AC Yellowhead alfalfa, AC Mountainview sainfoin, and Veldt cicer milkvetch legume species, had the highest amount of fixed N (286.9 kg N ha$^{-1}$). This was followed by M21 (226.1 kg N ha$^{-1}$), including AC Yellowhead alfalfa and AC Mountainview sainfoin; and M2 (159.4 kg N ha$^{-1}$), which included only AC Yellowhead alfalfa. The lowest amount of fixed N was found in M18 (97.1 kg N ha$^{-1}$), which contained AC Yellowhead alfalfa and AC Mountainview sainfoin (Table 6.4).

### 6.2.3 Nitrogen transfer

The proportion of N transferred to grasses was not significantly different among the treatments ($P$ = 0.82). The percentage of N transferred to grasses ranged from 49.5% for AC Success hybrid bromegrass to 60% for greenleaf pubescent wheatgrass (Table 6.5). Notably, there was a significant difference in the percentage of N transferred among the treatments with AC Success hybrid bromegrass species ($P$ = 0.032) (Table 6.5). Similarly, the amount of N transferred (kg N ha$^{-1}$) was significantly different among the grass species, ranging from 18.1 to 30.4 kg N ha$^{-1}$. The amount of N transferred was higher for AC Success hybrid bromegrass in M2 (30.4 kg N ha$^{-1}$) and M21 (29.7 kg N ha$^{-1}$), whereas it was lowest in M10 (19.6 kg N ha$^{-1}$) and M18 (18.1

kg N ha$^{-1}$) for both AC Success hybrid bromegrass and greenleaf pubescent wheatgrass (Table 6.5).

The total amount of N transferred was significantly different among treatments with grass species ($P$ = 0.015). The highest amount of N transferred was observed in M18 (36.2 kg N ha$^{-1}$), which contained AC Success hybrid bromegrass and greenleaf pubescent wheatgrass (Table 6.5). Both M2 (30.4 kg N ha$^{-1}$) and M21 (29.7 kg N ha$^{-1}$) were similar, whereas the lowest was recorded in M8 (23.9 kg N ha$^{-1}$) and M10 (19.6 kg N ha$^{-1}$) (Table 6.5). According to the total amount of N that was fixed versus the amount of N that was transferred, 37.3% of the total N fixed by the legumes in M18 was transferred to grasses, whereas less (6.8%) was transferred to the grasses in M10.

## 6.3 Discussion

*Proportion of N derived from the atmosphere and the amount of fixed N*

As expected, we observed a high %Ndfa with alfalfa, which was inoculated and is known to have a higher $N_2$-fixing capacity than other legumes (Pirhofer-Walzl et al., 2012). We were surprised to also find a high $N_2$ fixation capacity in sainfoin (>97%) and cicer milkvetch (>98%), as these have a lower $N_2$ fixation capacity and were not inoculated in our trials because of the lack of commercially available inoculants. In addition, sainfoin and cicer milkvetch are not widely grown in the area, and thus *rhizobia*-forming bacteria appropriate to these species were not expected to be present in these soils.

Evidently, our results for %Ndfa from biological N fixation were higher than the typical %Ndfa values reported in the literature: 88% for alfalfa, 80% for sainfoin, and 70% for cicer milkvetch (Kozhemyakov and Tikhonovich 1998). This is explained in part by how AC Success hybrid bromegrass and greenleaf pubescent wheatgrass were used as the reference plants in this study. This is because the N uptake patterns in these grasses are different from those in legumes and can affect the %Ndfa values. As posited by Goh (2007), %Ndfa values are highly dependent on the reference plants. To avoid disparities in the %Ndfa values between the $N_2$-fixing legumes and the non-fixing reference plants, the reference plant should be selected on basis of their close similarity to the $N_2$-fixing plant in terms of the phenology, root profile, and pattern of utilizing soil N pools (Ibewiro et al., 2000). However, it is difficult to ascertain whether the reference plant can obtain N from the same soil sources as the $N_2$-fixing plant. Therefore, the estimates and comparisons of %Ndfa between these two distinct plants will be compromised. This explains why the $N_2$ fixation by the legumes in our study did not conform to what had been reported from the literature. Nonetheless, alfalfa consistently fixed a higher amount of N (kg N $ha^{-1}$) within the treatments compared with both sainfoin and cicer milkvetch. This was reflected in the overall yield of alfalfa at harvest (Table 6.3). Kumar et al. (2021) postulated that N is imperative for vegetative growth and the expansion of crop plants, including synthesis of starch in leaves and the synthesis of protein, which affect crop yield. This is also consistent with Gierus et al. (2012), who stated that there is a positive correlation between the amount of N fixed and legumes' DM yield. The high N fixed by alfalfa compared with sainfoin and cicer milkvetch can be linked to the efficiency of the association between alfalfa and *Rhizobium meliloti* used in our study. This is because the *Rhizobium meliloti*–alfalfa association is one of the most efficient associations between $N_2$-fixing bacteria and legumes (de Oliveira et al., 2004; Issah et al., 2020).

The total amount of N fixed (kg N $ha^{-1}$) by legume species was higher in treatments with a high number of legume species. This was also influenced by the proportion of legumes in the treatments. For instance, a complex grass–legume mixture (M10), consisting of 25% alfalfa, 25% sainfoin, and 25% cicer milkvetch (i.e., a total of 75% legumes), had the highest total amount of fixed N compared with the simple mixture (M2), consisting of only 50% alfalfa. Interestingly, M8 (containing 33.3% alfalfa and 33.3% sainfoin; i.e., 66.6% legumes), and M18 (containing 25% alfalfa and 25% sainfoin; i.e., 50% legumes) had the lowest total amount of fixed N compared with M2 (i.e., 50% alfalfa). This indicates that although N fixation is partly influenced by the number of legume species, it is also linked to the intrinsic characteristics of the legume species, as well as their proportions within the mixtures. Our results are in line with Mulder et al. (2002), who found that the proportion of legumes planted in a mixture had a stronger effect on N fixation.

The estimated amount of N fixed by alfalfa (up to 210 kg N $ha^{-1}$) during the third year of forage production in our study is similar to the fixation rates reported in other studies in temperate regions of North America (Burity et al., 1989; Kelner et al., 1997; McCaughey and Chen, 1999; Issah et al., 2020). For instance, Burity et al. (1989) estimated that under field conditions in Eastern Canada, alfalfa plants typically fixed 93 kg N $ha^{-1}$ in the first year, but this substantially increased to 258 and 227 kg N ha$^{-1}$ in the second and third years, respectively, whereas Issah et al. (2020) reported that ~200 kg N ha$^{-1}$ was fixed by alfalfa in Saskatoon.

The estimated N fixation rates of sainfoin (37–52.9 kg N ha$^{-1}$) and cicer milkvetch (23.9 kg N ha$^{-1}$) in this study are comparatively lower than values from other studies (130–160 kg N ha$^{-1}$ for sainfoin and 40–65 kg N ha$^{-1}$ for cicer milkvetch) (Provorov and Tikhonovich, 2003; Issah, et al., 2020). The lower amount of N fixed by sainfoin and cicer milkvetch can be partly attributed to our inability to inoculate them before seeding because of the lack of commercially available inoculants. Although sainfoin and cicer milkvetch achieved >95 %Ndfa, this high proportion did not translate to enhanced DM yield relative to alfalfa's performance (Table 6.3), and hence their rates of N fixation were limited.

*Proportion and amount of N transferred*

More than 50% of the N used by grasses was transferred from the legumes within the forage mixtures (i.e., AC Success hybrid bromegrass and greenleaf pubescent wheatgrass). This agrees with Soussana and Hartwig (1996), who reported that the transfer of N from legumes constituted up to 50% of the N used by the companion non-legume plants. Notably, the variations in N transfer were not always commensurate with the number of legumes present in the mixture as expected. Our results revealed that although a high proportion of legumes in grass–legume mixtures may increase the amount of N fixed, this did not mean that a higher proportion of N was transferred to the neighboring grasses. This is because the N fixed by legumes can be impeded from reaching the neighbouring grasses by both soil and plant conditions. This is in agreement with Fierer and Schimel (2002), who posited that drought conditions can severely affect the fixation and transfer of N by restricting the movement of N, as it is water-soluble and requires water to move through the soil column. In line with De Silva et al. (2023), the low availability of water in the root zone can significantly reduce the transfer and bioavailability of nutrients, resulting in nutrient deficiency. In addition, the spatial arrangement and proximity of roots affect N transfer; more N compounds are shared among plant roots that are in close contact, and this decreases as the distance between them increases (Rasmussen et al., 2013; Thilakarathna et al., 2016a). This was noted in M8, which contained 33.3% alfalfa and 33.3% sainfoin, where 50% of the N was used by AC Success hybrid bromegrass, whereas in M18 (25% alfalfa + 25% sainfoin), 52% of the N in AC Success hybrid bromegrass originated from N fixation by legumes, but 60% of the N in greenleaf pubescent wheatgrass came from legumes (Table 6.5). Moreover, in contrast to both M8 and M18, it is plausible that in some cases, the higher presence of legumes in the mixtures further increased the proportion of N transferred to companion grasses, as noted in M10 (Table 6.5).

Mycorrhizal fungi that interconnect the roots can also influence the transfer of N to the neighbouring grasses. According to Newman (1988), up to 80% of the N can be transferred to non-$N_2$-fixing plants through mycorrhizal fungal networks. This indicates that a better connection between the $N_2$-fixing plant and fungi is crucial for enhancing the N transfer process, and vice versa. However, the fungi function best with sufficient water within the soil. Although we did not monitor the presence of mycorrhizal fungi, we speculate that the N transfer under most treatments was enabled by the fungi.

This study demonstrated that the amount of N transferred to grasses was higher in treatments containing over 50% alfalfa legumes. This is contrary to the findings of Pirhofer-Walzl et al. (2012), who reported low N transfer in alfalfa because of its deep tap roots and the small number of secondary roots. The total amount of N transferred was also greater in treatments with more grass species. This was expected, because individual grasses take up N differently and the sum may supersede that in treatments with a single grass. For instance, although M18 contained 25% AC Success hybrid bromegrass and 25% greenleaf pubescent wheatgrass (i.e., 50% grass), and M2 contained 50% AC Success hybrid bromegrass, M18 still had a greater total amount of transferred N than M2. This was also confirmed by the botanical composition of the grasses in the treatments. During 2023, when our N transfer measurements were conducted, the botanical composition of AC Success hybrid bromegrass and greenleaf pubescent wheatgrass was 19.9% and 21.3%, respectively, in M18 (i.e., 40.3%), whereas 31.8% AC Success hybrid bromegrass was found in M2 (unpublished data by Gyamfi et al.). This substantiates the higher amount of N transferred when two grasses are present (M18) than when only one grass is in the forage mixture.

## 6.4 Conclusion

This study highlights that perennial legumes within forage mixtures are effective in acquiring N via $N_2$ fixation, and that N is transferred to the neighbouring grasses under field conditions. However, the amount of N that is fixed is influenced by the quantity, proportions, and individual characteristics of the legumes in the mixtures. The total amount of fixed N was generally associated with treatments that had a high proportion of legume species; thus, N fixation was directly proportional to species richness. Similarly, forage mixtures with different legume species enhanced both biomass yield and N fixed. High amounts of N were transferred in grass–legume mixtures containing multiple grass species. However, this was not evidently influenced by the quantity of N fixed, but probably by the differing ability of individual grass species to take up N. This indicates that the non-fixing plant (grass) is crucial in N transfer. In summary, the fixation and transfer of N were higher under complex grass–legume mixtures than in simple mixtures. Though this study was based on only one production year of forages (the third harvest year), these findings could still guide the decision-making process of growers who seek to use perennial forage species to improve their soil and the sustainability of livestock–forage systems.

**Statement for data availability**

The authors intend to release the data used for this research upon request.

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**Conflict of interest**

The authors declare no conflict of interest.

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## 6.5 References


Alberta Climate Information Service (2020). Current and historical Alberta weather station data viewer. Accessed on 21 May 2021 from https://acis.alberta.ca/acis/weather-data-viewer.jsp

Ashworth, A. J., Allen, F. L., Warwick, K. S., Keyser, P. D., Bates, G. E., Tyler, D. D., Lambdin, P. L., and Pote, D. H. (2017). $N_2$ fixation of common and hairy vetches when intercropped into switchgrass. *Agronomy*, 7, 39, 1–10. https://doi.org/10.3390/agronomy7020039

Bullied, W. J., Entz, M. H., Smith, S. R., and Bamford, K. C. (2002). Grain yield and N benefits to sequential wheat and barley crops from single-year alfalfa, berseem and red clover, chickling vetch and lentil. *Canadian Journal of Plant Science,* 82(1), 53–65. https://doi.org/10.4141/P01-044

Burity, H. A., Ta, T. C., Faris, M. A., and Coulman, B. E. (1989). Estimation of nitrogen fixation and transfer from alfalfa to associated grasses in mixed swards under field conditions. *Plant and Soil*, 114, 249–255.

Campillo, R., Urquiaga, S., and Undurraga, P., Pino, I., and Boddey, R. M. (2005). Strategies to optimise biological nitrogen fixation in legume and grass pastures in the southern region of Chile. *Plant and Soil*, 273, 57–67. https://doi.org/10.1007/s11104-004-6717-4

Carlsson, G., and Huss-Danell, K. (2003). Nitrogen fixation in perennial forage legumes in the field. Plant and Soil, 253, 353–372.

Carranca, C. (2013). Legumes: properties and symbiosis. In: Camisão, A.H. and Pedroso, C.C. (Eds). *Symbiosis: Evolution, Biology and Ecological Effects*. Nova Science Publishers, New York. pp. 67–94.

Chai, R., Ye, X., Ma, C., Wang, Q., Tu, R., Zhang, L., and Gao, H. (2019). Greenhouse gas emissions from synthetic nitrogen manufacture and fertilization for main upland crops in China. *Carbon Balance and Management,* 14(1), 1–10. https://doi.org/10.1186/s13021-019-0133-9

Danso, S. K. A. (1986). Review: Estimation of $N_2$- fixation by isotope dilution: an appraisal of techniques involving $^{15}N$ enrichment and their application. *Soil Biology and Biochemistry*, 18, 243–244.

de Oliveira, W. S., Oliveira, P. P. A., Corsi, M., Duarte, F. R. S., and Tsai, S. M. (2004). Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with *Sinorhizobium meliloti*. *Science of Agriculture*, 61, 433–438.

De Silva, C., Rathor, P., Poudel, H. P., and Thilakarathna, M. S. (2023). Effects of drought stress on red clover–grass mixed stands compared to grass monoculture stands in nitrogen-deficient systems. *Nitrogen*, *4*, 382–396. https://doi.org/10.3390/nitrogen4040027

Fageria, N. K. (2014). Nitrogen harvest index and its association with crop yields. *Journal of Plant Nutrition*, *37*(6), 795–810. https://doi.org/10.1080/01904167.2014.881855

Fierer, N., and Schimel, J. P (2002). Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, 34, 777–787. doi:10.1016/S0038-0717(02)00007-X

Fried, M., and Middelboe, V. (1977). Measurement of the amount of nitrogen fixed by a legume crop. *Plant and Soil*, 47, 713–715. https://doi.org/10.1007/BF00011042

Fustec, J., Lesuffleur, F., Mahieu, S., and Cliquet, J.-B. (2010). Nitrogen rhizodeposition of legumes. A review. *Agronomy for Sustainable Development*, 30, 57–66. https://doi.org/10.1051/agro/2009003

Garg, N., and Geetanjali, M. (2007). Symbiotic nitrogen fixation in legume nodules: process and signaling. A review. *Agronomy for Sustainable Development*, 27 (1), 59–68.

Gierus, M., Kleen, J., Loges, R., and Taube, F. (2012). Forage legume species determine the nutritional quality of binary mixtures with perennial ryegrass in the first production year. *Animal and Feed Science Technology*, 172:150–161. doi:10.1016/j.anifeedsci.2011.12.026

Goh, K. M. (2007). Effects of multiple reference plants, season, and irrigation on biological nitrogen fixation by pasture legumes using the isotope dilution method. *Community in Soil Science and Plant Analysis*, 38, 1841–1860.

Government of Alberta (2020). Agricultural region of Alberta soil inventory database. Accessed on 29 February 2024 at https://www.alberta.ca/agricultural-regions-of-alberta-soil-inventory-database

Hardarson, G., and Atkins, G. (2003). Optimizing biological $N_2$ fixation by legumes in farming system. *Plant and Soil*, 252: 41–54.

Hardarson, G., and Danso, S. (1993). Methods for measuring biological nitrogen fixation in grain legumes. *Plant And Soil*, 152: 19–23.

Heichel, G. H., Barnes, D. K., and Vance, C. P. (1981). Nitrogen fixation of alfalfa in the seeding year. *Crop Science*, 21, 330–335 https://doi.org/10.2135/cropsci1981.0011183X002100020032x

Høgh-Jensen, H., and Schjoerring, J. K. (1997). Interactions between white clover and ryegrass under contrasting nitrogen availability: $N_2$ fixation, N fertilizer recovery, N transfer and water use efficiency. *Plant and Soil*, 197:187–199.

Høgh-Jensen, H., Schjoerring, J.K. (2000). Below-ground nitrogen transfer between different grassland species: direct quantification by $^{15}N$ leaf feeding compared with indirect dilution of soil $^{15}N$. *Plant and Soil*, 227, 171–183. https://doi.org/10.1023/A:1026535401773

Hutton, G., Berg, B., Najda, H., Johns, M., Cole, D., and Yoder, C. (2005). Perennial forage establishment in Alberta. Accessed on 21 March 2024 at https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9682/$file/120_22-3.pdf?OpenElement

Ibewiro, B., Sanginga, N., Vanlauwe, B., and Merckx, R. (2000). Evaluation of symbiotic dinitrogen inputs of herbaceous legumes into tropical cover-crop systems. *Biology and Fertility of Soils*, 32, 234–242.

Islam A, M., and Adjesiwor, A. T. (2018). Nitrogen fixation and transfer in agricultural production systems. In Amanullah and Fahad, S. (Eds.) *Nitrogen In Agriculture – Updates*. Accessed on 21 March 2024 at https://doi.org/DOI: 10.5772/intechopen.71766

Issah, G., Schoenau, J. J., Lardner, H. A., and Knight, J. D. (2020). Nitrogen fixation and resource partitioning in alfalfa (*Medicago sativa* L.), cicer milkvetch (*Astragalus cicer* L.) and sainfoin (*Onobrychis viciifolia* Scop.) using $^{15}N$ enrichment under controlled environment conditions. *Agronomy*, 10, 1438. doi:https://doi.org/10.3390/agronomy10091438

Kelner, D. J., Vessey, J. K., Entz, M. H. (1997). The nitrogen dynamics of 1-, 2- and 3-year stands of alfalfa in a cropping system. *Agriculture, Ecosystem and Environment*, 64, 1–10.

Kozhemyakov, A. P., and Tikhonovich, I. A. (1998). Application of the legume inoculants and the biopreparations of complex action in agriculture. [in Russian.] *C.R. Russian Academy of Agricultural Science.* 6, 7–10.

Kumar, S., Kumar, S., and Mohapatra, T. (2021). Interaction between macro- and micro-nutrients in plants. *Frontiers in Plant Science*, 12, 665583. https://doi.org/10.3389/fpls.2021.665583

Ledgard, S. F. (2001) Nitrogen cycling in low input legume-based agriculture, with emphasis on legume–grass pastures. *Plant and Soil*, 228, 43–59. doi:10.1023/A:1004810620983

Lee, G. F., and Jones-Lee, A. (2005). Eutrophication (excessive fertilization), water surface and agricultural water. In: Lehr, J. H., Keeley, J. W., Lehr, J. K. and Kingery, T. B. (Ed.). *Water Encyclopedia*. John Wiley & Sons, Hoboken, NJ. Pp. 535–541. https://doi.org/10.1002/047147844x.sw1606

Mallarino, A. P., Wedin, W. F., Perdomo, R. S., Goyenola, R. S., and West, C. P. (1990). Nitrogen transfer from white clover, red clover, and birdsfoot trefoil associated grass. *Agronomy Journal*, 82, 790–795.

Mårtensson, A. M., and Ljunggren, H. D. (1984). A comparison between the acetylene reduction method, the isotope dilution method, and the total nitrogen difference method for measuring nitrogen fixation in lucerne (*Medicago sativa* L.). *Plant and Soil*, 81, 177–184. https://doi.org/10.1007/BF02197149

McCaughey, W. P., and Chen, W. (1999). Benefits of including forage legumes in pastures and their management. In Proceedings of the Western Canadian Forage and Grazing Conference, Saskatoon, SK, Canada, 8–10 December 1999. Saskatchewan Stock Grower Association, Saskatoon, SK, Canada; pp. 97–115.

Moyer-Henry, K. A., Burton, J. W., Israel, D. W., and Rufty, T. W. (2006). Nitrogen transfer between plants : A $^{15}N$ natural abundance study with crops and weed species. *Plant and Soil*, 282, 7–20. https://doi.org/10.1007/s11104-005-3081-y

Mulder, C. P. H., Jumpponen, A., Högberg, P., and Huss-Danell, K. (2002). How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. *Oecologia*, 133, 412–421.

Munroe, J. W., and Isaac, M. E. (2014). $N_2$-fixing trees and the transfer of fixed-N for sustainable agroforestry: a review. 34, 417–427. https://doi.org/10.1007/s13593-013-0190-5

Newman, E. I. (1988). Mycorrhizal links between plants: their functioning and ecological significance. *Advances in Ecological Research*, 18, 243–71.

Nyfeler, D., Huguenin-Elie, O., Suter, M., and Frossard, E. (2011). Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystem and Environment*, 140, 155–163. https://doi.org/10.1016/j.agee.2010.11.022

Papastylianou I. (1988). The $^{15}N$ methodology in estimating N: fixation by vetch and pea grown in pure stand or in mixtures with oat. *Plant and Soil*, 107, 183–188.

Papastylianou I., and Danso S. K. A. (1989). Effect of nitrogen fertilization and cropping system of the reference crop on estimation of $N_2$ fixation by vetch using $^{15}N$ methodology. *Plant and Soil*, 114, 227–233.

Papastyliasou, I., and Danso, S. K. A. (1990). Nitrogen vetch–oat mixtures. *Soil Biology and Biochemistry*, 23(5), 447–452.

Paynel, F., Lesuffleur, F., Bigot, J., Diquélou, S., and Cliquet, J.-B. (2008). A study of $^{15}N$ transfer between legumes and grasses. *Agronomy for Sustainable Development*, 28, 281–290.

Peoples, M. B., Boddey, R. M., and Herridge, D. F. (2002). Quantification of nitrogen fixation. In Leigh, G. J. (Ed.) *Nitrogen Fixation at the Millennium* (pp. 357–389). Elsevier.

Peoples, M. B., Chalk, P. M., Unkovich, M. J., and Boddey, R. M. (2015). Can differences in $^{15}N$ natural abundance be used to quantify the transfer of nitrogen from legumes to neighboring non-legume plant species? *Soil Biology and Biochemistry*, 87, 97–109. https://doi.org/10.1016/j.soilbio.2015.04.010

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. (2020). Nlme: linear and nonlinear mixed effects models. R package version 4–149. Accessed on 29 February 2024 at https://CRAN.R-project.org/package=nlme

Pirhofer-Walzl, K., Rasmussen, J., Høgh-Jensen, H., Eriksen, J., Søegaard, K., and Rasmussen, J. (2012). Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil*, 350(1–2), 71–84. https://doi.org/10.1007/s11104-011-0882-z

Prosser, J., Ignacio Rangel-Castro, J., and Killham, K. (2006). Studying plant–microbe interactions using stable isotope technologies. *Current Opinion in Biotechnology*, 17, 98–102.

Provorov, N., and Tikhonovich, I. (2003). Genetic resources for improving nitrogen fixation in legume–rhizobia symbiosis. *Genetic Resources and Crop Evolution*, 50, 89–99.

Rasmussen, J., Eriksen, J., Jensen, E. S., Esbensen, K. H., and Høgh-Jensen, H. (2007). In situ carbon and nitrogen dynamics in ryegrass–clover mixtures: transfers, deposition, and leaching. *Soil Biology and Biochemistry*, 39, 804–815.

Rasmussen, J., Gylfadóttir, T., Loges, R., Eriksen, J., and Helgadóttir, A. (2013). Spatial and temporal variation in N transfer in grass–white clover mixtures at three Northern European field sites. *Soil Biology and Biochemistry*, 57, 654–662. doi:10.1016/j.soilbio.2012.07.004

Rütting, T., Aronsson, H., and Delin, S. (2018). Efficient use of nitrogen in agriculture. *Nutrient Cycling in Agroecosystems*, 110(1), 1–5. https://doi.org/10.1007/s10705-017-9900-8

Schipanski, M. E., and Drinkwater, L. E. (2012). Nitrogen fixation in annual and perennial legume–grass mixtures across a fertility gradient. *Plant and Soil*, 357(1), 147–159. https://doi.org/10.1007/s11104-012-1137-3

Soussana, J. F., and Hartwig, U. A. (1996). The effects of elevated $CO_2$ on symbiotic $N_2$-fixation: a link between the carbon and nitrogen cycles in grassland ecosystems. *Plant and Soil*, 187, 321–332.

Spehn, E. M., Schmid, B., Hector, A., Caldeira, M. C., Dimitrakopoulos, P. G., Finn, J. A., Jumpponen, A., Donnovan, G. O., Pereira, J. S., Schulze, E., Troumbis, A. Y., and Ko, C. (2002). The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. *Oikos,* 98, 205–218.

Stein, L. Y., and Klotz, M. G. (2016). The nitrogen cycle. *Current Biology*, *26*(3), R94–R98.

Ta, T. C., and Faris, M. A. (1987). Species variation in the fixation and transfer of nitrogen from legumes to associated grasses. *Plant and Soil*, 98, 265–274.

Thilakarathna, M. S., Mcelroy, M. S., Chapagain, T., Papadopoulos, Y. A., and Raizada, M. N. (2016a). Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. *Agronomy for Sustainable Development*. https://doi.org/10.1007/s13593-016-0396-4

Thilakarathna, M. S., Papadopoulos, Y. A., Rodd, A.V. et al. (2016b) Nitrogen fixation and transfer of red clover genotypes under legume–grass forage based production systems. *Nutrient Cycling In Agroecosystems*, 106, 233–247. doi:10.1007/s10705-016-9802-1

Thilakarathna, R. M. M. S., Papadopoulos, Y. A., Rodd, A. V., Gunawardena, A. N., Fillmore, S. A. E., and Prithiviraj, B. (2012). Characterizing nitrogen transfer from red clover populations to companion bluegrass under field conditions. *Canadian Journal of Plant Science,* 92(6): 1163–1173. https://doi.org/10.4141/cjps2012-036

Thilakarathna, S.K., Hernandez-Ramirez, G. (2021). How does management legacy, nitrogen addition and nitrification inhibition impact soil organic matter priming and nitrous oxide production? *Journal of Environmental Quality*, 50, 78–93. https://doi.org/10.1002/jeq2.20168

Unkovich, M. J., and Pate, J. S. (2000). An appraisal of recent field measurements of symbiotic $N_2$ fixation by annual legumes. Field Crops Research, 65, 2–3.

Unkovich, M. J., Pate, J. S., and Sanford, P. (1993). Preparation of plant samples for high precision nitrogen isotope ratio analysis. *Communications in Soil Science and Plant Analysis*, 24, 2093–2106.

Walker, R., Agapakis, C. M., Watkin, E., and Hirsch, A. M. (2015). Symbiotic nitrogen fixation in legumes: perspectives on the diversity and evolution of nodulation by *Rhizobium* and *Burkholderia* species. In de Bruijn, F. J. (Ed.) Biological Nitrogen Fixation (pp. 913–923). Wiley. https://doi.org/10.1002/9781119053095.ch89

Watt, M. S., Clinton, P. W., Whitehead, D., Richardson, B., Mason, E. G., and Leckie, A. C. (2003). Above-ground biomass accumulation and nitrogen fixation of broom (*Cytisus scoparius* L.) growing with juvenile *Pinus radiata* on a dryland site. *Forest Ecology and Management, 184*, 93–104. https://doi.org/10.1016/S0378-1127(03)00151-8

Yong, T., Liu, X., Yang, F., Song, C., Wang, X., Liu, W., Su, B., Zhou, L., and Yang, W. (2015). Characteristics of nitrogen uptake, use and transfer in a wheat–maize–soybean relay intercropping system. Plant Production Science, *18*, 388–397.

## 6.6 Tables

Table 6. 1 Rainfall, maximum and minimum temperatures with their long-term average during the growing season (2021–2023) at Fairview, Alberta, Canada.

| Month | 2020 | 2021 | 2022 | 2023 | Long-term average |
|---|---|---|---|---|---|
| Rainfall (mm month$^{-1}$) | | | | | |
| May | 19.1 | 13.3 | 53.4 | 12.7 | 35.1 |
| June | 67.2 | 43.3 | 60.9 | 60.9 | 94.5 |
| July | 89.8 | 23.5 | 18.6 | 3.5 | 161.4 |
| August | 53.9 | 40.5 | 31.4 | 1.7 | 203.1 |
| Cumulative | 230.0 | 120.6 | 164.3 | 40.8 | 494.1 |
| Maximum air temperature (°C) | | | | | |
| May | 24.8 | 16.6 | 13.8 | 23.2 | 14.8 |
| June | 26.8 | 20.2 | 21.0 | 22.9 | 14.0 |
| July | 29.2 | 22.2 | 23.9 | 23.4 | 15.9 |
| August | 29.2 | 21.3 | 25.5 | 22.5 | 14.6 |
| Minimum air temperature (°C) | | | | | |
| May | –2.3 | 3.1 | 2.6 | 6.4 | 10.3 |
| June | 2.6 | 7.5 | 8.9 | 9.0 | 9.4 |
| July | 7.0 | 9.2 | 10.3 | 10.7 | 11.1 |
| August | 0.3 | 7.7 | 10.8 | 9.7 | 13.7 |

Table 6. 2 A complete list and seeding rates of perennial forage species selected for the study in Fairview, Alberta, Canada.

| Simple mixture and pure grasses | Seeding rate (kg ha$^{-1}$) | Complex mixtures | Seeding rates (kg ha$^{-1}$) |
|---|---|---|---|
| M2 (50% AC Yellowhead alfalfa + 50% AC Success hybrid bromegrass) | 10.1 | M8 (33.3% AC Success hybrid bromegrass +33.3% AC Yellowhead alfalfa +33.3% AC Mountainview sainfoin) | 20.2 |
| AC Success hybrid bromegrass (100%) | 12.3 | M10 (25% AC Success hybrid bromegrass +25% AC Yellowhead alfalfa +25% AC Mountainview sainfoin + 25% Veldt cicer milkvetch) | 7.2 |
| Greenleaf pubescent wheatgrass (100%) | 11.2 | | |
| | | M18 (25% AC Success hybrid bromegrass +25% greenleaf pubescent wheatgrass + AC Yellowhead alfalfa + 25% AC Mountainview sainfoin) | 17.4 |
| | | M21 (30% AC Success hybrid bromegrass + 50% AC Yellowhead alfalfa + 20% AC Mountainview sainfoin) | 15.6 |

Table 6. 3 The N concentration in plant tissues and dry matter (DM) of legume and grass species under simple and complex grass–legume mixtures during the third production season (2023).

| Treatments | AC Yellowhead alfalfa | AC Mountainview sainfoin | Veldt cicer milkvetch | AC Success hybrid bromegrass | Greenleaf pubescent wheatgrass |
|---|---|---|---|---|---|
| | % N in plant tissue | | | | |
| M2 | 2.7 a | - | - | 1.9 | - |
| M8 | 2.7 a | 2.0 a | - | 1.6 | - |
| M10 | 2.9 a | 2.1 a | 1.7 | 1.5 | - |
| M18 | 1.7 b | 1.5 b | - | 1.7 | 1.5 |
| M21 | 2.5 a | 2.3 a | - | 2.0 | - |
| *P*-value | 0.006 | 0.017 | nc | 0.021 | nc |
| Treatments | DM (g DM $m^{-2}$) | | | | |
| M2 | 85.3 b | - | - | 38.3 | - |
| M8 | 50.3 c | 35.0 a | - | 37.5 | - |
| M10 | 103.3 a | 35.0 a | 14.3 | 27.3 | - |
| M18 | 31.8 d | 23.3 c | - | 30.8 | 28.0 |
| M21 | 100.8 a | 29.8 b | - | 33.3 | - |
| *P* value | 0.041 | 0.011 | nc | 0.037 | nc |

nc, no comparison; M2, simple mixtures; M8–M21, complex grass–legume mixtures. Lowercase letters indicate significant differences within a column.

Table 6. 4 The proportion of N derived from the atmosphere (%Ndfa) and the total amount of N fixed by legume species under simple and complex grass–legume mixtures during the third production growing season (2023).

| Treatments | % Ndfa | | |
|---|---|---|---|
| | AC Yellowhead alfalfa | AC Mountainview sainfoin | Veldt cicer milkvetch |
| M2 | 95.74 a | - | - |
| M8 | 97.29 a | 98.47 a | - |
| M10 | 99.02 a | 99.48 a | 98.3 |
| M18 | 94.6 a | 97.12 a | - |
| M21 | 98.14 a | 99.41 a | - |
| *P*-value | 0.143 | 0.228 | nc |

| Treatments | N fixed by legumes (kg N ha$^{-1}$) | | | Total |
|---|---|---|---|---|
| | AC Yellowhead alfalfa | AC Mountainview sainfoin | Veldt cicer milkvetch | |
| M2 | 159.4 b | - | - | 1594 c |
| M8 | 93.5 d | 50.0 a | - | 143.5 c |
| M10 | 210.1 a | 52.9 a | 23.9 | 286.9 a |
| M18 | 60.1 e | 37.0 b | - | 97.1 d |
| M21 | 176.1 c | 50.0 a | - | 226.1 b |
| *P*-value | 0.024 | 0.033 | nc | 0.018 |

nc, no comparison; M2, simple mixtures; M8–M21, complex grass–legume mixtures. Lowercase letters indicate significant differences within a column.

Table 6. 5 The amount of N transferred (as a percentage and in kg N $ha^{-1}$) by legumes to two grass species under simple and complex grass–legume stands.

| Treatments | % N transferred | |
|---|---|---|
| | AC Success hybrid bromegrass | Greenleaf pubescent wheatgrass |
| M2 | 53.27 b | - |
| M8 | 49.52 c | - |
| M10 | 65.96 a | - |
| M18 | 52.67 b | 60 |
| M21 | 56.25 b | - |
| *P*-value | 0.022 | nc |

| Treatments | N transferred (kg N $ha^{-1}$) | | | Total transferred N relative to total fixed N (%) |
|---|---|---|---|---|
| | AC Success hybrid bromegrass | Greenleaf pubescent wheatgrass | Total | |
| M2 | 30.4 a | - | 30.4 b | 19.1 b |
| M8 | 23.9 b | - | 23.9 c | 16.8 b |
| M10 | 19.6 c | - | 19.6 c | 6.8 d |
| M18 | 18.1 c | 18.1 | 36.2 a | 37.3 a |
| M21 | 29.7 a | - | 29.7 b | 13.1 c |
| *P*-value | 0.032 | nc | 0.015 | 0.012 |

nc, no comparison; M2, simple mixtures; M8–M21, complex grass–legume mixtures. Lowercase letters indicate significant differences within a column.

**Chapter 7.**

# Predicting the performance of beef cattle on diverse perennial forage mixtures using the CowBytes ration-balancing software program

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## Study synopsis

Quality forage with the required proportions of nutrients impacts the performance of beef cattle by improving their body condition scores, growth, reproductive development, and functions. The objective of this paper was to evaluate twenty-nine perennial forage treatments consisting of five monoculture grasses as control, six simple mixtures, and eighteen complex mixtures using the CowBytes® ration-balancing software to balance rations and predict the performances of three different classes of beef cattle (steers, dry gestating, and lactating beef cows). The input data included forage dry matter (DM) yield and nutritional value of the forage treatments measured over three years of forage harvest (2021, 2022, and 2023). A target weight gain of 0.8 kg $day^{-1}$ by steers and limiting feed intake to 1.2% neutral detergent fiber (NDF) as % body weight (BW) were baseline parameters. Barley straw as a cheaper energy source was included in the model simulations as a filler to reduce feed cost as well as to limit energy and protein supplied to pregnant and lactating cows when feeding the different forage mixtures. Higher weight gains were predicted when single treatment feed intakes were limited to NDF criteria. Complex grass–legume mixtures, AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + AC Yellowhead alfalfa (M12), AC Yellowhead alfalfa + rugged alfalfa + Veldt cicer milkvetch + AC Mountainview sainfoin + Birdsfoot trefoil (M16), AC Success hybrid bromegrass + AC Yellowhead alfalfa + AC Mountainview sainfoin (M17), AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + AC Yellowhead alfalfa + AC Mountainview sainfoin (M18), and AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + AC Yellowhead alfalfa + Veldt cicer milkvetch + AC Mountainview sainfoin + Birdsfoot trefoil (M22) and simple mixtures of Fleet meadow bromegrass (MB) + AC Yellowhead alfalfa (M1) and AC Knowles hybrid bromegrass + AC Yellowhead alfalfa (M3) had the highest predicted gains in both 2021 and 2023. Conversely, in 2022, simple mixtures (M1) and AC Knowles hybrid bromegrass + Spredor 5 alfalfa (M6), timothy grass monoculture, and grass-only mixtures of AC Saltander wheatgrass + Tall fescue + Slender wheatgrass (M13) and AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + Kirk crested wheatgrass + Italian rye grass + Manchar smooth bromegrass (M15) were the better options. The ration parameters for dry gestating beef cows were set to obtain an average daily gain (ADG) of 0.10 kg $day^{-1}$, without exceeding NDF limits. In 2021, grass-only mixture (M13) and orchard grass monoculture were the best in supporting cows in obtaining an ADG of 0.10 kg $day^{-1}$. However, simple mixture of Fleet meadow bromegrass + Spredor 5 alfalfa (M4) and complex

grass–legume mixture of AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + rugged alfalfa + AC Yellowhead alfalfa + Veldt cicer milkvetch + AC Mountainview sainfoin (M20), as well as Fleet MB and greenleaf pubescent wheatgrass monocultures produced >0.4 kg day$^{-1}$ of ADG within the NDF limits in both 2022 and 2023 due to their relatively high energy content. In 2021, complex mixtures (M14, M12, and M18) were better at generating suitable ADG of 0.10 kg day$^{-1}$ for lactating beef cows, whereas orchard grass, Kirk crested wheatgrass and timothy grass monocultures and grass-only mixtures (M13 and M15) were optimal in 2022 and 2023. Partial substitution with barley straw in the stimulated diets based on NDF criterion substantially reduced daily feeding costs compared with feeding only forage crops. For instance, in 2023, M1 + barley straw reduced the cost of feeding gestating beef cows greatly by \$0.53 head$^{-1}$ day$^{-1}$, but in the case of lactating beef cows, the cost reduction was only \$0.04 head$^{-1}$ day$^{-1}$. Overall, our study highlights differences in predicted animal performance across yearly harvested forages as well as the need to assess and consider forage nutritional value to better inform the feed rations for beef cattle.

**Keywords**: CowBytes, average daily gain, cow performance, neutral detergent fiber, ration, dry matter yield

## 7.0 Introduction

The profitability of beef cattle herds is a primary goal of beef producers. However, in Canada, feeding accounts for a large portion of the cost of production (McCartney et al., 2004). Winter feeding accounts for approximately 60-65% of the total cost of cow-calf operations in Western Canada (Kaliel and Kotowich 2002; Larson, 2013; Damiran et al., 2016). While forage yield is of paramount importance, quality impacts the ability of beef cattle to use forage feed (Ball et al., 2001). Generally, poor quality forages are low in energy, protein, and minerals (Beaty et al., 1994), which negatively affects ruminal microbial function, leading to reduced feed intake and utilization (Bohnert et al., 2002). This may lead to poor nutrition in beef herds and reduce vital animal performance, such as poor animal body condition score (BCS) or a decrease in the number of offspring (Köster et al., 1996). Robinson et al. (2013) reported that nutrition during pregnancy influenced dam body weight at parturition (499 kg for high nutrition versus 372 kg for low nutrition), and birth weight (35.2 vs. 31.5 kg for high vs. low nutrition). Nevertheless, supplements such as grains, minerals, and additives can be utilized to augment poor-quality forages (Caton and Dhuyvetter, 1997; NASEM, 2016).

Beef cattle are fed forage via grazing, hay, or provided by silage, greenfeed or straw. The forage dry matter intake is the most important variable affecting animal performance (Weiss, 2015). Consequently, intake and digestibility of feeds by ruminants is dependent upon the interaction of the diet, animal and feeding environment. The quality of these forages is usually determined through laboratory analyses to ascertain their nutritional content and possible effects on cattle performance (Weiss and Hall, 2020; Ball et al., 2001). To effectively evaluate the impact of a specific forage type on cattle performance, forage can be directly grazed in the field or harvested and supplied as hay to cattle, and periodic or daily monitoring of weight, body condition score, or reproduction is undertaken (Peprah et al., 2021; Lardner et al., 2013). Alternatively, a plethora of computer simulations such as the NASEM models, Cornell Net Carbohydrate and Protein System (Russel et al., 1992; Fox et al., 1992), and CowBytes (Okine et al., 2003) can also be used to determine the performance of cattle receiving forage feed without necessarily feeding forage directly to cattle. The CowBytes® beef ration-balancing program was developed by Alberta Agriculture and Rural Development to provide nutritional requirement of animals (NRC, 2000), allow the development of balanced rations which meet nutritional and performance requirements and aid producers to save money by using various feeds in a ration. This further ensures cost-effectiveness by providing information on the frequency at which feed supplementation should be delivered (Gross et al., 2016). Nevertheless, a major challenge with the CowBytes® model is that it requires accurate estimates of the dietary energy and protein (degradable intake and undegradable intake protein) content to predict the dry matter intake (DMI), feed conversion ratios, and ADG of cattle (McKinnon et al. 2002).

CowBytes has been designed and used for the purpose of predicting animal performance when knowing forage quality parameters. However, there is limited published information on the use of CowBytes® software to evaluate forage-based feeding programs and predict the performance of different categories of cattle supplied with forage mixture-based hay alone, with and without feed supplements, and their cascading effect on beef cattle. This study aimed to evaluate ***in silico*** how perennial forage monocrops, simple and complex mixtures sole and with supplementation impact the performance of steers, dry gestating beef cows, and lactating beef cows. We further assessed forage mixtures that could provide adequate nutritive value for beef cattle diets, even after supplementation with a cheaper lower quality forage such as barley straw partial substitution, to reduce costs.

## 7.1 Materials and methods

### 7.1.1 Research study area and weather

A field experiment was conducted on perennial forages from June 2020 to July 2023 at the Peace Country Beef & Forage Association research farm in Fairview, northwestern Alberta, Canada (56°04′53″N, 118°26′05″W; 670 m above sea level). The field selected was a previously stubbled canola (*Brassica napus* L.) crop. The field had a history of several years of wheat (*Triticum aestivum*)- canola rotations for grain production. Soil classification was Eluviated Black Chernozem according to the Canadian System of Soil Classification (Government of Alberta, 2020). The soil characteristics of the site including a pH of 5.2 and an organic matter of 3.1% have been provided (unpublished by data Gyamfi et al.). The site's weather information during the growing seasons and their long-term averages (Table 7.1) were obtained from on-site Alberta Climate Information Service.

### 7.1.2 Experimental design and treatments

The experimental design for the trial was a randomized complete block design (RCBD) with four replications ($n = 4$). The treatments consisted of six simple grass–legume mixtures (i.e., one grass and one legume) and 18 complex diverse perennial forage species blends (i.e., three or more grass–legume), thus three grass-only mixtures, two legume-only mixtures and 13 grass–legume mixtures (Table 7.2). In addition, five pure stands of perennial grasses were used as controls. The grass species used in monoculture and mixtures were wheatgrass (*Thinopyrum intermedium*; varieties- Kirk crested and greenleaf pubescent), orchardgrass (*Dactylis glomerata*), timothy grass (*Phleum pratense*), and meadow bromegrass (*Bromus commutatus*; variety- Fleet, Table 7.2). The seeding and management of plots were carried out as described by Gyamfi et al. (unpublished data).

### 7.1.3 Plant growth stages, harvest procedure and forage quality analysis

On 3 July 2021, 30 June 2022, and 22 June 2023, an area of 3 m encompassing four inner rows of the plots was harvested at 10-15% of alfalfa blossom (Bonin and Tomlin, 1968). As provided by Gyamfi et al. (unpublished data), the above ground biomass was harvested at a stubble height of 7.5 cm using a custom-made self-propelled small plot forage harvester (Swift Machine and Welding Ltd., Swift Current-SK, Canada). The harvested biomass was weighed fresh and sub-samples of (not more than 1000 g) were air-dried to determine forage dry matter content, which was used to calculate forage yield on a dry matter basis in (Kg ha$^{-1}$). The dried forage sub-samples were analyzed in a commercial laboratory (A&L Canada Laboratory, London, Ontario, Canada) for feed quality parameters, including crude protein (CP) and neutral detergent fiber (NDF) content. Total nitrogen (N) concentration was determined using the micro-Kjeldahl method (AOAC, 1984), and the total N was multiplied by 6.25 to determine the CP content (Schroeder, 1994). The neutral detergent fiber (NDF) was determined using an ANKOM$^{2000}$ fiber analyzer (Model 2000; ANKOM; Fairport, NY, USA). Total digestible nutrients were calculated based on equations provided by (Weiss and Hall, 2020) whereas forage minerals (calcium, phosphorus, potassium, magnesium) by using near infrared spectroscopy (NIRS) (Amari and Abe, 1997). The *in vitro* NDFD was determined by using a 48-h incubation buffered rumen fluid (Goering and Van Soest, 1970). See Appendices 4a- 4c for DM yield and nutritional value from 2021 to 2023.

### 7.1.4 Beef cattle information and ration formulation using the CowBytes® simulation program

Based on the energy requirement for young and mature cows to meet their expected ADG, rations were formulated using the CowBytes® software (version 5.2) for steers, dry gestating, and lactating beef cows, using the DM yield and nutrient content of the various forage treatments obtained from 2021 to 2023. Standard animals were: a 318 kg steer, with an expected slaughter weight of 658 kg and predicted ADG of 0.8 kg $day^{-1}$. A 680 kg beef cow, 8 months in gestation with expected slaughter weight of 771 kg. The cow required an ADG of 0.10 kg $day^{-1}$, with a birth weight of 39 kg. The lactating cow was of the same size and required growth rate. Calf birth weight was 41 kg. Cow was in the second month of lactation with a peak milk yield of 9 kg $day^{-1}$ (Table 7.10). Furthermore, a dry-clean hair depth of 1.30 cm with average hide thickness and no heat stress was assigned to all categories. For climatic conditions, temperatures between -20°C and 20°C, and a wind speed of 5 km $h^{-1}$ were used for all the categories of cows. We recognize that during cold stress, maintenance requirements can increase by 28 to 38% (Stanton, 1995). Below the critical temperature, energy requirements increase and feed intake normally increases in parallel (Degen and Young, 2002). The increase in energy needs at low temperature is to augment heat production in order to maintain a state of thermal equilibrium.

### 7.1.5 Ration formulation

Based on the information gathered on forage DM yield, nutritional value, animal type, and climatic conditions, rations were formulated for beef cattle. The quality parameters entered by treatments include DM yield, TDN, CP, NDF, and minerals (phosphorus, magnesium, calcium, sodium, and chlorine), as well as animal characteristics by type (e.g., initial weight, expected slaughter weight, average daily gain) and climatic conditions (e.g., temperature and windspeed). As reported by Alberta Agri-News (2023), in 2021, monoculture grass bales were valued at \$0.170 per kg, \$0.230 per kg in 2022, and \$0.150 per kg in 2023. In addition, grass-only mixtures bales were priced \$0.170 per kg in 2021, \$0.230 per kg in 2022, and \$0.150 per kg in 2023 while grass–legume haybales cost \$0.185 per kg in 2021, \$0.240 per kg in 2022, and \$0.180 per kg in 2023. The prices of barley straw was valued averagely at \$0.085 per kg in 2021, 2022, and 2023. Ballet et al. (2000) stated that generally most forage treatments may be deficient in vitamins, salts, and some trace minerals (iodine, cobalt, selenium, and iron); hence, 0.0507 kg of 19:9 minerals, 0.030

kg of Fortified Trace Mineral Salt with Selenium, 0.007 kg ADE 10 million vitamins, and 0.009 kg of Vitamin E 50000 with a total cost of $ 0.10 were added to have minerals, trace minerals and vitamins to meet requirements. Any feed that met the energy requirement of the animal to help it achieve the desired ADG showed a 'green color' for both total net energy (NEmTot) and net energy for gain (NEg) in the results panel that displays "supplied from ration." In some cases, an observed yellow color may indicate that the feed was approximately 5-10% off the required energy. A red color indicates that the feed did not meet the energy requirement of the animal type selected and could be supplemented with grains such as barley (*Hordeum vulgare*), oat (*Avena sativa*). Barley straw, a lower energy feed was added to some of the treatment forage when supplied energy was in excess of requirement.

### 7.1.6 Statistical analysis

Using forage treatments (feed) and animal attributes as input variables in CowBytes models, DMI and ADG for different beef cattle categories were predicted. The data of formulated ration obtained from the Cowbytes® software were analyzed using analysis of variance (ANOVA) with block replicates as random effects on a yearly basis (2021, 2022, and 2023) and ration x year interaction as fixed effects using the NLME program (Pinheiro et al., 2020) in the R statistical program (Version 4.2.3)(R Core Team 2020). All treatments were used in the analysis and the best performers were selected based on DMI, ADG, and daily feeding cost per head ($ head$^{-1}$ day$^{-1}$).

## 7.2 Results

### 7.2.1 Steers

Forage DMI results for steers were significantly different for 2021, 2022, and 2023 ($p$= 0.025, 0.031, and 0.004, respectively). However, the ADG of steers was unaffected ($p< 0.05$) by the forage treatments from 2021 to 2023 (Table 7.3). In the first year of forage production (2021), the top three forage treatments with respect to DMI were M16 (8.4 kg), followed by M22 (8.3 kg) and then M21 (8.1 kg) (Table 7.3). However, the corresponding daily feeding costs per head for these forage treatments indicated that M16, M21, and M22 had $1.29, $1.21, and $1.26 head$^{-1}$

day$^{-1}$, respectively. Although steers performed better in all three treatments, M21 was the best in terms of DMI and cost to the producer (Table 7.3).

In 2022, a total of 15 treatments, including four simple mixtures, two legume-only mixtures, two grass-only mixtures, seven complex grass–legume mixtures, and one monoculture grass, met the energy requirement of steers to obtain the target ADG (Table 7.3). The DMI of forage treatments ranged between 7.7 kg for timothy grass monoculture (TG) and 8.6 kg for M5. Generally, TG and grass-only mixtures (M13 and M15) recorded the lowest DMI of 7.7, 7.6, and 7.8 kg, respectively. The top five treatments with the lowest daily feeding cost were M6, TG, M13, M15, and M1 (Table 7.3). For instance, although M6 had a greater DMI (8.4 kg), it had the lowest daily feeding cost of \$0.69 head$^{-1}$ day$^{-1}$ among the top five treatments. This was approximately 0.7 kg more of DMI but \$0.72 head$^{-1}$ day$^{-1}$ less of daily feeding cost than TG, which had the lowest DMI of 7.7 kg.

Eight of the 29 treatments, consisting of two simple mixtures (M1 and M3), one legume-only mixture (M16), four complex grass–legume mixtures (M12, M17, M18, and M22), and orchard grass monoculture (OG), provided steers with sufficient energy to attain its expected ADG in 2023. The DMI ranged from 7.7 kg for OG to 8.4 kg for M1, M3, M16, and M22 (Table 7.3). OG had the lowest DMI (7.7 kg) and daily feeding cost of \$0.58 head$^{-1}$ day$^{-1}$ making it the best treatment among the eight during the year. This was approximately 0.7 kg of DMI less compared to the treatments with the highest DMI (8.4 kg). In addition, the daily feeding cost per head was \$0.20 head$^{-1}$ day$^{-1}$ less than M1 and M16, but \$0.22 head$^{-1}$ day$^{-1}$ less than M3 and M22 (Table 7.3).

### 7.2.2 Dry gestating beef cows

The DMI results for dry gestating beef cows over the three forage production years (2021-2023) were significant ($p$ = 0.038, 0.042, and 0.002, respectively). In 2021, M9 > M13 > OG were better at leading the performance of dry gestating beef cows to meet the expected ADG of 0.10 kg day$^{-1}$ (Table 7.4). Among these treatments, M9 had the highest DMI (15.7 kg), followed by OG (14.7 kg), whereas the lowest DMI was recorded in M13 (14.2 kg). As observed, M13 had the lowest daily feeding cost of \$1.41 head$^{-1}$ day$^{-1}$ while M9 and M13 cost \$2.15 and \$1.47 head$^{-1}$ day$^{-1}$, respectively. In both 2022 and 2023 all forage treatments supplied at 1.2 NDF as %BW produced ADG between 0.4 to 1.0 kg day$^{-1}$. This was more than the required ADG for dry gestating beef cow performance according to our study. For instance, in 2022, Fleet MB (0.4 kg day$^{-1}$) and greenleaf pubescent wheatgrass (GPWG) (0.5 kg day$^{-1}$) had ADG closer to the required 0.10 kg day$^{-1}$ (Table 7.4). The only treatment that met the expected ADG was M23. However, it recorded a 1.4 NDF as %BW. By 2023, M4 (0.4 kg day$^{-1}$), M20 (0.5 kg day$^{-1}$), and GPWG (0.4 kg day$^{-1}$) had the closest ADG to what was expected. All other treatments produced ADG higher than the required for dry gestating beef cows (Table 7.4). Hence, there was a need to substitute part of the forage feed with a cheaper energy source, such as barley straw, to reduce costs and obtain the expected ADG (0.10 kg day$^{-1}$).

Generally, forage treatments combined with barley straw resulted in a lower cost than feeding sole forage treatments, albeit with a higher DMI. In 2021, the top five treatments combined with barley straw were M3 (\$0.98 head$^{-1}$ day$^{-1}$), M0 (\$1.06 head$^{-1}$ day$^{-1}$), M1 (\$1.33 head$^{-1}$ day$^{-1}$), M5 (\$1.34 head$^{-1}$ day$^{-1}$), and M17 (\$1.41 head$^{-1}$ day$^{-1}$). For instance, M3 was \$0.80, \$0.35, \$0.36, and \$0.43 head$^{-1}$ day$^{-1}$ cheaper than M0, M1, M5, and M17, respectively (Table 7.5). In 2022, M0 + barley straw had the lowest daily feeding cost (\$1.45 head$^{-1}$ day$^{-1}$). Compared with the M0 sole forage treatment, M0 + barley straw was \$0.57 head$^{-1}$ day$^{-1}$ cheaper. Similarly, M15 + barley straw had a \$0.93 head$^{-1}$ day$^{-1}$ decrease in cost compared with sole forage of M15. The three other treatments with lower daily feeding cost were Fleet MB, TG, and GPWG combined with barley straw (Table 7.6). In 2023, all forage treatments combined with barley straw produced the right 1.2 NDF as %BW and ADG of 0.10 kg day$^{-1}$. However, the top five treatments with significantly lower daily feeding cost for dry gestating beef cows were monoculture grasses of OG (\$0.76 head$^{-1}$ day$^{-1}$), GPWG and Kirk crested wheatgrass (KCG) (\$0.77 head$^{-1}$ day$^{-1}$, each), and Fleet MB (\$0.81 head$^{-1}$ day$^{-1}$), and grass-only mixtures such as M0 (\$0.82 head$^{-1}$ day$^{-1}$). These top five treatments were cheaper than their sole forage counterparts of OG, GPWG, KCG, Fleet MB, and M0 (Table 7.7).

### 7.2.3 Lactating beef cows

The DMI results for lactating cows were significant for the forage treatments ($p = 0.042$, 2021; $p = 0.038$, 2022; and $p = 0.001$, 2023, respectively). Among the treatments, M17 had the highest forage DMI (16.9 kg) in 2021 for lactating beef cows whereas M6 (17.4 kg), M5, M8, and M10 (16 kg each) had the highest DMI in both 2022 and 2023 (Tables 7.8 and 7.9). Although there were variations in DMI across the three forage production years (2021-2023), the effects on ADG were indifferent ($p > 0.05$) for all treatments with 0.10 kg $day^{-1}$ in ADG. In 2021, one simple mixture (M3) and six complex grass–legume mixtures (M8, M12, M17, M18, and M23) were found to be better predictors of lactating beef cow performance on those forage treatments. Among these treatments, M17 had the lowest daily feeding cost of $1.44 $head^{-1}$ $day^{-1}$, followed by M3 ($1.98 $head^{-1}$ $day^{-1}$) (Table 7.8). In 2022, it was apparent that M6, monoculture grasses (KCG and TG), and grass-only mixtures (M0, M13, and M15) were the best treatments to predict the performance of lactating beef cows. Based on the DMI, M6 had the highest (17.4 kg), while both TG and M0 had the lowest (15.4 kg each). This makes TG and M0 the best treatments in terms of DMI supplied. However, based on the daily feeding cost per head, M6 ($1.25 $head^{-1}$ $day^{-1}$) had the lowest followed by M0 ($2.13 $head^{-1}$ $day^{-1}$). KCG had the highest daily feeding costs ($2.66 $head^{-1}$ $day^{-1}$). This implies that M6 was $1.41 $head^{-1}$ $day^{-1}$ cheaper than KCG. In 2023, several treatments adequately met the energy requirements for the performance of lactating beef cows. The top five treatments based on DMI and daily feeding cost per head were monoculture grasses (KCG and TG) and grass-only mixtures (M0, M13, and M15). M13 and M15 recorded the highest DMI of 15.4 kg each while KCG had the lowest DMI of 14.8 kg (Table 7.9). The corresponding daily feeding cost per head also showed that KCG had the lowest value ($0.98 $head^{-1}$ $day^{-1}$). This makes KCG the best among the top five treatments, followed by M0, M13, and M15, with $1.10 each.

The quantities of forage supplied to lactating cows and the daily feeding costs of treatments were the major difference across the three years. For instance, by comparing monoculture grasses and grass-only mixtures, more forage from both simple and complex grass–legume mixtures had to be supplied to cows to meet their expected ADG. This subsequently increased the feeding costs. As the feeding of the sole forage treatments had a higher cost, it was necessary to substitute part of the ration with a cheaper energy source (barley straw) as noted above.

Forage treatments combined with barley straw showed lower costs across all years, particularly in 2023. In 2021, the cost of feeding sole M3 was $1.98 $head^{-1}$ $day^{-1}$ but decreased to $1.10 $head^{-1}$ $day^{-1}$, when supplemented with barley straw. This implies a $0.88 $head^{-1}$ $day^{-1}$ decrease in cost. In addition, M8+ barley straw decreased by $1.29 $head^{-1}$ $day^{-1}$ compared with feeding sole M8 (Table 7.8). In 2022, TG + barley straw had a DMI of 15.8 kg and a decrease of

$0.11 head$^{-1}$ day$^{-1}$ in feeding cost. Generally, M13+ barley straw had a $0.21 head$^{-1}$ day$^{-1}$ decrease in feeding cost compared with sole M13. In 2023, supplementing the top five forage treatments (M1, M12, M19, KCG, and OG) with barley straw resulted in a substantial decrease in feeding costs. For instance, feeding sole OG cost $1.10 head$^{-1}$ day$^{-1}$ and decreased to $0.99 head$^{-1}$ day$^{-1}$ ($0.11 difference) after combining it with barley straw. In addition, M19 + barley straw and M12 + barley straw recorded $0.50 and $0.10 head$^{-1}$ day$^{-1}$, respectively, compared to their sole treatment (Table 7.9).

## 7.3 Discussion

### 7.3.1 Legume-dominated mixtures better supports steer performance

Forage treatments of M16, M21, and M22 which are legume-dominated mixtures, were superior in meeting the nutritional needs of steers; hence, they are better at sustaining their performance under our model simulations (i.e. ADG) during the first year of forage production (2021). This was because of their high forage quality. This can be explained by the relatively high proportions of legumes in the mixtures. For instance, M16 contained 100% legumes, M21 contained 70% legumes, and M22 contained 90% legumes. Bélanger et al. (2017), found that legumes generally improve the overall forage quality of mixtures. In addition, our study showed that the high TDN (63.8–64.9%) influenced the ability of these forages to supply adequate energy levels to meet the needs of steers. Alfredo (2019), posited that a small frame steer with expected ADG of 0.79 kg day$^{-1}$ and weight between 272 – 318 kg requires TDN of 63%. This indicates that the above-mentioned mixtures were superior in achieving this goal compared with the other treatments. Although the results of other forage treatments from the first year of forage production (2021) could predict the expected ADG (0.8 kg day$^{-1}$), the 1.2 NDF as %BW could not be achieved. For example, most treatments had NDF as %BW greater than 1.3. This indicates that the intake of forage materials was affected, thereby impeding their effectiveness in supporting the energy needs of steers. As CowBytes® stimulates forage feeding in the real world, it is assumed that cattle consume forage until they are filled. Hence, the 1.2 NDF as %BW targeted in the CowBytes® software equates to the forage intake by steers until they are filled. Our results are supported by the findings of Allison (1985), who reported that when cattle are offered either hay or dried grass, there is evidence that they eat to a constant rumen fill. Furthermore, the DMI of the formulated rations indicated that forage treatments had to be supplied to steers in different quantities to meet their requirements. In our simulations, this was as a result of the different feed qualities. Although the DMI and daily feeding cost per head varied, a targeted ADG of 0.8 kg day$^{-1}$ was often achieved. This suggests that treatment M21 which was supplied in lower quantities, had the lowest cost. From a broader perspective, this treatment M21 would be the best for a farm based on our results, because it could be fed over an extended period of time and at a lower overall cost to the producer.

In 2022, the ability of rations formulated with grass-only mixtures (M13 and M15), simple mixtures (M1 and M6), and monoculture grass (OG) and (TG) to adequately sustain the best performance (i.e., attain the expected ADG) of steers emanated from the sufficient energy content in forage treatments (Appendix 5b). Here, the DMI of grass-only mixtures and monoculture grasses was supplied in lower quantities than that of the simple mixtures. However, both supported steers in obtaining the required ADG. We confirmed that TG, OG, M13, and M15 were generally higher in TDN; hence, a lower quantity supplied was sufficient to meet the nutritional needs of steers. Conversely, simple mixtures had lower TDN, and as such, it appeared that more quantities had to be supplied to meet the needs of the steers. Our findings agree with those of Kunkle et al. (1999), who stated that in feeding animals, forage materials with lower energy may require supplements to meet the requirements for better performance.

Furthermore, the DMI of the rations formulated from OG and other highly legume-dominated mixtures (M1, M3, M12, M16, M17, M18, and M22) sustained steer performance (i.e., ADG of 0.8 kg day$^{-1}$) during the third year of forage production (2023). Among these forage treatments, the OG monoculture was supplied at a lower quantity compared with other treatments. This was because the OG had a relatively higher energy level. This is consistent with Gyamfi et al. (unpublished data) who found that orchard monoculture grass was high in TDN during the final year of forage production. This was explained by the ability of the orchard grass to efficiently use available soil resources for better structural growth. To compare OG to legume-dominated mixtures, it becomes evident that higher amount of forage biomass needs to be supplied to steers in order to obtain the expected ADG. This is because the legume-dominated mixtures were slightly lower in energy than OG in 2023. This indicates that a producer would need to cultivate more acreage of land to produce the desired quantity of these legume-dominated forage mixtures, to guarantee a constant supply throughout the season. This could substantially increase the producers' production costs.

### 7.3.2 Dry gestating beef cows' performance on straight forage versus forage plus barley straw and cost implications

In formulating rations for dry gestating beef cows, monoculture grass (OG) or their mixtures (M13), and M9 were dominant in enabling ADG of 0.10 kg day$^{-1}$. As evident from the nutritional value (TDN and NDF) in 2021, these treatments were adequate compared to the other treatments in this study. They supplied the energy content necessary for the performance of dry gestating beef cows for body maintenance and functions. Yurchak and Orkine (2004), reported that the TDN rule of thumb for mature dry gestating beef cows is 55% during mid-pregnancy, 60% in late pregnancy, and 65% post calving. This indicates that the forage treatments sufficiently met the requirements of the dry gestating beef cows, registered as eight months pregnant. Furthermore, our results showed that DMI increased (almost twice) compared with feeding steers in the same year. This is due to disparities in function between steers and dry gestating beef cows. One could conceptualize that a gestating beef cow may need more energy for maintenance as well as for the development and functions of the fetus, hence the high DMI. This is consistent with Sguizzato et al. (2020), who reported an increase in energy requirement by dry gestating beef cows compared with steers, particularly during the last trimester of pregnancy. However, when feeding dry gestating beef cows with higher energy forages, as opposed to the results presented in this study, the DMI may decrease.

Between 2022 and 2023, the formulated rations produced a higher expected ADG than required (0.10 kg day$^{-1}$). This was partly because of the higher forage TDN content recorded in 2022 and 2023. This suggests that feeding these forage treatments would result in unwanted ADG, which could affect the performance of cows and augment the producer's cost. Nevertheless, these forage treatments were supplemented with a cheaper source of energy (barley straw) to help achieve the required ADG and reduce costs drastically. Across the 3-yr study, the daily feeding cost per head was reduced upon supplementation with barley straw as anticipated. However, these costs differed from year to year. For instance, the daily feeding cost per head for forage treatments combined with barley straw was generally higher in 2022 than in 2021 and 2023 (Tables 7.5, 7.6, and 7.7). This was dependent on the yearly market prices of forage feeds and barley straw.

### 7.3.3 Variability in dry matter intake does not impact the performance of lactating beef cows

In 2021, rations formulated with high legume-dominated mixtures were superior in sustaining the performance of lactating beef cows. This was due to the ability of forage treatments containing legumes to adequately meet the energy needs of cows. Gadberry (1996), suggested a 59.7% TDN for a 680 kg lactating beef cow at 2 months of lactation, producing about 9 kg of milk daily. Notably, the results from our study showed that legume-dominated mixtures supported lactating beef cows to achieve its ADG. However, the variability in the DMI of treatments did not necessarily influence the targeted ADG of 0.10 kg day$^{-1}$. This could be explained by the varying degrees of available nutrients among forage treatments. For instance, M3 and M12 were better in TDN, NDF, and minerals than either M18 or M23; hence, DMI was supplied at lower quantities and was sufficient to support the needs of lactating beef cows. Similar to steers and dry gestating beef cows in both 2022 and 2023, monoculture grasses and grass-only mixtures were superior in meeting the nutritional requirements of lactating beef cows, partly because of the higher energy content reported in our study. As posited by Linn and Kuehn (1993), legumes have higher energy concentrations than grasses. However, the findings of our study are contradictory. Furthermore, the cost of feeding per head across treatments and years was also different due to the quantity of DMI supplied and variations in market prices during a specific year. For instance, in 2022 feeding M13 costs \$2.43 head$^{-1}$ day$^{-1}$ while same costs \$1.10 head$^{-1}$ day$^{-1}$ in 2023. These differences in costs were influenced by the prices of monoculture grasses in 2022 (\$0.230 per kg) and 2023 (\$0.150 per kg). A thorough observation of rations formulated with sole forage treatments showed a higher cost of feeding per head across the three years (Tables 7.8 and 7.9). Therefore, to reduce the producer's cost, an additional cheaper source of barley straw, which could aid lactating beef cows achieve the expected ADG (0.10 kg day$^{-1}$) was supplemented. Generally, prices decreased for treatments with barley straw, particularly for those that met the needs of lactating beef cows. For example, in 2021, feeding sole M18 cost the producer \$2.25 head$^{-1}$ day$^{-1}$, while M18 + barley straw cost \$2.20 head$^{-1}$ day$^{-1}$. This implies a \$0.05 head$^{-1}$ day$^{-1}$ decrease in the daily feeding cost to the producer. In addition, in 2023, the rations formulated with sole M1 cost \$1.39 head$^{-1}$ day$^{-1}$ while M1+ barley straw was valued at \$1.35 head$^{-1}$ day$^{-1}$, indicating a \$0.04 head$^{-1}$ day$^{-1}$ decrease in cost. Realistically, \$0.05 and \$0.04 head$^{-1}$ day$^{-1}$ seems marginal. However, \$0.05 head$^{-1}$ day$^{-1}$ or \$0.04 head$^{-1}$ day$^{-1}$ savings on 1000 herds are a substantial reduction in cost to the producer.

### 7.4 Conclusion

Based on simulations and predictions of the CowBytes® software, rations formulated with complex legume-dominated mixtures, monoculture grasses, and grass-only mixtures delivered the ideal performance of steers, dry gestating, and lactating beef cows. The predicted DMI of these treatments by category of cattle varied depending on the needs of the cattle. For instance, observations have shown that more forage treatments (almost twice) are offered to dry gestating beef cows than to both steers and lactating beef cows. However, the effects of the quantities supplied on ADG were dependent on the nutritional value of the forage. Thus, to achieve the required cow performance, the assessed forage treatments contained the appropriate nutrients and energy levels. Sole forage treatments achieved higher (>0.4 kg day$^{-1}$) ADG for dry gestating cows than the required (0.10 kg day$^{-1}$) in both the second (2022) and third (2023) years of forage production, when energy was relatively high in these treatments. Furthermore, the estimated costs from combining forage treatments with barley straw as an energy source supplement showed that the daily feeding costs per head decreased substantially. This strategy benefits cattle producers by reducing the production costs. In cases where producers have grains (oats and barley) instead of crop straw, this could be another option. However, with the current prices of grains, the cost of feed formulation would largely increase, detrimentally affecting the producer's profits. In summary, this study has demonstrated how feeding of high-quality forage can be beneficial compared with low quality forage and their cost implications. For future studies, research could be carried out to supplement sole forages with grains to determine their impact on beef cattle ADG and costs.

## 7.5 References

Alberta Agri-News (2023). Alberta hay prices. https://www.alberta.ca/agri-news-alberta-hay-prices (accessed on 14 January 2024).

Alberta Climate Information Service (2023). Current and historical Alberta weather station data viewer. Data provided by Alberta Agriculture and Forestry. Retrieved on Sept 10, 2023, from https://acis.alberta.ca/acis/weather-data-viewer.jsp

Alfredo, D. (2019). Basic Nutrient Requirements of Beef Cattle. Oregon State University Extension Services.https://extension.oregonstate.edu/animals-livestock/beef/basic-nutrient-requirements-beef-cattle (accessed on December 17, 2023).

Allison, C. D. (1985). Factors affecting forage intake by range ruminants: A review. Journal of Range Management, 38(4), 305–311.

Amari, M., and Abe, A. (1997). Application of near infrared reflectance spectroscopy to forage analysis and prediction of TDN contents. *Japan Agricultural Research Quarterly*, *31*(1), 55–63.

AOAC. (1984). Official Methods of Analysis, 14th Edition. AOAC.

Ball, D., Collins, M., Lacefield, G., Martin, N., Mertens, D., Olson, K., Putnam, D., Undersander, D., and Wolf, M. (2001). Understanding forage quality. American Farm Bureau Federation Publication 1-01, Park Ridge, IL. Pp 2–5.

Ballet, N., Robert, J. C., and Williams, P. E. V. (2000). Vitamins in forages. In : Forage evaluation in ruminant nutrition, eds. D. I. Givens, E. Owen, H. M. Omed, and R. F. E. Axford, 399–431. Wallingford, UK: CABI.

Beaty, J. L., .Cochran, R. C, Lintzenich, B. A., Vanzant, ., E. S., Morrill, J. L., Brandt, R. T. Jr., and Johnson, D. E. (1994). Effect of frequency of supplementation and protein concentration in supplements on performance and digestion characteristics of beef cattle consuming low–quality forages. Journal of Animal Science. 72, 2475–2486 . doi:10.2527/1994.7292475

Bélanger, G., Tremblay, G. F., Papadopoulos, Y. A., Duynisveld, J., Lajeunesse, J., Lafrenière, C., and Fillmore, S. A. E. (2017). Yield and nutritive value of binary legume–grass mixtures under grazing or frequent cutting. Canadian Journal of Plant Science, 98(2), 395–407. https://doi.org/10.1139/CJPS-2017-0183

Bohnert, D.W., Schauer, C.S. and DelCurto, T. (2002). Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage: cow performance and efficiency of nitrogen use in wethers. Journal of Animal Science. 80, 1629–1637.

Bonin, S.G. and Tomlin, D.C. (1968). Effects of Nitrogen on Herbage Yields of Timothy Harvested at Various Stages. Canadian Journal of Plant Science , 48, 501–509. https://doi.org/10.4141/cjps68-097

Caton, J. S., and Dhuyvetter, D. V. (1997). Influence of energy supplementation of grazing ruminants: requirements and response. Journal of Animal Science. 75, 533–542.

Dagen, A. A. and Young, B. A. (2002). Effect of air temperature and energy intake on body mass, body composition and energy requirements in sheep. Journal of Agricultural Science. 138, 221–226.

Damiran, D., Lardner, H. A., Larson, K., and McKinnon J. (2016). Effects of supplementing spring-calving beef cows grazing barley crop residue with canola meal and wheat-based dry distillers' grains with solubles on performance, reproductive efficiency, and system cost. Professional Animal Scientist. 32, 400–410.

Fox, D. G., Tedeschi, L. O., Tylutki, T. P., Russell, J. B., Van Amburgh, M. E., Chase, L. E., Pell, A.N. and Overton, T. R. (2004). The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Animal Feed Science Technology. 112, 29–78.

Gadberry, S. (1996). Beef cattle nutrition series part 3: Nutrient requirement tables. University of Arkansas Division of Agriculture: Little Rock, AR, USA.

Government of Alberta (2020). Agricultural region of Alberta soil inventory database. Accessed on 29 February 2024 at https://www.alberta.ca/agricultural-regions-of-alberta-soil-inventory-database

Gross, S. M., Neville, B. W., Brummer, F. A., and Undi, M. (2016). Frequency of feeding DDGS as a supplement to beef cows grazing corn residue. Joint Annual Meeting. Salt Lake City, Utah. Abstract # 612. Journal of Animal Science. Vol. 94, Suppl. https://academic.oup.com/

Gyamfi, H., Hernandez Ramirez, G., Omokanye, A., and Thilakarathna, M. S. (2023). Does diversity of perennial forage species affect soil quality. [Unpublished manuscript]. University of Alberta, Edmonton, AB.

Gyamfi, H., Hernandez Ramirez, G., Omokanye, A., and Thilakarathna, M. S. (2023). Forage dry matter yield, nutritive value, and botanical composition of diverse forage species mixtures. [Manuscript submitted for publication]. University of Alberta, Edmonton, AB.

Kaliel, D. and Kotowich, J. (2002). Economic evaluation of cow wintering systems – Provincial swath grazing survey analysis. Alberta Production Economics Branch, Alberta Agriculture Food and Rural Development, Edmonton, AB.

Köster, H. H., Cochran, R. C., Titgemeyer, E. C., Vanznat, E.S., Abdelgadir, I., and St. Jean, G. (1996). Effect of increasing degradable intake protein on intake and digestion of low quality, tall grass prairie forage by beef cows. Journal Animal Science. 74, 2473–2481.

Kunkle, W. E., Johns, J. T., Poore, M. H., and Herd, D. B. (1999). Designing supplementation programs for beef cattle fed forage-based diets. Journal of Animal Science. (E. Suppl.) (1999), p. E12. DOI:10.2527/jas2000.00218812007700ES0012x

Larson, K. (2013). 2012 Saskatchewan cow-calf cost of production analysis. Fact Sheet #2013–02. Western Beef Development Centre.

Linn, J., and Kuehn, C. (1993). The effects of forage quality on performance and cost of feeding lactating dairy cows. University of Minnesota, Department of Animal Science, 1364. Eckles Avenue, St – Paul, Minnesota, USA, 5508 – 6120.

McCartney, D., Basarab, J. A., Okine, E. K., Baron, V. S., and Depalme, A. J. (2004). Alternative fall and winter feeding systems for spring calving beef cows. Canadian Journal of Animal Science, 84(3), 511–522. https://doi.org/10.4141/A03-069

McKinnon, J. J., Block, H. C., Schenher, D. and Christensen, D. A. (2002). Predicting the performance of feedlot cattle — Do we have the right tools? Pages 195–204 in D. Korver and J. Morrison, eds. Proceedings of the 23rd Western Nutrition Conference. Edmonton, AB.

National Academies of Sciences Engineering Medicine (2016). Nutrient Requirements of Beef Cattle. Eighth revised edition. Animal Nutrition Series. National Academies press, Washington, DC. pp 72– 73.

National Research Council (NRC) (2000). Nutrient Requirements of Beef Cattle. 7th Rev. Edition, The National Academies Press, Washington DC.

National Research Council. (1984). Nutrient requirements of beef cattle. 6th ed. National Academy Press, Washington, DC.

National Research Council. (1996). Nutrient requirements of beef cattle. 7th ed. National Academy Press, Washington, DC.

Okine, E. K., McCartney, D., and Basarab, J. A. (2003). Evaluation of the National Research Council (NRC) nutrient requirements for beef cattle: Predicting feedlot performance. Canadian Journal of Animal Science. 83, 787–797.

Peprah, S., Damiran, D., Biligetu, B., Iwaasa, A. D., Larson, K., and Lardner, H. A. (2021). Evaluation of cool-season binary mixtures as pasture: Herbage yield, nutritive value, and beef cattle performance. Livestock Science, 248, 104–501. https://doi.org/10.1016/j.livsci.2021.104501

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. (2020). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-149, URL: https://CRAN.R-project.org/package=nlme

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Robinson, D. L., Cafe, L. M., and Greenwood, P. L. (2013). Meat science and muscle biology symposium: Developmental programming in cattle: Consequences for growth, efficiency, carcass, muscle, and beef quality characteristics. Journal of Animal Science, 91, 1428 –

1442. https://doiorg.login.ezproxy.library.ualberta.ca/10.2527/jas.2012-5799

Russel, J. B., O'Conner, J. D., Fox, D. G., Van Soest, P. J. and Sniffen, C. J. (1992). A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. Journal of Animal Science. 70, 3551–3561.

Schroeder, J. W. (1994). Interpreting Forage Analysis. Extension Dairy Specialist (NDSU), AS-1080, North Dakota State University. Available online: https://library.ndsu.edu/ir/bitstream/handle/10365/9133/AS-1080-1994.pdf?sequence=2&isAllowed=y (accessed on 6 June 2023).

Sguizzato, A. L. L., Marcondes, M. I., Dijkstra, J., Valadares, S. C., Filho, Campos, M. M., Machado, F. S., Silva, B. C., Rotta, P. P. (2020). Energy requirements for pregnant dairy heifers. PLoS One, 15 (7), 10.1371/journal.pone.0235619 e0235619

Stanton, T. L. (1995). Damage control strategies for cattle exposed to cold stress. Intake by feedlot cattle symposium, Pp 289 – 298.

Weiss, B. (2015). Optimizing and Evaluating Dry Matter Intake of Dairy Cows. WCDS Advances in Dairy Technology, 27, 189–200.

Weiss, W. P., and Hall, M. B. (2020). Laboratory methods for evaluating forage quality. Forages: Science Grassland Agriculture, 2, 659–672. doi: 10.1002/9781119436669.ch36

Yurchak, T., and Orkine, E. (2004). Beef ration rules of thumb. Alberta Agriculture, Food and Rural Development Publication., agdex (420/52-4).1–2. www.agric.gov

## 7.6 Tables

Table 7. 1 Rainfall, maximum and minimum temperatures with their long-term average during the growing season (2021–2023) at Fairview, Alberta, Canada.

| Month | 2020 | 2021 | 2022 | 2023 | Long-term average |
|---|---|---|---|---|---|
| | | | Rainfall (mm month$^{-1}$) | | |
| May | 19.1 | 13.3 | 53.4 | 12.7 | 35.1 |
| June | 67.2 | 43.3 | 60.9 | 60.9 | 94.5 |
| July | 89.8 | 23.5 | 18.6 | 3.5 | 161.4 |
| August | 53.9 | 40.5 | 31.4 | 1.7 | 203.1 |
| Cumulative | 230.0 | 120.6 | 164.3 | 40.8 | 494.1 |
| | | | Maximum air temperature (°C) | | |
| May | 24.8 | 16.6 | 13.8 | 23.2 | 14.8 |
| June | 26.8 | 20.2 | 21.0 | 22.9 | 14.0 |
| July | 29.2 | 22.2 | 23.9 | 23.4 | 15.9 |
| August | 29.2 | 21.3 | 25.5 | 22.5 | 14.6 |
| | | | Minimum air temperature (°C) | | |
| May | –2.3 | 3.1 | 2.6 | 6.4 | 10.3 |
| June | 2.6 | 7.5 | 8.9 | 9.0 | 9.4 |
| July | 7.0 | 9.2 | 10.3 | 10.7 | 11.1 |
| August | 0.3 | 7.7 | 10.8 | 9.7 | 13.7 |

Table 7. 2 Complete list of forage monocultures and mixtures grown at Fairview, Alberta, Canada.

| Monoculture grasses and simple mixtures | Complex mixtures | |
|---|---|---|
| | 3-species | 4 or more species |
| OG | M7: MB (Fleet) + AL (y) + SF | M0: MB (Fleet) + HB (AC Success) + HB (AC Knowles) + GPWG + CWG |
| GPWG | M8: HB (AC Success) + AL (y) + SF | |
| MB | M11: MB (Fleet) + GPWG + AL (y) | M9: MB (Fleet) + AL (y) + SF + CMV |
| CWG | M12: HB (AC Success) + GPWG + AL (y) | M10: HB (AC Success) + AL(y) + SF + CMV |
| TG | M14: SF + CMV + AL (s5) | |
| M1: MB (Fleet) + AL(s5) | M17: HB (AC Success) + AL (y) + SF | M15: HB (AC Success) + GPWG + CWG + IR + SB (Manchar) |
| M2: HB (AC Success) + AL(y) | M13: TF + SW + STL | M16 : AL(y) + AL (R) + CMV + SF +BT |
| M3: HB (AC Success) + AL (s5) | M21: HB (AC Success) + SF + AL(y) | M18: HB (AC Success) + GPWG + AL (y) + SF |
| M4: MB (Fleet) + AL (s5) | | M19: HB (AC Success) + GPWG + IR + AL (y) + SF |
| M5: HB (AC Success) + AL(s5) | | M20: HB (AC Success) + GPWG + AL(R) + AL (y) + CMV + SF |
| M6: HB (AC Knowles) + AL(s5) | | |
| | | M22: HB (AC Success) + GPWG + AL (y) + CMV + SF + BT |
| | | M23: HB (AC Success) + GPWG + CWG + IR + AL (y) + AL (R) + CMV + SF |

M, mixture; OG, orchardgrass; GPWG, greenleaf pubescent wheatgrass; HB, hybrid bromegrass; CWG, crested wheatgrass; TG, timothy grass; MB, meadow bromegrass; AL(s5), Spredor 5 alfalfa; AL(y), yellowhead alfalfa; AL(R), Rugged alfalfa; SF, sainfoin; CMV, cicer milkvetch; BT, birdsfoot trefoil; IR, Italian ryegrass; SB, smooth bromegrass; TF, tall fescue; SW, slender wheatgrass; STL, Saltander grass.

Table 7. 3 ANOVA of sole forage treatments predicting the performance of steers based on dry matter intake (DMI), average daily gain (ADG), and daily feeding cost per head during three years of forage harvests.

| Treatments | DMI (kg day$^{-1}$) | ADG (kg day$^{-1}$) | DFC (\$ head$^{-1}$ day$^{-1}$) |
|---|---|---|---|
| | 2021 | | |
| M16 | 8.4 a | 0.8 | 1.29 a |
| M21 | 8.1 c | 0.8 | 1.21 c |
| M22 | 8.3 b | 0.8 | 1.26 b |
| *p* value | 0.025 | **NS* | 0.045 |
| Treatments | 2022 | | |
| M1 | 8.4 bc | 0.8 | 1.74 de |
| M3 | 8.6 bc | 0.8 | 1.92 a |
| M5 | 8.3 c | 0.8 | 1.80 d |
| M6 | 8.4 bc | 0.8 | 0.69 e |
| M8 | 8.2 d | 0.8 | 1.88 b |
| M9 | 8.2 d | 0.8 | 1.86 c |
| M12 | 8.2 d | 0.8 | 1.90 ab |
| M13 | 7.6 ef | 0.8 | 1.26 fg |
| M14 | 8.7 a | 0.8 | 1.92 a |
| M15 | 7.8 e | 0.8 | 1.34 f |
| M16 | 8.3 c | 0.8 | 1.88 b |
| M17 | 8.1 d | 0.8 | 1.82 d |
| M18 | 8.4 bc | 0.8 | 1.88 b |
| M20 | 8.2 d | 0.8 | 1.80 d |
| TG | 7.7 ef | 0.8 | 1.41 ef |
| *p* value | 0.031 | **NS* | 0.014 |
| Treatments | 2023 | | |
| M1 | 8.4 a | 0.8 | 0.78 b |
| M3 | 8.4 a | 0.8 | 0.80 a |
| M12 | 8.2 b | 0.8 | 0.78 b |
| M16 | 8.4 a | 0.8 | 0.79 ab |
| M17 | 8.2 b | 0.8 | 0.78 b |
| M18 | 8.3 ab | 0.8 | 0.79 ab |
| M22 | 8.4 a | 0.8 | 0.80 a |
| OG | 7.7 c | 0.8 | 0.58 c |
| *p* value | 0.004 | **NS* | 0.043 |

**NS* represents not significance, DFC stands for the daily feeding cost per head in \$ head$^{-1}$ day$^{-1}$, TG; timothy grass, OG; orchard grass.

Table 7. 4 ANOVA of sole forage treatments predicting the performance of dry gestating beef cows based on dry matter intake (DMI), average daily gains (ADG), and daily feeding cost per head for three years of forage harvests (2021- 2023).

| Treatments | 2021 | | |
|---|---|---|---|
| | DMI (kg day$^{-1}$) | ADG (kg day$^{-1}$) | DFC (\$ head$^{-1}$ day$^{-1}$) |
| M9 | 15.7 a | 0.1 | 2.15 a |
| M13 | 14.2 c | 0.1 | 1.41 c |
| OG | 14.7 b | 0.1 | 1.47 b |
| *p* value | 0.038 | **NS* | 0.013 |
| Treatments | 2022 | | |
| Fleet MB | 13.6 c | 0.4 b | 2.2 bc |
| GPWG | 13.9 b | 0.5 a | 2.35 b |
| M23 | 19.9 a | 0.1 c | 4.04 a |
| *p* value | 0.042 | 0.024 | 0.035 |
| Treatments | 2023 | | |
| M4 | 14.8 b | 0.4 b | 1.28 b |
| M20 | 15.5 a | 0.5 a | 1.33 a |
| GPWG | 13.2 c | 0.4 b | 0.89 c |
| *p* value | 0.002 | 0.045 | 0.022 |

*NS connotes not significant, DFC represents the daily feeding cost per herd in \$ head$^{-1}$ day$^{-1}$, OG; orchard grass, GWPG; greenleaf pubescent wheatgrass, and FMB; Fleet meadow bromegrass.

Table 7. 5 ANOVA of forage treatments mixed with barley straw for dry gestating beef cows based on dry matter intake (DMI), average daily gain (ADG), and daily feeding cost per head during the 2021 year of forage harvest.

| Treatments | Treatment + barley straw DMI (Kg $day^{-1}$) | 2021 | | | DFC of sole treatment ($ $head^{-1}$ $day^{-1}$) |
|---|---|---|---|---|---|
| | | DMI straw (kg $day^{-1}$) | ADG (kg $day^{-1}$) | DFC($ $head^{-1}$ $day^{-1}$) | |
| M0 | 9.8 bc | 4.30 | 0.10 | 1.06 f | 1.32 j |
| M1 | 10.0 b | 4.30 | 0.10 | 1.33 g | 1.78 h |
| M3 | 9.1 f | 5.20 | 0.10 | 0.98 h | 1.96 fg |
| M5 | 9.8 bc | 5.20 | 0.10 | 1.34 g | 1.78 h |
| M6 | 10.4 ab | 4.10 | 0.10 | 1.57 ab | 2.13 e |
| M7 | 10.5 ab | 4.00 | 0.10 | 1.55 ab | 2.05 f |
| M8 | 9.7 c | 4.50 | 0.10 | 1.53 b | 2.26 c |
| M11 | 9.9 b | 4.50 | 0.10 | 1.47 d | 2.03 f |
| M12 | 9.5 d | 4.60 | 0.10 | 1.43 e | 2.12 e |
| M14 | 9.9 b | 4.30 | 0.10 | 1.52 bc | 2.19 d |
| M16 | 9.8 bc | 4.50 | 0.10 | 1.60 a | 2.44 a |
| M17 | 9.8 bc | 4.60 | 0.10 | 1.41 e | 2.01 f |
| M18 | 9.8 bc | 4.30 | 0.10 | 1.51 c | 2.15 e |
| M19 | 10.6 a | 4.10 | 0.10 | 1.54 b | 1.98 fg |
| M22 | 9.2 e | 5.00 | 0.10 | 1.48 d | 2.38 b |
| M23 | 9.2 e | 5.30 | 0.10 | 1.32 g | 1.40 i |
| *p* value | 0.008 | ** | *NS | 0.003 | 0.006 |

*NS, not significant; GPWG, green leaf pubescent wheatgrass; FBM, Fleet meadow bromegrass; KCG, kirk crested grass, while DFC represents the daily cost of feeding per herd in $ $head^{-1}$ $day^{-1}$.

Table 7. 6 ANOVA of sole forage treatments and forage treatments mixed with barley straw for dry gestating beef cows based on dry matter intake (DMI), average daily gain (ADG), and daily feeding cost per herd during the 2022 year of forage harvest.

| Treatments | Treatment + barley straw DMI (Kg $day^{-1}$) | 2022 | | | Cost of straight treatment ($ $head^{-1}$ $day^{-1}$) |
|---|---|---|---|---|---|
| | | DMI straw (kg $day^{-1}$) | ADG (kg $day^{-1}$) | DFC($ $head^{-1}$ $day^{-1}$) | |
| M0 | 9.3 e | 4.00 | 0.10 | 1.45 g | 2.02 q |
| M1 | 10.1 ab | 4.30 | 0.10 | 2.17 e | 3.31 i |
| M2 | 9.7 c | 4.30 | 0.10 | 2.20 d | 3.36 g |
| M4 | 9.9 b | 4.20 | 0.10 | 2.16 e | 3.20 k |
| M7 | 10.0 b | 3.90 | 0.10 | 2.22 d | 3.25 j |
| M10 | 9.5 d | 4.40 | 0.10 | 2.18 e | 3.38 h |
| M11 | 10.3 a | 4.40 | 0.10 | 2.28 b | 3.57 e |
| M14 | 10.0 b | 4.70 | 0.10 | 2.30 b | 3.75 ab |
| M15 | 9.0 fg | 4.70 | 0.10 | 1.63 f | 2.56 e |
| M17 | 9.4 d | 4.80 | 0.10 | 2.21 d | 3.66 c |
| M18 | 9.7 c | 4.60 | 0.10 | 2.26 c | 3.60 d |
| M19 | 9.4 d | 4.60 | 0.10 | 2.20 d | 3.46 f |
| M20 | 9.7 c | 4.40 | 0.10 | 2.21 d | 3.45 f |
| M22 | 10.1 ab | 4.30 | 0.10 | 2.44 a | 3.78 a |
| OG | 8.9 fg | 4.50 | 0.10 | 1.78 i | 2.71 l |
| Fleet MB | 9.7 c | 3.70 | 0.10 | 1.70 k | 2.20 p |
| GPWG | 9.3 e | 4.45 | 0.10 | 1.74 j | 2.35 o |
| KCG | 10.3 a | 3.50 | 0.10 | 1.86 h | 2.55 n |
| TG | 9.1 f | 4.40 | 0.10 | 1.74 j | 2.66 m |
| *p* value | 0.005 | ** | **NS* | 0.040 | 0.007 |

*NS; not significant, GPWG; greenleaf pubescent wheatgrass, Fleet MB; Fleet meadow bromegrass, KCG; kirk crested grass, OG; orchard grass, TG; timothy grass, while DFC represents the daily feeding cost per herd in $ $head^{-1}$ $day^{-1}$.

Table 7. 7 ANOVA of sole forage treatments and treatments mixed with barley straw for dry gestating beef cows based on dry matter intake (DMI), average daily gain (ADG), and daily feeding cost per head during the 2023 year of forage harvest.

| Treatments | Treatment + barley straw DMI (Kg day$^{-1}$) | 2023 | | | DFC of sole treatment (\$ head$^{-1}$ day$^{-1}$) |
|---|---|---|---|---|---|
| | | DMI straw (kg day$^{-1}$) | ADG (kg day$^{-1}$) | DFC (\$ head$^{-1}$ day$^{-1}$) | |
| M0 | 8.9 g | 4.70 | 0.10 | 0.82 e | 1.02 j |
| M1 | 9.3 c | 4.80 | 0.10 | 0.87 c | 1.39 d |
| M2 | 9.5 ab | 4.60 | 0.10 | 0.87 c | 1.38 d |
| M3 | 9.4 b | 4.90 | 0.10 | 0.99 b | 1.46 a |
| M4 | 9.6 a | 5.00 | 0.10 | 1.01 a | 1.28 h |
| M5 | 9.5 ab | 5.20 | 0.10 | 1.01 a | 1.38 d |
| M6 | 9.6 a | 5.10 | 0.10 | 1.01 a | 1.36 e |
| M7 | 9.4 b | 5.00 | 0.10 | 1.00 a | 1.31 g |
| M8 | 9.6 a | 5.10 | 0.10 | 1.01 a | 1.40 c |
| M9 | 9.1 e | 5.20 | 0.10 | 0.98 b | 1.40 c |
| M10 | 9.4 b | 5.30 | 0.10 | 1.00 a | 1.36 e |
| M11 | 9.1 e | 5.20 | 0.10 | 0.98 b | 1.42 b |
| M12 | 8.9 g | 5.20 | 0.10 | 0.96 bc | 1.43 ab |
| M13 | 9.0 ef | 5.00 | 0.10 | 0.83 d | 1.05 i |
| M14 | 9.5 ab | 5.10 | 0.10 | 1.01 a | 1.43 ab |
| M15 | 8.7 h | 5.20 | 0.10 | 0.82 e | 1.05 i |
| M16 | 9.2 d | 5.10 | 0.10 | 0.98 b | 1.43 ab |
| M17 | 8.8 h | 5.40 | 0.10 | 0.96 bc | 1.45 a |
| M18 | 8.9 g | 5.50 | 0.10 | 0.97 bc | 1.43 ab |
| M19 | 9.0 ef | 5.60 | 0.10 | 0.98 b | 1.37 e |
| M20 | 9.6 a | 5.00 | 0.10 | 1.01 a | 1.33 f |
| M21 | 9.3 c | 5.30 | 0.10 | 1.00 a | 1.40 c |
| M22 | 9.1 e | 5.10 | 0.10 | 0.98 b | 1.43 ab |
| M23 | 9.1 e | 5.30 | 0.10 | 0.98 b | 1.40 c |
| OG | 8.5 i | 5.30 | 0.10 | 0.76 h | 1.00 k |
| Fleet MB | 8.6 i | 5.00 | 0.10 | 0.81 f | 1.01 j |
| GPWG | 9.3 c | 4.00 | 0.10 | 0.77 g | 0.89 m |
| KCG | 9.3 c | 4.00 | 0.10 | 0.77 g | 0.98 l |
| TG | 9.0 ef | 4.50 | 0.10 | 0.83 d | 1.05 i |
| *p* value | 0.010 | ** | **NS* | 0.003 | 0.020 |

*NS; not significant, GPWG; greenleaf pubescent wheatgrass, Fleet MB; fleet meadow bromegrass, KCG; kirk crested grass, OG; orchard grass, TG; timothy grass, while represents the daily feeding cost per herd in \$ head$^{-1}$ day$^{-1}$.

Table 7. 8 ANOVA of sole forage treatments and forage treatments combined with barley straw for predicting the performance of lactating beef cows based on dry matter intake (DMI) and daily feeding cost per head in two years of forage harvests (2021-2022).

| | | | 2021 | | | |
|---|---|---|---|---|---|---|
| Treatments | DMI (kg day$^{-1}$) | ADG (kg day$^{-1}$) | DFC of sole treatment ($ head$^{-1}$ day$^{-1}$) | Treatment + barley straw DMI (kg day$^{-1}$) | DMI barley straw added (kg day$^{-1}$) | DFC of treatment + straw ($ head$^{-1}$ day$^{-1}$ |
| M3 | 16.6 c | 0.10 | 1.98 e | 15.9 c | 1.10 | 1.93 e |
| M8 | 16.6 c | 0.10 | 2.29 c | 16.0 b | 1.00 | 2.25 a |
| M12 | 16.3 e | 0.10 | 2.15 d | 15.7 d | 1.10 | 2.09 c |
| M14 | 16.8 b | 0.10 | 2.32 a | 16.0 b | 1.35 | 2.25 a |
| M17 | 16.9 a | 0.10 | 1.44 f | 16.2 a | 1.25 | 2.06 d |
| M18 | 16.5 d | 0.10 | 2.25 d | 15.9 c | 1.10 | 2.20 b |
| M23 | 16.6 c | 0.10 | 2.31 b | 15.9 c | 1.15 | 2.25 a |
| *p* value | 0.042 | **NS* | 0.033 | 0.006 | **NS* | 0.044 |
| Treatments | | | 2022 | | | |
| M0 | 15.4 d | 0.10 | 2.13 e | 16.0 c | 1.00 | 2.09 e |
| M6 | 17.4 a | 0.10 | 1.25 f | 17.0 a | 1.50 | 1.16 f |
| M13 | 15.8 c | 0.10 | 2.43 d | 16.0 c | 2.00 | 2.22 d |
| M15 | 16.0 b | 0.10 | 2.56 c | 16.1 b | 1.80 | 2.35 c |
| KCG | 15.9 b | 0.10 | 2.66 a | 16.4 b | 1.00 | 2.59 a |
| TG | 15.4 d | 0.10 | 2.65 ab | 15.8 d | 1.20 | 2.54 b |
| *p* value | 0.038 | **NS* | 0.001 | 0.005 | *NS | 0.043 |

*NS; not significant, TG; timothy grass, while DFC represents the daily feeding cost per head in $ head$^{-1}$ day$^{-1}$.

Table 7. 9 ANOVA results of sole forage treatments combined with barley straw for predicting the performance of lactating beef cows based on dry matter intake (DMI) and daily feeding cost per head in 2023.

| Treatment | DMI (kg $day^{-1}$) | ADG (kg $day^{-1}$) | DFC of sole treatment ($ $head^{-1}$ $day^{-1}$) | Treatment + barley straw DMI (kg $day^{-1}$) | DMI barley straw added (kg $day^{-1}$) | DFC of treatment + straw ($ $head^{-1}$ $day^{-1}$) |
|---|---|---|---|---|---|---|
| M0 | 15.3 e | 0.10 | 1.10 f | 16.0 d | 1.35 | 1.09 e |
| M1 | 16.5 d | 0.10 | 1.39 e | 16.7 c | 1.10 | 1.35 d |
| M2 | 16.4 d | 0.10 | 1.40 d | 16.9 c | 1.10 | 1.38 b |
| M5 | 16.9 b | 0.10 | 1.44 b | 17.3 ab | 1.40 | 1.42 a |
| M6 | 17.2 a | 0.10 | 1.46 a | 17.7 a | 1.00 | 1.43 a |
| M7 | 16.4 d | 0.10 | 1.40 d | 17.0 c | 1.25 | 1.38 b |
| M8 | 16.9 b | 0.10 | 1.44 b | 17.4 a | 1.30 | 1.42 a |
| M9 | 16.4 d | 0.10 | 1.40 d | 16.9 c | 1.20 | 1.38 b |
| M10 | 16.9 b | 0.10 | 1.44 b | 17.3 ab | 1.10 | 1.43 a |
| M11 | 16.6 d | 0.10 | 1.42 c | 17.0 c | 1.00 | 1.38 b |
| M13 | 15.4 e | 0.10 | 1.10 f | 15.9 d | 1.30 | 1.09 e |
| M14 | 16.8 c | 0.10 | 1.43 c | 17.3 ab | 1.40 | 1.41 ab |
| M15 | 15.4 e | 0.10 | 1.10 f | 16.0 d | 1.15 | 1.09 e |
| M19 | 16.6 d | 0.10 | 1.42 c | 16.8 c | 1.15 | 1.37 c |
| M21 | 16.5 d | 0.10 | 1.41 d | 17.1 c | 1.30 | 1.39 b |
| M23 | 16.4 d | 0.10 | 1.40 d | 16.9 c | 1.30 | 1.37 c |
| OG | 15.3 e | 0.10 | 1.10 f | 15.7 e | 1.20 | 0.99 g |
| KCG | 14.8 f | 0.10 | 0.98 g | 15.3 f | 1.00 | 0.97 g |
| TG | 15.3 e | 0.10 | 1.11 f | 15.8 e | 1.00 | 1.00 f |
| *p* value | 0.001 | **NS* | 0.014 | 0.006 | **NS* | 0.039 |

*NS; not significant, KCG; kirk crested grass, OG; orchard grass, TG; timothy grass, while DFC represents the daily feeding cost per herd in $ $head^{-1}$ $day^{-1}$.

Table 7. 10 Requirements for categories of cattle and environmental conditions used for ration formulation in the Cowbytes® program.

| Category of cattle | Initial body weight (kg) | Final body weight (kg) | Average daily gain (kg day$^{-1}$) | Temperature (°C) | NDF as % body weight | Birth weight (kg) | Month of lactation |
|---|---|---|---|---|---|---|---|
| Steers | 318 | 658 | 0.80 | -20 to 20°C | 1.2 | 0 | 0 |
| Dry gestation beef cow | 680 | 771 | 0.10 | -20 to 20°C | 1.2 | 39 | 0 |
| Lactating beef cow | 680 | 771 | 0.10 | -20 to 20°C | 1.2 | 41 | 2 |

*NDF; neutral detergent fiber. The average daily gain is an expected constant weight gain input of the ration simulation.

## Chapter 8. Identifying top performers of temperate perennial forages based on cluster analysis

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## Study synopsis

Perennial forage is vital to the sustainability of the beef cattle industry. Although producers cultivate a plethora of forage species, there is still skepticism about the right combinations of forage plant species (mixtures) that can substantially improve their forage-livestock systems. This study was conducted to evaluate the nutritive values and physiological traits of 29 perennial forage species comprising monoculture grasses and grass–legume mixtures using correlation and clustering analysis. A field trial was established in 2020 using a randomized complete block design with four replications at the Peace Country Beef & Forage Association, Fairview, Canada, with data collected over three consecutive production years: 2021, 2022, and 2023. Analysis of variance revealed significant differences among forage treatments for dry matter yield (DMY), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN), neutral detergent fiber digestibility (NDFD-48h), calcium, potassium, phosphorus, magnesium, water use efficiency (WUE), and crude protein water use efficiency ($WUE_{CP}$) ($p < 0.05$). The DMY for perennial forages ranged from 1.4 to 3.6 Mg $ha^{-1}$, CP ranged from 11.4 to 16.3%, TDN ranged from 61.2 to 65.1%, NDFD-48h ranged from 51.8 to 60.9, WUE ranged from 29.1 to 71.7 kg of DM $ha^{-1}$ $mm^{-1}$ whereas $WUE_{CP}$ ranged from 362.3 to 1086.3 kg of CP $ha^{-1}$ $mm^{-1}$. Forage DMY was positively correlated with CP (r = 0.62, $p < 0.001$), whereas forage DMY was negatively correlated with NDFD-48h (r = -0.64, $p < 0.001$), ADF (r = -0.40, $p < 0.01$), and NDF (r = -0.47, $p < 0.01$). Multivariate analysis grouped our perennial forage options into three main clusters: I, II, and III; based on their physiological traits and nutritive values. Overall, cluster I encompassed monoculture grasses and grass-only mixtures, cluster II contained grass–legume mixtures with high proportions of legumes, whereas cluster III included grass–legume mixtures but with lower legumes proportions. With notable advantages, forage mixtures within cluster II were generally higher in DMY, CP, WUE, $WUE_{CP}$, TDN, and calcium content. Several forage grass–legume mixtures from within cluster II were identified as superior, including AC Knowles hybrid bromegrass + Spredor 5 alfalfa (M6), AC Mountainview sainfoin + AC Veldt cicer milkvetch + Spredor 5 alfalfa (M14), AC Success hybrid bromegrass + AC yellowhead alfalfa + AC Mountainview sainfoin (M17), AC Success hybrid bromegrass + AC yellowhead alfalfa + Rugged alfalfa + AC Mountainview sainfoin (M21), and AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + Kirk crested wheatgrass + Italian ryegrass + AC yellowhead alfalfa + AC Veldt cicer milkvetch + AC Mountainview sainfoin (M23). These selected mixtures achieved better forage yield, CP, WUE, $WUE_{CP}$, and lower NDF and ADF, and are considered suitable for temperate climatic conditions comparable to western Canada.

**Keywords:** nutritive value, perennial forage, physiological traits, grass–legume mixtures

## 8.0 Introduction

Perennial forage production is an important component of beef cattle production worldwide (Sheppard et al., 2015; Gillian et al., 2021). Forages often contribute 80% of the beef cattle diet during their entire lifetime (McAllister et al., 2020; BCRC, 2023). In western Canada, beef cattle producers depend primarily on perennial alfalfa (*Medicago sativa* L.) -grass mixtures and seldom on grain supplements for feeding (Klinger et al., 2007; Foster et al., 2014; Darambazar et al., 2022). Nevertheless, beef cattle producers continue to search for perennial forage mixtures that can substantially improve their livestock-forage systems.

Since forage systems are seen as having benefits to both agricultural production and environmental sustainability, there is a need to identify forage mixtures that score high for both physiological traits and nutritive values. This can be achieved through the use of multi-criteria analysis. This is a structured approach used to determine the overall preferences among alternative treatments which present several characteristics (Dean, 2020). This approach is crucial in resolving problems with conflicting criteria and objectives and allows the researcher to identify a single preferred alternative and also explore trade-offs between different options (Dooley et al., 2009). For instance, Dhakal et al. (2020) evaluated the trade-offs between nutritive improvements water use efficiency for an alfalfa-grass system through various criteria approach and found that the lower densities and characteristics of alfalfa minimizes the trade-off between forage improvement and soil water consumption.

A cluster analysis can also be utilized as a multi-criteria optimization approach to simplify decision making about selecting and grouping forage based on their similar traits. Villalba et al. (2016), evaluated the nutritive value of forages based on their ability to fulfil requirements of growing beef calves and clustered forage treatments into categories based on hay (low quality), and pastures (high quality). This indicates that by using cluster analysis, forage establishments can be tailored to the needs of the beef cattle.

A number of common grass species cultivated in Westen Canada include timothy (*Phleum pratense*), bromegrasses (*Bromus* spp.), orchardgrass (*Dactylis glomerata*), ryegrass (*Lolium perenne*) and fescues (*Festuca* spp.), whereas the legume species include alfalfa, sainfoin (*Onobrychis viciifolia* Scop), cicer milkvetch (*Astragulus cicer* L.), red clover (*Trifolium* *pratense*), and birdsfoot trefoil (*Lotus corniculatus* L.). Several studies have reported on the yield and nutritive value of grass-alfalfa mixtures (Foster et al., 2014; Aponte et al., 2019; Darambazar et al., 2022), grass-sainfoin mixtures (Wang et al., 2006; Nielsen, 2021), and grass-vetch mixtures (Foster et al., 2019; Peprah et al., 2021). Although these forage mixtures may benefit the beef cattle industry, they present some challenges. For instance, grazing cattle on alfalfa-grass mixtures have been reported to cause bloat (Majak et al., 2003; Khatiwada et al., 2020). However, sainfoin-grass mixtures reduce the incidence of bloat (Lees, 1992; Bhattarai et al., 2016) due to its high concentration of condensed tannins (Sottie et al., 2014; Kelln et al., 2020).

By comparing grass–legume mixtures to monoculture grasses, it is also notable that they are diverse in their use of resources, particularly water within cropping systems, which affects their overall performance. Gyamfi et al. (unpublished data) found that grass–legume mixtures are largely water use efficient than monoculture grasses. Roscher et al (2016) stated that a well-defined complementary relationship between mixtures is crucial in increasing forage yield in drought stress conditions, by providing supportive ecological functions.

Although previous studies have focused on forage yield and nutritive values (Schellenberg et al., 2012; Serajchi et al., 2017; Foster et al., 2019), and water use-efficiencies (Jefferson and Cutforth, 2005) of perennial forages in western Canada, no studies have investigated and selected the best performing perennial forage species (either in mixtures or monocultures) based on combining their nutritive values and physiological traits. Therefore, the objectives of this study were (i) to holistically evaluate the nutritive values and physiological traits of 29 perennial forage treatments under northwestern Alberta growing conditions, and (ii) to identify the best forage mixtures to improve forage-livestock systems.

## 8.1 Materials and method

### 8.1.1 Site description and experimental design

A perennial forage field experiment was established in June 2020 at the research farm of the Peace Country Beef & Forage Association in Fairview, northwestern Alberta, Canada (lat. 56°04′53′ N, long. 118°26′05′W, 670 m above sea level) and was evaluated for three production years (2021, 2022, and 2023). The field had a history of several years of wheat (*Triticum* *aestivum*)- canola (*Brassica napus* L.) rotation for grain production. The soil at the experimental site was classified as Eluviated Black Chernozem according to the Canadian System of Soil Classification (Agricultural Region of Alberta Soil Inventory Database AGRASID; Government of Alberta, 2020). The weather information during the growing seasons of the field experiment and their long-term averages for the site (Table 8.1) was obtained from the Alberta Climate Information Service (ACIS). The maximum air temperature during the entire study period was higher than the long-term mean whereas the minimum air temperatures were lower than the long-term average. Cumulative precipitation during the 2020 growing season was higher than the subsequent years (2021-2023) but lower than long-term average. A randomized complete block design (RCBD) with four replications ($n$ = 4) was used in this trial. Forage treatments comprised six grass–legume (one grass and one legume) and 18 complex diverse perennial forage species (i.e., three or more grass–legume) mixtures. Within the complex mixtures were three grass-only mixtures, two legume-only mixtures and 13 grass–legume mixtures. In addition, there were five pure stands of perennial grasses which were used (Table 8.2 and Appendix 1). On 26 June 2020, all grasses and legume seeds were seeded at 1.3 cm depth with a six-row Fabro plot drill (Fabro Ltd Swift Current, SK, Canada) equipped with disc-type openers at 23 cm row spacing on experimental plots, measuring 2 m wide by 8 m in length with 2 m alleys between replicates. The seeding rates were estimated based on the monoculture seeding rate recommendations of perennial forage by Hutton et al. (2005). Before seeding, alfalfa seeds were inoculated with Nitragin Gold alfalfa granule inoculants from Northstar Seeds, Edmonton, Canada at a rate of 1.5 g per 250 g of seeds, whereas birdsfoot trefoil, cicer milkvetch, and sainfoin were not inoculated due to the lack of commercially available inoculants. In the establishment year (2020) and first year of production (2021), the plots were sprayed with Basagran Forte at 4.05 mL $ha^{-1}$ for in-crop weed control. Additionally, dandelions (*Taraxacum officinale*), volunteer wheat and canola were occasionally handpicked. Subsequently, from 2022–2023, no further weed control measures (chemical spraying or manual) were carried out.

### 8.1.2 Plant harvest procedure and forage quality analysis

On 3 July 2021, 30 June 2022, and 22 June 2023, an area of 3 m encompassing four inner rows of the plots were harvested at 10-15% of the alfalfa legume blossom (Bonin and Tomlin, 1968; Saskatchewan Forage production guide). The above ground biomass was harvested at a stubble height of 7.5 cm using a custom-made self-propelled small plot forage harvester (Swift Machine and Welding Ltd., Swift Current SK, Canada). The harvested biomass was weighed fresh and sub-samples (up to1000 g) were air-dried to determine forage dry matter content, which was used to calculate forage yield on a dry matter (DM) basis in (Mg $ha^{-1}$). The dried forage sub-samples were analyzed in a commercial laboratory (A&L Canada Laboratory, London, Ontario, Canada) for feed quality parameters, including CP, ADF, and NDF. In addition, the total nitrogen (N) concentration was determined using the micro-Kjeldahl method (AOAC, 1984), and the total N was multiplied by 6.25 to determine the CP content (Schroeder, 1994). The NDF and ADF were determined using an $ANKOM^{2000}$ fiber analyzer (Model 2000; ANKOM; Fairport, NY, USA). Total digestible nutrients were calculated based on equations provided by (Weiss and Hall, 2020) whereas forage minerals (calcium, phosphorus, potassium, magnesium) by using near infrared spectroscopy (NIRS) (Amari and Abe, 1997). The *in vitro* NDFD was determined by using a 48-h incubation buffered rumen fluid (Goering and Van Soest, 1970).

### 8.1.3 Water-use efficiency determination

Water use efficiency was calculated using the formula postulated by Gao et al. (2009).

$$\text{WUE} = \frac{Y(Kg\ \text{ha}-1\ )}{ET(mm)}$$

Evapotranspiration (ET) is expressed as:

$$\text{ET} = \Delta\text{S} + \text{P} + \text{I} + \text{U} - \text{R} - \text{D}w$$

Where **ΔS** denotes the seasonal changes in water content in the soil determined by using volumetric water content (VWC) measurement. The volumetric water content was measured weekly from vegetative stage of forage plants to early or mid -blossom stage prior to harvest (mid-May to early July 2021). This was done using the Time Domain Reflectometry (TDR- 350) method with a rod length of 0 to 15.2 cm. The changes in the VWC were converted from percentages (%) value into the water column (mm) by multiplying the measured VWC to the soil depth of 0 to 15.2 cm. In both 2022 and 2023, the VWC reading was done using a rod length of 0 to 25.4 cm due to an anticipated development in root systems of the treatments.

The **P** represents the total rainfall in (mm) during the entire growing season in each study year. For instance, the rainfall for each growing season was accounted for from May to the last week of June or early week of July before the forage was harvested. Data was obtained from the AICS (2021-2023) weather station. Cumulative seasonal rainfall in 2021 was 120.6 mm, and in 2022 was 164.3 and in 2023 was 40.8 mm (Table 8.1). **I** connotes irrigation and since the plots were not irrigated, **I** was not considered in the calculation. Run off (**R**) was considered negligible in the equation due to the flat topography of the experimental site at the Fairview research farm. The upward capillary (**U**) flow to root and downward (**D***w*) drainage was also assumed negligible based on Darcy's law (De Medeiros et al., 2005; Kar et al., 2007).

Y represents the biomass yield or CP yield. This comprises the forage dry matter measured in kg $ha^{-1}$ as ($WUE_{BM}$) and the CP yield obtained by multiplying the DM yield and CP of the forage as the Y for 'CP WUE ($WUE_{CP}$)'.

### 8.1.4 Data analysis

Research data was subjected to analysis of variance (ANOVA) with randomized complete block design (RCBD) using the NLME program (Pinheiro et al., 2020). The forage treatments and variables (DMY, CP, ADF, NDF, NDFD-48h, TDN, calcium, phosphorus, potassium, magnesium, WUE, and $WUE_{CP}$) were considered as fixed effect whereas the block replication and year were taken as a random effect. Before ANOVA, normality of residuals was tested using the Shapiro-Wilk statistic and homogeneity of variances was tested using Levene's test. No data transformations were required, as residuals of all data were homogeneous and normally distributed. Following ANOVA, Tukey's honestly significant difference (HSD) test was used to separate treatment means when significant ($P < 0.05$). In addition, Pearson correlations among traits of forage treatments were conducted using the performance analytic program, while the coefficient of variance was estimated using complete pair observation of the traits. Based on the 12 physiological traits and nutritive values, clustering of forage treatments was performed with 3-yr means whereas principal component analysis (PCA) was also conducted for the 29 forage treatments. We used R statistical software (Version 4.2.3) (R Core Team 2020).

## 8.2 Results

### 8.2.1 Variations in physiological traits and nutritive values of perennial forages

Across the 12 physiological traits and nutritive forage attributes assessed in our study, the coefficient of variation was highest for $WUE_{CP}$ (29.2%), WUE (24.3%), and DMY (22.5%) and lowest for TDN (1.4%), phosphorus (3.20%), ADF (4.74%), and NDF (4.87%). The DMY was low in 4 of the 29 forage treatments (Table 8.3). DMY of treatments ranged from 1.4 Mg ha$^{-1}$ for timothy grass (TG) to 3.6 Mg ha$^{-1}$ for M14 (Table 8.3). The forage treatments with high DMY were found in M4, M14, M21, and M23. Averaged across the three years of production, the CP of forage treatments ranged from 11.4% for Fleet meadow bromegrass (FMB) and greenleaf pubescent wheatgrass (GPWG) to 16.3% for M3, M14, M16, and M21, with an average of 14.4% (Table 8.3). The top five forage treatments with the highest CP were M3, M14, M16, M21, and M22. For example, M3 has 50% legume, M14 has 100% legume, M16 has 100% legume, M21 has 70% legume, and M22 has 90%. In general, monoculture grasses and their mixtures were the poorest in CP, whereas legume-dominated mixtures were the highest in CP, except for M3. ADF ranged from 38.5 to 45.6% with a mean value of 40.8%, whereas NDF ranged from 41.8 to 50.2%, with a mean value of 45.1% (Table 8.3). The five treatments with the best (i.e., lowest) ADF included M12, M16, M22, M17, and M3, whereas those for NDF included M21, M16, M14, M17, and M3. TDN varied slightly among the treatments ranging from 61.2% (M4) to 65. 1% (M0), with an average of 62.7%. The NDFD-48h ranged from 51.8 (M20) to 60.9 (M0) with an average of 54.3 (Table 8.3).

Calcium content ranged from 0.8% (M0) to 1.5% (M14 and M16) with a mean value of 1.2%. Phosphorus and magnesium contents were not significantly different among forage treatments. However, potassium content varied significantly among forage treatments ranging from 1.8 to 2.1% with an average of 1.8%. The treatments with the highest potassium were FMB, GPWG, M21, and M4 (Table 8.3).

The WUE ranged from 29.1 (FMB) to 71.7 kg of DM ha$^{-1}$ mm$^{-1}$ (M14) with an average of 47.8 kg of DM ha$^{-1}$ mm$^{-1}$. The five treatments with the highest WUE values were M14, M4, M21, M23, and M17 (Table 8.3). The $WUE_{CP}$ also ranged from 362.3 kg of CP ha$^{-1}$ mm$^{-1}$ (FMB) to

1086.3 kg of CP ha$^{-1}$ mm$^{-1}$ (M14) with an average value of 685 kg of CP ha$^{-1}$ mm$^{-1}$. The top five treatments with the highest $WUE_{CP}$ were M14, M4, M23, M21, and M6 (Table 8.3).

### 8.2.2 Associations among physiological traits and nutritive values

Forage dry matter had a significant positive correlation with crude protein (r = 0.62, $p < 0.001$), calcium (r = 0.067, $p < 0.001$), WUE (r = 0.90, $p < 0.001$), and $WUE_{CP}$ (r = 0.91, $p < 0.001$) (Table 8.4). However, dry matter yield was negatively correlated with NDFD-48h (r = -0.64, $p < 0.001$), ADF (r = -0.40, $p < 0.01$), and NDF (r = -0.47, $p < 0.01$). Crude protein was negatively correlated with ADF, NDF, and NDFD-48h (r = -0.93, $p < 0.001$; r = -0.92, $p < 0.001$; r = -0.82, $p < 0.001$, respectively), whereas a positive correlation was observed for calcium (r = 0.89, $p < 0.001$), WUE (r = 0.065, $p < 0.001$), and $WUE_{CP}$ (r = 0.79, $p < 0.001$). ADF was strongly positively correlated with NDF and NDFD-48h (r = 0.93, $p < 0.001$ and r = 0.79, $p < 0.001$, respectively). Similarly, NDF had a significant positive correlation with NDFD-48h (r = 0.73, $p < 0.001$), and $WUE_{CP}$ (r = 0.69, $p < 0.001$). However, a negative correlation was found in calcium with NDF (r = -0.78, $p < 0.001$) and NDF with WUE (r = -0.56, $p < 0.01$). Furthermore, TDN was positively correlated with NDFD-48h (r = 0.41, $p < 0.01$). In contrast, NDFD-48h correlated negatively with calcium (r = -0.87, $p < 0.001$), WUE (r = -0.67, $p < 0.001$), and $WUE_{CP}$ (r = -0.75, $p < 0.001$). Calcium also showed a significant positive correlation with both WUE (r=0.62, $p$<0.001) and $WUE_{CP}$ (r = -0.81, $p < 0.001$). No correlation was observed between the minerals (calcium, potassium, magnesium, and phosphorus). The WUE was positively correlated with $WUE_{CP}$ (r = 0.94, $p < 0.001$).

### 8.2.3 Clustering of perennial forage treatments based on physiological traits and nutritive values

The PCA showed that the first two components had an eigen value more than 1 and the first two components of the PCA explained 68.2% of the total observed variation (Table 8.5). The assessment of eigen vectors also revealed that CP, NDFD-48h, calcium, and $WUE_{CP}$ were the most important contributors to PC1, whereas DMY, TDN, and potassium were strongly associated with PC2. Analysis conducted showed that most of the variation in means is expressed by the first two PCs. The positive X axis is highly influenced by calcium, CP, and $WUE_{CP}$ traits, while the negative

X axis is highly influenced by NDFD-48h, ADF, and NDF traits (Figure 8.1). Similarly, the positive Y axis is highly influenced by TDN and phosphorus traits and the negative Y axis is highly influenced by potassium and DMY (Figure 8.1).

Additionally, the PCA biplot depicted trends in traits associated with perennial forage (Figure 8.1). FMB and GPWG had the highest ADF, NDF, and potassium content, but were also lowest in CP. Notably, the ADF and NDF were strongly correlated. The NDFD-48h and TDN were highest in TG, Kirk crested wheatgrass (KCG), M0, M13, and M15 but lowest in WUE, $WUE_{CP}$, DMY, and calcium. Crude protein was highest in M12, M16, M17, M21, and M22, but ADF, NDF, and potassium were lowest in these treatments. Although various treatments were high in DMY, WUE, $WUE_{CP}$, and calcium, treatments M14 and M4 contained the highest, but were also deficient in NDFD-48h.

The cluster analysis based on the 12 physiological traits and nutritive values of perennial forage is shown in three clusters (Figure 8.2). The first cluster comprised eight treatments, which were characterized by high ADF, NDF, TDN, and NDFD-48h (Table 8.6). The second cluster included 12 treatments characterized by high dry matter yield, CP, calcium, WUE, and $WUE_{CP}$. Nine treatments were present in cluster III and demonstrated the second-best performance to cluster II in terms of dry matter yield, CP, TDN, NDFD-48h, and $WUE_{CP}$. Cluster I consists of monoculture grasses and a complete blend of grass-only mixtures whereas Cluster II and III are either pure legume mixtures or grass–legume dominated mixtures. However, the majority of treatments in Cluster II contain higher proportions of legumes than those of Cluster III.

### 8.3 Discussion

Perennial forage treatments were grouped into three main clusters based on their physiological traits and nutritive values. The relationship between forage treatments as revealed by physiological traits and nutritive values in this study is supported by Gyamfi et al. (unpublished data). In their findings, the physiological traits (WUE and $WUE_{CP}$) of perennial forage treatments particularly those in cluster I showed similar patterns to those of our results. All treatments in cluster I were either monoculture grasses or grass mixtures suggesting similar phenotypic characteristics and performance. In addition, treatments in cluster I were the poorest in dry matter yield, crude protein, and crude fibers (NDF and ADF). This observation agrees with the findings of Foster et al. (2021) which reported lower dry matter yield, crude protein, and higher ADF in pure grasses compared with grass–legume mixtures. Nevertheless, treatments in cluster I also showed superior TDN and NDFD-48h which agrees with the findings of Gyamfi et al. (unpublished data) which reported high TDN and NDFD-48h values in monoculture grasses and grass-only mixtures. The treatments in clusters II and III performed similarly, although those of cluster II were slightly higher than in cluster III. This was expected because treatments in both clusters had legumes at varying proportions. For instance, treatments grouped into cluster II had higher dry matter yield, crude protein, WUE, and $WUE_{CP}$, and lower ADF and NDF. This is in part attributed to the higher proportions of legumes within mixtures compared to those of cluster III. For example, M14 and M16 were pure legume mixtures (100%) whereas M22 had as high as 90% of legumes. In contrast, this observation was absent from any of the treatments in cluster III. The only treatment with a high proportion of legumes was the M10 treatment (75%). In addition, treatments in cluster II had a higher number of plant species than those of cluster III. Tokatlidis et al. (2022) posited that high plant densities optimize resource use and promote water and nutrient utilization, which eventually increases crop yield. Similarly, Sanderson et al. (2004) reported that there is a direct relationship between plant species diversity and forage yield. This implies that it is necessary to consider treatments with high plant species (with legumes) to attain better forage yield and nutritive value. However, high plant diversity does not always guarantee a high forage yield. This confirmed our observation in a 3-way mixture (M14) and 6-way mixture (M22), where M14 had higher yield (3.6 Mg $ha^{-1}$) than M22 (2.9 Mg $ha^{-1}$).

The potential total forage dry matter yield after establishment is an important selection criterion for forage producers. However, in an attempt to achieve high forage productivity, nutritive values of the established forage crop may be compromised. This is because forage dry matter increases with advancing maturity whereas forage quality declines. Ball et al. (2001) posited that grass digestibility is often above 80% during the first 2 to 3 weeks after growth initiations in the spring but declines between ⅓ and ½ % per day until it reaches a digestibility below 50%. Based on this notion, we anticipated that nutritive value such as CP would be affected as the plant ages, as reported by previous studies (Horrocks and Vallentine, 1999; Nair et al., 2018). In contrast, our study showed a significant positive correlation between dry matter yield and crude protein (Figure 8.3). This may be due to individual characteristics of various plant species within treatments. Several mixtures with a high proportion of legumes within stands showed high CP content. The M3, M14, M16, M21, and M23 treatments had the highest CP contents. Nevertheless, this did not translate consistently into high forage biomass. For instance, M14 (100% legume) had a CP of 16.3% and forage dry matter of 3.6 Mg $ha^{-1}$ whereas M16 (100% legume) also had CP of 16.3%, but a lower forage dry matter of 2.7 Mg $ha^{-1}$. Similarly, having a high proportion of legumes did not commensurate with high CP content. This is true for M20 which has 70% legume but had similar CP (15.8%) as orchard grass monoculture and M12 which had 33.3% legume. Although treatments were harvested at the same period, our observations in CP may also have been driven by the phenological stage of the various plant species across treatments. This is because, in mixtures, individual species attain maturity at different times.

Dry matter yield was positively correlated with WUE and $WUE_{CP}$ as anticipated (Figure 8.3). This suggests that water is a major driving force behind high forage yield. This is similar to findings by Gyamfi et al (unpublished data) who reported positive effects of water availability on dry matter yield in a dry growing season. Negative correlations between dry matter yield and crude fibers (NDF and ADF) seem to suggest that as plants mature, crude fibers decrease, which contradicts the findings of previous studies (Horrocks and Vallentine, 1999; Ball et al., 2001; Atis et al., 2012; Moore et al., 2020). For example, as plant matures there is a decline in leaf:stem ratio leading to an increase in fibers and decrease CP. A significant positive correlation between NDF, ADF, and NDFD-48h indicates that as the NDF concentration increases affecting the potential forage intake, the ADF and NDFD-48h which measures the forage digestibility decrease. This phenomenon is key to understanding the balance between yield and quality, particularly when cultivating various forages for different purposes. Our results also showed that crude fiber content was negatively correlated with crude protein content. This suggests that maturity of forage plants results in more stems and higher fibers and less CP. In contrast, NDFD-48h was negatively correlated with WUE and $WUE_{CP}$. This indicated that under water stress conditions coupled with higher temperatures, such as the 2021 production season, poor-quality forages resulted in lower NDFD-48h. This is because forages tend to be lower in quality if produced in warm conditions than in cool conditions, which is consistent with the findings of Gardarin et al. (2014) and Lee et al. (2017).

In conclusion, the 29 perennial forage treatments showed a high degree of variation in all measured traits. A plethora of promising forage treatments including  mixtures of AC Knowles hybrid bromegrass + spredor 5 alfalfa (M6), AC Mountainview sainfoin + Veldt cicer milkvetch + spredor 5 alfalfa (M14), AC Success hybrid bromegrass + AC Yellowhead alfalfa + AC Mountainview sainfoin (M17), AC Success hybrid bromegrass + AC Yellowhead alfalfa + AC Mountainview sainfoin (M21), and AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + Kirk crested wheatgrass + Italian rye grass + AC Yellowhead alfalfa + rugged alfalfa + Veldt cicer milkvetch + AC Mountainview sainfoin (M23) have been identified via clustering while selecting for high forage yield, crude protein, WUE, and $WUE_{CP}$, and lower NDF and ADF. In further details, the selection of high forage dry matter yield and nutritive values may be accomplished by choosing diverse forage mixtures highly dominated by legumes.

## 8.4 References


Alberta Climate Information Service (2020). Current and historical Alberta weather station data viewer. Data provided by Alberta Agriculture and Forestry. Retrieved on 21 May 2021, from https://acis.alberta.ca/acis/weather-data-viewer.jsp

Amari, M., and Abe, A. (1997). Application of near infrared reflectance spectroscopy to forage analysis and prediction of TDN contents. *Japan Agricultural Research Quarterly*, *31*(1), 55–63.

AOAC. (1984). Official Methods of Analysis, 14th Edition. AOAC.

Aponte, A., Samarappuli, D., and Berti, M. T. (2019). Alfalfa–grass mixtures in comparison to grass and alfalfa monocultures. *Agronomy Journal*, *111*(2), 628–638 https://doi.org/10.2134/agronj2017.12.0753

Atis, I., Konuskan, O., Duru, M., Gozubenli, H., and Yilmaz, S. (2012). Effect of harvesting time on yield, composition and forage quality of some forage sorghum cultivars. International Journal of Agriculture & Biology. 14, 1814–9596. http://www.fspublishers.org

Ball, D., Collins, M., Lacefield, G., Martin, N., Mertens, D., Olson, K., Putnam, D., Undersander, D., and Wolf, M. (2001). Understanding forage quality. 2–5.

Beef Cattle Research Council, BCRC. (2023). Forage species. Accessed on May 7, 2023, from https://www.beefresearch.ca/topics/forage-species/

Bhattarai, S., Coulman, B., and Biligetu, B. (2016). Sainfoin (*Onobrychis viciifolia* Scop.): Renewed interest as a forage legume for Western Canada. Canadian Journal of Plant Science, 96(5), 748–756. https://d oi.org/10.1139/cjps-2015-0378

Bonin, S.G. and Tomlin, D.C. (1968). Effects of nitrogen on herbage yields of timothy harvested at various stages. Canadian Journal of Plant Science , 48, 501–509. https://doi.org/10.4141/cjps68-097

Darambazar, E., Larson, K., Schoenau, J., Wang, G., Biligetu, B., Damiran, D., Lardner, H. A. (2022). Evaluation of alfalfa and grass species in binary and complex mixtures on performance under soil salinity conditions. *Agronomy*, *12*, 1672.

https://doi.org/10.3390/agronomy12071672

Dean, M. (2020). Multi-criteria analysis. In Advances in Transport Policy and Planning (Vol. 6, pp. 165-224). Academic Press.

De Medeiros, G. A., Arruda, F. B., and Sakai, E. (2005). Crop coefficient for irrigation beans derived using three reference evaporation methods. Agricultural and Forest Meteorology. 115, 135–143.

Dhakal, M., West, C. P., Villalobos, C., Sarturi, J. O., and Deb, S. K. (2020). Trade-offs between nutritive value improvement and crop water use for alfalfa-grass systems. Crop Science, 60, 1711–1723.

Dooley A. E., Smeaton D. C., Sheath G. W., and Ledgard S. F. (2009). Application of multiple criteria decision analysis in the New Zealand agricultural industry. Journal of Multi-Criteria Decision Analysis, 16(1–2), 39–53.

Foster, A., Biligetu, B., and Darambazar, E. (2019). Forage accumulation, nutritive value, and botanical composition of grass–cicer milkvetch mixtures under two harvest managements. *Crop Science*, 59(6), 2876–2885. https://doi.org/10.2135/cropsci2019.03.0198

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.Volume 94. pp. 41–50. https://doi.org/10.4141/CJPS2013-095

Gao, Y, Duan, A., Sun, J., Li, F., Liu, Z., Liu, H., and Liu, Z. (2009). Crop coefficient and water-use efficiency of winter wheat/spring maize strip intercropping. Field Crops Research, 111, 65–73,

Gardarin, A., Garnier, E. et al. (2014). Plant trait-digestibility relationships across management and climate gradients in permanent grasslands. Journal of Applied Ecology, 51 (5) 1207–1217. https://doi.org/10.1111/1365-2664.12293

Gillian, B., Ali Mohamed, A., Oladokun, S., Wang, J., and Davis, H. (2021). Forage-fed cattle point the way forward for beef? Future Foods, Volume 3, 2021,100012, ISSN 2666-8335, https://doi.org/10.1016/j.fufo.2021.100012

Goering, H. K., and Van, P. J. (1970). Forage fiber analyses. *U.S.* Agricultural Research Service, 379, 387–598.

Government of Alberta (2020). Agricultural region of Alberta soil inventory database. Accessed on 29 February 2024 at https://www.alberta.ca/agricultural-regions-of-alberta-soil-inventory-database

Gyamfi, H., Hernandez Ramirez, G., Omokanye, A., and Thilakarathna, M. S. (2024). Biomass and crude protein water use efficiencies of perennial forage mixtures [Unpublished manuscript]. University of Alberta, Edmonton, AB.

Gyamfi, H., Omokanye, A., Hernandez Ramirez, G., and Thilakarathna, M. S. (2024). Forage dry matter yield, nutritive value, and botanical composition of diverse forage species. [Manuscript submitted for publication]. University of Alberta, Edmonton, AB.

Horrocks, R. D. and Vallentine, J. F. (1999). Harvested Forages. Academic Press, London, UK. Pages 245–277, ISBN 9780123562555. https://doi.org/10.1016/B978-012356255-5/50035-3

Hutton, G., Berg, B., Najda, H., Johns, M., Cole, D., and Yoder, C. (2005). Perennial forage establishment in Alberta. Government of Alberta. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9682/$file/120_22-3.pdf?OpenElement

Jefferson, P. G., and Cutforth, H. W. (2005). Comparative forage yield, water use, and water use efficiency of alfalfa, crested wheatgrass and spring wheat in a semiarid climate in southern Saskatchewan. *Canadian Journal of Plant Science*, *85*(4), 877–888. https://doi.org/10.4141/P04-115

Kar, G., Kumar, A., and Martha, M. (2007). Water use efficiency and crop coefficients of dry season oilseed crops. Agricultrual Water Management, 87, 73–82.

Kelln, B. M., Penner, G. B., Acharya, S. N., McAllister, T. A., and Lardner, H. A. (2020). Impact of condensed tannin-containing vegetables on ruminal fermentation, nutrition, and performance in ruminants: A review. Canadian Journal of Animal Science, 101, 210–223.

Khatiwada, B., Acharya, S. N., Larney, F. J., Lupwayi, N. Z., Smith, E. G., Islam, M. A., and Thomas, J. E. (2020). Benefits of mixed grass–legume pastures and pasture rejuvenation using bloat-free legumes in western Canada: A review. *Canadian Journal of Plant Science*, *100*(5), 463–476. https://doi.org/10.1139/cjps-2019-0212

Klinger, S. A., Campbell, J., Wildeman, B., Block, H., and McKinnon, J. J. (2007). The effects of limit feeding a high-energy barley-based diet to backgrounding cattle in western Canada. Canadian Journal of Animal Science. 87(3), 385–391. https://doi.org/10.4141/A06-069

Lee, M. A., Davis, A. P., Chagunda, M. G. G., and Manning, P. (2017). Forage quality declines with rising temperatures, with implications for livestock production and methane emissions. *Biogeosciences*, *14*(6), 1403–1417. https://doi.org/10.5194/bg-14-1403-2017

Lees, G. L. (1992). Condensed tannins in some forage legumes: Their role in the prevention of ruminant pasture bloat. In: Hemingway, R. W. and Laks, P. S. (Eds.), Plant polyphenols: Synthesis, properties, significance, Plenum Press, New York, NY (1992) pp.915–934.

Majak, W., McAllister, T. A., McCartney, D., Stanford, K., and Cheng, K. J. (2003). Bloat in cattle. Agriculture and Agri-Food Canada/Alberta Agriculture, Food and Rural Development. AGDEX 420/60-1. 28 pp

McAllister, T. A., Stanford, K., Chaves, A. V., Evans, P. R., Eustaquio de Souza Figueiredo, E., and Ribeiro, G. (2020). Nutrition, feeding, and management of beef cattle in intensive and extensive production systems. Animal Agriculture, 75–98. doi: 0.1016/B978-0-12-817052-6.00005-7

Moore, K. J., Curtiss, C. F., Lenssen, A. W., Fales, S. L. (2020). Factors Affecting Forage Quality. In Forages Volume II: The Science of Grassland Agriculture; Moore, K. J., Collins, M., Nelson, C. J., Redfearn, D. D., Eds.; Wiley: West Sussex, UK, 2020; pp. 701–717

Nair, J., Beattie, A. D., Christensen, D., Yu, P., McAllister, T., Damiran, D., and McKinnon, J. J. (2018). Effect of variety and stage of maturity at harvest on nutrient and neutral detergent fiber digestibility of forage barley grown in western Canada. Canadian Journal of Animal Science. 98(2): 299-310. https://doi.org/10.1139/cjas-2017-0060

Nielsen, K. (2021). Agronomic analysis of the forage yield, nutritional value, species compatibility, and winter survival rates of sainfoin (*Onobrychis viciifolia* Scop.) and grasses mixtures (Thesis, University of Saskatchewan). University of Saskatchewan. Retrieved from https://harvest.usask.ca/handle/10388/13459

Peprah, S., Damiran, D., Biligetu, B., Iwaasa, A. D., Larson, K., and Lardner, H. A. (2021). Evaluation of cool-season binary mixtures as pasture: Herbage yield, nutritive value, and beef cattle performance. *Livestock Science*, 248, 104501. https://doi.org/10.1016/j.livsci.2021.104501

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. (2020). nlme: linear and nonlinear mixed effects models. R package version 4-149. Accessed 29 February 2024 at https://CRAN.R-project.org/package=nlme

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Roscher, C., Schmid, B., Kolle, O., and Schulze, E. D. (2016). Complementarity among four highly productive grassland species depends on resource availability. *Oecologia*, 181(2), 571–582. https://doi.org/10.1007/s00442-016-3587-4

Sanderson, M. A., Skinner, R. H., Barker, D. J., Edwards, G. R., Tracy, B. F., and Wedin, D. A. (2004). Plant species diversity and management of temperate forage and grazing land ecosystems. *Crop Science*, *44*(4), 1132–1144. https://doi.org/10.2135/cropsci2004.1132

Saskatchewan Forage Production Guide. https://publications.saskatchewan.ca/#/products/75246

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. https://doi.org/10.4141/CJPS2011-273

Serajchi, M., Schellenberg, M. P., Mischkolz, J. M., and Lamb, E. G. (2017). Mixtures of native perennial forage species produce higher yields than monocultures in a long-term study. *Canadian Journal of Plant Science*, *98*(3), 633–647. https://doi.org/10.1139/cjps-2017-0087

Sheppard, S. C., Bittman, S., Donohoe, G., Flaten, D., Wittenberg, K. M., Small, J. A., Berthiaume, R., McAllister, T. A., Beauchemin, K. A., McKinnon, J., Amiro, B. D., Macdonald, D., Mattos, F., and Ominski, K. H. (2015). Beef cattle husbandry practices across Ecoregions of Canada in 2011. *Canadian Journal of Animal Science*, 95(2), 305–321. https://doi.org/10.4141/CJAS-2014-158

Sottie, E. T., Acharya, S. N., McAllister, T., Thomas, J., Wang, Y., and Iwaasa, A. (2014). Alfalfa pasture bloat can be eliminated by intermixing with newly-developed sainfoin population. Agronomy Journal, 106, 1470–1478. https://doi.org/10.2134/agronj13.0378

Tokatlidis, I., Chauhan, Y., and Assefa, Y. (2022). Crop response to density: Optimization of resource use to promote sustainability. Frontiers in Plant Science, 13, 969332.

Villalba, D., Molina, E., and Alvarez-Rodriguez, J. (2016). Clustering forage types according to their nutritive value. In book: Mountain pastures and livestock farming facing uncertainty: environmental, technical, and socio-economic challenges (Proceedings of the 19th Meeting of the Sub-Network on Mediterranean Pastures of the FAO-CIHEAM International Network) (pp.123-126). Chapter: Options Méditerranéennes, Series A: Mediterranean Seminars, No. 116 Publisher: CIHEAM. Editors: I. Casasús, G. Lombardi

Wang, Y., Berg, B. P., Barbieri, L. R., Veira, D. M., and McAllister, T. A. (2006). Comparison of alfalfa and mixed alfalfa-sainfoin pastures for grazing cattle: Effects on incidence of bloat, ruminal fermentation, and feed intake. *Canadian Journal of Animal Science*, *86*(3), 383–392. https://doi.org/10.4141/A06-009

Weiss, W. P., and Hall, M. B. (2020). Laboratory methods for evaluating forage quality. Forages: Science Grassland Agriculture, 2, 659–672. doi: 10.1002/9781119436669.ch36

Yungblut, D. (2012). National Forage and Grassland Assessment. *European University Institute*, *2*, 2–5. https://eur-lex.europa.eu/legal-/PT/TXT/PDF/?uri=CELEX:32016R0679&from=PT%0Ahttp://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:52012PC0011:pt:NOT

## 8.5 Tables

Table 8. 1 Rainfall, maximum and minimum temperatures with their long-term average during the growing season 2020, 2021, 2022, and 2023 at Fairview, Alberta, Canada.

| Month | 2020 | 2021 | 2022 | 2023 | Long-term average |
|---|---|---|---|---|---|
| Rainfall (mm month$^{-1}$) | | | | | |
| May | 19.1 | 13.3 | 53.4 | 12.7 | 35.1 |
| June | 67.2 | 43.3 | 60.9 | 60.9 | 94.5 |
| July | 89.8 | 23.5 | 18.6 | 3.5 | 161.4 |
| August | 53.9 | 40.5 | 31.4 | 1.7 | 203.1 |
| Cumulative | 230.0 | 120.6 | 164.3 | 40.8 | 494.1 |
| Maximum air temperature (°C) | | | | | |
| May | 24.8 | 16.6 | 13.8 | 23.2 | 14.8 |
| June | 26.8 | 20.2 | 21.0 | 22.9 | 14.0 |
| July | 29.2 | 22.2 | 23.9 | 23.4 | 15.9 |
| August | 29.2 | 21.3 | 25.5 | 22.5 | 14.6 |
| Minimum air temperature (°C) | | | | | |
| May | –2.3 | 3.1 | 2.6 | 6.4 | 10.3 |
| June | 2.6 | 7.5 | 8.9 | 9.0 | 9.4 |
| July | 7.0 | 9.2 | 10.3 | 10.7 | 11.1 |
| August | 0.3 | 7.7 | 10.8 | 9.7 | 13.7 |

Table 8. 2 A complete list of forage monocultures and mixtures grown at Fairview, Alberta, Canada.

| Monoculture grasses and simple mixtures | Complex mixtures<br>3-species | 4 or more species |
|---|---|---|
| OG | M7: MB (Fleet) + AL (y) + SF | M0: MB (Fleet) + HB (AC Success) + HB (AC Knowles) + GPWG + CWG |
| GPWG | M8: HB (AC Success) + AL (y) + SF | |
| MB | M11: MB (Fleet) + GPWG + AL (y) | M9: MB (Fleet) + AL (y) + SF + CMV |
| CWG | M12: HB (AC Success) + GPWG + AL (y) | M10: HB (AC Success) + AL(y) + SF + CMV |
| TG | M14: SF + CMV + AL (s5) | |
| M1: MB (Fleet) + AL(s5) | M17: HB (AC Success) + AL (y) + SF | M15: HB (AC Success) + GPWG + CWG + IR + SB (Manchar) |
| M2: HB (AC Success) + AL(y) | M13: TF + SW + STL | M16: AL(y) + AL (R) + CMV + SF +BT |
| M3: HB (AC Success) + AL (s5) | M21: HB (AC Success) + SF + AL(y) | M18: HB (AC Success) + GPWG + AL (y) + SF |
| M4: MB (Fleet) + AL (s5) | | M19: HB (AC Success) + GPWG + IR + AL (y) + SF |
| M5: HB (AC Success) + AL(s5) | | M20: HB (AC Success) + GPWG + AL(R) + AL (y) + CMV + SF |
| M6: HB (AC Knowles) + AL(s5) | | |
| | | M22: HB (AC Success) + GPWG + AL (y) + CMV + SF + BT |
| | | M23: HB (AC Success) + GPWG + CWG + IR + AL (y) + AL (R) + CMV + SF |

M, mixture; OG, orchardgrass; GPWG, greenleaf pubescent wheatgrass; HB, hybrid bromegrass; CWG, crested wheatgrass; TG, timothy grass; MB, meadow bromegrass; AL(s5), Spredor 5 alfalfa; AL(y), yellowhead alfalfa; AL(R), Rugged alfalfa; SF, sainfoin; CMV, cicer milkvetch; BT, birdsfoot trefoil; IR, Italian ryegrass; SB, smooth bromegrass; TF, tall fescue; SW, slender wheatgrass; STL, AC Saltlander wheatgrass.

Table 8. 3 3-yr means of physiological traits and nutritive values of 29 treatments harvested in 2021, 2022, and2023 at Fairview, Canada.

| Treatment | DMY | CP | ADF | NDF | TDN | NDFD-48h | Calcium | Phosphorus | Potassium | Magnesium | WUE | $WUE_{CP}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (Mg ha$^{-1}$) | (%) | (%) | (%) | (%) | | (%) | (%) | (%) | (%) | (kg of DM ha$^{-1}$ mm$^{-1}$) | (kg CP ha$^{-1}$ mm$^{-1}$) |
| **M0** | 2.3 | 11.7 | 45.0 | 47.9 | 65.1 | 60.9 | 0.8 | 0.25 | 1.8 | 0.33 | 47.7 | 534.2 |
| **M1** | 3.0 | 16.0 | 39.7 | 44.2 | 63.0 | 53.7 | 1.3 | 0.25 | 1.8 | 0.36 | 54.8 | 840.3 |
| **M2** | 2.8 | 15.0 | 40.3 | 45.5 | 62.1 | 53.1 | 1.3 | 0.24 | 1.8 | 0.33 | 48.4 | 737.6 |
| **M3** | 2.6 | 16.3 | 38.8 | 42.6 | 63.2 | 52.4 | 1.4 | 0.25 | 1.8 | 0.34 | 44.7 | 719.5 |
| **M4** | 3.5 | 14.4 | 42.3 | 46.1 | 61.2 | 52.2 | 1.3 | 0.26 | 1.9 | 0.33 | 64.6 | 922.7 |
| **M5** | 3.0 | 15.1 | 40.1 | 45.0 | 62.2 | 52.1 | 1.3 | 0.25 | 1.8 | 0.34 | 56.9 | 822.9 |
| **M6** | 3.1 | 15.8 | 40.4 | 43.8 | 61.9 | 50.9 | 1.4 | 0.25 | 1.7 | 0.32 | 57.4 | 878.6 |
| **M7** | 2.4 | 13.8 | 41.5 | 46.0 | 62.6 | 54.0 | 1.2 | 0.25 | 1.7 | 0.33 | 41.9 | 572.9 |
| **M8** | 2.1 | 15.0 | 39.2 | 43.7 | 63.1 | 52.3 | 1.3 | 0.25 | 1.7 | 0.32 | 36.8 | 541.8 |
| **M9** | 2.0 | 14.3 | 40.1 | 44.8 | 62.5 | 52.9 | 1.2 | 0.25 | 1.7 | 0.31 | 37.7 | 524.1 |
| **M10** | 2.5 | 14.2 | 41.0 | 46.2 | 61.5 | 52.3 | 1.3 | 0.25 | 1.7 | 0.35 | 50.5 | 687.5 |
| **M11** | 2.8 | 15.2 | 40.1 | 45.3 | 62.2 | 52.8 | 1.3 | 0.25 | 1.8 | 0.32 | 53.8 | 816.1 |
| **M12** | 2.4 | 15.8 | 38.5 | 42.9 | 64.3 | 54.0 | 1.3 | 0.26 | 1.8 | 0.33 | 46.2 | 724.5 |
| **M13** | 1.6 | 12.3 | 42.3 | 48.1 | 63.6 | 58.8 | 0.9 | 0.25 | 1.7 | 0.35 | 34.2 | 411.8 |
| **M14** | 3.6 | 16.3 | 39.2 | 42.7 | 62.5 | 52.0 | 1.5 | 0.25 | 1.7 | 0.32 | 71.7 | 1086.3 |
| **M15** | 1.5 | 12.7 | 42.0 | 46.7 | 63.8 | 57.8 | 1.0 | 0.25 | 1.7 | 0.31 | 32.4 | 417.2 |
| **M16** | 2.7 | 16.3 | 38.6 | 42.1 | 63.8 | 52.7 | 1.5 | 0.25 | 1.7 | 0.35 | 47.8 | 759.3 |
| **M17** | 2.9 | 15.1 | 38.9 | 43.1 | 63.9 | 52.9 | 1.3 | 0.25 | 1.7 | 0.32 | 60.0 | 872.2 |
| **M18** | 2.6 | 15.5 | 39.4 | 43.9 | 63.6 | 53.6 | 1.3 | 0.25 | 1.8 | 0.33 | 51.6 | 744.7 |
| **M19** | 2.7 | 13.7 | 40.9 | 45.2 | 62.5 | 52.9 | 1.2 | 0.25 | 1.8 | 0.35 | 50.7 | 707.7 |
| **M20** | 2.7 | 13.7 | 40.8 | 45.3 | 61.8 | 51.8 | 1.3 | 0.23 | 1.6 | 0.40 | 55.9 | 748.6 |
| **M21** | 3.4 | 16.3 | 39.1 | 41.8 | 63.9 | 53.5 | 1.4 | 0.26 | 1.9 | 0.36 | 61.8 | 957.4 |
| **M22** | 2.9 | 16.1 | 38.7 | 42.9 | 63.5 | 52.8 | 1.4 | 0.25 | 1.6 | 0.33 | 52.6 | 830.1 |
| **M23** | 3.4 | 15.4 | 39.7 | 43.8 | 63.1 | 53.7 | 1.3 | 0.27 | 1.7 | 0.33 | 61.7 | 930.4 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **OG** | 2.0 | 13.3 | 41.8 | 47.6 | 63.1 | 57.0 | 1.0 | 0.25 | 1.8 | 0.37 | 40.8 | 404.9 |
| **FMB** | 2.5 | 11.4 | 45.0 | 50.2 | 62.0 | 58.7 | 1.0 | 0.24 | 2.0 | 0.31 | 29.1 | 362.3 |
| **GPWG** | 2.2 | 11.4 | 45.6 | 50.2 | 62.2 | 59.5 | 1.1 | 0.26 | 2.1 | 0.32 | 31.8 | 456.3 |
| **KCG** | 1.9 | 13.5 | 41.4 | 44.5 | 63.4 | 57.0 | 1.0 | 0.27 | 1.8 | 0.30 | 37.1 | 400.1 |
| **TG** | 1.4 | 13.4 | 42.2 | 45.7 | 63.3 | 57.8 | 1.2 | 0.26 | 1.7 | 0.37 | 24.4 | 452.2 |
| **Mean** | 2.6 | 14.4 | 40.8 | 45.1 | 62.9 | 54.3 | 1.2 | 0.30 | 1.8 | 0.34 | 47.8 | 685.0 |
| **SEM** | 0.11 | 0.28 | 0.36 | 0.41 | 0.17 | 0.51 | 0.03 | 0.02 | 0.02 | 0.01 | 2.16 | 37.19 |
| **CV** | 22.53 | 10.6 | 4.74 | 4.87 | 1.44 | 5.03 | 14.08 | 3.20 | 6.06 | 6.55 | 24.33 | 29.24 |

DMY: dry matter yield (Mg $ha^{-1}$); CP: crude protein (%); ADF: acid detergent fiber (%); NDF: neutral detergent fiber (%); TDN: total detergent fiber (%); NDFD-48h: neutral detergent fiber digestibility; WUE: water use efficiency (kg of DM $ha^{-1}$ $mm^{-1}$); $WUE_{CP}$: crude protein water use efficiency (kg CP $ha^{-1}$ $mm^{-1}$); SEM; standard error of means; OG: orchard grass; FMB: fleet meadow bromegrass; GPWG: greenleaf pubescent wheatgrass; KCG: Kirk crested grass; TG: timothy grass; CV: coefficient of variance.

Table 8. 4 Coefficient of correlation (r) among 12 traits measured on 29 treatments.

| | DMY (Mg ha$^{-1}$) | CP (%) | ADF (%) | NDF (%) | TDN (%) | NDFD-48h | Calcium (%) | Phosphorus (%) | Potassium (%) | Magnesium (%) | WUE (Kg of DM ha$^{-1}$ mm$^{-1}$) | WUE$_{CP}$ (Kg CP ha$^{-1}$ mm$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DMY** | 1 | | | | | | | | | | | |
| **CP** | 0.62*** | 1 | | | | | | | | | | |
| **ADF** | -0.40** | -0.93*** | 1 | | | | | | | | | |
| **NDF** | -0.47** | -0.92*** | 0.93*** | 1 | | | | | | | | |
| **TDN** | -0.30* | 0 | 0 | 0.20* | 1 | | | | | | | |
| **NDFD-48h** | -0.64*** | -0.82*** | 0.79*** | 0.73*** | 0.41** | 1 | | | | | | |
| **Calcium** | 0.67*** | 0.89*** | -0.87*** | -0.78*** | 0 | -0.87** | 1 | | | | | |
| **Phosphorus** | 0 | 0 | 0 | 0 | 0.27 | 0 | 0 | 1 | | | | |
| **Potassium** | 0 | -0.36* | 0.54** | 0.48** | 0 | 0.41** | 0 | 0.23* | 1 | | | |
| **Magnesium** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -0.30* | 0.22* | 1 | | |
| **WUE** | 0.90*** | 0.65*** | -0.51** | -0.56** | 0 | -0.67*** | 0.62*** | 0 | 0 | 0.10* | 1 | |
| **WUE$_{CP}$** | 0.91** | 0.79*** | -0.63*** | 0.69*** | 0 | -0.75*** | 0.81*** | 0 | 0 | 0.11* | 0.94*** | 1 |

ADF: acid detergent fiber; CP: crude protein; DMY: dry matter yield; NDF: neutral detergent fiber; NDFD; neutral detergent fiber digestibility; TDN: total digestible nutrients; WUE: water use efficiency; WUE$_{CP}$; crude protein water use efficiency. Coefficient of correlation (r); significant at $P$ = 0.05, **0.01 and ***0.001, respectively.

Table 8. 5 Eigenvectors from the first two principal components (PC) for 12 traits of perennial forage treatments at Fairview, Canada.

| **Variables** | **PC1** | **PC2** |
|---|---|---|
| Dry matter yield | 0.31 | **-0.37** |
| Crude protein | **0.37** | 0.15 |
| Acid detergent fiber | -0.34 | -0.31 |
| Neutral detergent fiber | -0.35 | -0.33 |
| Total digestible nutrient | -0.05 | **<u>0.59</u>** |
| Neutral detergent fiber digestibility-48h | **-0.36** | 0.10 |
| Calcium | **0.36** | -0.05 |
| Phosphorus | -0.01 | 0.21 |
| Potassium | -0.16 | **-0.38** |
| Magnesium | 0.05 | -0.01 |
| Water use efficiency | 0.33 | -0.23 |
| Water use efficiency (Crude protein) | **0.36** | -0.17 |
| Eigenvalue | 6.36 | 1.82 |
| Proportion (%) | 53.00 | 15.00 |
| Cumulative (%) | 53.00 | 68.00 |

The bold and underlined value had the highest significant coefficient with the relevant component in the PCA.

Table 8. 6 Comparison of the 12 traits means among the three clusters of forage treatments.

| Traits | Mean | | |
|---|---|---|---|
| | Cluster I (n=8) | Cluster II (n=12) | Cluster III (n=9) |
| **Dry matter yield (Mg/ha)** | 1.9c | 3.0a | 2.6b |
| **Crude protein (%)** | 12.5c | 15.8a | 14.4b |
| **Acid detergent fiber (%)** | 43.2a | 39.4b | 40.4b |
| **Neutral detergent fiber (%)** | 47.6a | 43.3c | 45.2b |
| **Total digestible nutrient (%)** | 63.3a | 63.2ab | 62.3b |
| **Neutral detergent fiber digestibility-48h** | 58.4a | 52.9b | 52.7ab |
| **Calcium (%)** | 1.0b | 1.4a | 1.3ab |
| **Phosphorus (%)** | 0.3a | 0.3a | 0.2ab |
| **Potassium (%)** | 1.8a | 1.8a | 1.7ab |
| **Magnesium (%)** | 0.3a | 0.3a | 0.3a |
| **Water use efficiency (kg of DM $ha^{-1}$ $mm^{-1}$)** | 34.7c | 56.2a | 48.1b |
| **Water use efficiency(CP) (kg of CP $ha^{-1}$ $mm^{-1}$)** | 429.9c | 855.5a | 684.4b |

Means with same letters within the row for each trait are not significantly different at $P = 0.05$

## 8.6 Figures

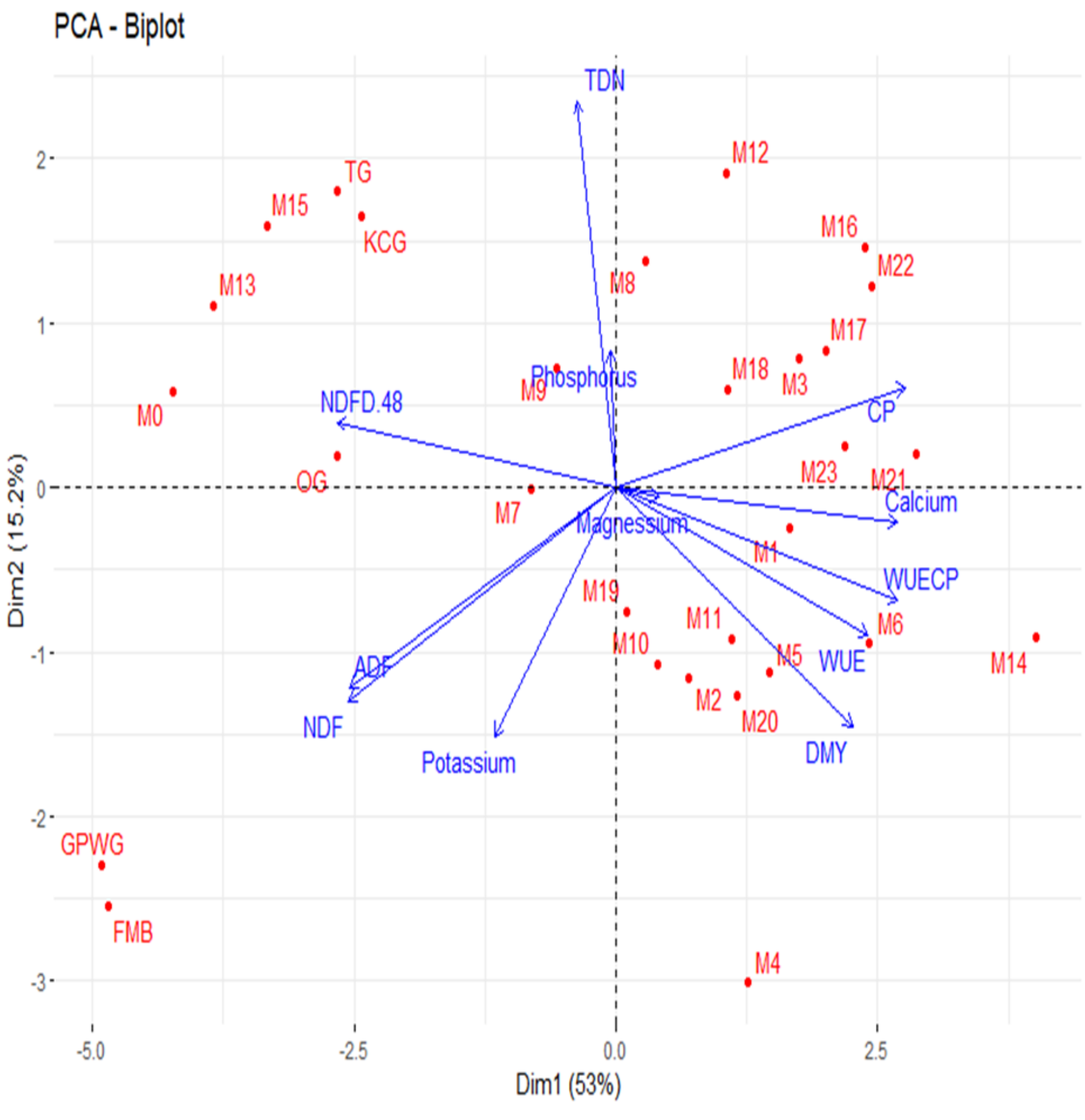



Figure 8. 1 PCA biplot for 29 treatments associated with 12 physiological trait and nutritive value (based on 3-year means).

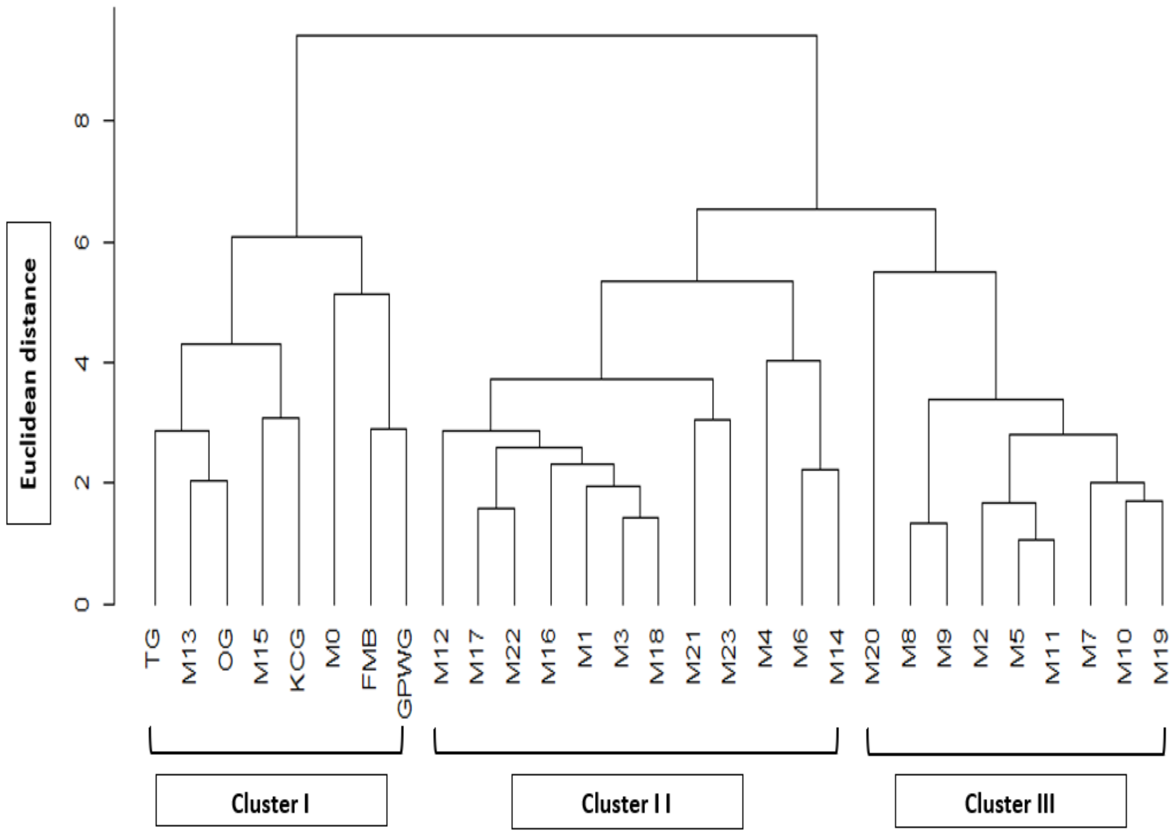



Figure 8. 2 Dendrogram of the 29 perennial forage treatments revealed by cluster analysis based on 12 physiological traits and nutritive values (based on 3-year means).

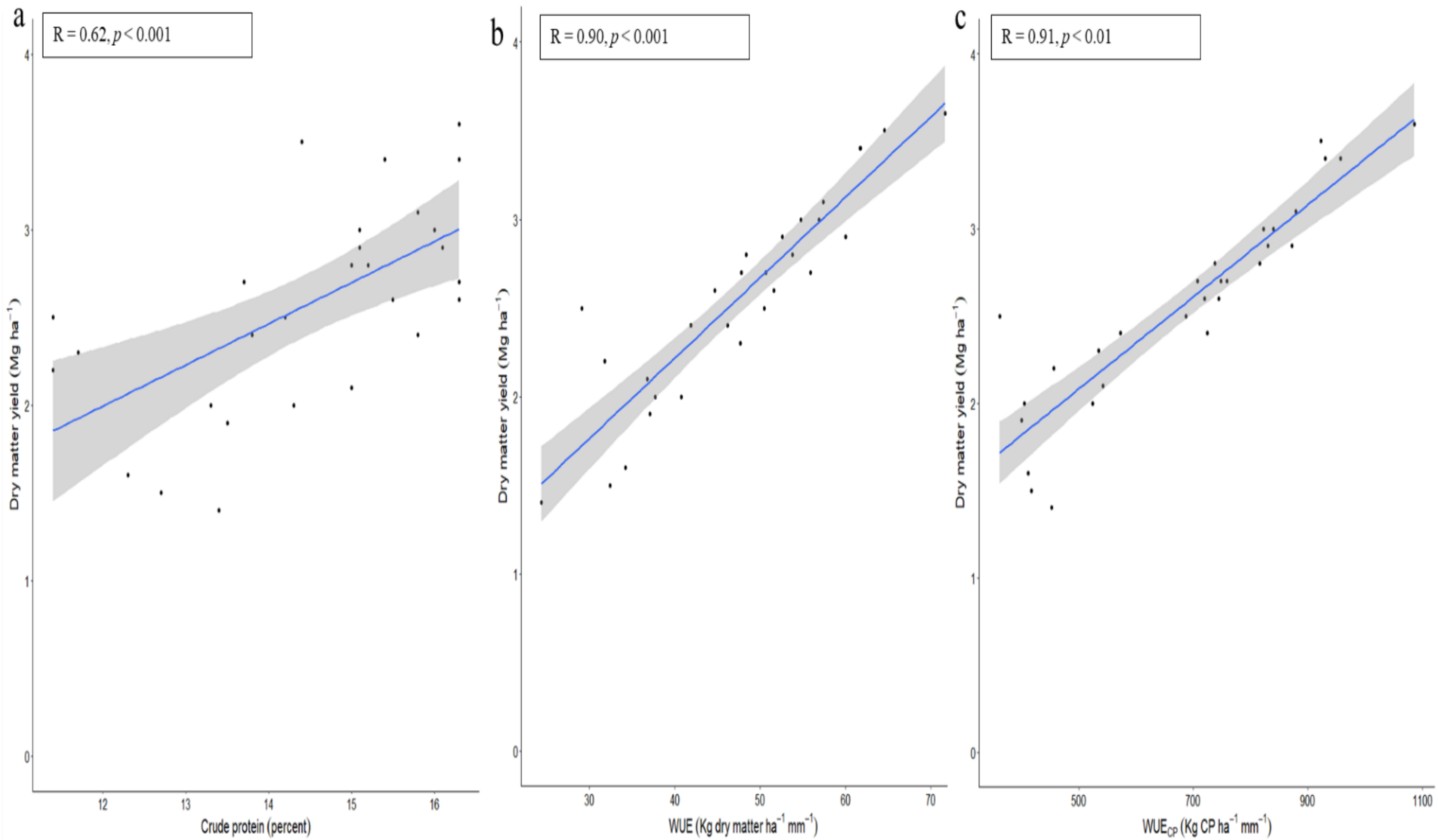



Figure 8. 3 Correlations between traits (a) dry matter yield and crude protein, (b) dry matter yield and water use efficiency, (c) dry matter yield and crude protein water use efficiency. Panel "a" shows positive correlation (r = 0.62), whereas both panel "b" and "c" also show positive correlations (r = 0.90, r = 0.91, respectively).

## Chapter 9. General conclusion

With regenerative agriculture gaining traction in Alberta, the results (Chapter 3) have provided various perennial forage mixtures that are superior in improving the physical, chemical, and biological properties of the soil. Our major findings showed that complex grass–legume mixtures improved surface soil infiltration rate, soil compaction, and total nitrogen accumulation but were ineffective in enhancing soil carbon sequestration and microbial activity. We believe that the improvement in surface soil infiltration and soil compaction was influenced by the varying degree of root systems and growth patterns of diverse forage species. Grass monocultures were better at enhancing carbon sequestration and microbial activity owing to the high carbon content in their tissue and the large root surface contact for microbes. This indicates that substantial changes in the soil ecosystem can occur by using various plant species. This reduces the additional costs incurred by purchasing synthetic fertilizers and mechanical tillage of the soil. This information is valuable when considering the combinations of perennial forage species for establishment.

This research has highlighted the contribution of perennial grass–legume mixtures in livestock-forage systems in terms of productivity and quality, and has resolved concerns of beef producers regarding mixtures that are viable options for establishment in Alberta. The environmental conditions, in particular precipitation can significantly alter forage productivity as observed (in Chapter 4). During a relatively dry first year of forage production, grass–legume mixtures generally had greater dry matter yields than monoculture grasses or grass-only mixtures. This was attributed to the complementary effects that existed within the grass–legume systems. During the second year of forage production, all forage dry matter yields increased by 50% compared with the first year of forage production. This was associated with adequate precipitation, better root biomass development and distribution, and nutrient bioavailability. However, the monoculture of Fleet meadow bromegrass produced the highest dry matter yield. This indicates that when field conditions are favorable, some grasses can potentially outyield grass–legume mixtures. Although grass–legume mixtures had greater dry matter yields in the third year of forage production, monoculture grasses decreased drastically. This was attributable to a probable decline in nitrogen in soils under grasses. Grass–legume mixtures had lower neutral detergent fiber and acid detergent fiber contents (only in the first year of production), whereas grass-only mixtures and monoculture grass had lower acid detergent fiber content (in the second and third years of forage production). In addition, grass–legume mixtures had higher crude protein content and total digestible nutrients (only in the first year of forage production), whereas monoculture grasses and grass-only mixtures had greater neutral digestibility fiber digestibility and total digestible nutrients (in the second and third years of forage production). The high crude protein content in grass–legume mixtures and lower neutral detergent fiber and acid detergent fiber contents were due to the presence of legumes within the mixtures. Legumes are self-reliant in acquiring nitrogen for use and contain less hemicellulose which is easily digestible by ruminal microbes. Monoculture grasses had higher total digestible nutrients during the second and third years of forage production because of their low acid detergent fiber content. This means that the forage had high digestibility, resulting in better energy levels. Forage calcium and phosphorus contents were higher in grass–legume mixtures, whereas monoculture grasses had a high potassium content. This is because grasses can extract higher amounts of potassium during the growing season than legumes. Although perennial grass–legume mixtures are superior to monoculture grasses, our findings further revealed that certain legume species such as sainfoin within mixtures decreased in the final year of forage production. This indicates that growers should conduct seasonal botanical composition measurements to ascertain the proportions of individual species within mixtures. Regardless of the environmental conditions during the growing season, grass–legume mixtures have superior productivity and nutritive value. These results can support the beef cattle industry by providing producers with mixtures that are high in nutritional value and productivity to extend the feeding or grazing days of their livestock, which has a cascading effect on performance (in terms of average daily gains, body condition scores, and carcass quality).

By evaluating the water use efficiency ($WUE_{BM}$), we were able to examine the dynamics of grass–legume mixtures compared to monoculture grass systems (Chapter 5). $WUE_{BM}$ was higher in grass–legume mixtures in the first year of forage production which was attributed to the supportive functions that existed within the system during the drought-affected year. In contrast, monoculture grasses did not obtain these supportive functions, resulting in low yield. In the second year of production, grasses were better in $WUE_{BM}$ due to adequate precipitation during the growing season, whereas this advantage receded in the third year of forage production. Our key finding of $WUE_{BM}$ was that during the drought-affected first year of forage production, $WUE_{BM}$ was higher than in both the moist second and third years of forage production . This is because forage plants consumed water at a reduced rate during the drought-affected year. In terms of crude protein yield, protein WUE ($WUE_{CP}$) was measured. Across the three years of forage production, legume-dominated mixtures were superior to monoculture grasses and grass-only mixtures. This was anticipated because of the presence of legumes within the mixtures. We believe that monoculture grasses or grass-only mixtures were poor at $WUE_{CP}$ particularly in the first year of forage production, because plants expended their energies scavenging the soil for water and nutrients other than building plant structures that can influence biomass and subsequently crude protein production. Although there was an improvement in $WUE_{CP}$ for monoculture grasses or grass-only mixtures during both the second and third years of forage production due to a probable availability of water and nutrients, grass–legume mixtures were still superior. With the ongoing effects of climate change that detrimentally affect forage production globally, this study has reinforced the need for polyculture in cropping systems by providing the first report in the literature that documents how perennial grass–legume forage polyculture systems can result in better WUE than grass monocrops though the study did not explore deeper soil depths. In addition, future research should be conducted to quantify the impact of individual species within mixtures and their contributions to overall WUE.

We examined the N fixation and transfer under simple and complex grass–legume mixtures under field conditions (Chapter 6), where we found that all legumes derived more than 95% of their N from the atmosphere. Alfalfa consistently had greater amount of N fixed in kg N $ha^{-1}$ than both sainfoin and cicer milkvetch. Moreover, more than 50% of the N derived from the atmosphere was transferred to grass species for use. Forage grass–legume mixtures with many grass species had greater N transferred owing to the ability of grasses to uptake nitrogen differently. We also found that the amount of N fixed and transferred can be influenced by legume and grass species richness, though N fixed can be hindered from reaching the grass due to dry soil conditions and proximity of their rooting systems. In effect, N fixation and transfer are higher under complex than in simple grass–legume mixtures. This study can serve as a guide for growers who wish to use legumes to improve their forage-livestock systems. This is because N fixed can reduce the application of synthetic fertilizers which is an additional cost to the producer and reduce environmental problems such as eutrophication of water, groundwater contamination, and mitigate ozone layer depletion. This study can also provide a guide to scientists conceptualizing the dynamics of source and sink relationships for N within diverse perennial legume–grass mixtures.

Although inoculating legume species is vital for enhancing atmospheric nitrogen fixation, our study was limited in this aspect due to the lack of commercially available inoculants for sainfoin, cicer milkvetch, and birdsfoot trefoil. Efforts should be made by legume seed industries to expand the production and sales of inoculants to aid growers in obtaining optimal benefits.

As observed in the CowBytes ration simulations (Chapter 7), yearly forage production can vary greatly in terms of productivity and quality. This means that seasonal forage quality analysis is imperative to ascertain their effects on ration formulation and their ability to meet the requirements of beef cattle categories. In the first year of forage harvest, grass–legume mixtures were superior at supporting steers and lactating beef cows in obtaining average daily gain (ADG) of 0.80 and 0.10 kg day$^{-1}$, respectively, while monoculture grass and grass-only mixture supported gestating beef cows to obtain 0.10 kg day$^{-1}$ within the neutral detergent fiber limits (1.2% body weight). In both the second and third years of forage harvests, grass–legume mixtures were optimal for steers while grass monocultures and grass-only mixtures were superior at supporting lactating beef cows in obtaining their required ADG of 0.10 kg day$^{-1}$. However, forage ration for gestating cows supplied ADG which was in excess of the required due to their relatively high energy content; hence a portion of the ration was substituted with a cheaper source of energy (barley straw) to obtain the desired ADG and reduce cost. Similarly, portions of the ration for lactating beef cows were substituted to cut costs and simultaneously achieve an ADG of 0.10 kg day$^{-1}$ across the three years of forage harvests. The supplementation with barley straw showed that dry matter intake was reduced for both gestating and lactating cows while obtaining ADG within the neutral detergent fiber limits. This substantially reduced the daily feeding costs. Our key observation was that gestating beef cows required almost twice the feed required by both steers and lactating cows, particularly during the last trimester of pregnancy.

Multivariate analysis which included clustering of forage species based on physiological traits and nutritive values (Chapter 8), indicated that perennial forage mixtures were grouped into three main clusters. Notably, cluster I contained monoculture grasses and grass-only mixtures characterized by poor dry matter, crude protein, crude fibers, WUE, and $WUE_{CP}$ but were superior in total digestible nutrients and neutral detergent fiber digestibility. Cluster II contained forage mixtures with slightly higher proportion of legumes than cluster III. Overall forage mixtures in cluster II were superior in dry matter yield, crude protein, WUE, and $WUE_{CP}$. This was attributed to the high plant densities which optimized resource use and promoted forage yield. Based on the three clusters (I, II, and III), it was evident that the functional roles of individual species are crucial to the agroecosystem. For example, treatments in cluster I are grasses which can stabilize the soil's structure through their fibrous rooting systems whereas both cluster II and III are legume-dominated which can fix atmospheric N into the soil. This becomes useful in planning and designing perennial forage mixture establishments. We also observed correlations between various physiological traits and nutritive values. Dry matter yield was positively correlated with WUE and WUECP. In addition, we found negative correlations between dry matter yield, crude protein, neutral detergent fiber, and acid detergent fiber. These observations are attributed to the individual characteristics of various plant species and forage attaining maturity at different times. Overall, our findings showed that perennial forage mixtures containing (i) AC Knowles hybrid bromegrass + spredor 5 alfalfa, (ii) AC Mountainview sainfoin + Veldt cicer milkvetch + spredor 5 alfalfa, (iii) AC Success hybrid bromegrass + AC Mountainview sainfoin + AC yellowhead alfalfa, (iv) AC Success hybrid bromegrass + greenleaf pubescent wheatgrass + Kirk crested wheatgrass + Italian ryegrass +AC yellowhead alfalfa + rugged alfalfa + Veldt cicer milkvetch + AC Mountainview sainfoin, and (v) AC Success hybrid bromegrass + AC yellowhead alfalfa + AC Mountain sainfoin are viable options for cultivation in Alberta. However, to obtain better results, growers need soil tests before seeding, understand species compatibility, and undertake best field management practices. In the future a comprehensive economic report on this study will be released to guide growers on the best alternative in terms of investment and returns.

### 9.1 Future directions

This research has demonstrated that cultivating perennial forage mixtures can support beef cattle production and provide ecosystem benefits. Although the Cowbytes® balancing software was used to predict the performance of category of beef cattle, further research can focus on feeding forage directly to mature cows to ascertain its impact on livestock performance. Additionally, by identifying that nitrogen fixed within mixtures can be transferred to neighbouring plants when available, we can explore how the "clipping" of forage plants by cattle during grazing can augment and enhance the bioavailability of nitrogen within forage-livestock systems. I believe that by expanding on these findings, we would be able to have a more sustainable forage-livestock system which would eliminate the cost of input and ensure a closed system.

## Literature cited


AAFC, Agriculture and Agri-Food Canada (2021). Beef and veal exports by country - year-to-date. Prepared by Animal Industry Division, Market Information Section. [Accessed on 23-02-2022] at https://www.agr.gc.ca/eng/animal-industry/red-meat-and-livestockmarket-information/trade/red-meat-exports-by-country/?id=1419965032803

Aasen, A., and Bjorge, M. (2009). Forage. Alberta Agriculture and Rural Development, Information Management Services. https://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/agdex16/$FILE/120_20-1_2009.pdf (accessed 20 March 2021).

Agriculture Canada. (1987). Growing and managing alfalfa in Canada. Agriculture Canada.

Agriculture Canada. (1993). Meadow bromegrass. Research Program Service. Agriculture Canada.

Alam, S. M. (1994). Nutrient by plant under stress condition. In: Pessarakli M. (ed.): Handbook of Plant and Crop Stress (pp. 227–246). Marcel Dekker, New York.

Alamooti, M. Y. and Navabzadeh, M. (2007). Investigation of plowing depth effect on some soil physical properties. Pakistan Journal of Biological Sciences 10 (24): 4510–4514 https://doi.org/10.3923/pjbs.2007.4510.4514

Albayrak, S., and Ekiz, H. (2005). An investigation on the establishment of artificial pasture under Ankara's ecological conditions. *Turkish Journal of Agriculture and Forestry*, *29*(1), 69–74. https://doi.org/10.3906/tar-0310-6

Alberta Agri-News (2023). Alberta hay prices. https://www.alberta.ca/agri-news-alberta-hay-prices (accessed on 14 January 2024).

Alberta Beef Producers Annual Report (2020). https://www.albertabeef.org/files/annual-reports/d5aN2ubsRRwNZvwA6PaAc2rSDn5mZvwdnbAZNvLu.pdf (accessed 6 October, 2021).

Alberta Climate Information Service (2020). Current and historical Alberta weather station data viewer. Accessed on 21 May 2021 from https://acis.alberta.ca/acis/weather-data-viewer.jsp

Alemu, A. W., Doce, R. R., Dick, A. C., Basarab, J. A., Kröbel, R., Haugen-Kozyra, K., and Baron, V. S. (2016). Effect of winter-feeding systems on farm greenhouse gas emissions.

*Agricultural Systems*, *148*, 28–37. https://doi.org/10.1016/j.agsy.2016.06.008

Alexandratos, N., and Bruinsma, J. (2012). World agriculture towards 2030/2050: The 2012 review. *Food and Agriculture Organization of the United Nations (FAO)*, *30*(Jun 11 2012). https://doi.org/10.22004/ag.econ.288998

Alfredo, D. (2019). Basic Nutrient Requirements of Beef Cattle. Oregon State University Extension Services.https://extension.oregonstate.edu/animals-livestock/beef/basic-nutrient-requirements-beef-cattle (accessed on December 17, 2023).

Alkorta I, Garbisu C. (2001). Phytoremediation of organic contaminants in soils. Bioresource Technology, 79, 273–276.

Allison, C. D. (1985). Factors affecting forage intake by range ruminants: A review. Journal of Range Management, 38(4), 305–311.

Altman, D. G., and Bland, J. M. (2011). Statistics notes: How to obtain the P value from a confidence interval. *British Medical Journal (Online)*, *343*(7825), 1–2. https://doi.org/10.1136/bmj.d2304

Amari, M., and Abe, A. (1997). Application of near infrared reflectance spectroscopy to forage analysis and prediction of TDN contents. *Japan Agricultural Research Quarterly*, *31*(1), 55–63.

Amiro, B. D., Tenuta, M., Gervais, M., Glenn, A. J., and Gao, X. (2017). A decade of carbon flux measurements with annual and perennial crop rotations on the Canadian Prairies. Agricultural and Forest Meteorology, 247, 491–502.

AOAC. (1984). Official Methods of Analysis, 14th Edition. AOAC.

Aponte, A., Samarappuli, D., and Berti, M. T. (2019). Alfalfa–grass mixtures in comparison to grass and alfalfa monocultures. *Agronomy Journal*, *111*(2), 628–638 https://doi.org/10.2134/agronj2017.12.0753

Araus, V., Swift, J., Alvarez, J. M., Henry, A., and Coruzzi, G. M. (2020). A balancing act: How plants integrate nitrogen and water signals. Journal of Experimental Botany, 71(15), 4442–4451. https://doi.org/10.1093/jxb/eraa054

Arelovich, H. M., Abney, C. S., Vizcarra J. A., and Galyean, M. L. (2008). Effects of dietary neutral detergent fiber on intakes of dry matter and net energy by dairy and beef cattle: analysis of published data. *Professional Animal Scientist*, *24*, 375–383.

Arshad, M. A., Franzluebbers, A. J., and Azooz, R. H. (2004). Surface-soil structural properties under grass and cereal production on a Mollic Cyroboralf in Canada. *Soil and Tillage Research*, *77*(1), 15–23. https://doi.org/10.1016/j.still.2003.10.004

Arshad, M. A., Soon, Y. K., and Ripmeester, J. A. (2011). Quality of soil organic matter and C storage as influenced by cropping systems in northwestern Alberta, Canada. *Nutrient Cycling in Agroecosystems*, *89*(1), 71–79. https://doi.org/10.1007/s10705-010-9377-1

Arzani, H., Khatibi, F., Basiri, M., and Fish, E. (2003). Comparison of forage quality of 22 Zagros Mountain rangeland species. In: Kirilov, A., Todorov, N., and Katerov, I., eds., Proceedings of the 12th Symposium of the European Grassland Federation Pleven, Bulgaria, 26–28 May (pp. 222–225). British Grassland Society.

Ashworth, A. J., Allen, F. L., Warwick, K. S., Keyser, P. D., Bates, G. E., Tyler, D. D., Lambdin, P. L., and Pote, D. H. (2017). $N_2$ fixation of common and hairy vetches when intercropped into switchgrass. *Agronomy*, 7, 39, 1–10. https://doi.org/10.3390/agronomy7020039

Atis, I., Konuskan, O., Duru, M., Gozubenli, H., and Yilmaz, S. (2012). Effect of harvesting time on yield, composition and forage quality of some forage sorghum cultivars. International Journal of Agriculture & Biology. 14, 1814–9596. http://www.fspublishers.org

Attia-Ismail, S. (2019). Impacts of climate change on forages production, world wide efforts for mitigation and adaptation and the concept of smart-climate agriculture ARIDLANDS: Biodiversity, Management and Conservation, Nova Science Publisher, Inc, New York, USA, 2019, pp.207–233.

Attram, J. (2015). Determination of water use efficiency for forage legume in Southern Alberta with emphasize on dryland and irrigated alfalfa cultivars. University of Lethbridge, Canada.(Publication No. 1585934)[Masters dissertation].

Ball, D., Collins, M., Lacefield, G., Martin, N., Mertens, D., Olson, K., Putnam, D., Undersander, D., and Wolf, M. (2001). Understanding forage quality. American Farm Bureau Federation Publication.

Ball, D., Ballard, E.N., Kennedy, M., Lacefield, G.D., and Undersander, D. (2008). Extending Grazing and Reducing Stored Feed Needs. Publication 8-01. Grazing Lands Conservation Initiative.

Ballet, N., Robert, J. C., and Williams, P. E. V. (2000). Vitamins in forages. In : Forage evaluation in ruminant nutrition, eds. D. I. Givens, E. Owen, H. M. Omed, and R. F. E. Axford, 399–431. Wallingford, UK: CABI.

Beaty, J. L., .Cochran, R. C, Lintzenich, B. A., Vanzant, ., E. S., Morrill, J. L., Brandt, R. T. Jr., and Johnson, D. E. (1994). Effect of frequency of supplementation and protein concentration in supplements on performance and digestion characteristics of beef cattle consuming low–quality forages. Journal of Animal Science. 72, 2475–2486 . doi:10.2527/1994.7292475

Beauchemin, K. A. (1996). Using ADF and NDF in dairy cattle diet formulation – A western Canadian perspective. *Animal Feed Science and Technology, 58*, 101–111. https://doi.org/10.1016/0377-8401(95)00877-2

Beef Cattle Research Council. (2016). Cover Crops as Forage for Beef Cattle. 1–2. https://www.beefresearch.ca/files/pdf/BCRC_Cover_Crops_Fact_Sheet.pdf (accessed on 25 December 2022).

Beef Cattle Research Council. (2023a). Nutrition in beef Cattle. Accessed on 10 May 2023 at https://www.beefresearch.ca/topics/nutrition-in beef cattle/

Beef Cattle Research Council. (2023b). Forage species. Accessed on 7 May 2023, at https://www.beefresearch.ca/topics/forage-species/

Bélanger, G., Castonguay, Y., and Lajeunesse, J. (2014). Benefits of mixing timothy with alfalfa for forage yield, nutritive value, and weed suppression in northern environments. *Canadian Journal of Plant Science, 94,* 51–60.

Bélanger, G., Tremblay, G. F., Papadopoulos, Y. A., Duynisveld, J., Lajeunesse, J., Lafrenière, C., and Fillmore, S. A. E. (2017). Yield and nutritive value of binary legume–grass mixtures under grazing or frequent cutting. *Canadian Journal of Plant Science, 98*(2), 395–407. https://doi.org/10.1139/CJPS-2017-0183

Belyea, R. L., Steevens, B. J., Garner, G. B., Whittier, J. C., and Sewell, H. B. (1993). Using NDF and ADF to Balance Diets. Extension Publications. http://hdl.handle.net/10355/3631

Berard, N. C., Wang, Y., Wittenberg, K. M., Krause, D. O., Coulman, B. E., McAllister, T. A., and Ominski, K. H. (2011). Condensed tannin concentrations found in vegetative and mature forage legumes grown in western Canada. *Canadian Journal of Plant Science*, *91*(4), 669–675.

Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology, 84*(1),11–8. doi: 10.1007/s00253-009-2092-7

Berkenkamp, B., and Meeres, J. (1987). Mixtures of Annual Crops for Forage in Central Alberta. *Canadian Journal of Plant Science*, *67*(1), 175–183. https://doi.org/10.4141/cjps87-021

Bertrand, A., Tremblay, G. F., Pelletier, S., Castonguay, Y., and Bélanger, G. (2008). Yield and nutritive value of timothy as affected by temperature, photoperiod, and time of harvest. *Grass and Forage Science*, *63*(4), 421–432. https://doi.org/10.1111/j.1365-2494.2008.00649.x

Bhandari, K. B., West, C. P., Acosta-Martinez, V., Cotton, J., and Cano, A. (2018). Soil health indicators as affected by diverse forage species and mixtures in semi-arid pastures. Applied Soil Ecology, 132, 179–186. https://doi.org/10.1016/j.apsoil.2018.09.002

Bhattarai, S., Coulman, B., and Biligetu, B. (2016). Sainfoin (*Onobrychis viciifolia* Scop.): Renewed interest as a forage legume for Western Canada. Canadian Journal of Plant Science, 96(5), 748–756. https://d oi.org/10.1139/cjps-2015-0378

Biederbeck, V.O. and Bourman, O. T. (1994). Water use by annual green manure legumes in dryland cropping systems. Agronomy Journal, 86:543–549.

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.

https://doi.org/10.4141/CJPS2013-269

Bittman, S., Waddington, J., and McCartney, D. H. (1991). Performance of alfalfa strains grown in mixture with smooth bromegrass as affected by management. *Canadian Journal of Plant Science*, *71*(4), 1029–1037. https://doi.org/10.4141/cjps91-145

Bohnert, D.W., Schauer, C.S. and DelCurto, T. (2002). Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage: cow performance and efficiency of nitrogen use in wethers. Journal of Animal Science. 80, 1629–1637.

Bolinder, M. A., Angers, D. A., Bélanger, G., Michaud, R., and Laverdière, M. R. (2022). Root biomass and shoot to root ratios of perennial forage crops in eastern Canada. *Canadian Journal of Plant Science, 82*, 731–737.

Bonin, C. L., and Tracy, B. F. (2012). Diversity influences forage yield and stability in perennial prairie plant mixtures. *Agriculture, Ecosystems and Environment*, *162*, 1–7. https://doi.org/10.1016/j.agee.2012.08.005

Bonin, S. G., and Tomlin, D. C. (1968). Effects of nitrogen on herbage yields of timothy harvested at various stages. *Canadian Journal of Plant Science, 48*, 501–509. https://doi.org/10.4141/cjps68-097

Bonnefield Research. (2016). Canada's forage crop: The Overlooked Cornerstone of Canadian Agriculture. Accessed on 7 May 2023 at https://bonnefield.com/wp-content/uploads/2020/04/Forage_review.pdf

Bork, E. W., Gabruck, D. T., McLeod, E. M., and Hall, L. M. (2017). Five-year forage dynamics arising from four legume–grass seed mixes. *Agronomy Journal*, *109*(6), 2789–2799. https://doi.org/10.2134/agronj2017.02.0069

Bower, C. E. and Holm-Hansen, T. (1980). A salicylate–hypochlorite method for determining ammonium in seawater. Canadian Journal of Fisheries and Aquatic Sciences, 37, 794–798.

Bramley, H., Turner, N., and Siddique, K. (2013). Water Use Efficiency. In Genomics and Breeding for Climate-resilient Crops. (Vol. 2, pp. 225–268). https://doi.org/10.1007/978-3-

642-37048-9_6

Brown, W. F, Lai, Z. Q., and Pitman, W. D. (1991). In vitro fibre digestion: Associative effects in tropical grass–legume mixtures. *Tropical Grasslands, 25,* 297–304.

Bullied, W. J., Entz, M. H., Smith, S. R., and Bamford, K. C. (2002). Grain yield and N benefits to sequential wheat and barley crops from single-year alfalfa, berseem and red clover, chickling vetch and lentil. *Canadian Journal of Plant Science,* 82(1), 53–65. https://doi.org/10.4141/P01-044

Bürgmann, H., Meier, S., Bunge, M., Widmer, F., and Zeyer, J. (2005). Effects of model root exudates on structure and activity of a soil diazotroph community. Environmental Microbiology, 7, 1711–1724.

Burity, H. A., Ta, T. C., Faris, M. A., and Coulman, B. E. (1989). Estimation of nitrogen fixation and transfer from alfalfa to associated grasses in mixed swards under field conditions. *Plant and Soil*, 114, 249–255.

Buxton, D. R. (1996). Quality related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science Technology*, 59, 37–49. https://doi.org/10.1002/9781118860359.ch2

Buxton, D. R. and Redfearn, D. D. (1997). Plant limitations to fiber digestion and utilization. *Journal of Nutrition, 127*, 814–818.

Campbell, C. A., Zentner, R. P. and Steppuhn, H. (1987). Effect of crop rotations and fertilizers on moisture conserved and moisture use by spring wheat in southwestern Saskatchewan. Canadian Journal of Soil Science, 67: 457–472.

Campillo, R., Urquiaga, S., and Undurraga, P., Pino, I., and Boddey, R. M. (2005). Strategies to optimise biological nitrogen fixation in legume and grass pastures in the southern region of Chile. *Plant and Soil*, 273, 57–67. https://doi.org/10.1007/s11104-004-6717-4

Canadell, P., Tian, H., Patra, P., and Thompson, R. (2019). Nitrogen fertilisers are incredibly efficient, but they make climate change a lot worse. *CSIROscope*, *November*, 2019–2021. https://blog.csiro.au/nitrogen-fertilisers-make-climate-change-worse/

Canadian Roundtable for Sustainable Beef. (2016). National Beef Assessment and Strategy Summary Report the Go-To Forum on Sustainable. Accessed on 17 October 2022 at https://crsb.ca/wp-content/uploads/2021/12/Assessment-and-Strategy_summary_report_2016.pdf

Canan, T., Levent, C., and Koc, F. (2004). Determination of nutritional value of some legume and grasses. *Pakistan Journal of Biological Science, 7*(10), 1750–1753.

Carlsson, G., and Huss-Danell, K. (2003). Nitrogen fixation in perennial forage legumes in the field. Plant and Soil, 253, 353–372.

Carlsson, G., Huss-Danell K. (2014). Does nitrogen transfer between plants confound $^{15}$N-based quantifications of $N_2$ fixation? Plant Soil, 374, 345–358. doi:10.1007/s11104-013-1802-1

Carranca, C. (2013). Legumes: properties and symbiosis. In: Camisão, A.H. and Pedroso, C.C. (Eds). *Symbiosis: Evolution, Biology and Ecological Effects*. Nova Science Publishers, New York. pp. 67–94.

Caton, J. S., and Dhuyvetter, D. V. (1997). Influence of energy supplementation of grazing ruminants: requirements and response. Journal of Animal Science. 75, 533–542.

Cecen, S., Erdurmus, C., and Oten, M. (2005). Determination of suitable forage plants that can be used in artificial pasture areas in Antalya conditions. In: Kokten K, Celiktas N, Atis I, Hatipoglu R, Tukel T, eds., VI Turkey Field Crop Congress, 5–9 September 2005. Antalya, pp. 386–390.

Chai, R., Ye, X., Ma, C., Wang, Q., Tu, R., Zhang, L., and Gao, H. (2019). Greenhouse gas emissions from synthetic nitrogen manufacture and fertilization for main upland crops in China. *Carbon Balance and Management,* 14(1), 1–10. https://doi.org/10.1186/s13021-019-0133-9

Clément, C., Sleiderink, J., Fiil, S., George, A., Efstathios, S., Bodin, D., Kristian, D., and Kristensen, T. (2022). Comparing the deep root growth and water uptake of intermediate wheatgrass (Kernza®) to alfalfa. Plant and Soil 472, 369–390. https://doi.org/10.1007/s11104-021-05248-6

Cochrane, H. R., and Aylmore, L.A.G. (1994). The effects of plant roots on soil structure. In:

Clarke, C. J., George, R. J., Bell, R. W., Beetson, G. R., Mauger, G. W., and Hobbs. R. J., Eds., Proceedings of the Third Triennial Conference "Soils 94". (pp 207–212). Australian Society of Soil Science.

Coleman, S. W., and Moore, J. E. (2003). Feed quality and animal performance. Field Crops Research, 84(1–2), 17–29. https://doi.org/10.1016/S0378-4290(03)00138-2

Conant, R. T., Cerri, C. E. P., Osborne, B. B., and Paustian, K. (2017). Grassland management impacts on soil carbon stocks: a new synthesis. Applied Ecology, 27, 662–668. https://doi.org/10.1002/eap.1473

Crotty, F. V., Fychan, R., Scullion, J., Sanderson, R., and Marley, C. L. (2015). Assessing the impact of agricultural forage crops on soil biodiversity and abundance. Soil Biology and Biochemistry, 91, 119–126. https://doi.org/10.1016/j.soilbio.2015.08.036

Crowder, L.V. (1985). Pasture management for optimum ruminant production. In: McDowell, L.R. (ed). Nutrition of Grazing Ruminants in Warm Climates (pp. 103–128). Academic Press.

Cutforth, H. W., Jefferson, P. G., Campbell, C. A., and Ljunggren, R. H. (2013). Yield, water use, and protein content of spring wheat grown after six years of alfalfa, crested wheatgrass, or spring wheat in semiarid southwestern Saskatchewan. Canadian Journal of Plant Science, 93(5), 983. https://doi.org/10.4141/CJPS2013-503

Dabney, S. M., Delgado, J. A., and Reeves, D. W. (2001). Using winter cover crops to improve soil and water quality. *Communications in Soil Science and Plant Analysis*, *32*(7–8), 1221–1250. https://doi.org/10.1081/CSS-100104110

Dagen, A. A. and Young, B. A. (2002). Effect of air temperature and energy intake on body mass, body composition and energy requirements in sheep. Journal of Agricultural Science. 138, 221–226.

Daly, E. J., Kim, K., Hernandez-Ramirez, G., and Klimchuk, K. (2023). The response of soil physical quality parameters to a perennial grain crop. Agriculture, Ecosystems and Environment, 343, 108265. https://doi.org/10.1016/j.agee.2022.108265

Damiran, D., Lardner, H. A., Larson, K., and McKinnon J. (2016). Effects of supplementing spring-calving beef cows grazing barley crop residue with canola meal and wheat-based dry distillers' grains with solubles on performance, reproductive efficiency, and system cost.

Professional Animal Scientist. 32, 400–410.

Danso, S. K. A. (1986). Review: Estimation of $N_2$- fixation by isotope dilution: an appraisal of techniques involving $^{15}N$ enrichment and their application. *Soil Biology and Biochemistry*, 18, 243–244.

Darambazar, E., Larson, K., Schoenau, J., Wang, G., Biligetu, B., Damiran, D., Lardner, H. A. (2022). Evaluation of Alfalfa and Grass Species in Binary and Complex Mixtures on Performance under Soil Salinity Conditions. *Agronomy*, *12*, 1672. https://doi.org/10.3390/agronomy12071672

Dean, M. (2020). Multi-criteria analysis. In Advances in Transport Policy and Planning (Vol. 6, pp. 165-224). Academic Press.

De Medeiros, G. A., Arruda, F. B., and Sakai, E. (2005). Crop coefficient for irrigation beans derived using three reference evaporation methods. Agriculture and Forest Meterorology. 115, 135–143.

de Oliveira, W. S., Oliveira, P. P. A., Corsi, M., Duarte, F. R. S., and Tsai, S. M. (2004). Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with *Sinorhizobium meliloti*. *Science of Agriculture*, 61, 433–438.

De Silva, C., Rathor, P., Poudel, H. P., and Thilakarathna, M. S. (2023). Effects of drought stress on red clover–grass mixed stands compared to grass monoculture stands in nitrogen-deficient systems. *Nitrogen*, *4*, 382–396. https://doi.org/10.3390/nitrogen4040027

Dhakal, D., and Anowarul Islam, M. (2018). Grass–legume mixtures for improved soil health in cultivated agroecosystem. Sustainability, 10, 2718. https://doi.org/10.3390/su10082718

Dhakal, M., West, C. P., Villalobos, C., Sarturi, J. O., and Deb, S. K. (2020). Trade-offs between nutritive value improvement and crop water use for alfalfa-grass systems. Crop Science, 60, 1711–1723.

Dooley A. E., Smeaton D. C., Sheath G. W., and Ledgard S. F. (2009). Application of multiple criteria decision analysis in the New Zealand agricultural industry. Journal of Multi-Criteria Decision Analysis, 16(1–2), 39–53.

Doran, J. W., and Zeiss, M. R. (2000). Parent material and soil physical properties. Encyclopedia of Earth Sciences Series, 15, 3–9. https://doi.org/10.1007/978-90-481-3585-1_107

Doran, J.W. and Parkin, T.B. (1994). Defining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F. and Stewart, B.A., Eds., Defining Soil Quality for a Sustainable Environment. (pp. 3–21). Soil Science Society of America. http://dx.doi.org/10.2136/sssaspecpub35.c1

Doran, J.W. and Parkin, T.B. (1996). Qualitative indicators of soil quality: a minimum data set. In: Doran, J.W. and Jones, A. J., eds., Methods for Assessing Soil Quality. (pp. 25–37). Soil Science Society of America. https://doi.org/10.2136/sssaspecpub49.c2

Drenovsky, R. E., and James, J. J. (2010). Designing invasion-resistant plant communities. The role of plant functional traits. *Rangelands, 32,* 32–37.

Dunham, R. J., and Nye, P. H. (1973). Influence of soil-water content on uptake of ions by roots. Soil-water content gradients near a plane of onion roots. *Journal of applied ecology* 10:585–598.

Durack, P.J., Wijffels, S.E., and Matear, R. J. (2014). Ocean salinities reveal strong global water cycle intensification during 1950 to 2000. Science 2012, 336, 455–458.

Durunna, O. N., Baron, V., Scott, S. L., Robins, C., Khakbazan, M., and Block, H. C. (2015). Effects of resting perennial pastures during the sensitive pre-dormancy period in western Manitoba: Pasture productivity and beef cattle performance. *Canadian Journal of Animal Science*, *95*(2), 129–141. https://doi.org/10.4141/CJAS-2014-046

Dyer, A. R., and Rice, K. J. (1999). Effects of competition on resource availability and growth of a California bunchgrass. Ecology *80*(8), 2697–2710.

Egger, M., Smith, G. D., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. British Medical Journal, 315, 629–634.

Elizalde, J. C., Merchen, N. R., and Faulkner, D. B. (1994). Fractionation of fiber and crude protein in fresh forages during the spring growth. *Journal of Animal Science, 77*, 476–484.

Elmy, K. R. (2020). Cover Cropping in Western Canada, 5th ed. Friesen Press.

Entz, M. H., Bullied, W. J., and Katepa-Mupondwa, F. (1995). Rotational benefits of forages in Canadian Prairies cropping systems. Journal of Production Agriculture, 4,521–528.

https://doi.org/10.2134/jpa1995.0521

Fageria, N. K. (2014). Nitrogen harvest index and its association with crop yields. *Journal of Plant Nutrition*, *37*(6), 795–810. https://doi.org/10.1080/01904167.2014.881855

FAO. (2012). Sustainability Pathways Share of Livestock Production in Global Land Surface Did You Know? Why Does Livestock Matter for Sustainability? *Livestock and Landscapes*, All. http://www.fao.org/docrep/018/ar591e/ar591e.pdf

FAO. (2017). Strategic work of FAO for Sustainable Food and Agriculture. *Food and Agriculture Organization of the United Nations (FAO)*, 28. http://www.fao.org/documents/card/en/c/c021f962-c228-4c99-b8b0-01f70ed85293/

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development, 29, 185–212.

Fierer, N., and Schimel, J. P (2002). Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, 34, 777–787. doi:10.1016/S0038-0717(02)00007-X

Fisher, D. S., Mayland, H. F., and Burns, J. C. (1999). Variation in ruminants' preference for tall fescue hays cut either at sundown or at sunup. *Journal of Animal Science*, 77, 762–768.

Fornara, D. A. and Tilman, D. (2008). Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology, 96, 314–322. https://doi.org/10.1111/j.1365-2745.2007.01345.x

Foster, A., Biligetu, B., and Darambazar, E. (2019). Forage accumulation, nutritive value, and botanical composition of grass–cicer milkvetch mixtures under two harvest managements. *Crop Science*, 59(6), 2876–2885. https://doi.org/10.2135/cropsci2019.03.0198

Foster, A., Biligetu, B., Malhi, S. S., Gill, K. S., Mollison, B., and Leach, D. (2021). Harvest time and fertility effects on yield and quality of forage from alfalfa, hybrid bromegrass and their mixture. *Agricultural Sciences, 12*(04), 325–338. https://doi.org/10.4236/as.2021.124021

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.Volume 94. pp. 41–50. https://doi.org/10.4141/CJPS2013-095

Fox, D. G., Tedeschi, L. O., Tylutki, T. P., Russell, J. B., Van Amburgh, M. E., Chase, L. E., Pell, A.N. and Overton, T. R. (2004). The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Animal Feed Science Technology. 112, 29–78.

Franzluebbers, A. J. (2012). Grass roots of soil carbon sequestration. Carbon Management, 3, 9–11. https://doi.org/10.4155/cmt.11.73

Fraser, M. D., Moorby, J. M., Vale, J. E., and Evans, D. M. (2014). Mixed grazing systems benefit both upland biodiversity and livestock production. PLoS ONE, 9(2). https://doi.org/10.1371/journal.pone.0089054.

Fried, M., and Middelboe, V. (1977). Measurement of the amount of nitrogen fixed by a legume crop. *Plant and Soil*, 47, 713–715. https://doi.org/10.1007/BF00011042

Fustec, J., Lesuffleur, F., Mahieu, S., and Cliquet, J.-B. (2010). Nitrogen rhizodeposition of legumes. A review. *Agronomy for Sustainable Development*, 30, 57–66. https://doi.org/10.1051/agro/2009003

Gadberry, S. (1996). Beef cattle nutrition series part 3: Nutrient requirement tables. University of Arkansas Division of Agriculture: Little Rock, AR, USA.

Gao, Y, Duan, A., Sun, J., Li, F., Liu, Z., Liu, H., and Liu, Z. (2009). Crop coefficient and water-use efficiency of winter wheat/spring maize strip intercropping. Field Crops Research, 111, 65–73.

Gardarin, A., Garnier, E. et al. (2014). Plant trait-digestibility relationships across management and climate gradients in permanent grasslands. Journal of Applied Ecology, 51 (5) 1207–1217. https://doi.org/10.1111/1365-2664.12293

Garg, N., and Geetanjali, M. (2007). Symbiotic nitrogen fixation in legume nodules: process and signaling. A review. *Agronomy for Sustainable Development*, 27 (1), 59–68.

Gelley, C., Ashworth, A., Keyser, P., Nave, R., and Rhinehart, J. (2020). Water- use efficiency of forage crops in the Southeastern United States, Agronomy, 10, 1377. https://doi.org/10.3390/agronomy10091377

Gierus, M., Kleen, J., Loges, R., and Taube, F. (2012). Forage legume species determine the nutritional quality of binary mixtures with perennial ryegrass in the first production year. *Animal and Feed Science Technology*, 172:150–161. doi:10.1016/j.anifeedsci.2011.12.026

Gijsman, A. J., and Thomas, R. J. (1996). Evaluation of some physical properties of an oxisol after conversion of native sannana into legume-based or pure grass pastures. Tropical Grassland, 30, 237–248.

Gillian, B., Ali Mohamed, A., Oladokun, S., Wang, J., and Davis, H. (2021). Forage-fed cattle point the way forward for beef? Future Foods, Volume 3, 2021,100012, ISSN 2666-8335, https://doi.org/10.1016/j.fufo.2021.100012

Goering, H. K., and Van, P. J. (1970). Forage fiber analyses. *U.S.* Agricultural Research Service, 379, 387–598.

Goh, K. M. (2007). Effects of multiple reference plants, season, and irrigation on biological nitrogen fixation by pasture legumes using the isotope dilution method. *Community in Soil Science and Plant Analysis*, 38, 1841–1860.

Government of Alberta (2020). Agricultural region of Alberta soil inventory database. Accessed on 29 February 2024 at https://www.alberta.ca/agricultural-regions-of-alberta-soil-inventory-database

Gregory, M. M., Shea, K. L., and Bakko, E. B. (2005). Comparing agroecosystems: effects of cropping and tillage patterns on soil, water, energy use and productivity. Renewable Agriculture and Food Systems, 20, 81–90.

Grosbellet, C., Vidal-Beaudet, L., Caubel, V., and Charpentier, S. (2011). Improvement of soil structure formation by degradation of coarse organic matter. *Geoderma*, *162*(1–2), 27–38. https://doi.org/10.1016/j.geoderma.2011.01.003

Gross, S. M., Neville, B. W., Brummer, F. A., and Undi, M. (2016). Frequency of feeding DDGS as a supplement to beef cows grazing corn residue. Joint Annual Meeting. Salt Lake City, Utah. Abstract # 612. Journal of Animal Science. Vol. 94, Suppl. https://academic.oup.com/

Gülser, C. (2006). Effect of forage cropping treatments on soil structure and relationships with fractal dimensions. Geoderma, 131, 33–44. https://doi.org/10.1016/j.geoderma.2005.03.004

Gyamfi, H., Hernandez Ramirez, G. Omokanye, A., and Thilakarathna, M. S. (2024). Productivity and nutritive value of perennial grass–legume mixtures in the Canadian Prairies: a synthesis. [Manuscript submitted for publication]. University of Alberta, Edmonton, AB.

Gyamfi, H., Hernandez Ramirez, G., Omokanye, A., and Thilakarathna, M. S. (2024). Does diversity of perennial forage species affect soil quality. [Unpublished manuscript]. University of Alberta, Edmonton, AB.

Gyamfi, H., Hernandez Ramirez, G., Omokanye, A., and Thilakarathna, M. S. (2024). Forage dry matter yield, nutritive value, and botanical composition of diverse forage species mixtures. [Manuscript submitted for publication]. University of Alberta, Edmonton, AB.

Gyamfi, H., Hernandez Ramirez, G., Omokanye, A., and Thilakarathna, M. S. (2024). Biomass and crude protein water use efficiencies of perennial forage mixtures [Unpublished manuscript]. University of Alberta, Edmonton, AB.

Hamza, M. A., and Anderson, W. K. (2005). Soil compaction in cropping systems: a review of the nature, causes and possible solutions. Soil and Tillage Research 82, 121–145. https://doi.org/10.1016/j.still.2004.08.009

Hansen, J. C., Schillinger, W. F., Sullivan, T. S., and Paulitz, T. C. (2019). Soil microbial biomass and fungi reduced with canola introduced into long-term monoculture wheat rotations. Frontiers in Microbiology, 19, 1045–1059. https://doi.org/10.3389/fmicb.2019.01488

Hardarson, G., and Atkins, G. (2003). Optimizing biological $N_2$ fixation by legumes in farming system. *Plant and Soil*, 252: 41–54.

Hardarson, G., and Danso, S. (1993). Methods for measuring biological nitrogen fixation in grain legumes. *Plant and Soil*, 152: 19–23.

Hatfield, J. L., and Dold, C. (2019). Water-use efficiency: Advances and challenges in a changing climate. *Frontiers in Plant Science*, *10*, 1–14. https://doi.org/10.3389/fpls.2019.00103

Havlin, J. L., Beaton, J. D., Tisdale, S. L. and Nelson, W. L. (1999). Soil fertility and fertilizers: an introduction to nutrient management. 6th ed. Prentice Hall Inc., Upper Saddle River, NJ. 499 pp.

Heichel, G. H., Barnes, D. K., and Vance, C. P. (1981). Nitrogen fixation of alfalfa in the seeding year. *Crop Science*, 21, 330–335 https://doi.org/10.2135/cropsci1981.0011183X002100020032x

Helgadóttir, Á., Suter, M., Gylfadóttir, T., Kristjánsdóttir, T. A., and Lüscher, A. (2018). Grass–legume mixtures sustain strong yield advantage over monocultures under cool maritime growing conditions over a period of 5 years. *Annals of Botany, 122*(2), 337–345. https://doi.org/10.1093/aob/mcy074

Hendrickson, J. R., Schmer, M. R., and Sanderson, M. A. (2013). Water Use Efficiency by Switchgrass Compared to a Native Grass or a Native Grass Alfalfa Mixture. Bioenergy Research, 6(2), 746–754. https://doi.org/10.1007/s12155-012-9290-3

Hillel, D. (1982). Introduction to soil physics. Academic Press.

Høgh-Jensen, H., and Schjoerring, J. K. (1997). Interactions between white clover and ryegrass under contrasting nitrogen availability: $N_2$ fixation, N fertilizer recovery, N transfer and water use efficiency. *Plant and Soil*, 197, 187–199.

Høgh-Jensen, H., Schjoerring, J.K. (2000). Below-ground nitrogen transfer between different grassland species: direct quantification by $^{15}N$ leaf feeding compared with indirect dilution of soil $^{15}N$. *Plant and Soil*, 227, 171–183. https://doi.org/10.1023/A:1026535401773

Holechek, J. L., Pieper, R. D., and Herbel, C. H. (1998). Range management: principles and practices. 3rd ed. Prentice Hall Inc., Upper Saddle River, NJ. 542 pp.

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., Schmid, B., Setälä, H., Symstad, A. J., Vandermeer, J., and Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, *75*(1), 3–35. https://doi.org/10.1890/04-0922

Horrocks, R. D. and Vallentine, J. F. (1999). Harvested Forages. Academic Press, London, UK. Pages 245–277, ISBN 9780123562555. https://doi.org/10.1016/B978-012356255-5/50035-3

Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W., and Mortensen, D. A. (2017). Agriculture in 2050: Recalibrating targets for sustainable intensification. *BioScience*, *67*(4), 386–391. https://doi.org/10.1093/biosci/bix010

Huntington, G. B., and Burns, J. C. (2007). Afternoon harvest increases readily fermentable carbohydrate concentration and voluntary intake of gamagrass and switch grass baleage by beef steers. *Journal of Animal Science*, 85, 276–284.

Hurisso, T. T., Norton, J. B., and Norton, U. (2013). Soil profile carbon and nitrogen in prairie, perennial grass–legume mixture and wheat–fallow production in the central High Plains, USA. Agriculture, Ecosystems and Environment, 181,179–187. https://doi.org/10.1016/j.agee.2013.10.008

Hussain, T., Hussain, N., Tahir, M., Raina, A., Ikram, S., Maqbool, S., Fraz Ali, M., and Duangpan, S. (2022). Impacts of drought stress on water use efficiency and grain productivity of rice and utilization of genotypic variability to combat climate change. *Agronomy*, 12(10), 2518. https://doi.org/10.3390/agronomy12102518

Hutton, G., Berg, B., Najda, H., Johns, M., Cole, D., and Yoder, C. (2005). Perennial forage establishment in Alberta. Accessed on 21 March 2024 at https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9682/$file/120_22-3.pdf?OpenElement

Ibewiro, B., Sanginga, N., Vanlauwe, B., and Merckx, R. (2000). Evaluation of symbiotic dinitrogen inputs of herbaceous legumes into tropical cover-crop systems. *Biology and Fertility of Soils*, 32, 234–242.

Iheshiulo, E. M. A., Larney, F. J., Hernandez-Ramirez, G., Luce, M. St., Liu, K., and Chau, H.W. (2023a). Crop rotations influence soil hydraulic and physical quality under no-till on the Canadian Prairies. Agriculture, Ecosystem, and Environment, 361, 108820. https://doi.org/10.1016/j.agee.2023.108820

Iheshiulo, E. M. A., Larney, F. J., Hernandez-Ramirez, G., Luce, M. St., Liu, K., and Chau, H.W. (2023b). Do diversified crop rotations influence soil physical health? A meta-analysis. Soil and Tillage Research 233, 105781 https://doi.org/10.1016/j.still.2023.105781

Imani, M., Mirzaei, M., Baghbanzadeh-Nobari, B., and Ghaffari, M. H. (2017). Effects of forage provision to dairy calves on growth performance and rumen fermentation: A meta-analysis and meta-regression. *Journal of Dairy Science*, *100*(2), 1136–1150. https://doi.org/10.3168/jds.2016-11561

IPCC. (2014). Climate Change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change / R. Pachauri and L. Meyer (editors), Geneva, Switzerland, IPCC, 151p., ISBN: 978-92-9169-143-2.

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid. B., et al. (2015). Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature, 526, 574–77.

Islam A, M., and Adjesiwor, A. T. (2018). Nitrogen fixation and transfer in agricultural production systems. In Amanullah and Fahad, S. (Eds.) *Nitrogen In Agriculture – Updates*. Accessed on 21 March 2024 at https://doi.org/DOI: 10.5772/intechopen.71766

Issah, G., Schoenau, J. J., Lardner, H. A., and Knight, J. D. (2020). Nitrogen fixation and resource partitioning in alfalfa (*Medicago sativa* L.), cicer milkvetch (*Astragalus cicer* L.) and sainfoin (*Onobrychis viciifolia* Scop.) using $^{15}$N enrichment under controlled environment conditions. *Agronomy*, 10, 1438. doi:https://doi.org/10.3390/agronomy10091438

Jaleel, C. A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H. J., Somasundaram, R., and Panneerselvam, R. (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. International Journal of Agriculture & Biology, 11, 100–105.

Jedel, P. E., and Salmon, D. F. (1994). Forage potential of Wapiti triticale mixtures in central Alberta. Canadian Journal of Plant Science, 74(3), 515–519. https://doi.org/10.4141/cjps94-093

Jefferson, P. G., and Cutforth, H. W. (2005). Comparative forage yield, water use, and water use efficiency of alfalfa, crested wheatgrass and spring wheat in a semiarid climate in southern Saskatchewan. *Canadian Journal of Plant Science*, *85*(4), 877–888. https://doi.org/10.4141/P04-115

Jefferson, P. G., Lyons, G., Pastl, R., and Zentner, R. P. (2005). Companion crop establishment of short-lived perennial forage crops in Saskatchewan. *Canadian Journal of Plant Science*, *85*(1), 135–146. https://doi.org/10.4141/P03-220.

Jefferson, P. G., Zentner, R. P., Selles, F. and Lemke, R. (2007). Performance of short-lived perennial grasses grown with and without alfalfa at a semiarid location in southern Saskatchewan. *Canadian Journal of Plant Science*, 87(1), 59–65. https://doi.org/10.4141/P05-103

Jing, J., Søegaard, K., Cong, W. F., and Eriksen, J. (2017). Species diversity effects on productivity, persistence, and quality of multispecies swards in a four-year experiment. *PLoS ONE*, *12*(1) https://doi.org/10.1371/journal.pone.0169208

Judy, J. V., Jenkins, K. H., Klopfenstein, T. J., Stalker, L. A., and Volesky, J. D. (2015). Effects of stocking rate on forage nutrient composition of Nebraska sandhills upland range when grazed in early summer. *Journal of Animal Science*, *93*(9), 4343–4349. https://doi.org/10.2527/jas.2015-9185

Jung, H. G., and Vogel, K. P. (1992). Lignification of switchgrass (*Panicum virgatum*) and big bluestem (*Andropogon gerardii*) plant parts during maturation and its effect on fibre degradability. *Journal of the Science of Food and Agriculture*, *59*(2), 169–176. https://doi.org/10.1002/jsfa.2740590206

Kaliel, D. and Kotowich, J. (2002). Economic evaluation of cow wintering systems – Provincial swath grazing survey analysis. Alberta Production Economics Branch, Alberta Agriculture Food and Rural Development, Edmonton, AB.

Kar, G., Kumar, A., and Martha, M. (2007). Water use efficiency and crop coefficients of dry 453 season oilseed crops. Agricultural Water Management. 87, 73–82.

Keeney, D. R. (1970). Nitrates in plants and waters. Journal of Milk and Food Technology. (10),425–432.

Kelley, O. J., Hardman, J. A., and Jennings, D. S. (1947). A soil-sampling machine for obtaining two, three, and four inches cores of undisturbed soil to a depth of six feet. Soil Science, American Proceedings 12,85–87.

Kelling, K., Peters, J., Rankin, M., and Undersander, D. (2014). Potassium in forages. Focus on Forage, 4, 1–2

Kelln, B. M., Penner, G. B., Acharya, S. N., McAllister, T. A., and Lardner, H. A. (2020). Impact of condensed tannin-containing vegetables on ruminal fermentation, nutrition, and performance in ruminants: A review. Canadian Journal of Animal Science, 101, 210–223.

Kelner, D. J., Vessey, J. K., Entz, M. H. (1997). The nitrogen dynamics of 1-, 2- and 3-year stands of alfalfa in a cropping system. *Agriculture, Ecosystem and Environment*, 64, 1–10.

Kemper, W.D., Rosenau, R.C. (1986). Aggregate stability and size distribution. In: Klute, A., (ed.) Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. Agronomy Monographs No. 9. (pp. 425–442). Society of Agronomy and Soil Science Society of America.

Khangura, R., Ferris, D., Wagg, C., and Bowyer, J. (2023). Regenerative agriculture—a literature review on the practices and mechanisms used to improve soil health. Sustainability, 15, 1–41. https://doi.org/10.3390/su15032338

Khatiwada, B., Acharya, S. N., Larney, F. J., Lupwayi, N. Z., Smith, E. G., Islam, M. A., and Thomas, J. E. (2020). Benefits of mixed grass–legume pastures and pasture rejuvenation using bloat-free legumes in western Canada: A review. *Canadian Journal of Plant Science, 100*, 463–476. https://doi.org/10.1139/cjps-2019-0212

Kim, K., Daly, E. J., Gorzelak, M., and Hernandez-Ramirez, G. (2022). Soil organic matter pools response to perennial grain cropping and nitrogen fertilizer. Soil and Tillage Research. 220,105376. 105376 https://doi.org/10.1016/j.still.2022.105376

Kim, K., Daly, E. J., Gorzelak, M., Hernandez-Ramirez, G. (2022). Soil organic matter pools response to perennial grain cropping and nitrogen fertilizer. Soil and Tillage Research, 220, 105376. https://doi.org/10.1016/j.still.2022.105376

Kim, S., Williams, A., Kiniry, J. R., and Hawkes, C. V. (2016). Simulating diverse native C4 perennial grasses with varying rainfall. *Journal of Arid Environments*, 134, 97–103. https://doi.org/10.1016/j.jaridenv.2016.07.004

Kimura, E., Collins, H. P., and Fransen, S. (2015). Biomass production and nutrient removal by switchgrass under irrigation. Agronomy Journal, 107, 204–210. https://doi.org/10.2134/agronj14.0259

Klinger, S. A.,  Campbell, J., Wildeman, B., Block, H., and McKinnon, J. J. (2007). The effects of limit feeding a high-energy barley-based diet to backgrounding cattle in western Canada. Canadian Journal of Animal Science. 87, 385–391. https://doi.org/10.4141/A06-069

Köster, H. H., Cochran, R. C., Titgemeyer, E. C., Vanznat, E.S., Abdelgadir, I., and St. Jean, G. (1996). Effect of increasing degradable intake protein on intake and digestion of low quality, tall grass prairie forage by beef cows. Journal Animal Science. 74, 2473–2481.

Kozhemyakov, A. P., and Tikhonovich, I. A. (1998). Application of the legume inoculants and the biopreparations of complex action in agriculture. [in Russian.] *C.R. Russian Academy of Agricultural Science.* 6, 7–10.

Küchenmeister, K., Küchenmeister, F., Kayser, M., Wrage-Mönnig, N., and Isselstein, J. (2013). Influence of drought stress on nutritive value of perennial forage legumes. *International Journal of Plant Production* 7(4), 693–710.

Kulathunga, D. G. R. S., Penner, G. B., Schoenau, J. J., Damiran, D., Larson, K., and Lardner, H. A. (2016). Effect of perennial forage system on forage characteristics, soil nutrients, cow performance, and system economics. *Professional Animal Scientist*, *32*(6), 784–797. https://doi.org/10.15232/pas.2015-01490

Kulshreshtha, S., and Wheaton, E. (2013). Climate change adaptation and food production in Canada: Some research challenges. In WIT Transactions on Ecology and the Environment (Vol. 170). https://doi.org/10.2495/FENV130101

Kumar, S., Kumar, S., and Mohapatra, T. (2021). Interaction between macro- and micro-nutrients in plants. *Frontiers in Plant Science*, 12, 665583. https://doi.org/10.3389/fpls.2021.665583

Kunelius, H. T., and Durr, G. H., McRae, K. B., and Fillmore, A. E. (2006). Performance of timothy-based grass / legume mixtures in cold winter region. *Journal of Agronomy and Crop Science, 192*, 159–167.

Kunkle, W. E., Johns, J. T., Poore, M. H., and Herd, D. B. (1999). Designing supplementation programs for beef cattle fed forage-based diets. Journal of Animal Science. (E.

Suppl.) (1999), p. E12. DOI:10.2527/jas2000.00218812007700ES0012x

Lambers, H., Chapin, F., and Pons, T. (2008). Plant physiological ecology. Springer, New York, p 540.

Lange, M., Eisenhauer, N., Sierra, C. et al. (2017). Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications, 6, 6707. https://doi.org/10.1038/ncomms7707

Larson, K. (2013). 2012 Saskatchewan cow-calf cost of production analysis. Fact Sheet #2013–02. Western Beef Development Centre.

Lean, I. J., Rabiee, A. R., Duffield, T. F., and Dohoo, I. R. (2009). Invited review: Use of meta-analysis in animal health and reproduction: Methods and applications. *Journal of Dairy Science,* 92, 3545–3565.

Ledgard, S. F. (2001) Nitrogen cycling in low input legume-based agriculture, with emphasis on legume–grass pastures. *Plant and Soil*, 228, 43–59. doi:10.1023/A:1004810620983

Lee, A., Omokanye, A., and Hernandez-Ramirez, G. (2022). Oat- pea and pea-canola intercropping with and without nitrogen fertilization alter biomass and crude protein water-use efficiencies [Masters dissertation, University of Alberta] Accessed on May 28, 2023, at https://era.library.ualberta.ca

Lee, G. F., and Jones-Lee, A. (2005). Eutrophication (excessive fertilization), water surface and agricultural water. In: Lehr, J. H., Keeley, J. W., Lehr, J. K. and Kingery, T. B. (Ed.). *Water Encyclopedia*. John Wiley & Sons, Hoboken, NJ. Pp. 535–541. https://doi.org/10.1002/047147844x.sw1606

Lee, M. A., Davis, A. P., Chagunda, M. G. G., and Manning, P. (2017). Forage quality declines with rising temperatures, with implications for livestock production and methane emissions. *Biogeosciences*, *14*(6), 1403–1417. https://doi.org/10.5194/bg-14-1403-2017

Leen J., and Martin N. P. (2004). Forage quality tests and interpretation. Agriculture, Food and Environment Sciences. 6, 67–80.

Lees, G. L. (1992). Condensed tannins in some forage legumes: Their role in the prevention of ruminant pasture bloat. In: Hemingway, R. W. and Laks, P. S. (Eds.), Plant polyphenols:

Synthesis, properties, significance, Plenum Press, New York, NY (1992) pp.915–934.

Lees, G. L., Howarth, R. E. and Goplen, B. P. (1982). Morphological characteristics of leaves from some legume forages: Relation to digestion and mechanical strength. Canadian Journal of Botany, 60(10), 2126–2132.

Lenth, R. (2020). Emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.2-1. https://CRAN.R-project.org/package=emmeans

Li, J. C., Hernandez-Ramirez, G., Kiani, M., Quideau, S., Smith, E., Janzen, H., Larney, F., Puurveen, D. (2018). Soil organic matter dynamics in long-term temperate agroecosystems: rotation and nutrient addition effects. Canadian Journal of Soil Science, 98, 232–245.

Li, Y., Iwaasa, A. D., Wang, Y., Jin, L., Han, G., and Zhao, M. (2014). Condensed tannins concentration of selected prairie legume forages as affected by phenological stages during two consecutive growth seasons in western Canada. *Canadian Journal of Plant Science*, 94(5), 817–826. https://doi.org/10.4141/CJPS2013-234

Lindenmayer, B., Hansen, N., Crookston, M., Brummer, J., and Jha, A. (2008). Strategies for reducing alfalfa consumptive water use. p. 52–61. In: Proc. America Geophysical Union Hydrology Days. Colorado State University.

Linn, J., and Kuehn, C. (1993). The effects of forage quality on performance and cost of feeding lactating dairy cows. University of Minnesota, Department of Animal Science, 1364. Eckles Avenue, St – Paul, Minnesota, USA, 5508 – 6120.

Luo, C., Deng, Y., Inubushi, K., Liang, J., Zhu, S., Wei, Z., et al. (2018). Sludge biochar amendment and alfalfa revegetation improve soil physicochemical properties and increase diversity of soil microbes in soils from a rare earth element mining wasteland. International Journal of Environmental Research and Public Health 15, 965–986. https://doi.org/10.3390/ijerph15050965

Majak, W., McAllister, T. A., McCartney, D., Stanford, K., and Cheng, K. J. (2003). Bloat in cattle. Agriculture and Agri-Food Canada/Alberta Agriculture, Food and Rural Development, Edmonton, Alberta. AGDEX 420/60-1. 28 pp

Makenzie Applied Research Association (MARA). (2018). Perennial Forage Research Report. 1–16.

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*, 43–62.

Malhi, S. S., Zentner, R. P., and Heier, K. (2002). Effectiveness of alfalfa in reducing fertilizer N input for optimum forage yield, protein concentration, returns and energy performance of bromegrass–alfalfa mixtures. *Nutrient Cycling in Agroecosystems, 62*(3), 219–227. https://doi.org/10.1023/A:1021229824357

Mallarino, A. P., Wedin, W. F., Perdomo, R. S., Goyenola, R. S., and West, C. P. (1990). Nitrogen transfer from white clover, red clover, and birdsfoot trefoil associated grass. *Agronomy Journal*, 82, 790–795.

Mårtensson, A. M., and Ljunggren, H. D. (1984). A comparison between the acetylene reduction method, the isotope dilution method, and the total nitrogen difference method for measuring nitrogen fixation in lucerne (*Medicago sativa* L.). *Plant and Soil*, 81, 177–184. https://doi.org/10.1007/BF02197149

Matejovic, I. (1997). Determination of carbon and nitrogen in samples of various soils by the dry combustion. Communications in Soil Science and Plant Analysis, 28, 1499–1511. https://doi.org/10.1080/00103629709369892

McAllister, T. A., Stanford, K., Chaves, A. V., Evans, P. R., Eustaquio de Souza Figueiredo, E., and Ribeiro, G. (2020). Nutrition, feeding, and management of beef cattle in intensive and extensive production systems. Animal Agriculture, 75–98. doi: 10.1016/B978-0-12-817052-6.00005-7

McAloon, C. G., Whyte, P., More, S. J., Green, M. J., O'Grady, L., Garcia, A. B., and Doherty, M. L. (2016). The effect of paratuberculosis on milk yield-A systematic review and meta-analysis. *Journal of Dairy Science*, 99(2), 1449–1460. https://doi.org/10.3168/jds.2015-10156

McCartney, D., and Fraser, J. (2010). The potential role of annual forage legumes in Canada: A review. *Canadian Journal of Plant Science*, *90*(4), 403–420. https://doi.org/10.4141/CJPS07182.

McCartney, D., Basarab, J. A., Okine, E. K., Baron, V. S., and Depalme, A. J. (2004). Alternative fall and winter feeding systems for spring calving beef cows. Canadian Journal of Animal Science, 84(3), 511–522. https://doi.org/10.4141/A03-069

McCaughey, W. P., Chen, W. (1999). Benefits of including forage legumes in pastures and their management. In Proceedings of the Western Canadian Forage and Grazing Conference, Saskatoon, SK, Canada, 8–10 December 1999; Saskatchewan Stock Grower Association: Saskatoon, SK, Canada; pp. 97–115

McElrone, A. J., Choat, B., Gambetta, G. A., and Brodersen, C. R. (2013). Water Uptake and Transport in Vascular Plants. Nature Education Knowledge 4(5):6

McGeough, E. J., Cattani, D. J., Koscielny, Z., Hewitt, B., and Ominski, K. H. (2017). Annual and perennial forages for fall/winter grazing in western Canada. *Canadian Journal of Plant Science, 98*(2), 247–254. https://doi.org/10.1139/cjps-2017-0228

McKinnon, J. J., Block, H. C., Schenher, D. and Christensen, D. A. (2002). Predicting the performance of feedlot cattle — Do we have the right tools? Pages 195–204 in D. Korver and J. Morrison, eds. Proceedings of the 23rd Western Nutrition Conference. Edmonton, AB.

Meier, I. C., Finzi, A. C., and Phillips, R. P. (2017). Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. Soil Biology and Biochemistry, 106, 119–128.

Mercier, K. M., Teutsch, C. D., Fike, J. H., Munsell, J. F., Tracy, B. F., and Strahm, B. D. (2020). Impact of increasing shade levels on the dry-matter yield and botanical composition of multispecies forage stands. *Grass and Forage Science, 75*, 291–302. https://doi.org/10.1111/gfs.12489

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. https://doi.org/10.4141/CJPS2012-261

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.

Mocek, A., and Owczarzak, W. (2011). Parent material and soil physical properties. In Gliński, J., Horabik, J., Lipiec, J. (eds), Encyclopedia of Earth Sciences Series (pp. 543–547). https://doi.org/10.1007/978-90-481-3585-1_107

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. and PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Medicine 6, e1000097. https://doi.org/10.1371/journal.pmed.1000097

Moore, A. D., and Ghahramani, A. (2013). Climate change and broadacre livestock production across southern Australia. Impacts of climate change on pasture and livestock productivity, and on sustainable levels of profitability. Global Change Biology. 2013, 19, 1440–1455.

Moore, K. J., Curtiss, C. F., Lenssen, A. W., Fales, S. L. (2020). Factors affecting forage quality. In Forages Volume II: The Science of Grassland Agriculture; Moore, K. J., Collins, M., Nelson, C. J., Redfearn, D. D., Eds.; Wiley: West Sussex, UK, 2020; pp. 701–717

Moore, J. M., Klose, S., and Tabatabai, M. A. (2000). Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biology and Fertility of Soils, 31, 200–210.

Moot, D. J., Brown, H. E., Pollock, K., and Mills, A. (2008). Yield and water use of temperate pastures in summer dry environments. Proceedings of the New Zealand Grassland Association, 70, 51–57. https://doi.org/10.33584/jnzg.2008.70.2724

Moyer-Henry, K. A., Burton, J. W., Israel, D. W., and Rufty, T. W. (2006). Nitrogen transfer between plants : A $^{15}N$ natural abundance study with crops and weed species. *Plant and Soil*, 282, 7–20. https://doi.org/10.1007/s11104-005-3081-y

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. https://doi.org/10.1111/j.1365-2494.2011.00806.x

Mulder, C. P. H., Jumpponen, A., Högberg, P., and Huss-Danell, K. (2002). How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. *Oecologia*, 133, 412–421.

Muñoz-Rojas, M. (2018). Soil quality indicators: critical tools in ecosystem restoration. Current Opinion in Environmental Science and Health, 5, 47–52. https://doi.org/10.1016/j.coesh.2018.04.007

Munroe, J. W., and Isaac, M. E. (2014). $N_2$-fixing trees and the transfer of fixed-N for sustainable agroforestry: a review. 34, 417–427. https://doi.org/10.1007/s13593-013-0190-5

Murphy, D. V., Sparling, G. P., Fillery, I. R. P. (1998). Stratification of microbial biomass C and N and gross N mineralization with soil depth in two contrasting Western Australian agricultural soils. Australian Journal of Soil Research, 36, 45–55.

Nair, J., Beattie, A. D., Christensen, D., Yu, P., McAllister, T., Damiran, D., and McKinnon, J. J. (2018). Effect of variety and stage of maturity at harvest on nutrient and neutral detergent fiber digestibility of forage barley grown in western Canada. Canadian Journal of Animal Science. 98(2): 299-310. https://doi.org/10.1139/cjas-2017-0060

Naqvi, M., Kumar, D., De, K., and Sejian, V. (2015). Climate change and water availability for livestock: Impact of both quality and quantity. In Climate Change impact on livestock: Adaptation and Mitigation; Sejian, V., Gaughan, J., Baumgard, L., Prasad, C.S., Eds.; Springer-Verlag GMbH Publisher: New Delhi, India, 2015; pp. 81–96. https://doi.org/10.1007/978-81-322-2265-1_6

Nasrollahi, S.M., Imani M., and Zebeli, Q. A. (2015). Meta-analysis and meta-regression of the effect of forage particle size, level, source, and preservation method on feed intake, nutrient digestibility, and performance in dairy cows. *Journal of Dairy Science*. 98(12), 8926-8939. doi: 10.3168/jds.2015-9681

National Academies of Sciences Engineering Medicine (2016). Nutrient Requirements of Beef Cattle. Eighth revised edition. Animal Nutrition Series. National Academies press, Washington, DC. pp 72– 73.

National Research Council (2000). Nutrient Requirements of Beef Cattle, 7th Revised Edition. The National Academies Press.

Naydenova, Y. and Vasileva, V. (2016). Analysis of Forage Quality of Grass Mixtures- Grasses with Subterranean Clover. Page 534–538.

Nelson, C.J. and L.E. Moser. 1994. Plant factors affecting forage quality. p. 115–154. In: G. C. Fahey, Jr. (ed.) Forage Quality, Evaluation, and Utilization. ASA, CSSA, SSSA, Madison, Wisc.

Newman, E. I. (1988). Mycorrhizal links between plants: their functioning and ecological significance. *Advances in Ecological Research*, 18, 243–71.

Nielsen, K. (2021). Agronomic analysis of the forage yield, nutritional value, species compatibility, and winter survival rates of sainfoin (*Onobrychis viciifolia* Scop.) and grasses mixtures (Thesis, University of Saskatchewan). University of Saskatchewan. Retrieved from https://harvest.usask.ca/handle/10388/13459

Nyfeler, D., Huguenin-Elie, O., Suter, M., and Frossard, E. (2011). Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystem and Environment*, 140, 155–163. https://doi.org/10.1016/j.agee.2010.11.022

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., and Lüscher, A. (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology, 46,* 683–691. *Journal of Applied Ecology*. 46:683–691. doi:https://doi-org

O'Dell, B. L. (1984). Bioavailability of trace elements. *Nutrition Reviews, 42*, 301–308.

Oba, M., and Allen, M. S. (1999). Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. *Journal of Dairy Science, 82*, 589–591.

Okine, E. K., McCartney, D., and Basarab, J. A. (2003). Evaluation of the National Research Council (NRC) nutrient requirements for beef cattle: Predicting feedlot performance. Canadian Journal of Animal Science. 83, 787–797.

Olmstead, J., and Brummer, E. C. (2008). Benefits and barriers to perennial forage crops in Iowa corn and soybean rotations. Renewable Agriculture and Food Systems, 2*3*(2), 97–107. https://doi.org/10.1017/S1742170507001937

Omokanye, A., Hernandez-Ramirez, G., Lardner, H.A., Al-Maqtari, B., Gill, K.S., and Lee, A.

(2021). Alternative forage feeds for beef cattle in Northwestern Alberta, Canada: Forage yield and nutritive value of forage brassicas and forbs. Journal of Applied Animal Research. DOI: 10.1080/09712119.2021.1933990

Papastylianou I. (1988). The $^{15}N$ methodology in estimating N: fixation by vetch and pea grown in pure stand or in mixtures with oat. *Plant and Soil*, 107, 183–188.

Papastylianou I., and Danso S. K. A. (1989). Effect of nitrogen fertilization and cropping system of the reference crop on estimation of $N_2$ fixation by vetch using $^{15}N$ methodology. *Plant and Soil*, 114, 227–233.

Papastyliasou, I., and Danso, S. K. A. (1990). Nitrogen vetch–oat mixtures. *Soil Biology and Biochemistry*, 23(5), 447–452.

Paynel, F., Lesuffleur, F., Bigot, J., Diquélou, S., and Cliquet, J.-B. (2008). A study of $^{15}N$ transfer between legumes and grasses. *Agronomy for Sustainable Development*, 28, 281–290.

Peoples, M. B., Boddey, R. M., and Herridge, D. F. (2002). Quantification of nitrogen fixation. In Leigh, G. J. (Ed.) *Nitrogen Fixation at the Millennium* (pp. 357–389). Elsevier.

Peoples, M. B., Chalk, P. M., Unkovich, M. J., and Boddey, R. M. (2015). Can differences in $^{15}N$ natural abundance be used to quantify the transfer of nitrogen from legumes to neighboring non-legume plant species? *Soil Biology and Biochemistry*, 87, 97–109. https://doi.org/10.1016/j.soilbio.2015.04.010

Peprah, S., Damiran, D., Biligetu, B., Iwaasa, A. D., Larson, K., and Lardner, H. A. (2021). Evaluation of cool-season binary mixtures as pasture: Herbage yield, nutritive value, and beef cattle performance. *Livestock Science*, *248*, 104501. https://doi.org/10.1016/j.livsci.2021.104501

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 Agricultural Food Systems, 26*, 317–327.

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*, 331–342.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. (2020). nlme: linear and nonlinear mixed effects models. R package version 4-149. Accessed 29 February 2024 at https://CRAN.R-project.org/package=nlme

Pirhofer-Walzl, K., Rasmussen, J., Høgh-Jensen, H., Eriksen, J., Søegaard, K., and Rasmussen, J. (2012). Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil*, 350(1–2), 71–84. https://doi.org/10.1007/s11104-011-0882-z

Poeplau, C., and Don, A. (2015). Carbon sequestration in agricultural soils via cultivation of cover crops – a meta-analysis. Agriculture, Ecosystems and Environment, 200, 33–41. https://doi.org/10.1016/j.agee.2014.10.024

Pogue, S. J., Kröbel, R., Janzen, H. H., Beauchemin, K. A., Legesse, G., de Souza, D. M., Iravani, M., Selin, C., Byrne, J., and McAllister, T. A. (2018). Beef production and ecosystem services in Canada's prairie provinces: A review. *Agricultural Systems*, *166*(August), 152–172. https://doi.org/10.1016/j.agsy.2018.06.011

Prakasa Rao, E. V. S. and Puttanna, K. (2000). Nitrates, agriculture and environment. Current Science, 79,1163–1168.

Prosser, J., Ignacio Rangel-Castro, J., and Killham, K. (2006). Studying plant–microbe interactions using stable isotope technologies. *Current Opinion in Biotechnology*, 17, 98–102.

Provorov, N., and Tikhonovich, I. (2003). Genetic resources for improving nitrogen fixation in legume–rhizobia symbiosis. *Genetic Resources and Crop Evolution*, 50, 89–99.

Quilichini, S. M., Schellenberg, M. P., and Biligetu, B. (2021). Introduction of four native legumes of the Canadian prairies in monoculture and in mixtures with meadow bromegrass (Bromus riparius). *Canadian Journal of Plant Science*, 101(5), 714–729. https://doi.org/10.1139/cjps-2020-0249

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for

Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rasmussen, J., Eriksen, J., Jensen, E. S., Esbensen, K. H., and Høgh-Jensen, H. (2007). In situ carbon and nitrogen dynamics in ryegrass–clover mixtures: transfers, deposition, and leaching. *Soil Biology and Biochemistry*, 39, 804–815.

Rasmussen, J., Gylfadóttir, T., Loges, R., Eriksen, J., and Helgadóttir, A. (2013). Spatial and temporal variation in N transfer in grass–white clover mixtures at three Northern European field sites. *Soil Biology and Biochemistry*, 57, 654–662. doi:10.1016/j.soilbio.2012.07.004

Rayburn, E. B. (2002) Forage management. West Virginia University Extension Service, 5817–5820.

Ren, L., Bennett, J. A., Coulman, B., Liu, J., and Biligetu, B. (2021). Forage yield trend of alfalfa cultivars in the Canadian prairies and its relation to environmental factors and harvest management. *Grass and Forage Science*, *76*(3), 390–399. https://doi.org/10.1111/gfs.12513

Robinson, D. L., Cafe, L. M., and Greenwood, P. L. (2013). Meat science and muscle biology symposium: Developmental programming in cattle: Consequences for growth, efficiency, carcass, muscle, and beef quality characteristics. Journal of Animal Science, 91, 1428 – 1442. https://doiorg.login.ezproxy.library.ualberta.ca/10.2527/jas.2012-5799

Roscher, C., Schmid, B., Kolle, O., and Schulze, E. D. (2016). Complementarity among four highly productive grassland species depends on resource availability. *Oecologia, 181*(2), 571–582. https://doi.org/10.1007/s00442-016-3587-4

Rotaru, V., and Sinclair, T. R. (2009). Interactive influence of phosphorus and iron on nitrogen fixation by soybean. *Environmental and Experimental Botany, 66*(1), 94–99.

Rothstein, H. R., Sutton, J. A., and Borenstein, M. (2005). *Publication bias in meta-Analysis: prevention, assessment, and adjustments*. John Wiley & Sons.

Russel, J. B., O'Conner, J. D., Fox, D. G., Van Soest, P. J. and Sniffen, C. J. (1992). A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. Journal of Animal Science. 70, 3551–3561.

Rütting, T., Aronsson, H., and Delin, S. (2018). Efficient use of nitrogen in agriculture. *Nutrient Cycling in Agroecosystems*, 110(1), 1–5. https://doi.org/10.1007/s10705-017-9900-8

Sanderson, M. A., Skinner, R. H., Barker, D. J., Edwards, G. R., Tracy, B. F., and Wedin, D. A. (2004). Plant species diversity and management of temperate forage and grazing land ecosystems. *Crop Science*, *44*(4), 1132–1144. https://doi.org/10.2135/cropsci2004.1132

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*(5), 1465–1471. https://doi.org/10.2134/agronj2005.0032

Sanderson, M.A., Brink, G., Stout, R., and Ruth, L. (2013). Grass–legume proportions in forage seed mixtures and effects on herbage yield and weed abundance. *Agronomy Journal*. 105:1289–1297.

Saskatchewan Forage Council (2011). An economic assessment of feed costs within the cow/calf sector. Western Canadian Feed Innovation Network, University of Saskatchewan, Saskatoon, SK.

Saskatchewan Forage Production Guide. https://publications.saskatchewan.ca/#/products/75246

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. https://doi.org/10.4141/CJPS2011-273

Schipanski, M. E., and Drinkwater, L. E. (2012). Nitrogen fixation in annual and perennial legume–grass mixtures across a fertility gradient. *Plant and Soil*, 357(1), 147–159. https://doi.org/10.1007/s11104-012-1137-3

Schroeder, J. W. (1994). Interpreting forage analysis. Extension Dairy Specialist, North Dakota State University. Accessed on 6 June 2023 at: https://library.ndsu.edu/ir/bitstream/handle/10365/9133/AS-1080 1994.pdf?sequence=2&isAllowed=y (accessed on 6 June 2023).

Serajchi, M., Schellenberg, M. P., Mischkolz, J. M., and Lamb, E. G. (2017). Mixtures of native perennial forage species produce higher yields than monocultures in a long-term study. *Canadian Journal of Plant Science*, *98*(3), 633–647. https://doi.org/10.1139/cjps-2017-0087

Sguizzato, A. L. L., Marcondes, M. I., Dijkstra, J., Valadares, S. C., Filho, Campos, M. M., Machado, F. S., Silva, B. C., Rotta, P. P. (2020). Energy requirements for pregnant dairy heifers. PLoS One, 15 (7), 10.1371/journal.pone.0235619 e0235619

Sheaffer, C. C., Ehlke, N. J., Albrecht, K. A., Jungers, J. M., and Goplen, J. J. (2018). Forage legumes: clovers, birdsfoot trefoil, cicer milkvetch, crownvtech and alfalfa. Station Bulletin 608-2018. St. Paul, MN: Minnesota Agricultural Experiment Station.

Sheaffer, C. C., Mathison, R. D., Martin, N. P., Rabas, D. L., Ford, H. J., and Swanson, D. R. (1993). Forage legumes: clovers, birdsfoot trefoil, cicer milkvetch, crownvtech, sainfoin and alfalfa. Station Bulletin 597-1993. St. Paul, MN: Minnesota Agricultural Experiment Station, University of Minnesota

Sheaffer, C. C., Miller, D.W. and Marten, G. C. (1990). Grass dominance and mixture yield quality in perennial grass–alfalfa mixtures. *Journal of Production Agriculture, 3*, 480–485.

Sheppard, S. C., Bittman, S., Donohoe, G., Flaten, D., Wittenberg, K. M., Small, J. A., Berthiaume, R., McAllister, T. A., Beauchemin, K. A., McKinnon, J., Amiro, B. D., Macdonald, D., Mattos, F., and Ominski, K. H. (2015). Beef cattle husbandry practices across Ecoregions of Canada in 2011. *Canadian Journal of Animal Science*, 95(2), 305–321. https://doi.org/10.4141/CJAS-2014-158

Siddique K. H. M., Regan, K. L, Tennant, D., and Thomson B. D. (2001). Water use and water use efficiency of cool season grain legumes in low rainfall Mediterranean-type environments. European Journal of Agronomy, 15:267–280.

Silva, S., Barros, N., Costa, L., and Leite, F. (2008). Soil compaction and eucalyptus growth in response to forwarder traffic intensity and load. Revista Brasileira Ciencia do Solo 32, 921–932.

Sleugh, B., Moore, J. K., George, R., and Brummer, E. C. (2000). Binary legume mixtures improve forage yield, quality, and seasonal distribution. *Agronomy Journal, 92,* 24–29.

Snyder, E. M., Karsten, H. D., Curran, W. S., Malcolm, G. M., and Hyde, J. A. (2016). Green manure comparison between winter wheat and corn: weeds, yields, and economics. Agronomy Journal, 108(5), 2015–2025. https://doi.org/10.2134/agronj2016.02.0084

Sottie, E. T., Acharya, S. N., McAllister, T., Thomas, J., Wang, Y., and Iwaasa, A. (2014). Alfalfa pasture bloat can be eliminated by intermixing with newly-developed sainfoin population. Agronomy Journal, 106, 1470–1478. https://doi.org/10.2134/agronj13.0378

Soussana, J. F., and Hartwig, U. A. (1996). The effects of elevated $CO_2$ on symbiotic $N_2$-fixation: a link between the carbon and nitrogen cycles in grassland ecosystems. *Plant and Soil*, 187, 321–332.

Spehn, E. M., Schmid, B., Hector, A., Caldeira, M. C., Dimitrakopoulos, P. G., Finn, J. A., Jumpponen, A., Donnovan, G. O., Pereira, J. S., Schulze, E., Troumbis, A. Y., and Ko, C. (2002). The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. *Oikos,* 98, 205–218.

Stanton, T. L. (1995). Damage control strategies for cattle exposed to cold stress. Intake by feedlot cattle symposium, Pp 289 – 298.

Statistics Canada. (2019). Livestock estimates, July 1, 2019. Government of Canada, July 2019, 1–5. https://www150.statcan.gc.ca/n1/daily-quotidien/190822/dq190822d-eng.pdf (Accessed on 29 August, 2021).

Statistics Canada. (2022). Production of principal field crops, November 2022. Government of Canada. Accessed on 29 December 2023 at: https://www150.statcan.gc.ca/n1/daily-quotidien/221202/dq221202b-eng.htm

Statistics Canada. (2023). July 1, 2023 Livestock Inventory Estimates, *Government of Canada*, *July 2023*, 1–5. https://www150.statcan.gc.ca/n1/daily-quotidien/230823/dq230823d-eng.htm (Accessed 15 September 2023).

Stein, L. Y., and Klotz, M. G. (2016). The nitrogen cycle. *Current Biology*, *26*(3), R94–R98.

Sturludóttir, E., Brophy, C., Bélanger, G., Gustavsson, A. M., Jørgensen, M., Lunnan, T., and Helgadóttir, Á. (2014). Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. *Grass and Forage Science*, *69*(2), 229–240. https://doi.org/10.1111/gfs.12037

Su, Y. Z. (2007). Soil carbon and nitrogen sequestration following the conversion of cropland to alfalfa forage land in northwest China. Soil and Tillage Research, 92, 181–189.

Suhartanto, B., Widodo, S., Kustantinah, and Lestari, E. S. (2019). Potential of forage production on dry land agriculture with mixed cropping pattern. IOP Conference Series: Earth and Environmental Science, 387, 012061. https://doi.org/10.1088/1755-1315/387/1012061

Ta, T. C., and Faris, M. A. (1987). Species variation in the fixation and transfer of nitrogen from legumes to associated grasses. *Plant and Soil*, 98, 265–274.

Taylor, J. P., Wilson, B., Mills, M. S., and Burns, R. G. (2002). Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biology and Biochemistry, 34, 387–401. https://doi.org/10.1016/S0038-0717(01)00199-7

Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B., and Buchmann, N. (2007). Positive interactions between nitrogen-fixing legumes and four different neighboring species in a biodiversity experiment. *Oecologia, 151*(2), 190–205. https://doi.org/10.1007/s00442-006-0576-z

Thilakarathna, M. S., Mcelroy, M. S., Chapagain, T., Papadopoulos, Y. A., and Raizada, M. N. (2016a). Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. *Agronomy for Sustainable Development*. https://doi.org/10.1007/s13593-016-0396-4

Thilakarathna, M. S., Papadopoulos, Y. A., Rodd, A.V. et al. (2016b) Nitrogen fixation and transfer of red clover genotypes under legume–grass forage based production systems. *Nutrient Cycling In Agroecosystems*, 106, 233–247. doi:10.1007/s10705-016-9802-1

Thilakarathna, R. M. M. S., Papadopoulos, Y. A., Rodd, A. V., Gunawardena, A. N., Fillmore, S. A. E., and Prithiviraj, B. (2012). Characterizing nitrogen transfer from red clover populations to companion bluegrass under field conditions. *Canadian Journal of Plant Science,* 92(6): 1163–1173. https://doi.org/10.4141/cjps2012-036

Thilakarathna, S.K., Hernandez-Ramirez, G. (2021). How does management legacy, nitrogen addition and nitrification inhibition impact soil organic matter priming and nitrous oxide production? *Journal of Environmental Quality*, 50, 78–93.

https://doi.org/10.1002/jeq2.20168

Thilakarathna, S. K., and Hernandez-Ramirez, G., Puurveen, D., Kryzanowski, L., Lohstraeter, G., Powers, L., Quan, N., and Tenuta, M. (2020). Nitrous oxide emissions and nitrogen use efficiency in wheat: Nitrogen fertilization timing and formulation, soil nitrogen, and weather effects. *Soil Science Society America Journal*. 2020, 84, 1910–1927.

Thorvaldsson, G., Tremblay, G. F., and Kunelius, H. T. (2007). The effects of growth temperature on digestibility and fibre concentration of seven temperate grass species. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, *57*(4), 322–328. https://doi.org/10.1080/09064710600984221

Tilman, D., and J. A. Downing. (1994). Biodiversity and stability in grasslands. *Nature* 367:363–365.

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*, 843–845.

Tilman, D., Wedin, D., and Knops, J. (1996). Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379, 718–720.

Tokatlidis, I., Chauhan, Y., and Assefa, Y. (2022). Crop response to density: Optimization of resource use to promote sustainability. Frontiers in Plant Science, 13, 969332.

Tuna, C., Tuna, L. C., and Koc, F. (2004). Determination of nutritional value of some legumes and grasses. *Pakistan Journal of Biological Science, 7*, 1750–1753.

Unkovich, M. J., Pate, J. S., and Sanford, P. (1993). Preparation of plant samples for high precision nitrogen isotope ratio analysis. *Communications in Soil Science and Plant Analysis*, 24, 2093–2106.

Unkovich, M. J., and Pate, J. S. (2000). An appraisal of recent field measurements of symbiotic $N_2$ fixation by annual legumes. Field Crops Research, 65, 2–3.

USDA (2001). Soil infiltration: soil health – Guide for Educators. https://www.nrcs.usda.gov/sites/default/files/2022-10/Soil%20Infiltration.pdf (accessed 20 March 2021).

Vasques, I. C. F., Souza, A. A., Morais, E. G., Benevenute, P. A. N., Silva, L. de C. M. d., Homem, B. G. C., Casagrande, D. R., and Silva, B. M. (2019). Improved management increases carrying capacity of Brazilian pastures. *Agriculture, Ecosystems and Environment*, *282*(December 2018), 30–39. https://doi.org/10.1016/j.agee.2019.05.017

Vermeulen, S. J., Campbell, B. M., and Ingram, J. S. I. (2012). Climate change and food systems. Annual Review of Environment and Resources, 37, 195–222. https://doi.org/10.1146/annurev-environ-020411-130608

Villalba, D., Molina, E., and Alvarez-Rodriguez, J. (2016). Clustering forage types according to their nutritive value. In book: Mountain pastures and livestock farming facing uncertainty: environmental, technical, and socio-economic challenges (Proceedings of the 19th Meeting of the Sub-Network on Mediterranean Pastures of the FAO-CIHEAM International Network) (pp.123-126). Chapter: Options Méditerranéennes, Series A: Mediterranean Seminars, No. 116 Publisher: CIHEAM. Editors: I. Casasús, G. Lombardi

Volesky, D. J. and Berger, A. (2010). Forage Production with Limited Irrigation. NebGuide, Range and Forage Pasture Management, 4–7. http://extensionpublications.unl.edu./asssest/pdf/g2012.pdf

Waldie, O., Wright, S. B., and Cohen, R. D. H. (1983). The effects of advancing maturity on crude protein and digestibility of meadow foxtail (*Alopecurus pratensis*) and timothy (*Phleum pratense*). *Canadian Journal of Plant Science, 63*, 1083–1085. https://doi.org/10.4141/cjps83-139

Walker, R., Agapakis, C. M., Watkin, E., and Hirsch, A. M. (2015). Symbiotic nitrogen fixation in legumes: perspectives on the diversity and evolution of nodulation by *Rhizobium* and *Burkholderia* species. In de Bruijn, F. J. (Ed.) Biological Nitrogen Fixation (pp. 913–923). Wiley. https://doi.org/10.1002/9781119053095.ch89

Wang, M., Yang, J., Gao, H., Xu, W., Dong, M., Shen, G., Xu, J., Xu, X., Xue, J., Xu, C. Y., and Zhou, X. (2020). Interspecific plant competition increases soil labile organic carbon and nitrogen contents. Forest Ecology and Management, 462, 117991. https://doi.org/10.1016/j.foreco.2020.11799

Wang, Y., Berg, B. P., Barbieri, L. R., Veira, D. M., and McAllister, T. A. (2006). Comparison of alfalfa and mixed alfalfa–sainfoin pastures for grazing cattle: Effects on incidence of bloat, ruminal fermentation, and feed intake. *Canadian Journal of Animal Science*, *86*(3), 383–392. https://doi.org/10.4141/A06-009

Watt, M. S., Clinton, P. W., Whitehead, D., Richardson, B., Mason, E. G., and Leckie, A. C. (2003). Above-ground biomass accumulation and nitrogen fixation of broom (*Cytisus scoparius* L.) growing with juvenile *Pinus radiata* on a dryland site. *Forest Ecology and Management, 184*, 93–104. https://doi.org/10.1016/S0378-1127(03)00151-8

Wedin, D. A., and Russelle, M. P. (2020). Nutrient Cycling in Forage Production Systems. *Forages*, 215–225. https://doi.org/10.1002/9781119436669.ch11.

Weggler, K., and Elsäßer, M. (2023). The influence of nitrogen applications and low rainfall conditions on yield of mixed grass–legume grassland for 2 years. *Nitrogen* 2023, *4*, 194-208. https://doi.org/10.3390/nitrogen4020013

Weiss, B. (2015). Optimizing and Evaluating Dry Matter Intake of Dairy Cows. WCDS Advances in Dairy Technology, 27, 189–200.

Weiss, W. P., and Hall, M. B. (2020). Laboratory methods for evaluating forage quality. *Forages: Science Grassland Agriculture, 2*, 659–672. doi: 10.1002/9781119436669.ch36

Weißhuhn, P., Reckling, M., Stachow, U., and Wiggering, H. (2017). Supporting agricultural ecosystem services through the integration of perennial polycultures into crop rotations. Sustainability, 9, 2267. https://doi.org/10.3390/su9122267

Westerlund, D. (2019). Quantifiying yield quality of perennial varieties and mixtures: Perennial Forage Variety Evaluation and Demonstration at Multiple Sites in Alberta. 1–4.

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. *Australian Crop and Pasture Science, 60*(12), 1147–1155. https://doi.org/10.1071/CP09058

Wieland, G., Neumann, R., and Backhaus, H. (2001). Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. Applied and Environmental Microbiology, 67, 5849–5854. doi: 10.1128/AEM.67.12.5849-5854.2001.

Wortman, S. E., Francis, C. A., Bernards, M. L., Drijber, R. A., and Lindquist, J. L. (2012). Optimizing cover crop benefits with diverse mixtures and an alternative termination method. *Agronomy Journal*, *104*(5), 1425–1435. https://doi.org/10.2134/agronj2012.0185.

Wright, A. J., Mommer, L., Barry, K., and Van Ruijven, J. (2021). Stress gradients and biodiversity: monoculture vulnerability drives stronger biodiversity effects during drought years. *Ecology* 102(1):e03193.10.1002/ecy.3193

Yan, H., Gu, S., Li, S., Shen, W., Zhou, X., Yu, H., Ma, K., Zhao, Y., Wang, Y., Zheng, H., Deng, Y., and Lu, G. (2022). Grass–legume mixtures enhance forage production via the bacterial community. Agriculture, Ecosystems and Environment, 338, 108087. https://doi.org/10.1016/j.agee.2022.108087

Yong, T., Liu, X., Yang, F., Song, C., Wang, X., Liu, W., Su, B., Zhou, L., and Yang, W. (2015). Characteristics of nitrogen uptake, use and transfer in a wheat–maize–soybean relay intercropping system. Plant Production Science, *18*, 388–397.

Yu, P., Christensen, D. A., McKinnon, J. J., and Market, J. D. (2003). Effect of variety and maturity on chemical composition, carbohydrate and protein subfractions, in vitro rumen degradability and energy values of timothy and alfalfa. *Canadian Journal of Animal Science, 83*, 279–290. https://doi.org/10.4141/A02-053

Yüksel, O., and Balabanlı, C. (2021). Yield and some quality properties of binary alfalfa–grass mixtures in different mixture ratios. *Turkish Journal of Agriculture – Food Science and Technology, 9*(6), 1020–1029. https://doi.org/10.24925/turjaf.v9i6.1020-1029.40

Yungblut, D. (2012). National Forage and Grassland Assessment. *European University Institute*, *2*, 2–5. https://eur-lex.europa.eu/legal-content/PT/TXT/PDF/?uri=CELEX:32016R0679&from=PT%0Ahttp://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:52012PC0011:pt:NOT

Yurchak, T., and Orkine, E. (2004). Beef ration rules of thumb. Alberta Agriculture, Food and Rural Development Publication., agdex (420/52-4), 1–2. www.agric.gov

Zemenchik, R.A., Albrecht, K.A., and Shaver, R.D. (2002). Improved nutritive value of Kura clover and birdsfoot trefoil with grass monocultures. *Agronomy Journal, 94*, 1131–1138.

Zibilske, L. M. (1994). Carbon mineralization. In Weaver R. W., Angle S., Bottomley P., Bezdicek D., Smith S., Tabatabai A., Wollum A., and Mickelson S. H., (eds), Methods in Soil Analysis, Part 2. Microbiological and Biochemical Properties. (pp. 835–863) Soil Science Society of America.

## Appendices

**Appendix 1.** Species in perennial forage mixtures and monoculture grasses with the recommended seeding rates and the corresponding seeding rates in mixtures.

| Treatment | Target stand proportion (%) | Species | Variety | Normal monoculture seeding rate (kg ha$^{-1}$) | Actual seeding rate (kg ha$^{-1}$) |
|---|---|---|---|---|---|
| M0 | 20 | Meadow bromegrass *(Bromus riparius Rehm*) | Fleet | 13.5 | 2.6 |
| | 20 | Hybrid bromegrass (*Bromus* spp.) | AC Knowles | 12.3 | 2.5 |
| | 20 | Hybrid bromegrass | AC Success | 12.3 | 2.5 |
| | 20 | Crested wheatgrass (*Agropyron cristatum*) | Kirk | 6.7 | 1.3 |
| | 20 | Pubescent wheatgrass (*Agropyron trichophorum* (Link) Richt.) | Greenleaf | 11.2 | 2.2 |
| M1 | 50 | Meadow bromegrass | Fleet | 13.5 | 6.7 |
| | 50 | Alfalfa (*Medicago sativa subsp. falcata*) | AC Yellowhead | 8.9 | 4.5 |
| M2 | 50 | Hybrid bromegrass | AC Success | 12.3 | 5.6 |
| | 50 | Alfalfa | AC Yellowhead | 8.9 | 4.5 |
| M3 | 50 | Hybrid bromegrass | AC Success | 12.3 | 6.2 |
| | 50 | Alfalfa | AC Yellowhead | 8.9 | 4.5 |
| M4 | 50 | Meadow bromegrass | Fleet | 13.5 | 6.7 |
| | 50 | Alfalfa | Spredor 5 | 8.9 | 4.5 |
| M5 | 50 | Hybrid bromegrass | AC Success | 12.3 | 6.2 |
| | 50 | Alfalfa | Spredor 5 | 8.9 | 4.5 |
| M6 | 50 | Hybrid bromegrass | AC Knowles | 12.3 | 6.2 |
| | 50 | Alfalfa | Spredor 5 | 8.9 | 4.5 |
| M7 | 33.3 | Meadow bromegrass | Fleet | 13.5 | 4.5 |
| | 33.3 | Alfalfa | AC Yellowhead | 8.9 | 3 |
| | 33.3 | Sainfoin (*Onobrychis viciifolia*) | AC Mountain view | 39.2 | 12.3 |
| M8 | 33.3 | Hybrid bromegrass | AC Success | 12.3 | 4.1 |
| | 33.3 | Alfalfa | AC Yellowhead | 8.9 | 3 |
| | 33.3 | Sainfoin | AC Mountain view | 39.2 | 13.1 |

| Treatment | Target stand proportion (%) | Species | Variety | Normal monoculture seeding rate (kg ha$^{-1}$) | Actual seeding rate (kg ha$^{-1}$) |
|---|---|---|---|---|---|
| M9 | 25 | Meadow bromegrass | Fleet | 13.5 | 3.4 |
| | 25 | Alfalfa | AC Yellowhead | 8.9 | 2.2 |
| | 25 | Sainfoin | AC Mountain view | 39.2 | 9.9 |
| | 25 | Cicer milkvetch (*Astralagus cicer*) | Veldt | 4.5 | 1.1 |
| M10 | 25 | Hybrid bromegrass | AC Success | 4.5 | 1.5 |
| | 25 | Alfalfa | AC Yellowhead | 2.2 | 0.5 |
| | 25 | Sainfoin | AC Mountain view | 11.2 | 2.8 |
| | 25 | Cicer milkvetch | Veldt | 15.7 | 3.9 |
| M11 | 33.3 | Meadow bromegrass | Fleet | 12.7 | 4.5 |
| | 33.3 | Pubescent wheatgrass | Greenleaf | 11.2 | 3.7 |
| | 33.3 | Alfalfa | AC Yellowhead | 8.9 | 2.7 |
| M12 | 33.3 | Hybrid bromegrass | AC Success | 11 | 4.1 |
| | 33.3 | Pubescent wheatgrass | Greenleaf | 11.2 | 3.7 |
| | 33.3 | Alfalfa | AC Yellowhead | 8.9 | 2 |
| M13 | 45 | Wheatgrass | AC Saltlander | 11.2 | 24.7 |
| | 25 | Fescue | Tall | | |
| | 35 | Wheatgrass | Slender | | |
| M14 | 55 | Sainfoin | AC Mountain view | 24.7 | 24.7 |
| | 25 | Cicer milkvetch | Veldt | | |
| | 20 | Alfalfa | Spredor 5 | | |
| M15 | 20 | Hybrid bromegrass | AC Success | 12.3 | 2.5 |
| | 20 | Pubescent wheatgrass | Greenleaf | 11.2 | 2.4 |
| | 20 | Crested wheatgrass (*Agropyron cristatum*) | Kirk | 6.7 | 1.3 |
| | 20 | Italian ryegrass (*Lolium perenne*) | VNS | 10.1 | 2 |
| | 20 | Smooth bromegrass (*Bromus inermis Leyss*) | Manchar | 10.1 | 2 |
| M16 | 20 | Alfalfa | AC Yellowhead | 8.9 | 1.8 |
| | 20 | Alfalfa | Rugged | 8.9 | 1.8 |
| | 20 | Cicer milkvetch | Veldt | 15.7 | 3.1 |
| | 20 | Sainfoin | AC Mountain view | 39.2 | 7.8 |
| | 20 | Birdsfoot trefoil (*Lotus corniculatus*) | N/A | 1.01 | 2 |

| Treatment | Target stand proportion (%) | Species | Variety | Normal monoculture seeding rate (kg ha$^{-1}$) | Actual seeding rate (kg ha$^{-1}$) |
|---|---|---|---|---|---|
| M17 | 33.3 | Hybrid bromegrass | AC Success | 12.3 | 4.1 |
| | 33.3 | Alfalfa | AC Yellowhead | 6.7 | 2.2 |
| | 33.3 | Sainfoin | AC Mountain view | 39.2 | 13.1 |
| M18 | 25.0 | Hybrid bromegrass | AC Success | 12.3 | 3.1 |
| | 25.0 | Pubescent wheatgrass | Greenleaf | 11.2 | 2.8 |
| | 25.0 | Alfalfa | AC Yellowhead | 6.7 | 1.7 |
| | 25.0 | Sainfoin | AC Mountain view | 39.2 | 9.8 |
| Mix 19 | 20.0 | Hybrid bromegrass | AC Success | 12.3 | 2.5 |
| | 20.0 | Pubescent wheatgrass | Greenleaf | 11.2 | 2.2 |
| | 20.0 | Italian ryegrass (*Lolium perenne*) | VNS | 10.1 | 2.0 |
| | 20.0 | Alfalfa | AC Yellowhead | 6.7 | 1.3 |
| | 20.0 | Sainfoin | AC Mountain view | 35.0 | 7.0 |
| M20 | 15.0 | Hybrid bromegrass | AC Success | 12.3 | 1.9 |
| | 15.0 | Pubescent wheatgrass | Greenleaf | 11.2 | 1.7 |
| | 10.0 | Alfalfa | Rugged | 8.9 | 0.9 |
| | 5.0 | Alfalfa | AC Yellowhead | 8.9 | 0.4 |
| | 27.5 | Cicer Milk Vetch (*Astralagus cicer*) | Veldt | 15.7 | 4.4 |
| | 27.5 | Sainfoin | AC Mountain view | 39.2 | 10.8 |
| M21 | 30.0 | Hybrid bromegrass | AC Success | 11.0 | 3.3 |
| | 50.0 | Alfalfa | AC Yellowhead | 12.3 | 4.5 |
| | 20.0 | Sainfoin | AC Mountain view | 39.2 | 7.8 |

VSN, variety not supplied.

| Treatment | Target stand proportion (%) | Species | Variety | Normal monoculture seeding rate (kg $ha^{-1}$) | Actual seeding rate (kg $ha^{-1}$) |
|---|---|---|---|---|---|
| M22 | 5.0 | Hybrid bromegrass | AC Success | 12.3 | 0.7 |
| | 5.0 | Pubescent wheatgrass | Greenleaf | 11.2 | 0.6 |
| | 22.5 | Alfalfa | AC Yellowhead | 8.9 | 2.0 |
| | 22.5 | Cicer milkvetch | Veldt | 15.7 | 3.6 |
| | 22.5 | Sainfoin | AC Mountain view | 39.2 | 8.9 |
| | 22.5 | Birdsfoot trefoil | VNS | 10.1 | 2.2 |
| M23 | 12.5 | Hybrid bromegrass | AC Success | 12.3 | 1.6 |
| | 12.5 | Pubescent wheatgrass | Greenleaf | 11.2 | 1.5 |
| | 12.5 | Crested wheatgrass | Kirk | 6.7 | 0.9 |
| | 12.5 | Italian ryegrass | VNS | 10.1 | 1.2 |
| | 12.5 | Alfalfa | AC Yellowhead | 8.9 | 1.1 |
| | 12.5 | Alfalfa | AC Yellowhead | 8.9 | 1.1 |
| | 12.5 | Cicer milkvetch | Veldt | 15.7 | 2.0 |
| | 12.5 | Sainfoin | AC Mountain view | 39.2 | 4.9 |
| Monoculture grasses | | Pubescent wheatgrass | Greenleaf | 11.2 | 11.2 |
| | | timothy grass (*Phleum pratense)* | Grinstat | 4.5 | 4.5 |
| | | Hybrid bromegrass | Fleet | 12.3 | 12.3 |
| | | Orchard grass (*Dactylis glomerata)* | VNS | 11.2 | 11.2 |
| | | Crested Wheatgrass | Kirk | 6.7 | 6.7 |

VSN, variety not supplied.

**Appendix 2a.**


**Appendix 2a.** (a) Root profile of Fleet meadow bromegrass, showing vigorous growth and spread at a depth of 0–25 cm. This was sampled after harvest in the final year of production (2023). (b) Root profile of a complex grass–legume mixture, showing an alfalfa plant and multiple grasses of Kirk crested and Fleet meadow bromegrass sampled in the final year of production (2023). The taproot of the alfalfa plant is observed to extend more than 30 cm into the soil, whereas the roots of both grasses spread within the 0–23 cm layer.

**Appendix 2b.** Baseline data for soil chemical properties at 0–15 cm prior to seeding in June 2020 at Fairview, Alberta, Canada.

| Chemical property | Mean | SEM |
|---|---|---|
| pH | 5.2 | 0.2 |
| *Macronutrients* (*mg kg$^{-1}$*) | | |
| $NO_3$–N | 33.0 | 4.8 |
| Phosphorus | 27.3 | 4.1 |
| Potassium | 209.3 | 22.7 |
| Magnesium | 350.8 | 123.0 |
| Calcium | 1197.5 | 27.2 |
| *Micronutrients* (*mg kg$^{-1}$*) | | |
| Zinc | 4.5 | 0.5 |
| Manganese | 21.3 | 4.0 |
| Iron | 97.0 | 5.3 |
| Copper | 0.6 | 0.2 |
| Boron | 0.3 | 0.0 |

SEM, standard error of means

**Appendix 3a.** Analysis of variance *p*-values and mean groupings for the annual and 3-year cumulative dry matter yield of perennial forage mixtures and monoculture grasses for three production years (2021–2023). See Appendix 1 for a detailed list of the treatments in the study.

| Treatment | Dry matter yield (Mg ha$^{-1}$) | | | |
|---|---|---|---|---|
| | 2021 | 2022 | 2023 | 3-yr total |
| M0 | 1.95 abcde | 3.07 cdefgh | 1.95 ijkl | 7.0 efghi |
| M1 | 1.80 bcdefgh | 3.45 bcdef | 3.68 abcdefg | 8.9 abcde |
| M2 | 1.32 fghijkl | 3.07 cdefgh | 4.08 abcde | 8.5 bcde |
| M3 | 1.40 defghijk | 2.82 fgh | 3.50 bcdefgh | 7.7 efgh |
| M4 | 1.97 abcd | 4.07 ab | 4.35 abcd | 10.4 ab |
| M5 | 1.83 abcdefg | 2.87 efgh | 4.33 abcd | 9.0 abcde |
| M6 | 1.50 cdefghij | 2.97 defgh | 4.93 a | 9.4 abcd |
| M7 | 1.52 cdefghij | 3.07 cdefgh | 2.60 fghijkl | 7.2 efgh |
| M8 | 1.20 ijkl | 2.80 fgh | 2.23 hijkl | 6.2 fghij |
| M9 | 1.15 ijkl | 2.40 ghij | 2.43 ghijkl | 6.0 hij |
| M10 | 1.85 abcdefg | 2.72 fghi | 2.90 efghijk | 7.5 defgh |
| M11 | 1.80 bcdefgh | 2.80 fgh | 3.83 abcdef | 8.4 bcde |
| M12 | 1.57 bcdefghij | 2.45 ghij | 3.08 defghij | 7.1 efgh |
| M13 | 1.37 efghijk | 1.85 ij | 1.68 klm | 4.9 ij |
| M14 | 2.40 a | 3.80 bcd | 4.70 ab | 10.9 a |
| M15 | 1.22 hijkl | 1.62 j | 1.80 jklm | 4.6 j |
| M16 | 1.62 bcdefghij | 3.25 bcdefg | 3.10 cdefghij | 8.0 defgh |
| M17 | 2.40 a | 3.20 bcdefgh | 3.23 cdefghi | 8.8 abcde |
| M18 | 1.87 abcdef | 3.37 bcdef | 2.53 fghijkl | 7.8 defgh |
| M19 | 1.65 bcdefghij | 3.30 bcdefg | 3.23 cdefghi | 8.2 cdef |
| M20 | 2.15 ab | 3.10 cdefgh | 2.90 efghijk | 8.2 cdefg |
| M21 | 2.05 abc | 3.90 abc | 4.25 abcd | 10.2 abc |
| M22 | 1.70 bcdefghi | 3.50 bcdef | 3.58 bcdefg | 8.8 abcde |
| M23 | 2.05 abc | 3.75 bcde | 4.43 abc | 10.2 abc |
| FMB | 0.77 l | 4.77 a | 1.88 jkl | 7.4 efghi |
| GPWG | 1.15 ijkl | 3.90 abc | 1.63 klm | 6.7 ghij |
| KCG | 0.90 kl | 3.47 bcdef | 1.45 lm | 5.8 j |
| OG | 1.10 jkl | 2.92 defgh | 1.88 jkl | 5.9 ij |
| TG | 1.27 ghijkl | 2.32 hij | 0.53 m | 4.1 j |
| P-value | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Treatments with same letters in the columns indicate no significant difference based on Tukey HSD test. [TG denotes timothy grass, OG is orchardgrass, FMB connotes Fleet meadow bromegrass, KCG is Kirk crested wheatgrass and GPWG represents greenleaf pubescent wheatgrass]. M0, M13, and M15 are grass-only mixtures while M1 to M6 are simple grass–legume mixtures. *P*-value for treatments x year interaction is 0.014.

**Appendix 3b.** ANOVA p-values and mean grouping for crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) for mixtures and monoculture grasses during three production years (2021–2023).

| Treatments | CP (%) | | | ADF (%) | | | NDF (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2021 | 2022 | 2023 | 2021 | 2022 | 2023 | 2021 | 2022 | 2023 |
| M0 | 11.2 fghi | 12.30 gh | 11.7 i | 43.7 defg | 35.65 cd | 35.1 bcd | 54.5 abcde | 54.12 ab | 55.5 bc |
| M1 | 14.1 abc | 17.02 a | 16.8 abcd | 35.9 abcdefg | 35.72 cd | 34.4 bcd | 51.4 cdefghij | 46.92 efghij | 47.6 hij |
| M2 | 12.9 abcdefg | 16.07 abc | 15.9 abcd | 36.6 abcdefg | 35.52 cd | 35.7 bcd | 52.2 cdefgh | 48.62 cdefghi | 48.7 fghij |
| M3 | 14.2 abc | 16.62 ab | 18.0 a | 34.2 efg | 36.47 bc | 33.7 de | 47.8 hijk | 46.25 fghij | 45.6 j |
| M4 | 12.6 bcdefghi | 15.72 abcd | 14.8 def | 36.5 abcdefg | 37.17 abc | 38.4 a | 50.7 defghijk | 49.27 cdefgh | 53.1 cde |
| M5 | 12.8 bcdefgh | 16.35 abc | 16.2 abcd | 36.5 abcdefg | 35.15 cd | 35.7 bcd | 52.1 cdefgh | 47.12 efghij | 48.7 fghij |
| M6 | 13.5 abcde | 16.62 ab | 17.2 abc | 35.7 bcdefg | 36.15 cd | 36.3 abc | 50.1 efghijk | 45.12 hij | 49.2 fghi |
| M7 | 12.5 bcdefghi | 14.25 cdefg | 14.6 defgh | 36.1 abcdefg | 36.70 bc | 36.7 abc | 51.1 cdefghij | 50.10 cdef | 51.6 def |
| M8 | 13.3 abcdef | 15.27 abcde | 16.3 abcde | 34.3 efg | 35.62 cd | 35.4 bcd | 48.5 ghijk | 47.07 efghij | 47.8 hij |
| M9 | 11.9 cdefghi | 15.25 abcde | 15.8 abcd | 36.8 abcdefg | 35.50 cd | 34.7 bcd | 53.2 bcdefg | 46.62 fghij | 47.9 ghij |
| M10 | 11.7 efghi | 15.37 abcde | 15.6 bcde | 37.9 abcd | 35.85 cd | 35.8 bcd | 53.9 abcdef | 48.95 cdefgh | 49.2 fghi |
| M11 | 13.6 abcde | 15.20 abcde | 16.7 abcd | 35.0 defg | 38.07 ab | 35.1 bcd | 50.7 defghijk | 50.00 cdef | 47.3 hij |
| M12 | 14.5 ab | 16.67 a | 16.2 abcd | 33.9 g | 35.00 cd | 34.7 bcd | 48.8 fghijk | 45.17 ghij | 46.6 ij |
| M13 | 10.7 hi | 13.27 efg | 12.8 fghi | 38.9 ab | 34.20 d | 35.3 bcd | 59.2 a | 49.67 cdef | 53.8 bcde |
| M14 | 14.1 abcd | 17.02 a | 17.8 ab | 34.9 defg | 35.45 cd | 34.4 bcd | 49.6 efghijk | 44.20 j | 47.1 ij |
| M15 | 11.7 efghi | 13.80 defg | 12.5 ghi | 37.3 abcde | 35.00 cd | 35.2 bcd | 55.5 abcd | 49.40 cdefg | 53.7 bcde |
| M16 | 14.8 ab | 16.57 ab | 17.5 ab | 34.2 efg | 34.97 cd | 35.2 bcd | 46.7 ijk | 44.52 ij | 46.5 ij |
| M17 | 13.7 abcde | 15.70 abcde | 15.8 bcd | 35.5 cdefg | 35.05 cd | 34.3 bcd | 48.9 fghijk | 46.20 fghij | 46.2 ij |
| M18 | 14.2 abc | 15.85 abcde | 16.3 abcd | 35.5 cdefg | 35.67 cd | 34.8 bcd | 50.1 efghijk | 46.67 fghij | 47.1 ij |
| M19 | 11.9 cdefghi | 14.45 bcdef | 14.6 defgh | 37 abcdefg | 36.77 bc | 34.9 bcd | 51.9 cdefghij | 48.75 cdefghi | 48.9 fghij |
| M20 | 11.8 defghi | 14.35 cdefg | 15.0 cde | 36.3 abcdefg | 35.62 cd | 36.4 abc | 51.7 cdefghij | 47.75 defghij | 50.6 efgh |
| M21 | 15.2 a | 16.60 ab | 17.1 abc | 33.9 g | 35.80 cd | 34.5 bcd | 45.7 k | 45.22 ghij | 47.7 hij |
| M22 | 14.6 ab | 16.95 a | 16.6 abcd | 33.9 g | 35.52 cd | 35.6 bcd | 46.6 jk | 46.57 fghij | 46.8 ij |
| M23 | 13.3 abcdef | 15.45 abcde | 17.5 ab | 34.9 defg | 36.17 bcd | 34.2 cd | 49.5 efghijk | 47.82 defghij | 47.9 ghij |
| OG | 10.9 ghi | 12.70 gh | 16.3 abcd | 37.3 abcdef | 36.82 bc | 34.3 bcd | 56.3 abc | 52.27 bc | 51.4 efg |
| FMB | 11.5 efghi | 10.77 h | 12.0 i | 39. 0 a | 38.97 a | 35.0 bcd | 58.0 ab | 57.67 a | 56.9 b |
| GPWG | 10.4 i | 11.20 h | 12.5 hi | 38.8 abc | 38.02 ab | 35.3 bcd | 58.5 a | 56.65 a | 60 a |
| KCG | 12.6 bcdefghi | 13.30 efg | 14.7 defg | 34.0 fg | 37.17 abc | 31.3 e | 50.6 defghijk | 51.65 bcd | 53.1 cde |
| TG | 13.1 abcdefg | 13.52 efg | 13.5 efghi | 35.2 defg | 36.37 bcd | 34.4 bcd | 51.9 cdefghi | 50.92 bcde | 54.9 bcd |
| *P*-value | 0.0004 | <0.0001 | <0.0001 | 0.0266 | 0.0001 | 0.0162 | <0.0001 | < 0.0001 | <0.0001 |

Treatments with the same letters within a column indicate no significant difference according to Tukey's honestly significant difference test. TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures. *P*-value for treatments x year interaction of CP = 0.001, treatments x year interaction of ADF = 0.002, and treatments x year interaction of NDF = 0.013.

**Appendix 3c.** ANOVA *p*-values and mean grouping for total digestible nutrients (TDN) and neutral detergent fiber digestibility (NDFD-48h) for mixtures and monoculture grasses during three production years (2021–2023).

| **Treatments** | **TDN (%)** | | | **NDFD 48h** | | |
|---|---|---|---|---|---|---|
| | **2021** | **2022** | **2023** | **2021** | **2022** | **2023** |
| M0 | 63.0 abcd | 65.82 abc | 66.4 abcd | 57.07 a | 62.10 a | 63.4 abc |
| M1 | 61.8 abcdef | 63.60 cd | 63.7 fgh | 52.85 bc | 54.12 d | 54.1 def |
| M2 | 59.4 cdefgh | 63.92 cd | 63.1 fghi | 50.20 bcdefg | 55.50 cd | 53.5 def |
| M3 | 63.0 abcd | 63.00 de | 63.7 gh | 50.82 bcdefg | 53.90 d | 52.6 def |
| M4 | 59.9 bcdefg | 62.57 de | 61.2 i | 49.52 bcdefg | 54.35 d | 52.7 def |
| M5 | 60.6 abcdefg | 64.02 cd | 61.9 hi | 50.87 bcdefg | 54.00 d | 51.4 ef |
| M6 | 60.6 bcdefg | 63.80 cd | 61.4 i | 49.07 bcdefg | 52.55 d | 51.1 ef |
| M7 | 60.8 abcdefg | 63.90 cd | 63.1 fghi | 50.87 bcdefg | 55.85 cd | 55.3 d |
| M8 | 62.9 abcde | 64.52 cd | 62.0 hi | 51.95 bcde | 54.22 d | 50.6 f |
| M9 | 59.9 bcdefg | 64.52 cd | 63.2 fghi | 50.70 bcdefg | 55.10 cd | 52.8 def |
| M10 | 58.3 fgh | 64.05 cd | 62.1 ghi | 49.85 bcdefg | 55.27 cd | 51.8 def |
| M11 | 62.1 abcdef | 61.35 e | 63.1 fghi | 52.10 bcde | 54.27 d | 52.1 def |
| M12 | 63.5 abc | 64.57 bcd | 64.7 cdef | 51.45 bcde | 53.67 d | 55.1 d |
| M13 | 56.9 ghi | 67.62 a | 66.3 abcd | 53.20 b | 62.47 a | 62.5 bc |
| M14 | 62.3 abcdef | 62.67 de | 62.4 ghi | 51.72 bcde | 52.90 d | 51.4 ef |
| M15 | 58.7 efgh | 66.75 ab | 65.9 bcde | 51.87 bcde | 60.12 ab | 61.3 c |
| M16 | 63.8 ab | 64.22 cd | 63.5 fgh | 51.95 bcde | 54.32 d | 51.9 def |
| M17 | 62.0 abcdef | 64.95 bcd | 64.6 def | 49.65 bcdefg | 54.87 cd | 54.0 def |
| M18 | 62.9 abcde | 63.85 cd | 64.1 def | 52.50 bcde | 54.14 d | 54.3 de |
| M19 | 60.1 bcdefg | 63.67 cd | 63.6 fgh | 50.42 bcdefg | 54.50 cd | 53.9 def |
| M20 | 59.1 defgh | 64.27 cd | 61.9 hi | 48.40 cdefg | 54.80 cd | 52.1 def |
| M21 | 64.9 a | 63.87 cd | 62.9 fghi | 52.95 bc | 54.60 cd | 53.0 def |
| M22 | 64.1 ab | 62.75 de | 63.5 fgh | 52.00 bcde | 53.37 d | 53.0 def |
| M23 | 62.8 abcde | 62.85 de | 63.5 fgh | 52.62 bcd | 54.22 d | 54.4 de |
| OG | 55.3 hi | 67.17 a | 66.8 abc | 46.57 g | 62.60 a | 61.7 c |
| FMB | 53.9 i | 64.87 bcd | 67.3 ab | 48.00 efg | 62.37 a | 65.7 ab |
| GPWG | 55.4 hi | 64.75 bcd | 66.4 abcd | 49.97 bcdefg | 61.92 a | 66.5 a |
| KCG | 57.3 ghi | 64.70 bcd | 68.1 a | 47.02 fg | 58.25 a | 65.7 ab |
| TG | 56.6 ghi | 67.00 a | 66.2 abcd | 48.25 defg | 62.40 a | 62.7 bc |
| *P*-value | <0.0001 | <0.0001 | <0.0001 | 0.0001 | <0.0001 | <0.0001 |

Treatments with the same letters within a column indicate no significant difference according to Tukey's honestly significant difference test. TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures. *P*-value for treatments x year interaction of TDN = <0.001 while treatments x year interaction of NDFD- 48h = 0.046.

**Appendix 3d.** Botanical composition of individual perennial forage species in the first year of production (2021).

| Treatments | Forage treatments | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grasses (%DM) | | | | | | | | | | | Legumes (% DM) | | | |
| | Fleet MB | AC Success | AC Knowles | Greenleaf pubescent | Kirk crested | Tall fescue | Slender | AC Saltander | Italian rye | Manchar | Weeds | Birdsfoot | Alfalfa | CMV | Sainfoin |
| M0 | 11.8 | 21.8 | 12.7 | 11.8 | 25.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 16.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| M1 | 23.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 15.3 | 0.0 | 60.8 | 0.0 | 0.0 |
| M2 | 22.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 29.4 | 0.0 | 48.4 | 0.0 | 0.0 |
| M3 | 0.0 | 0.0 | 10.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 16.2 | 0.0 | 73.6 | 0.0 | 0.0 |
| M4 | 10.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.3 | 0.0 | 69.3 | 0.0 | 0.0 |
| M5 | 31.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.6 | 0.0 | 58.2 | 0.0 | 0.0 |
| M6 | 0.0 | 0.0 | 14.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.1 | 0.0 | 75.7 | 0.0 | 0.0 |
| M7 | 18.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 25.2 | 0.0 | 40.0 | 0.0 | 16.0 |
| M8 | 0.0 | 29.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.8 | 0.0 | 27.1 | 0.0 | 25.4 |
| M9 | 16.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.1 | 0.0 | 37.3 | 2.8 | 23.3 |
| M10 | 0.0 | 7.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 30.1 | 0.0 | 32.5 | 2.5 | 27.5 |
| M11 | 22.8 | 0.0 | 0.0 | 22.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 12.3 | 0.0 | 42.1 | 0.0 | 0.0 |
| M12 | 0.0 | 17.2 | 0.0 | 19.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 38.9 | 0.0 | 24.9 | 0.0 | 0.0 |
| M13 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 27.2 | 25.5 | 28.9 | 0.0 | 0.0 | 18.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| M14 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.1 | 0.0 | 61.7 | 5.2 | 27.0 |
| M15 | 0.0 | 14.6 | 0.0 | 22.5 | 16.1 | 0.0 | 0.0 | 0.0 | 28.5 | 18.3 | 3.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| M16 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 12.8 | 0.0 | 69.6 | 1.6 | 18.7 |
| M17 | 0.0 | 24.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.3 | 0.0 | 26.5 | 0.0 | 31.9 |
| M18 | 0.0 | 9.8 | 0.0 | 18.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.2 | 0.0 | 37.0 | 0.0 | 32.1 |
| M19 | 0.0 | 13.7 | 0.0 | 12.7 | 0.0 | 0.0 | 0.0 | 0.0 | 8.4 | 0.0 | 9.8 | 0.0 | 40.4 | 0.0 | 15.0 |
| M20 | 0.0 | 7.4 | 0.0 | 9.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 11.1 | 0.0 | 45.6 | 2.6 | 23.6 |
| M21 | 0.0 | 10.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.6 | 0.0 | 55.0 | 0.0 | 23.8 |
| M22 | 0.0 | 12.3 | 0.0 | 3.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 19.2 | 0.0 | 39.8 | 9.8 | 15.8 |
| M23 | 0.0 | 11.2 | 0.0 | 11.2 | 0.0 | 0.0 | 0.0 | 0.0 | 9.2 | 0.0 | 11.8 | 0.0 | 39.1 | 2.3 | 9.5 |

Grasses, legumes, and weeds are expressed as a percentage of dry matter (DM) weight. CMV, cicer milkvetch; FMB, Fleet meadow bromegrass. The average proportion of weeds as a percentage of DM was 16.1%.

**Appendix 3e:** Botanical composition of individual perennial forage species in the second year of production (2022).

| Mixture | Forage treatments | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grasses (%DM) | | | | | | | | | | Legumes (% DM) | | | | |
| | Fleet MB | AC Success | AC Knowles | Greenleaf pubescent | Kirk crested | Tall fescue | Slender | AC Saltander | Italian rye | Manchar | Weeds | Birdsfoot | Alfalfa | CMV | Sainfoin |
| M0 | 14.9 | 22.1 | 19.5 | 13.3 | 10.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 19.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| M1 | 30.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 39.9 | 0.0 | 29.7 | 0.0 | 0.0 |
| M2 | 0.0 | 34.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 37.0 | 0.0 | 28.3 | 0.0 | 0.0 |
| M3 | 0.0 | 0.0 | 44.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 33.6 | 0.0 | 22.4 | 0.0 | 0.0 |
| M4 | 35.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 36.1 | 0.0 | 28.8 | 0.0 | 0.0 |
| M5 | 0.0 | 37.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 35.0 | 0.0 | 27.1 | 0.0 | 0.0 |
| M6 | 0.0 | 0.0 | 35.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 32.2 | 0.0 | 32.3 | 0.0 | 0.0 |
| M7 | 22.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 30.9 | 0.0 | 17.8 | 0.0 | 29.2 |
| M8 | 0.0 | 19.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 32.5 | 0.0 | 25.8 | 0.0 | 21.9 |
| M9 | 23.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.8 | 0.0 | 20.4 | 16.4 | 18.7 |
| M10 | 0.0 | 20.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 14.7 | 0.0 | 17.3 | 26.2 | 21.7 |
| M11 | 27.3 | 0.0 | 0.0 | 18.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 33.1 | 0.0 | 21.4 | 0.0 | 0.0 |
| M12 | 0.0 | 27.4 | 0.0 | 26.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 24.5 | 0.0 | 21.8 | 0.0 | 0.0 |
| M13 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 21.3 | 30.7 | 21.4 | 0.0 | 0.0 | 26.6 | 0.0 | 0.0 | 0.0 | 0.0 |
| M14 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.4 | 0.0 | 16.3 | 31.5 | 31.8 |
| M15 | 0.0 | 34.0 | 0.0 | 9.4 | 12.8 | 0.0 | 0.0 | 0.0 | 0.0 | 21.9 | 21.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| M16 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 12.2 | 17.5 | 29.6 | 28.2 | 12.4 |
| M17 | 0.0 | 32.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 26.5 | 0.0 | 19.3 | 0.0 | 22.2 |
| M18 | 0.0 | 24.3 | 0.0 | 19.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 23.9 | 0.0 | 18.6 | 0.0 | 13.7 |
| M19 | 0.0 | 25.1 | 0.0 | 26.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 16.2 | 0.0 | 16.6 | 0.0 | 15.9 |
| M20 | 0.0 | 14.8 | 0.0 | 18.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 9.9 | 0.0 | 26.3 | 21.0 | 9.6 |
| M21 | 0.0 | 19.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 38.4 | 0.0 | 21.6 | 0.0 | 20.3 |
| M22 | 0.0 | 8.9 | 0.0 | 18.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.7 | 18.1 | 9.3 | 23.5 | 11.2 |
| M23 | 0.0 | 13.9 | 0.0 | 12.6 | 10.5 | 0.0 | 0.0 | 0.0 | 7.4 | 0.0 | 13.8 | 0.0 | 24.6 | 6.6 | 12.8 |

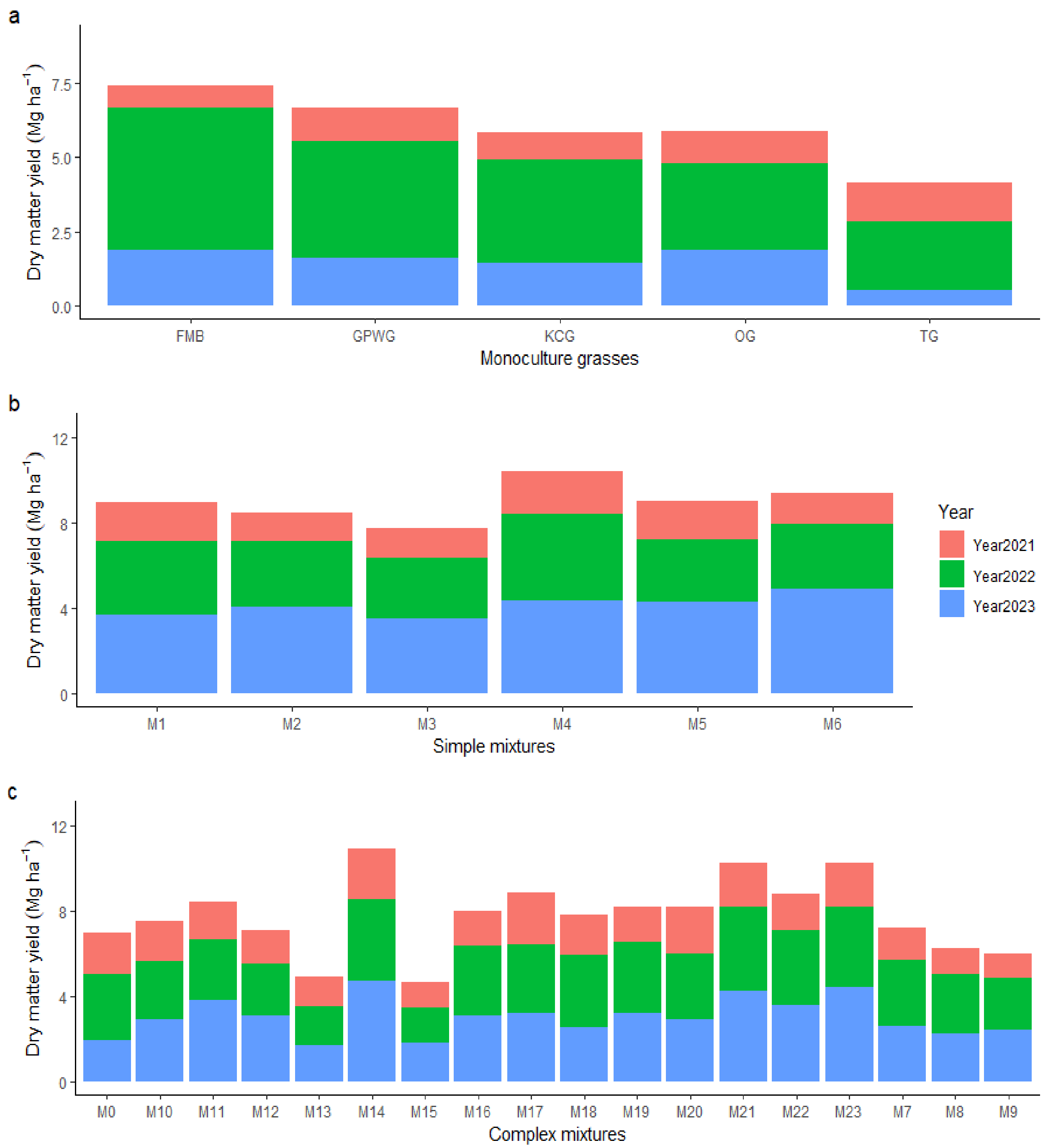
Grasses, legumes, and weeds are expressed as a percentage of dry matter (DM) weight. CMV, cicer milkvetch; FMB, Fleet meadow bromegrass. The average proportion of weeds as a percentage of DM was 25.4 %.

**Appendix 3f:** Botanical composition of individual perennial forage species in the third year of production (2023).

| Mixtures | Forage treatments | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grasses (%DM) | | | | | | | | | | | Legumes (% DM) | | | |
| | Fleet MB | AC Success | AC Knowles | Greenleaf pubescent | Kirk crested | Tall fescue | Slender | AC Saltander | Italian rye | Manchar | Weeds | Birdsfoot | Alfalfa | CMV | Sainfoin |
| M0 | 16.1 | 24.5 | 9.2 | 13.3 | 17.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 19.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| M1 | 33.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 40.2 | 0.0 | 26.7 | 0.0 | 0.0 |
| M2 | 0.0 | 31.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 42.9 | 0.0 | 25.3 | 0.0 | 0.0 |
| M3 | 0.0 | 0.0 | 45.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 23.3 | 0.0 | 30.9 | 0.0 | 0.0 |
| M4 | 0.0 | 38.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 29.5 | 0.0 | 32.3 | 0.0 | 0.0 |
| M5 | 0.0 | 32.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 44.2 | 0.0 | 23.5 | 0.0 | 0.0 |
| M6 | 0.0 | 0.0 | 38.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 32.1 | 0.0 | 29.0 | 0.0 | 0.0 |
| M7 | 30.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 18.8 | 0.0 | 19.4 | 0.0 | 31.4 |
| M8 | 0.0 | 28.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 23.5 | 0.0 | 22.1 | 0.0 | 25.9 |
| M9 | 22.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 23.3 | 0.0 | 20.4 | 10.5 | 21.7 |
| M10 | 0.0 | 23.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 29.2 | 0.0 | 19.2 | 16.8 | 11.6 |
| M11 | 26.1 | 0.0 | 0.0 | 33.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 22.1 | 0.0 | 18.5 | 0.0 | 0.0 |
| M12 | 0.0 | 28.0 | 0.0 | 15.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 28.5 | 0.0 | 28.4 | 0.0 | 0.0 |
| M13 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 31.4 | 33.1 | 30.3 | 0.0 | 0.0 | 5.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| M14 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 27.1 | 0.0 | 17.2 | 35.1 | 20.6 |
| M15 | 0.0 | 31.1 | 0.0 | 14.6 | 13.3 | 0.0 | 0.0 | 0.0 | 0.0 | 11.8 | 29.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| M16 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 24.1 | 0.0 | 43.7 | 9.0 | 23.2 |
| M17 | 0.0 | 26.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 25.0 | 0.0 | 20.1 | 0.0 | 28.6 |
| M18 | 0.0 | 19.9 | 0.0 | 21.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 19.8 | 0.0 | 19.0 | 0.0 | 20.1 |
| M19 | 0.0 | 20.6 | 0.0 | 17.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 23.8 | 0.0 | 15.1 | 0.0 | 23.1 |
| M20 | 0.0 | 16.8 | 0.0 | 7.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.3 | 0.0 | 29.3 | 18.6 | 10.2 |
| M21 | 0.0 | 27.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.0 | 0.0 | 21.6 | 0.0 | 31.3 |
| M22 | 0.0 | 10.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.0 | 6.8 | 17.0 | 24.3 | 24.4 |
| M23 | 0.0 | 10.5 | 0.0 | 3.0 | 24.5 | 0.0 | 0.0 | 0.0 | 4.0 | 0.0 | 18.2 | 0.0 | 29.8 | 3.1 | 6.8 |

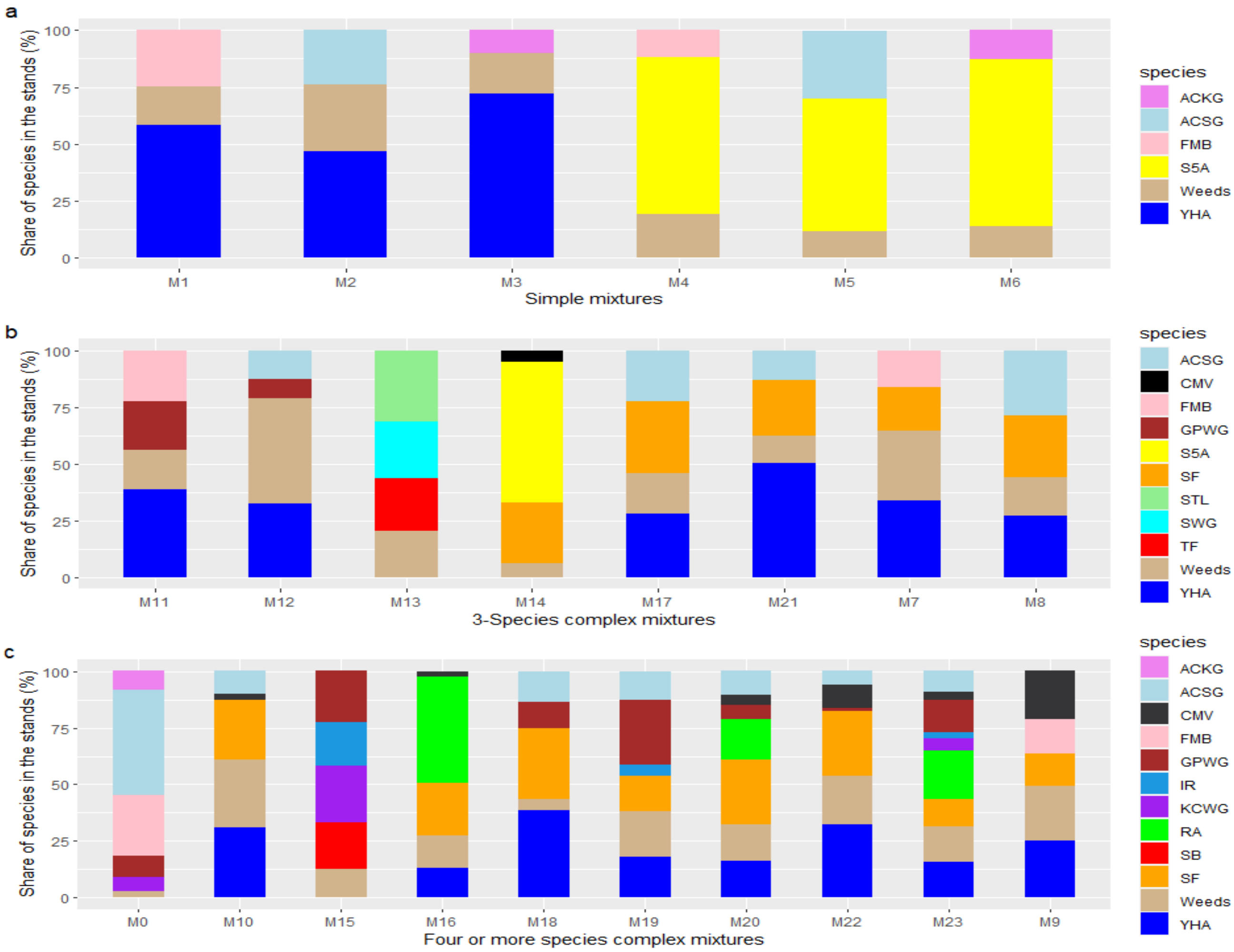
Grasses, legumes, and weeds are expressed as a percentage of dry matter (DM) weight. CMV, cicer milkvetch; FMB, Fleet meadow bromegrass. The average proportion of weeds as a percentage of DM was 25.2%.

**Appendix 3g.**



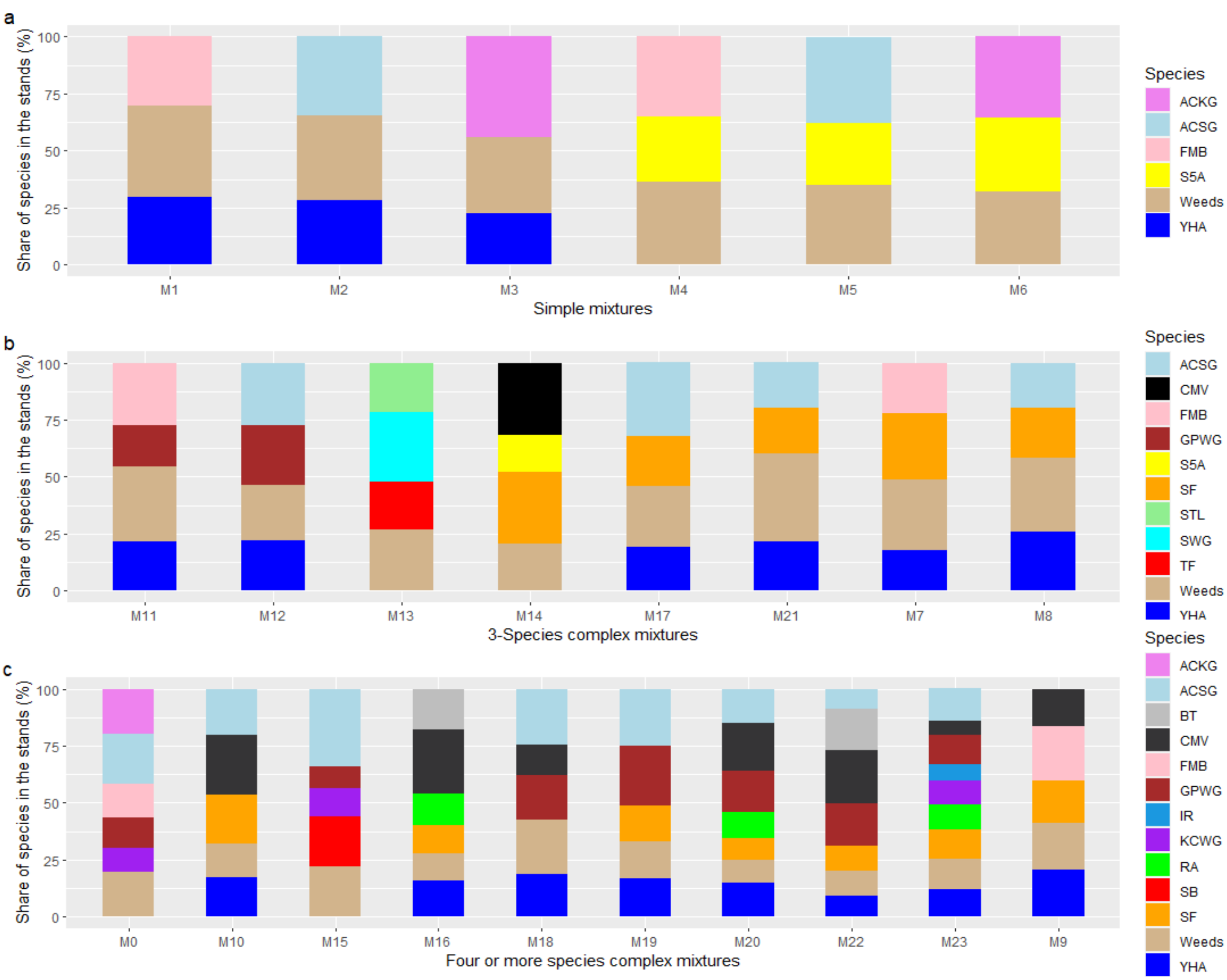

**Appendix 3g.** Cumulative dry matter (DM) yield of (a) monoculture grasses, (b) simple and (c) complex mixtures (2021–2023). $P < 0.0001$. TG, timothygrass; OG, orchardgrass; FMB, Fleet meadow bromegrass; KCG, Kirk crested wheatgrass; GPWG, greenleaf pubescent wheatgrass. M0, M13, and M15 are grass-only mixtures, whereas M1 to M6 are simple grass–legume mixtures. See Appendix 1 for a list of species combinations for each treatment.

**Appendix 3h.**




**Appendix 3h.** Botanical composition of individual species of grasses, legumes, and weeds expressed as a percentage of dry matter weight during the first year of production (2021). CMV, cicer milkvetch; FMB, Fleet meadow bromegrass; IR, Italian ryegrass; KCWG, Kirk crested wheatgrass; SB, smooth bromegrass; ACSG, AC Success hybrid bromegrass; ACKG, AC Knowles hybrid bromegrass; GPWG, greenleaf pubescent wheatgrass; RA, rugged alfalfa; SF, sainfoin; YHA, yellowhead alfalfa, SWG, slender wheatgrass; STL, AC Saltander wheatgrass; TF, tall fescue; s5A, Spredor 5 alfalfa. The average proportion of weeds as a percentage of dry matter was 16.1%. See Appendix 1 for a list of the species combinations for each treatment.

**Appendix 3i.**




**Appendix 3i**. Botanical composition of individual species of grasses, legumes, and weeds expressed as a percentage of dry matter weights during the second year of production (2022). CMV, cicer milkvetch; FMB, Fleet meadow bromegrass; BT, birdsfoot trefoil; IR, Italian ryegrass; KCWG, Kirk crested wheatgrass; SB, smooth bromegrass; ACSG, AC Success hybrid bromegrass; ACKG, AC Knowles hybrid bromegrass; GPWG, greenleaf pubescent wheatgrass; RA, rugged alfalfa; SF, sainfoin; YHA, yellowhead alfalfa; SWG, slender wheatgrass; STL, AC Saltander wheatgrass; TF, tall fescue; s5A, Spredor 5 alfalfa. The average proportion of weeds as a percentage of dry matter was 25.4 %. See Appendix 1 for a list of the species combinations for each treatment.

**Appendix 4.** ANOVA p-values and mean grouping annual biomass (BM) and crude protein (CP) water-use efficiency (WUE) for perennial forage mixtures and monoculture grasses (2021–2023). See Appendix 1 for a detailed list of the experiment treatments in the study.

| **Treatments** | **WUE** | | | | | |
|---|---|---|---|---|---|---|
| | **2021** | **2022** | **2023** | **2021** | **2022** | **2023** |
| | **Biomass (kg dry matter ha$^{-1}$ mm$^{-1}$)** | **Biomass(kg dry matter ha$^{-1}$ mm$^{-1}$)** | **Biomass(kg dry matter ha$^{-1}$ mm$^{-1}$)** | **CP (kg CP ha$^{-1}$ mm$^{-1}$)** | **CP (kg CP ha$^{-1}$ mm$^{-1}$)** | **CP (kg CP ha$^{-1}$ mm$^{-1}$)** |
| M0 | 91.4 abcd | 23.92 cdef | 27.7 jkl | 983.5 cdef | 294.60 efg | 324.4 mn |
| M1 | 81.8 abcd | 27.30 bcde | 55.2 bcdef | 1130.7 abcde | 464.32 abcde | 925.8 bcdefg |
| M2 | 62.3 cde | 23.87 cdef | 58.9 abcd | 890.5 cdef | 385.10 abcdef | 937.3 bcdefgh |
| M3 | 62.9 cde | 23.72 cdef | 47.4 cdefghi | 920.2 cdef | 395.90 abcdef | 842.3 bcdefgh |
| M4 | 93.4 abcd | 34.57 ab | 65.9 abc | 1230.9 abcd | 548.82 a | 988.3 abcde |
| M5 | 79.4 abcd | 23.35 cdef | 67.8 ab | 975.5 cdef | 397.70 abcdef | 1095.6 abc |
| M6 | 73.3 cde | 23.70 cdef | 75.2 a | 957.1 cdef | 395.62 abcdef | 1283.0 a |
| M7 | 67.1 cde | 24.15 cdef | 34.3 ghijkl | 864.3 cdefg | 344.77 defg | 509.6 ijklm |
| M8 | 55.9 de | 22.77 cdef | 31.6 hijkl | 750.9 defg | 344.65 defg | 529.7 hijklm |
| M9 | 56.1 de | 18.82 efg | 38.1 fghijkl | 679.8 efg | 286.05 fg | 606.5 ghijklm |
| M10 | 85.1 abcd | 22.67 cdef | 43.8 defghij | 1037.4 bcde | 346.82 defg | 678.3 efghijk |
| M11 | 82.1 abcd | 22.42 cdef | 57.0 abcde | 1163.9 abcde | 343.20 defg | 941.2 bcdef |
| M12 | 69.3 cde | 19.95 defg | 49.4 bcdefgh | 1035.0 bcde | 333.35 defg | 805.1 cdefghi |
| M13 | 63. 4 cde | 14.55 fg | 24.7 klm | 691.6 efg | 188.10 g | 355.7 lmn |
| M14 | 116.8 a | 31.82 abc | 66.5 ab | 1560.7 a | 537.22 ab | 1161.0 ab |
| M15 | 55.0 de | 13.25 g | 28.9 ijkl | 705.6 efg | 181.55 g | 364.3 klmn |
| M16 | 73.3 bcde | 26.80 bcde | 43.2 defghijk | 1071.6 abcde | 444.65 abcdef | 761.5 defghij |
| M17 | 109.8 ab | 25.57 bcde | 44.6 defghij | 1520.6 ab | 393.17 abcdef | 702.8 efghij |
| M18 | 89.3 abcd | 27.05 bcde | 38.4 efghijkl | 1178.2 abcde | 429.97 abcdef | 625.9 fghijklm |
| M19 | 74.3 bcde | 27.00 bcde | 50.9 bcdefgh | 987.1 cdef | 389.07 abcdef | 746.8 defghij |
| M20 | 97.6 abc | 26.02 bcde | 44.1 defghij | 1218.1 abcd | 372.65 bcdef | 655.0 fghijkl |
| M21 | 92.0 abcd | 31.82 abc | 61.7 abcd | 1298.2 abc | 537.40 ab | 1036.5 abcd |
| M22 | 77.9 bcd | 29.10 abcd | 50.8 bcdefgh | 1145.3 abcde | 493.97 abcd | 851.1 bcdefg |
| M23 | 93.2 abcd | 31.97 abc | 59.9 abcd | 1256.1 abc | 490.55 abcd | 1044.4 abcd |
| TG | 56.7 de | 38.42 a | 27.2 jklm | 713.3 efg | 528.75 abc | 114.4 n |
| OG | 37.0 e | 22.45 cdef | 8.5 m | 496.7 fg | 284.55 fg | 433.3 jklm |

| | | | | | | |
|---|---|---|---|---|---|---|
| FMB | 36.3 e | 34.37 ab | 27.8 jkl | 382.3 g | 371.62 bcdef | 332.9 mn |
| GPWG | 56.7 de | 30.75 abc | 24.8 klm | 713.3 efg | 343.22 defg | 312.5 mn |
| KCG | 37.0 e | 27.67 abc | 23.8 lm | 496.7 fg | 364.67 cdef | 338.8 lmn |
| *P*-value | 0.00464 | < 0.0001 | < 0.0001 | 0.000877 | < 0.0001 | < 0.0002 |

Treatments with same letters in the columns indicate no significant difference based on Tukey HSD test. [TG denotes timothy grass, OG is orchard grass, FMB connotes Fleet meadow bromegrass, KCG is Kirk crested wheatgrass and GPWG represents greenleaf pubescent wheatgrass] M0, M13, and M15 are grass-only mixtures while M1 to M6 are simple grass–legume mixtures. *P*-values for treatments x year of $WUE_{BM}$ and $WUE_{CP}$ were 0.016 and <0.001, respectively. Adequate seasonal water uptake by forage treatments influenced both biomass and crude protein water use efficiencies.

**Appendix 5a.** Forage dry matter (DM) yield and nutritional values for the first year of production (2021).

| Treatments | DMY (Mg ha$^{-1}$) | CP (%) | ADF (%) | NDF (%) | TDN (%) | NDFD 48hr | Ca (%) | P (%) | K (%) | Na (%) | Cl (%) | Mg (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M0 | 1.95 | 11.20 | 43.70 | 54.50 | 63.00 | 57.10 | 0.59 | 0.16 | 1.68 | 0.09 | 0.05 | 0.22 |
| M1 | 1.80 | 14.10 | 35.90 | 51.40 | 61.80 | 52.90 | 0.90 | 0.16 | 1.72 | 0.10 | 0.12 | 0.25 |
| M2 | 1.32 | 12.90 | 36.60 | 52.20 | 59.40 | 50.20 | 0.95 | 0.15 | 1.67 | 0.04 | 0.24 | 0.23 |
| M3 | 1.40 | 14.20 | 34.20 | 47.80 | 63.00 | 50.80 | 0.96 | 0.19 | 1.69 | 0.09 | 0.10 | 0.25 |
| M4 | 1.97 | 12.60 | 36.50 | 50.70 | 59.90 | 49.50 | 0.88 | 0.15 | 1.75 | 0.03 | 0.26 | 0.22 |
| M5 | 1.83 | 12.80 | 36.50 | 52.10 | 60.60 | 50.90 | 0.86 | 0.16 | 1.79 | 0.09 | 0.08 | 0.24 |
| M6 | 1.50 | 13.50 | 35.70 | 50.10 | 60.60 | 49.10 | 0.97 | 0.15 | 1.70 | 0.04 | 0.23 | 0.24 |
| M7 | 1.52 | 12.50 | 36.10 | 51.10 | 60.80 | 50.90 | 0.98 | 0.16 | 1.63 | 0.03 | 0.25 | 0.23 |
| M8 | 1.20 | 13.30 | 34.30 | 48.50 | 62.90 | 52.00 | 1.05 | 0.17 | 1.68 | 0.13 | 0.25 | 0.23 |
| M9 | 1.15 | 11.90 | 36.80 | 53.20 | 59.90 | 50.70 | 0.90 | 0.16 | 1.61 | 0.14 | 0.22 | 0.21 |
| M10 | 1.85 | 11.70 | 37.90 | 53.90 | 58.30 | 49.90 | 0.96 | 0.16 | 1.70 | 0.07 | 0.26 | 0.21 |
| M11 | 1.80 | 13.60 | 35.00 | 50.70 | 62.10 | 52.10 | 0.88 | 0.17 | 1.74 | 0.06 | 0.26 | 0.24 |
| M12 | 1.57 | 14.50 | 33.90 | 48.80 | 63.50 | 53.20 | 0.97 | 0.18 | 1.83 | 0.05 | 0.34 | 0.29 |
| M13 | 1.37 | 10.70 | 38.90 | 59.20 | 56.90 | 51.50 | 0.62 | 0.16 | 1.63 | 0.12 | 0.27 | 0.21 |
| M14 | 2.40 | 14.10 | 34.90 | 49.60 | 62.30 | 51.70 | 1.10 | 0.17 | 1.92 | 0.15 | 0.26 | 0.26 |
| M15 | 1.22 | 11.70 | 37.30 | 55.50 | 58.70 | 51.90 | 0.82 | 0.17 | 1.63 | 0.06 | 0.32 | 0.22 |
| M16 | 1.62 | 14.80 | 34.20 | 46.70 | 63.80 | 52.00 | 1.11 | 0.16 | 1.69 | 0.08 | 0.18 | 0.26 |
| M17 | 2.40 | 13.70 | 35.50 | 48.90 | 62.00 | 49.70 | 1.08 | 0.16 | 1.62 | 0.08 | 0.25 | 0.25 |
| M18 | 1.87 | 14.20 | 35.50 | 50.10 | 62.90 | 52.50 | 0.93 | 0.16 | 1.82 | 0.10 | 0.27 | 0.24 |
| M19 | 1.65 | 11.90 | 37.00 | 51.90 | 60.10 | 50.40 | 0.96 | 0.16 | 1.71 | 0.03 | 0.50 | 0.22 |
| M20 | 2.15 | 11.80 | 36.30 | 51.70 | 59.10 | 48.40 | 0.99 | 0.15 | 1.57 | 0.09 | 0.29 | 0.21 |
| M21 | 2.05 | 15.20 | 33.90 | 45.70 | 64.90 | 53.00 | 1.09 | 0.19 | 1.93 | 0.12 | 0.43 | 0.26 |
| M22 | 1.70 | 14.60 | 33.90 | 46.60 | 64.10 | 52.00 | 1.13 | 0.17 | 1.68 | 0.11 | 0.16 | 0.25 |
| M23 | 2.05 | 13.30 | 34.90 | 49.50 | 62.80 | 52.60 | 0.92 | 0.17 | 1.82 | 0.13 | 0.16 | 0.24 |
| OG | 1.10 | 10.90 | 37.30 | 56.30 | 55.30 | 46.60 | 0.90 | 0.17 | 1.51 | 0.10 | 0.37 | 0.20 |
| FMB | 0.77 | 11.50 | 39.00 | 58.00 | 53.90 | 48.00 | 0.69 | 0.14 | 1.76 | 0.02 | 0.31 | 0.18 |
| GPWG | 1.15 | 10.40 | 38.80 | 58.50 | 55.40 | 50.00 | 0.57 | 0.15 | 1.75 | 0.06 | 0.43 | 0.19 |
| KCG | 0.90 | 12.60 | 34.00 | 50.60 | 57.30 | 47.00 | 0.79 | 0.14 | 1.42 | 0.11 | 0.32 | 0.19 |
| TG | 1.27 | 13.10 | 35.20 | 51.90 | 56.60 | 48.30 | 1.07 | 0.14 | 1.42 | 0.05 | 0.28 | 0.19 |

**Appendix 5b.** Forage dry matter (DM) yield and nutritive values for second year of production (2022).

| Treatments | DMY (Mg ha$^{-1}$) | CP (%) | ADF (%) | NDF (%) | TDN (%) | NDFD-48 h | Ca (%) | P (%) | K (%) | Na (%) | Cl (%) | Mg (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M0 | 3.07 | 12.30 | 35.65 | 54.12 | 65.82 | 62.10 | 0.90 | 0.26 | 1.76 | 0.05 | 0.51 | 0.69 |
| M1 | 3.45 | 17.02 | 35.72 | 46.92 | 63.60 | 54.12 | 1.45 | 0.27 | 1.76 | 0.04 | 0.63 | 0.35 |
| M2 | 3.07 | 16.07 | 35.52 | 48.62 | 63.92 | 55.50 | 1.36 | 0.27 | 1.71 | 0.05 | 0.55 | 0.40 |
| M3 | 2.82 | 16.62 | 36.47 | 46.25 | 63.00 | 53.90 | 1.55 | 0.25 | 1.67 | 0.04 | 0.28 | 0.36 |
| M4 | 4.07 | 15.72 | 37.17 | 49.27 | 62.57 | 54.35 | 1.41 | 0.25 | 1.68 | 0.09 | 0.43 | 0.33 |
| M5 | 2.87 | 16.35 | 35.15 | 47.12 | 64.02 | 54.00 | 1.41 | 0.26 | 1.64 | 0.04 | 0.56 | 0.33 |
| M6 | 2.97 | 16.62 | 36.15 | 45.12 | 63.80 | 52.55 | 1.60 | 0.24 | 1.61 | 0.05 | 0.40 | 0.37 |
| M7 | 3.07 | 14.25 | 36.70 | 50.10 | 63.90 | 55.85 | 1.21 | 0.25 | 1.70 | 0.06 | 0.47 | 0.31 |
| M8 | 2.80 | 15.27 | 35.62 | 47.07 | 64.52 | 54.22 | 1.38 | 0.24 | 1.61 | 0.03 | 0.50 | 0.32 |
| M9 | 2.40 | 15.25 | 35.50 | 46.62 | 64.52 | 55.10 | 1.26 | 0.25 | 1.70 | 0.02 | 0.45 | 0.30 |
| M10 | 2.72 | 15.37 | 35.85 | 48.95 | 64.05 | 55.27 | 1.34 | 0.25 | 1.65 | 0.10 | 0.68 | 0.32 |
| M11 | 2.80 | 15.20 | 38.07 | 50.00 | 61.35 | 54.27 | 1.38 | 0.25 | 1.70 | 0.06 | 0.70 | 0.33 |
| M12 | 2.45 | 16.67 | 35.00 | 45.17 | 64.57 | 53.67 | 1.48 | 0.25 | 1.70 | 0.22 | 0.56 | 0.36 |
| M13 | 1.85 | 13.27 | 34.20 | 49.67 | 67.62 | 62.47 | 1.06 | 0.25 | 1.72 | 0.06 | 0.45 | 0.33 |
| M14 | 3.80 | 17.02 | 35.45 | 44.20 | 62.67 | 52.90 | 1.66 | 0.23 | 1.50 | 0.09 | 0.82 | 0.34 |
| M15 | 1.62 | 13.80 | 35.00 | 49.40 | 66.75 | 60.12 | 1.13 | 0.25 | 1.69 | 0.06 | 0.55 | 0.33 |
| M16 | 3.25 | 16.57 | 34.97 | 44.52 | 64.22 | 54.32 | 1.53 | 0.24 | 1.60 | 0.09 | 0.50 | 0.34 |
| M17 | 3.20 | 15.70 | 35.05 | 46.20 | 64.95 | 54.87 | 1.40 | 0.24 | 1.59 | 0.15 | 0.46 | 0.32 |
| M18 | 3.37 | 15.85 | 35.67 | 46.67 | 63.85 | 54.14 | 1.43 | 0.25 | 1.65 | 0.11 | 0.40 | 0.33 |
| M19 | 3.30 | 14.45 | 36.77 | 48.75 | 63.67 | 54.50 | 1.30 | 0.24 | 1.62 | 0.23 | 0.56 | 0.32 |
| M20 | 3.10 | 14.35 | 35.62 | 47.75 | 64.27 | 54.80 | 1.34 | 0.23 | 1.50 | 0.11 | 0.75 | 0.31 |
| M21 | 3.90 | 16.60 | 35.80 | 45.22 | 63.87 | 54.60 | 1.49 | 0.25 | 1.73 | 0.12 | 0.47 | 0.35 |
| M22 | 3.50 | 16.95 | 35.52 | 46.57 | 62.75 | 53.37 | 1.56 | 0.24 | 1.48 | 0.08 | 0.40 | 0.32 |
| M23 | 3.75 | 15.45 | 36.17 | 47.82 | 62.85 | 54.22 | 1.44 | 0.23 | 1.56 | 0.07 | 0.51 | 0.33 |
| OG | 2.92 | 12.70 | 36.82 | 52.27 | 67.17 | 62.60 | 1.12 | 0.23 | 1.49 | 0.21 | 0.65 | 0.29 |
| FMB | 4.77 | 10.77 | 38.97 | 57.67 | 64.87 | 62.37 | 1.43 | 0.22 | 1.67 | 0.13 | 0.70 | 0.23 |
| GPWG | 3.90 | 11.20 | 38.02 | 56.65 | 64.75 | 61.92 | 1.29 | 0.25 | 1.75 | 0.56 | 0.60 | 0.33 |
| KCG | 3.47 | 13.30 | 37.17 | 51.65 | 64.70 | 58.25 | 1.43 | 0.24 | 1.53 | 0.23 | 0.56 | 0.32 |
| TG | 2.32 | 13.52 | 36.37 | 50.92 | 67.00 | 62.40 | 1.41 | 0.25 | 1.73 | 0.08 | 0.75 | 0.30 |

**Appendix 5c.** Forage dry matter (DM) yield and nutritive values for the third year of production (2023).

| Treatments | DMY(Mg ha$^{-1}$) | CP (%) | NDF(%) | ADF (%) | TDN (%) | NDFD-48 h | Ca (%) | P (%) | K (%) | Na (%) | Cl (%) | Mg (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M0 | 1.95 | 11.70 | 55.50 | 35.10 | 66.40 | 63.40 | 0.82 | 0.24 | 2.03 | 0.03 | 0.45 | 0.33 |
| M1 | 3.68 | 16.80 | 47.60 | 34.40 | 63.70 | 54.10 | 1.50 | 0.27 | 2.04 | 0.02 | 0.41 | 0.37 |
| M2 | 4.08 | 15.90 | 48.70 | 35.70 | 63.10 | 53.50 | 1.53 | 0.25 | 1.89 | 0.03 | 0.48 | 0.31 |
| M3 | 3.50 | 18.00 | 45.60 | 33.70 | 63.70 | 52.60 | 1.72 | 0.26 | 1.90 | 0.02 | 0.47 | 0.32 |
| M4 | 4.35 | 14.80 | 53.10 | 38.40 | 61.20 | 52.70 | 1.50 | 0.23 | 2.14 | 0.02 | 0.43 | 0.30 |
| M5 | 4.33 | 16.20 | 48.70 | 35.70 | 61.90 | 51.40 | 1.66 | 0.25 | 1.84 | 0.02 | 0.45 | 0.32 |
| M6 | 4.93 | 17.20 | 49.20 | 36.30 | 61.40 | 51.10 | 1.71 | 0.26 | 1.80 | 0.02 | 0.38 | 0.33 |
| M7 | 2.60 | 14.60 | 51.60 | 36.70 | 63.10 | 55.30 | 1.33 | 0.24 | 1.89 | 0.03 | 0.33 | 0.36 |
| M8 | 2.23 | 16.30 | 47.80 | 35.40 | 62.00 | 50.60 | 1.61 | 0.25 | 1.69 | 0.02 | 0.36 | 0.33 |
| M9 | 2.43 | 15.80 | 47.90 | 34.70 | 63.20 | 52.80 | 1.47 | 0.25 | 1.88 | 0.02 | 0.46 | 0.34 |
| M10 | 2.90 | 15.60 | 49.20 | 35.80 | 62.10 | 51.80 | 1.49 | 0.24 | 1.78 | 0.02 | 0.37 | 0.33 |
| M11 | 3.83 | 16.70 | 47.30 | 35.10 | 63.10 | 52.10 | 1.62 | 0.25 | 1.95 | 0.02 | 0.30 | 0.34 |
| M12 | 3.08 | 16.20 | 46.60 | 34.70 | 64.70 | 55.10 | 1.58 | 0.26 | 1.83 | 0.03 | 0.30 | 0.32 |
| M13 | 1.68 | 12.80 | 53.80 | 35.30 | 66.30 | 62.50 | 0.91 | 0.25 | 1.84 | 0.03 | 0.30 | 0.33 |
| M14 | 4.70 | 17.80 | 47.10 | 34.40 | 62.40 | 51.40 | 1.78 | 0.25 | 1.77 | 0.02 | 0.43 | 0.32 |
| M15 | 1.80 | 12.50 | 53.70 | 35.20 | 65.90 | 61.30 | 0.95 | 0.25 | 1.83 | 0.02 | 0.39 | 0.31 |
| M16 | 3.10 | 17.50 | 46.50 | 35.20 | 63.50 | 51.90 | 1.76 | 0.25 | 1.82 | 0.02 | 0.36 | 0.35 |
| M17 | 3.23 | 15.80 | 46.20 | 34.30 | 64.60 | 54.00 | 1.51 | 0.25 | 1.79 | 0.02 | 0.42 | 0.32 |
| M18 | 2.53 | 16.30 | 47.10 | 34.80 | 64.10 | 54.30 | 1.51 | 0.25 | 1.78 | 0.03 | 0.30 | 0.33 |
| M19 | 3.23 | 14.60 | 48.90 | 34.90 | 63.60 | 53.90 | 1.36 | 0.25 | 1.97 | 0.02 | 0.33 | 0.35 |
| M20 | 2.90 | 15.00 | 50.60 | 36.40 | 61.90 | 52.10 | 1.53 | 0.23 | 1.87 | 0.03 | 0.36 | 0.40 |
| M22 | 4.25 | 17.10 | 47.70 | 34.50 | 62.90 | 53.00 | 1.57 | 0.26 | 1.92 | 0.02 | 0.37 | 0.36 |
| M22 | 3.58 | 16.60 | 46.80 | 35.60 | 63.50 | 53.00 | 1.64 | 0.25 | 1.74 | 0.02 | 0.41 | 0.33 |
| M23 | 4.43 | 17.50 | 47.90 | 34.20 | 63.50 | 54.40 | 1.59 | 0.27 | 1.72 | 0.01 | 0.46 | 0.33 |
| OG | 1.88 | 16.30 | 51.40 | 34.30 | 66.80 | 61.70 | 1.02 | 0.25 | 2.40 | 0.03 | 0.38 | 0.37 |
| FMB | 1.88 | 12.00 | 56.90 | 35.00 | 67.30 | 65.70 | 0.73 | 0.24 | 2.49 | 0.01 | 0.51 | 0.31 |
| GPWG | 1.63 | 12.50 | 60.00 | 35.30 | 66.40 | 66.50 | 1.30 | 0.26 | 2.73 | 0.02 | 0.40 | 0.32 |
| KCG | 1.45 | 14.70 | 53.10 | 31.30 | 68.10 | 65.70 | 0.78 | 0.27 | 2.51 | 0.02 | 0.39 | 0.30 |
| TG | 0.53 | 13.50 | 54.90 | 34.40 | 66.20 | 62.70 | 1.09 | 0.26 | 1.86 | 0.23 | 0.37 | 0.37 |