Alternatives to Timothy Grown with Alfalfa in Eastern Canada

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Abstract

Timothy (*Phleum pratense* L.) is the main forage grass species cultivated with alfalfa (*Medicago sativa* L.) in eastern Canada, yet its regrowth under dry and warm conditions is poor. Air temperature and water stress are predicted to increase in the near future, which could further reduce timothy's regrowth. We evaluated six alfalfa-grass binary mixtures at three contrasted sites in eastern Canada to find potential alternatives to the alfalfa-timothy mixture under current climatic conditions. Timothy, tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort.), meadow fescue (*Schedonorus pratensis* [Huds.] P. Beauv.), festulolium (× *Festulolium* Asch. & Graebn), perennial ryegrass (*Lolium perenne* L.), and meadow bromegrass (*Bromus biebersteinii* Roem. & Schult.) were evaluated with harvests either at the early bud or early flower stage of alfalfa. Dry matter yield, nutritive attributes, and the yield contribution of each species were determined. Alfalfa mixtures with festulolium (cv. Spring Green) and perennial ryegrass (cv. Remington) had inferior grass yield contributions due to winter damages, as well as inferior forage yield and estimated milk production per hectare; these cultivars are not currently viable alternatives to timothy in eastern Canada. In contrast, alfalfa-meadow fescue and alfalfa-meadow bromegrass mixtures produced comparable yields, nutritive value, and estimated milk production per hectare, and they are, therefore, possible alternatives to the alfalfa-timothy mixture. The alfalfa-tall fescue mixture also represents a possible alternative; its lower nutritive value was compensated by its slightly greater yield. Timothy, tall fescue, meadow fescue, and meadow bromegrass remained productive over the first three production years when cultivated in mixture with alfalfa.

Résumé

La fléole des prés (*Phleum pratense* L.) est la graminée principalement associée avec la luzerne (*Medicago sativa* L.) dans l'est du Canada, bien qu'elle ait un potentiel de repousse limité sous conditions de sécheresse. Une augmentation de la température et du stress hydrique en été sont prédits dans un futur proche, ce qui pourrait nuire à la repousse et persistance de la fléole des prés. Ce projet évaluait six associations binaires de graminées et de luzerne à trois sites contrastés dans l'est du Canada afin de trouver des alternatives au mélange luzerne-fléole des prés sous les conditions climatiques actuelles. La fléole des prés, la fétuque élevée (*Schedonorus arundinaceus* [Schreb.] Dumort.), la fétuque des prés (*Schedonorus pratensis* [Huds.] P. Beauv.), le festulolium (× *Festulolium* Asch. & Graebn), le ray-grass vivace (*Lolium perenne* L.) et le brome des prés (*Bromus biebersteinii* Roem. & Schult.) ont été évalués avec des récoltes au stade début boutons ou début floraison de la luzerne. Le rendement en matière sèche, des attributs de valeur nutritive et la contribution au rendement de chaque espèce semée ont été mesurés. Les mélanges de luzerne avec les cultivars évalués de festulolium (cv. Spring Green) et de ray-grass vivace (cv. Remington) ont eu des rendements totaux, une contribution au rendement de la graminée et des productions de lait estimées à l'hectare de fourrage inférieurs; ils ne sont présentement pas des alternatives envisageables dans l'est du Québec. Au contraire, les mélanges luzerne-fétuque des prés et luzerne-brome des prés ont produit des rendements, valeurs nutritives et productions de lait estimées à l'hectare comparables, et seraient donc des alternatives possibles au mélange luzerne-fléole des prés. Le mélange luzerne-fétuque élevée représente aussi une alternative possible au mélange luzerne-fléole des prés; sa valeur nutritive inférieure étaient

compensée par son rendement légèrement supérieur. La fléole des prés, la fétuque élevée, la fétuque des prés et le brome des prés sont demeurées productifs au cours des trois premières années de production lorsque cultivés en mélange avec la luzerne.

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List of Abbreviations

ADF	Acid detergent fibre
aNDF	Neutral detergent fiber assayed with a heat-stable amylase and sodium sulfite
CP	Crude protein
DM	Dry matter
GDD5	Growing degree-days calculated using a 5°C basis
IVTD	*in vitro* true digestibility
NDFd	*In vitro* neutral detergent fiber digestibility
NE_l	Net energy for lactation
NFC	Nonfiber carbohydrates
NSC	Non-structural carbohydrates
PCA	Principal component analysis
TDN	Total digestible nutrients
VNIRS	Visible and near-infrared reflectance spectroscopy

Preface and Contribution of Authors

This dissertation is ultimately based on a collaborative experiment between McGill University, Agriculture and Agri-Food Canada, and Université Laval. None of the text of the dissertation is taken directly from previously published articles; an article reporting the results from this thesis will be submitted for publication in a peer-reviewed journal. The protocol and experimental design presented in Chapter 3 (sections 3.1.1 and 3.1.2) were developed primarily by G. Bélanger in collaboration with P. Seguin, G. F. Tremblay, J. Lajeunesse, and É. Charbonneau. The execution of the methods presented in sections 3.1.1 and 3.1.2 were performed and supervised by P. Seguin and myself at Sainte-Anne-de-Bellevue; by G. Bélanger and G. F. Tremblay at Saint-Augustin-de-Desmaures; and by J. Lajeunesse at Normandin. The chemical analyses for the three sites (section 3.1.3) were completed under the guidance of G. F. Tremblay. The statistical analysis in section 3.1.4 are my original work, with suggestions from P. Seguin and G. F. Tremblay. Data interpretation in Chapter 4 (sections 4.1-4.3) was executed by myself, with contributions from all collaborators to this project, namely P. Seguin, G. F. Tremblay, G. Bélanger, J. Lajeunesse, and É. Charbonneau.

Chapter I Introduction

1.1 General Introduction

Alfalfa is the main perennial forage legume species grown worldwide and is an important forage crop in Canada, being grown on millions of hectares (Statistics Canada, 2017). In eastern Canada, alfalfa is mostly grown in mixtures with perennial forage grasses (CFIA, 2012). Growing alfalfa or other forage legume species in mixtures with grasses increases forage dry matter (DM) yield (Berdahl et al., 2001; Bélanger et al., 2014) and reduces weed invasion (Sanderson et al., 2012; Sturludóttir et al., 2013; Bélanger et al., 2014), without decreasing forage digestibility compared to alfalfa or forage legumes grown alone (Kunelius et al., 2006; Sturludóttir et al., 2013; Bélanger et al., 2014).

In eastern Canada, the main forage grass species associated with alfalfa is timothy (*Phleum pratense* L.) due to its tolerance to adverse winter conditions (Bélanger et al., 2006), but its regrowth under prolonged warm and dry summer conditions is poor (Bertrand et al., 2008; Cosgrove, 2009; Virkajärvi et al., 2012b). Climate change is predicted to result in warmer temperatures, more frequent drought stress events, and longer growing seasons (Qian et al., 2013). Summer air temperatures in Canada are expected to increase by 3.1°C in 2040-2069 relative to the current annual average temperature (Jing et al., 2013). Soil moisture deficit stress is also predicted to increase due to a decrease in summer precipitations (Qian et al., 2010; Jing et al., 2013) and an increase in evapotranspiration, especially in northernmost agricultural regions of Canada (Jing et al., 2013). The resulting warmer and drier summers could negatively affect the regrowth and nutritive value of timothy (Jing et al., 2013; Piva et al., 2013). In addition, a study looking at different climate change scenarios by Thivierge et al. (2016) has shown that annual yields of the alfalfa-timothy mixture could decrease under the predicted future climate of eastern Canada, especially in the currently warmer regions of southern Quebec and Ontario, due to greater water and temperature stresses.

Tall fescue, meadow fescue, festulolium, perennial ryegrass, and meadow bromegrass are five grass species known to have better regrowth potential and drought tolerance than timothy (Cosgrove, 2009). Based on their growth characteristics, they are expected to have a better regrowth under current and predicted summer conditions but they are less tolerant to adverse winter conditions (Cosgrove, 2009). The potential of those species for use in binary mixtures with alfalfa under current eastern Canadian climate conditions is not well known. The objective of this study was therefore to evaluate five grass species for use in binary mixtures, as alternatives to timothy in eastern Canada under current climatic conditions, with mixtures being harvested at two alfalfa developmental stages.

1.2 Objectives and Hypotheses of the Study

1.2.1 Broad Objective

To identify alternatives to timothy cultivated with alfalfa in order to maximize the productivity, nutritive value, and persistence of this type of forage mixture in the context of climate change. Ultimately, this project aims at contributing to the formulation of new forage recommendations for the province of Quebec.

1.2.2 Specific Objective

To evaluate five cool-season grass species (tall fescue, meadow fescue, perennial ryegrass, festulolium, and meadow bromegrass) harvested when alfalfa reaches two different stages of development, as possible alternatives to timothy for use in binary mixture with alfalfa, at three contrasted sites in QC, Canada (Sainte-Anne-de-Bellevue [2098 growing degree-days base 5°C, GDD5], Saint-Augustin-de-Desmaures [1712 GDD5], and Normandin [1359 GDD5]) under the actual climatic conditions. Comparisons between alfalfa-grass binary mixtures will be made in terms of:

a) Seasonal DM yield (total, seeded grass, others);
b) Regrowth;
c) Persistence;
d) Nutritive value.

1.2.3 Hypotheses

a. The six alfalfa-grass binary mixtures differ in seasonal DM yield, regrowth, persistence, or nutritive value.
b. The binary mixtures of alfalfa and at least one of the five alternative grasses perform as well as or better than the alfalfa-timothy mixture since the evaluated grasses have a greater drought tolerance and regrowth than timothy, and similar or superior establishment potential and persistence.

Chapter II Literature Review

2.1 Perennial Forage Crops

Perennial forage crops provide great advantages over annual crops. Indeed, perennial crops use soil water more efficiently than most annual crops (Porqueddu et al., 2005), require less pesticide and herbicide applications, have roots that protect the soil against erosion (Karlen et al., 2007) and leaching (Russelle, 2014), and reduce the expenses linked to soil preparation for new seeding. Perennial crops can be fed to animals under various forms: fresh forage, silage, hay, and pellets (Annicchiarico et al., 2015).

2.2 Introduction to Alfalfa

Alfalfa is the main perennial legume crop cultivated worldwide and the most important forage crop in Canada (CFIA, 2012), where it covers more than 3.7 million hectares (Statistics Canada, 2017). It is characterized by a long, persistent taproot that can reach six meters in depth under optimal conditions, as well as several lateral roots growing from the crown near the soil surface. Altogether, the root system of alfalfa improves soil structure where it establishes. Numerous stems grow from the crown as well, and they bear alternate leaves (Teuber and Brick, 1988; Barnes and Sheaffer, 1995). In spring, after winter dormancy, or following a harvest, buds develop from the crown or from axillary buds on the remaining portion of the stem. Vegetative growth further continues after alfalfa flowers. Moreover, by associating with a nodule-forming, nitrogen-fixing bacterium (*Sinorhizobium meliloti*), alfalfa roots contribute to soil nitrogen content.

Alfalfa can adapt to a wide range of conditions, although it will have a greater growth and persistence in non-acidic (pH > 6.1), well-drained, loam soils (CFIA, 2012; Ontario Ministry of Agriculture, Food and Rural Affairs [OMAFRA], 2016). It further provides a protein-rich and nutritive diet to ruminants by having a high protein concentration, intake potential, and digestibility (CFIA, 2012).

Under proper management, alfalfa has a high yield and can persist for 3-5 years in Quebec (Michaud and Allard, 2005). To reduce the risks of winterkill, producers should respect the critical fall harvest period for alfalfa; it should not be harvested in the 4-6 weeks preceding the first killing frost (about -3°C) in order for roots to accumulate carbohydrate reserves for winter survival and spring regrowth, and for plants to grow back to a height 20-25 cm in the following season (Goplen et al., 1987; Michaud and Allard, 2005). When alfalfa is cut in the first half of the critical fall harvest period, root reserves are depleted for regrowth, but do not have time to get replenished before the frost. When it is harvested right before the frost, alfalfa does not have time to use root reserves for regrowth, but the lack of new growth to retain a snow cover reduces root and crown insulation, and increases winterkill risks. Therefore, it seems worth losing a late-season harvest by respecting the critical fall harvest period in order to have better survival, regrowth, and yield in following seasons (Goplen et al., 1987).

2.3 Importance of Alfalfa-Grass Mixtures

In eastern Canada, alfalfa is mostly grown in mixture with perennial forage grasses (CFIA, 2012). Growing alfalfa or other forage legume species in mixtures with grasses increases forage dry matter (DM) yield (Berdahl et al., 2001; Bélanger et al., 2014) and reduces weed invasion (Sanderson et al., 2012; Sturludóttir et al., 2013; Bélanger et al., 2014), and this, without decreasing forage digestibility compared to alfalfa or forage legumes grown alone (Kunelius et al., 2006; Sturludóttir et al., 2013; Bélanger et al., 2014).

Alfalfa provides a high amount of proteins to ruminants, yet these proteins are degraded very rapidly in the rumen. Because there is not enough readily fermentable energy for microbial grow, a portion of the nitrogen contained in alfalfa amino acids is not used by the animals (Bélanger et al., 2014). This occurs because the amino acids assembled into proteins are deaminated to provide energy to microbes, and ammonia is released in the process (Kingston-Smith and Theodorou, 2000). Yet, a high concentration of non-structural carbohydrates (NSC) in crops improves the utilization of nitrogen by ruminants (Bélanger et al., 2014). Brito et al. (2008) have indeed shown that when alfalfa with a higher concentration of NSC is given to dairy cows, more fermentable energy in the form of fermentable organic matter is available to microbes. Ruminants can thus more effectively convert nitrogen-containing amino acids to microbial proteins.

Furthermore, alfalfa-grass mixtures have a comparable digestibility to alfalfa alone (Kunelius et al., 2006; Bélanger et al., 2014), and reduce the risk of bloating in ruminants, which occurs frequently when they are fed exclusively on legumes (Burggraaf et al., 2008). Such mixtures thus provide valuable alternatives to alfalfa monocultures as a food source for ruminants.

In addition to benefiting the health of ruminants, alfalfa-grass mixtures produce greater DM yield and require less nitrogen fertilization than monocultures. Various studies have shown that alfalfa-grass mixtures have a greater seasonal DM yield than either alfalfa or grass monocultures, and this greater DM yield persists for several consecutive years (e.g., Sleugh et al., 2000; Berdahl et al., 2001; Bélanger et al., 2014). Indeed, alfalfa fixes atmospheric nitrogen (N_2) and transfers some of this nitrogen to non-legumes. Nyfeler et al. (2009) have demonstrated that alfalfa-grass mixtures fertilized with 50 kg of N ha^{-1} yr^{-1} had similar DM yields than grass monocultures fertilized with nine times the amount of nitrogen (i.e., 450 kg of N ha^{-1}). Alfalfa-grass mixtures thus require considerably lower amounts of expensive nitrogen fertilizers than monocultures. Furthermore, because the grass in mixtures competes with alfalfa for the fixed nitrogen, alfalfa fixes greater levels of nitrogen when mixed with grasses than in monocultures (Nyfeler et al., 2011). Due to interactions between the grass and legume components, alfalfa-grass mixtures are therefore often more productive than monocultures.

Moreover, the increased species richness in alfalfa-grass mixtures over monocultures results in lower weed invasion. Wardle (2001) has reported two possible explanations for the reduced weed invasion in mixed forages: (1) stands containing high species diversity utilize complementary resources thus fewer resources remain for weeds to grow and invade; and (2) samples randomly taken from stands contain more of the most competitive species therefore have a relatively lower weed proportion. Various studies have demonstrated that when species richness increases in a field, weed invasion is significantly reduced (e.g., Tracy and Sanderson, 2004; Picasso et al., 2008; Finn et al., 2013). Indeed, in most alfalfa-grass binary mixtures, the number of weed is lower than in monocultures (Sanderson et al., 2012; Bélanger et al., 2014). Sanderson et al. (2012) further concluded that the proportion of both species in these binary mixtures is of lower importance than the species combination itself in reducing weed proportion. Therefore, although an increasing diversity in stands generally decreases weed invasion, carefully chosen grass companions in alfalfa-grass mixtures can maximize weed suppression.

2.4 Timothy as a Companion Grass

Timothy is the forage grass species most commonly grown with alfalfa in Quebec. Timothy is a late-maturing, highly palatable bunchgrass that easily establishes in new areas, and is especially adapted to cool and humid areas with precipitations above 900 mm a year (Tran and Lebas, 2015). It is considered a non-aggressive species when seeded in mixtures because of its limited tillering ability. Timothy thrives in clays, clay loams and loams with a variable drainage, and a slightly acidic pH of 5.5 to 7.0 (Ogle et al., 2011) and under temperatures of 18 to 22°C (Tran and Lebas, 2015). It further tolerates extreme winter conditions, including both cold temperatures and ice encasement, which is when roots and crown buds become trapped in ice; timothy is therefore tolerant to the Quebec winter climate. Yet, this shallow-rooted species has a limited regrowth potential over growing seasons (Cosgrove, 2009; Virkajärvi et al., 2012a), and a poor persistence under prolonged warm and dry summer conditions due to its long recovery periods after grazing or harvests (Cosgrove, 2009). Timothy is therefore susceptible to warm and dry climates.

2.5 Climate Change Trends

The Canadian climate is changing because of the increasing amount of greenhouse gas emissions (Solomon et al., 2007), resulting in warmer temperatures (Jing et al., 2013; Qian et al., 2013) and changes in annual precipitation patterns (Qian et al., 2010; Jing et al., 2013). Jing et al. (2013) have predicted that the summer air temperature in Canada will increase by 3.1°C by 2040-2069 relative to the current annual climate average. This implies that the number of growing degree days (°C day^{-1}) will increase, and the growing season will extend (Qian et al., 2013). In addition to changes in temperature, Jing et al. (2013) predicted that summer precipitation will decrease and evapotranspiration will increase, resulting in a greater soil moisture deficit stress, especially in northernmost regions of Canada. The resulting warmer, drier summers could negatively affect the summer regrowth and nutritive value of timothy (Jing et al., 2013; Piva et al., 2013). In addition, a study looking at different climate change scenarios by Thivierge et al. (2016) has shown that annual yields of the alfalfa-timothy forage mixture could decrease under the predicted climate of eastern Canada, especially in warmer regions, due to greater water and temperature stresses.

Simultaneously to these changes occurring over summers, the winter air temperature and precipitation patterns are also predicted to change. The winter temperature in Canada could increase by 4.0°C by 2040-2069, and winter precipitation in the form of rain could increase as well (Jing et al., 2013). These predicted changes could decrease winter survival of sensitive forages (Jing et al., 2013). Indeed, snow cover insulates crop roots and crown buds from sub-freezing temperatures, and this insulation is necessary for winter survival of forages (Bélanger et al., 2006). A minimum of 10 cm of snow cover is necessary to maintain the temperature around the crown of plants at 0°C (Leep et al., 2001). Yet, because of the predicted warmer winter temperatures predicted, this thin snow protection could melt. There might also be more rain falling, along with the melting of the snow cover, which would result in ice forming at the soil surface, and roots and crown buds becoming trapped in ice, a phenomenon called ice encasement

(Gudleifsson, 1993). Ice encasement reduces gas exchange between the soil and air; as microbes and plants consume the oxygen in the soil, it eventually switches to an anoxic environment. Then, anaerobic respiration by trapped plants results in the use of substrates necessary for plant regrowth and aerobic respiration, the reduction of carbon reserves, the release of CO_2, and the production of ethanol and lactic acid which are potentially toxic for plants (Andrews, 1996; Bertrand et al., 2001). In addition, when the winter temperature rises above 0°C, even for short periods of time, it can cause a loss of winter hardiness in plants (Sakai and Larcher, 1987; Eagles et al., 1997), making them more susceptible to the extreme winter conditions (Ouellet and Desjardins, 1981; Suzuki, 1981). For these reasons, the predicted changes in winter conditions due to climate change could result in greater winterkill risks; winter hardy forage species should be selected in Canada in the context of a changing climate.

Despite variabilities in temperature and precipitation currently observed in Canada, average seasonal and annual temperatures in the past 70 years illustrate a clear departure from the 1961-1990 climate normal (Figure 2.1; Government of Canada, 2016; 2017). In addition, there has been an increase in the duration of growing seasons (Barrow et al., 2004) and in the frequency of extreme warm days and nights and, conversely, a decrease in the frequency of extreme cold days and nights (Vincent and Mekis, 2006). An overall warming of the climate has therefore already started to take place across Canada, and current forage production practices must be adapted to respond to ongoing climatic changes.

2.6 Presentation of Alternative Grass Species

Other forage grasses are potentially better adapted to warm and dry conditions, and might be alternatives to timothy when grown in mixture with alfalfa in the current climatic context. This project explores five binary mixtures of alfalfa with one cool-season grass species as alternatives to the timothy-alfalfa mixture. The alternative grass species evaluated are tall fescue, meadow fescue, festulolium, perennial ryegrass, and meadow bromegrass.

2.6.1 Tall Fescue

Tall fescue grows in moist, cool soils with a pH between 5.5 and 7.0 (Henson, 2001), like timothy. Tall fescue has stiff, sharp blades that are thought to be relatively unpalatable for ruminants. When grown in certain regions, this grass can also sometimes reduce the performance of animals consuming it since it might contain an endophytic fungus producing toxic compounds. However, new cultivars have improved palatability or contain no toxic compounds (Cosgrove, 2009).

Tall fescue gets more easily established, and has better regrowth and persistence than timothy (Cosgrove, 2009; Table 2.1). Indeed, it germinates rapidly and has vigorous seedlings (Henson, 2001). A study directly comparing the regrowth of timothy and tall fescue in the United States (Cherney and Cherney, 2005) has shown that tall fescue produces a greater portion of its seasonal yield later in the season than timothy, suggesting a superior regrowth potential. Furthermore, tall fescue tolerates drought better than timothy, thus it might be more productive under warm and dry summer conditions (Cosgrove, 2009).

Tall fescue is known to survive harsh conditions and to be a long-lived species (Henson, 2001; OMAFRA, 2017). It further grows in many soil types, including those with imperfect drainage, and it retains nutritive quality late in the season (OMAFRA, 2017). However, it is more susceptible to harsh winter conditions than timothy thus it might suffer from a poorer winter survival (Cosgrove, 2009).

2.6.2 Meadow Fescue

Meadow fescue is deep-rooted and vigorous, and it rapidly recovers from harvests (Vinall, 1909). Although it prefers deep, fertile soils, it will grow in poorly-drained and low-fertility soils. It has a relatively good spring and fall growth. Similarly to tall fescue, meadow fescue retains its nutritive quality in the fall (OMAFRA, 2017).

Meadow fescue has the same establishment potential and drought tolerance as tall fescue (Table 2.1), but has a superior winter hardiness and persistence, yet an inferior regrowth potential. Meadow fescue is thus expected to have better winter survival than tall fescue, which might make it better adapted to the predicted climate in Canada. Also, meadow fescue has increased palatability and does not contain the toxins produced by endophytes sometimes present in tall fescue (Cosgrove, 2009).

2.6.3 Festulolium

Festulolium, is a hybrid grass between meadow fescue or tall fescue, and annual ryegrass or perennial ryegrass. As a result, festulolium has a greater persistence, resistance to cold, and drought tolerance than ryegrass as well as a superior forage quality, palatability, and digestibility than fescues. Under optimal conditions, festulolium is expected to have greater yields than both parental plants (DLF International Seeds, 2013).

Although festulolium cultivars have different characteristics since they are bred from different parental lines, general features are that festulolium gets established easily, has an excellent regrowth, and tolerates drought better than timothy, similarly to tall fescue, meadow fescue, and perennial ryegrass (Table 2.1). Its winter hardiness and persistence are similar to that of tall fescue. Festulolium's winter hardiness is inferior to that of meadow fescue and timothy, making it slightly more susceptible to winterkill (Cosgrove, 2009).

This project specifically uses the tetraploid festulolium cultivar 'Spring Green', which has been selected for enhanced winter hardiness. This cultivar is indeed adapted to the northcentral and northeastern United States, and southeastern Canada, where winters are generally mild, and the snow cover is reliable and sufficient to insulate forage crops. Due to the breeding from two existing festulolium populations, 'Spring green' festulolium has a complex pedigree made of four cultivars in various proportions: 18% 'Elmet', 15% 'Prior', 17% 'Tandem', and 50% 'Kemal'. While 'Elmet', 'Tandem', and 'Kemal' cultivars have Italian ryegrass, an annual grass, as their ryegrass parent, 'Prior' is bred from perennial ryegrass. The four cultivars have meadow fescue as their fescue parental line (Casler et al, 2001).

2.6.4 Perennial Ryegrass

Perennial ryegrass grows optimally under temperatures between 20 and 25°C. It tolerates soils with a wide pH range (i.e., 4.5 to 8.4), although it prefers dark and rich soils with a pH between 5.5 and 7.5 (Najda et al., 2004; Ogle et al., 2008). It is a highly palatable and high-quality grass that tolerates periodic flooding (Najda et al., 2004). Extended flooding along with dry and warm conditions negatively affect the yield of this species; it is expected to have lower yields in warm summer months (Najda et al., 2004; OMAFRA, 2017).

Perennial ryegrass has the same drought tolerance, establishment, and regrowth potential as tall fescue and meadow fescue (Table 2.1). It can therefore be harvested frequently. However, perennial ryegrass has poor persistence, like timothy, and poor winter hardiness (Cosgrove, 2009), making it more susceptible to winterkill than fescues and timothy (Najda et al., 2004). Therefore, in cold winter areas, it is sometimes treated as an annual crop that has to get re-established every year (Ogle et al., 2008).

2.6.5 *Meadow Bromegrass*

Meadow bromegrass is considered a palatable and long-lived species. It grows in many soils, but prefers deeper, fertile, and well-drained soils; it does not tolerate extended flooding. This grass species is known for its high palatability and is therefore used in forage production for pasture, hay, and haylage (Ogle et al., 2006).

Meadow bromegrass has inferior establishment and regrowth potentials to the five other alternatives, yet its regrowth remains superior to that of timothy (Table 2.1). However, meadow bromegrass grows earlier than other grasses in the spring, tolerates drought the best (Cosgrove, 2009), and retains a superior nutritive quality when it matures (OMAFRA, 2017). It has an excellent winter survival, similar to timothy and meadow fescue. Its persistence is also as good as that of meadow fescue, thus better than the other evaluated grasses, including timothy (Cosgrove, 2009).

2.7 Forage Development at Harvest

When evaluating binary forage mixtures, the developmental stage at harvest of each species should be considered. During photosynthesis, crops produce carbohydrates which are stored in their tissues in fall and throughout winter. In spring, these carbohydrates supply energy for plants to grow and develop, slowly at first, and then more rapidly. The stage at which plants grow the fastest is shortly before flowering. As plants mature further, their growth slows down as they spend energy producing flowers and seeds. They also synthesize excess carbohydrates via photosynthesis, which are stored in plant tissues and will provide energy to trigger regrowth after grazing or harvesting, or in the following spring. For this reason, the timing at which crops are harvested determines how quickly and efficiently they will regrow (Undersander et al., 2002).

However, the carbohydrate cycles are not the only important factor to consider when determining at which developmental stage plants should be harvested. Indeed, by harvesting later to maximize carbohydrate storage and yield, crop nutritive value and palatability decrease as more nutrients become incorporated into indigestible or partly digestible fibers (i.e., lignin, hemicellulose and cellulose; Figure 2.2; Bélanger et al., 2001; Yu et al., 2003). Therefore, the optimal time to harvest is when crops are tall enough to have a high DM yield, but prior to flowering and seed formation, when nutritive value decreases rapidly. Since not all six proposed grasses (i.e., timothy, tall fescue, meadow fescue, festulolium, perennial ryegrass, and meadow bromegrass), as well as alfalfa, grow and develop at the same rate (Undersander et al., 2002), the grass species in alfalfa-grass mixtures should be selected in order for all seeded components to have optimal yield and quality at harvest.

2.8 Assessment of Forage Nutritive Value

Forage quality is the potential of specific forages to produce a desired response in animal consuming them, and it is determined by a variety of factors. Indeed, forage quality encompasses multiple forage attributes, like palatability, intake potential, extent and rate of digestibility, nutrient concentration, and the presence of undesirable factors (Ball et al., 2001). Various laboratory analytic techniques can be performed on forages to estimate their overall nutritive value, including their concentrations in acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude protein (CP), as well as their *in vitro* true digestibility of dry matter (IVTD) and *in vitro* neutral detergent fiber digestibility (NDFd). Integrating attributes, such as the total digestible nutrient (TDN) concentration and estimated milk production per hectare, can further be calculated from measured nutritive value attributes.

2.8.1 Detergent Fiber Analysis

The NDF concentration estimates the total cell wall constituents of forages including hemicellulose, cellulose and lignin, plus bound minerals and proteins (Figure 2.3), by measuring forage residue that is insoluble in a neutral detergent solution. The NDF concentration can thus be used to predict the intake potential of a forage, which is an attribute of forage quality. A higher NDF concentration is associated with a lower intake (Ball et al., 2001; Tremblay et al., 2005). To determine the NDF concentration, a forage sample is boiled for 60 minutes in a neutral detergent solution. Plant compounds, except structural carbohydrates, become soluble in the solution; these compounds are called the neutral detergent solubles. The resulting insoluble fibers are the original cell wall constituents of the sample (Mertens, 2015).

The ADF concentration is used to determine the amount of cellulose and lignin, plus bound minerals and proteins that are present in plant cell walls (Figure 2.3). Precisely, it determines the least digestible portion of cell walls, which is insoluble in an acid detergent solution. As a result, it estimates the digestibility potential of a forage; the higher the ADF concentration, the lower the forage digestibility, resulting in a lower-quality forage (Ball et al., 2001; Tremblay et al., 2005). In order to determine the ADF concentration, the forage sample is boiled in an acid detergent solution for 60 minutes. In this acid solution, hemicellulose becomes soluble (Ball et al., 2001; Mertens, 2015).

Both the NDF and ADF concentrations increase with legume and grass maturity, resulting in lower-quality forages when forages are harvested at later developmental stages (Bélanger et al., 2001; Yu et al., 2003; Tremblay et al., 2005).

2.8.2 Crude Protein

The CP concentration is estimated by multiplying the total nitrogen (TN) concentration of a forage sample by a factor of 6.25 (Ball et al., 2001). The factor 6.25 is generally used because we consider that proteins from leaf and stem tissues usually contain 16% nitrogen (nitrogen : protein ratio of 1 : 6.25; University of Georgia, 2015). The total nitrogen of a sample comes from true proteins as well as non-protein nitrogen (Figure 2.3). Yet, once non-protein nitrogen reaches the rumen, microbes can convert it to microbial proteins, which are in turn used by ruminants (Ball et al., 2001).

In order to determine total nitrogen, all organic-bound nitrogen in a sample is first converted to ammonium (NH_4) using a solution of sulfuric acid (H_2SO_4), selenious acid

(H_2SeO_3), and hydrogen peroxide (H_2O_2). Samples are then digested at 380°C and NH_4 content is measured by spectrophotometry (Isaac and Johnson, 1976). The CP concentration decreases in both legumes and grasses as they mature (Tremblay et al., 2005).

2.8.3 In vitro *True Digestibility*

The IVTD consists of measuring the true digestibility of a forage in rumen fluid. The dry and ground forage samples are placed in filter bags and digestion jars filled with a buffer solution. These jars are inserted into an incubator. The temperature is maintained at 39°C, at which the buffer pH is 6.8; these conditions correspond to the rumen environment. Rumen fluid, which was maintained at 39°C from the moment of collection, is then added to the jars. Samples are digested for 24, 30, or 48 hours. The NDF concentration is further determined on the residue after incubation to remove remaining microbial debris and soluble plant fractions. This technique provides the amount of undigested fibers in the sample (Ball et al., 2001; University of Georgia, 2015). The IVTD (g kg^{-1} DM) is calculated as follows:

$$\mathrm{IVTD} = \left(1 - \frac{\text{postdigestion dry weight following aNDF weight}}{\text{predigestion dry weight}}\right) \times 1000$$

2.8.4 In vitro *Neutral Detergent Fiber Digestibility*

The NDFd corresponds to the digestible fraction by weight of the measured NDF concentration. The NDF is therefore allowed to get digested for 24, 30, or 48 hours in rumen fluid, and the remaining fibers are then weighed again. By subtraction, the digestible portion can be calculated. The NDFd is reported as a fraction of the NDF concentration of a forage sample (Ball *et al*., 2001; University of Georgia, 2015). With the both the NDFd and NDF concentration values, we can further estimate dry matter digestibility (Mertens, 2015). The NDFd (g kg^{-1} aNDF) is calculated as follows:

$$\mathrm{NDFd} = \left(1 - \frac{\text{postdigestion dry weight following aNDF wash}}{\text{predigestion dry weight of aNDF}}\right) \times 1000$$

2.8.5 Total Digestible Nutrient Concentration

The TDN concentration measures the total amount of energy available to ruminants through forage digestion. The TDN concentration of forages is determined via equations involving multiple components, precisely the CP, NDF, nonfiber carbohydrates (NFC), and crude fat (FA) concentrations, and their digestibilities (Milk2013; Undersander et al., 2013). The concentrations (% of DM) of truly digestible CP (tdCP), NDF (tdNDF), NFC (tdNFC), and FA(tdFA) are calculated as below (Weiss et al., 1992):

$$\mathrm{tdCP} = \mathrm{CP} \times \exp[-1.2 \times \left(\frac{\mathrm{ADICP}}{\mathrm{CP}}\right)]$$

$$\mathrm{tdNDF} = 0.75 \times (\mathrm{NDFn} - \mathrm{ADL}) \times [1 - \left(\frac{\mathrm{ADL}}{\mathrm{NDFn}}\right)^{0.667}]$$

$$\mathrm{tdNFC} = 0.98 \times (100 - [(\mathrm{NDF} - \mathrm{NDICP}) + \mathrm{CP} + \mathrm{EE} + \mathrm{Ash}]) \times \mathrm{PAF}$$

$$\mathrm{tdFA} = \mathrm{FA} \quad \text{Note: if EE} < 1, \text{then FA} = 0$$

In the above equations, NDICP = neutral detergent insoluble N × 6.25, PAF = processing adjustment factor, ADICP = acid detergent insoluble N × 6.25, ADL = acid detergent lignin, and NDFn = NDF – NDICP. The TDN concentration (% of DM) is further calculated as below (Weiss et al., 1992):

$$\mathrm{TDN} = \mathrm{tdNFC} + \mathrm{tdCP} + (\mathrm{tdFA} \times 2.25) + \mathrm{tdNDF} - 7$$

The resulting TDN concentration is an accurate and rapid integrating estimate (Ball et al., 2001; University of Georgia, 2015). The TDN concentration is negatively correlated to the maturity of the plant (Ball et al., 2001; Yu et al., 2003).

2.8.6 Estimated Milk Production per Hectare

The amount of milk produced per ton of forage can be calculated using a developed equation which incorporates the NDFd and the TDN concentration (Milk2013; Undersander et al., 2013). From the milk production per ton of forage, we can calculate the milk production per hectare of forage. This is done by multiplying the milk production per ton by the DM yield in tons per hectare. This value incorporates both the milk production potential of a forage as well as its yield in a single value; it is a powerful integrating variable.

2.9 Tables and Figures

Table 2.1. Comparison of characteristics from six cool-season grass species. Adapted from Cosgrove (2009).

Species	Establishment	Winter hardiness	Drought tolerance	Regrowth	Persistence
Timothy	Good	Excellent	Poor	Fair	Poor
Tall fescue	Excellent	Fair	Fair	Excellent	Fair
Meadow fescue	Excellent	Excellent	Fair	Excellent	Good
Perennial ryegrass	Excellent	Poor	Fair	Excellent	Poor
Festulolium	Excellent	Fair	Fair	Excellent	Fair
Meadow bromegrass	Good	Excellent	Good	Good	Good

Figure 2.1. Temperature departure from the 1961-1990 annual average annual (blue) and linear trend (red) in Canada (Government of Canada, 2017).

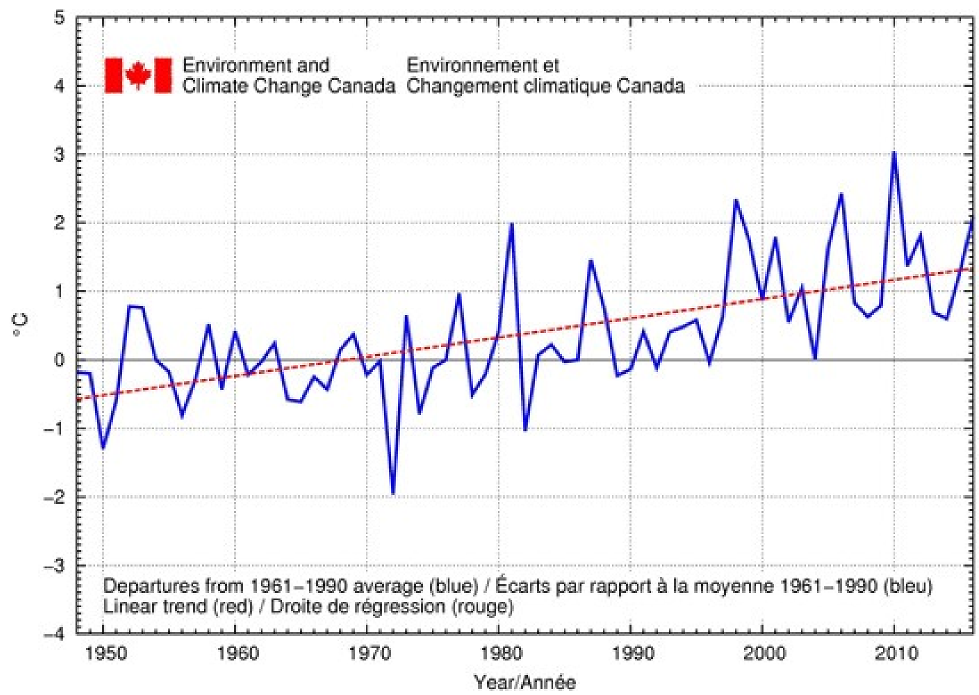


Figure 2.2. Effects of the developmental stage of plants on their yield and nutritive value (Pomerleau-Lacasse et al., 2017).

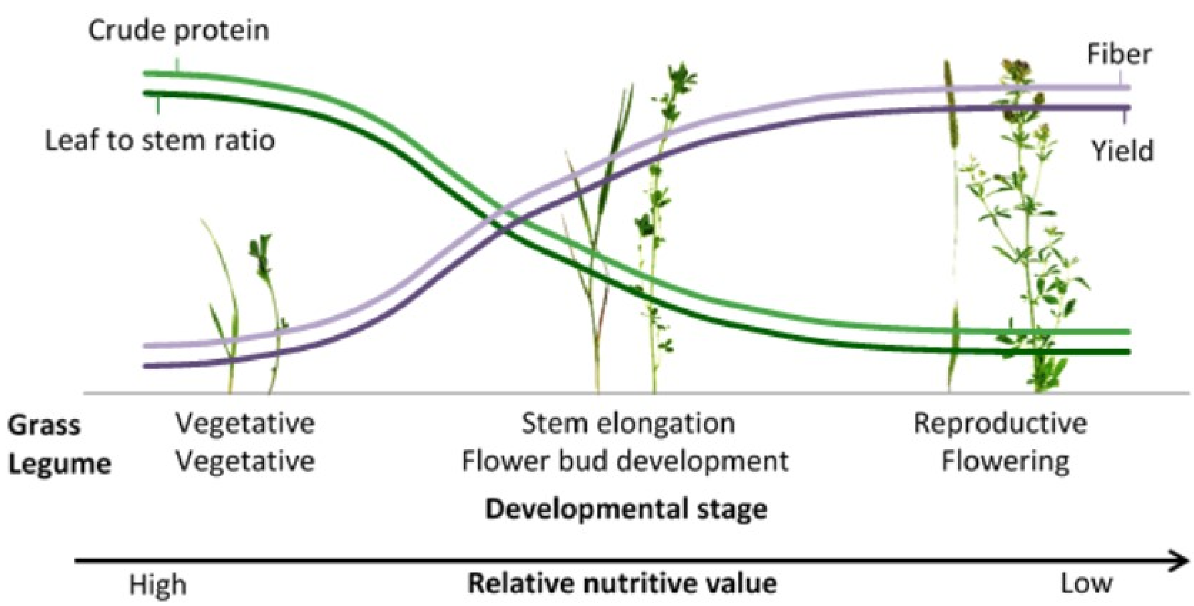


Figure 2.3. Forage analytic fractions and chemical constituents. Adapted from Ball et al. (2001).

Portion of forages

<table>
<tr><th colspan="4">Analytic fractions</th><th colspan="2">Chemical constituents</th></tr>
<tr><td colspan="4">Moisture</td><td colspan="2">Water</td></tr>
<tr><td rowspan="13">Dry matter</td><td colspan="3">Ash</td><td colspan="2">Minerals and sand</td></tr>
<tr><td rowspan="12">Organic matter</td><td rowspan="4">Neutral detergent fiber (NDF)</td><td rowspan="3">Acid detergent fiber (ADF)</td><td colspan="2">Cellulose</td></tr>
<tr><td colspan="2">Lignin</td></tr>
<tr><td colspan="2">Fiber-bound and heat-damaged nitrogen</td></tr>
<tr><td colspan="3">Hemicellulose</td></tr>
<tr><td rowspan="8">Neutral detergent solubles
(NDS)</td><td rowspan="4">Neutral detergent soluble carbohydrates (NDSC)</td><td colspan="2">Fructans
Glucans
Pectic acids</td></tr>
<tr><td rowspan="3">NSC</td><td>Sugars</td></tr>
<tr><td>Starches</td></tr>
<tr><td>Organic acids</td></tr>
<tr><td rowspan="3">Crude protein (CP)</td><td colspan="2">Non-protein nitrogen</td></tr>
<tr><td colspan="2">True proteins</td></tr>
<tr><td colspan="2">Fiber-bound and heat-damaged nitrogen</td></tr>
<tr><td>Ether extract</td><td colspan="2">Esterified fatty acids
Pigments
Waxes</td></tr>
</table>

Chapter III Methodology

3.1 Sites and Treatments Description

The experiment was conducted at three climatically-contrasted sites in QC, Canada: Sainte-Anne-de-Bellevue (45°25’ N; 73°55’ W, 2,100 cumulated growing degree days based on 5°C [GDD5]), Saint-Augustin-de-Desmaures (46°43’ N; 71°29’ W, 1,700 cumulated GDD5), and Normandin (48°50’ N; 72°33’ W, 1,350 cumulated GDD5). Plots were seeded on 21, 23, and 29 May 2014 at Saint-Augustin-de-Desmaures, Normandin, and Sainte-Anne-de-Bellevue, respectively, with binary mixtures of alfalfa and one of six cool-season grasses. Each mixture was harvested at two mean alfalfa developmental stages, specifically at the early bud (i.e., stage 3) and early flower (i.e., stage 5) (Fick and Muller, 1989; Pomerleau-Lacasse et al., 2017), resulting in a 2 × 6 factorial arrangement of 12 treatments. Treatments were assigned to a randomized complete block design with split-plot restriction and three replicates. The stage of development of alfalfa at harvest (hereafter called “Alfalfa stage”) was assigned to main-plots and the six alfalfa-grass mixtures (hereafter called “Mixture”) to sub-plots. Plot size varied at each site, but was a minimum of 1.3 × 5 m.

Alfalfa (cv. Calypso) was seeded at a rate of 9 kg ha^{-1} on a pure life seed basis (PLS) in mixture with one of six grass species: timothy (cv. AC alliance; 7 kg ha^{-1} PLS), tall fescue (cv. Carnival; 10 kg ha^{-1} PLS), meadow fescue (common seed; 10 kg ha^{-1} PLS), festulolium (cv. Spring Green; 10 kg ha^{-1} PLS), perennial ryegrass (cv. Remington; 12 kg ha^{-1} PLS), and meadow bromegrass (cv. Fleet; 12 kg ha^{-1} PLS). Cultivars and seeding rates were selected according to provincial recommendations (Centre de Référence en Agriculture en Agroalimentaire du Québec [CRAAQ], 2013) or, when cultivar recommendations were unavailable for the province of Québec (i.e., meadow fescue, perennial ryegrass, festulolium), according to recommendations for the province of Ontario (Ontario Forage Crop Committee, 2013). Species, for which no seeding rate recommendations were locally available (i.e., meadow fescue, perennial ryegrass, festulolium, meadow bromegrass), were compared to other species with a similar number of seeds per kg to determine their seeding rates. Seeding was done at a targeted depth of 5-10 mm using a Fabro 7-row seeder (Swift Current, SK, Canada) at Sainte-Anne-de-Bellevue and a Carter 5-row seeder (Brookston, IN) at Normandin, and by broadcast seeding immediately followed by a light raking for seed incorporation and rolling with a Brillion seeder at Saint-Augustin-de-Desmaures.

Phosphorus, K, and B fertilizers were applied before seeding and each year if needed based on soil tests and provincial recommendations (CRAAQ, 2010). In the establishment year (2014), plots were harvested once at Normandin and Saint-Augustin-de-Desmaures, and twice at Sainte-Anne-de-Bellevue, but no measurement was made. After the last harvest of the establishment year, plots were fertilized with 30 N ha^{-1} at Normandin and Sainte-Anne-de-Bellevue or 40 kg N ha^{-1} at Saint-Augustin-de-Desmaures. The insecticide Matador® 120EC (Syngenta Crop Protection Canada, Guelph, ON, Canada), with the active ingredient lambda-cyhalothrin (120 g L^{-1}), was applied twice during the third production year at Sainte-Anne-de-Bellevue to control a severe outbreak of potato leafhopper (*Empoasca fabae)*, which affected alfalfa plants. The pesticide was applied on 4 and 19 July 2017 using a field sprayer at the recommended rate of 83 mL ha^{-1}. No herbicide was applied; weeds were clipped once above the seeded species in the establishment year to avoid seed dispersal.

3.2 Harvests and Data Collection

Plots were harvested two to four times in the first, second, and third production years (i.e., 2015, 2016, and 2017); the number of harvests depending on the developmental stage of alfalfa at harvest and the site. Half of the plots were harvested every time alfalfa reached the early bud mean stage of development, and the other half when it reached the early flower mean stage. Harvest dates are provided in Table 3.1. The average developmental stage of the associated grass in each plot (Moore et al., 1991; Pomerleau-Lacasse et al., 2017) was also visually estimated at each harvest. Climatic conditions at each harvest date were retrieved from the closest Environmental Canada weather station, and the GDD5, from 1 April to the first harvest and between harvests, were calculated (Table 3.1).

Each plot was harvested at a height of 7 cm from the ground using a self-propelled flail forage harvester, and the fresh weight was recorded. A 500-g sample was collected, weighed, dried at 55°C in a forced-air oven for 72 h, and weighed again to determine the DM concentration. The dry samples were then ground to pass 1-mm sieve using a Wiley mill (Standard model 4, Arthur H. Thomas Co., Philadelphia, PA) for laboratory analyses.

In each plot, immediately after each harvest, the forage in a distinct 0.25-m^2 fixed quadrat was cut using clippers at a 7-cm height from the ground, and later separated by hand into alfalfa, seeded grass, and weed components; each component was then dried at 55°C for 72 h to determine their yield contributions and DM yields.

3.3 Laboratory Analyses

Ground forage samples were scanned by visible and near infrared reflectance spectroscopy (VNIRS) using a NIRsystem DS 2500 monochromator (Foss, Silver spring, MD). Laboratory analyses were performed on calibration (*n* = 92) and validation (*n* = 24) sets of samples selected by the WinISI IV (version 4.0) software (Infrasoft International, LLC, Silver Spring, MD) from each production year (2015, 2016, 2017) to determine the following nutritive attributes: neutral detergent fiber assayed with a heat-stable amylase and sodium sulfite (aNDF), acid detergent fiber (ADF), total nitrogen (TN), ash, crude fat, acid detergent lignin (ADL), neutral detergent insoluble nitrogen (NDIN), and acid detergent insoluble nitrogen (ADIN).

All laboratory analyses were performed as described in Simili da Silva et al. (2014). In summary, aNDF concentration was measured according to Mertens (2002) with the addition of a heat-stable α-amylase and sodium sulfite. The ADF concentration was determined following AOAC (1990). Both aNDF and ADF determinations were done using the ANKOM filter bag technique (ANKOM Technology, Macedon, NY; ANKOM Technology, 2017a; ANKOM Technology 2017b). The TN concentration was determined with an autoanalyser (QuikChem 8000 Lachat Zellweger Analytics Inc., Lachat Instruments, Milwaukee, WI) using the method 13-107-06-2-E (Lachat Instruments, 2011) following a mineralization in a mixture of sulfuric and selenious acids (Isaac and Johnson, 1976). The crude protein (CP) concentration was estimated from the TN concentration using the following formula: CP = TN × 6.25. The analytical DM and ash concentrations (Leco Corporation, 2009) were determined using a thermogravimetric analyser (model TGA701, Leco Corporation, St. Joseph, MI). Crude fat (ether extract) was determined using Ankom xt15 Extractor Technology Method (AOCS, 2003). The NDIN and ADIN concentrations were also chemically determined (Licitra et al., 1996) for the calibration and validation sets of samples.

The *in vitro* true digestibility of DM (IVTD) and *in vitro* NDF digestibility (NDFd) were determined using the method of Goering and Van Soest (1970). Rumen fluid was collected from a rumen-fistulated dairy cow, and samples were incubated for 48 h in buffered rumen fluid in an Ankom Daisy II incubator (Ankom Technology, Macedon, NY). The IVTD (g kg^{-1} DM) and NDFd (g kg^{-1} aNDF) were calculated as below:

$$\mathrm{IVTD} = \left(1 - \frac{\text{postdigestion dry weight following aNDF wash}}{\text{predigestion dry weight}}\right) \times 1000$$

$$\mathrm{NDFd} = \left(1 - \frac{\text{postdigestion dry weight following aNDF wash}}{\text{predigestion dry weight of aNDF}}\right) \times 1000$$

The ADF, aNDF, IVTD, and NDFd determinations were followed by ashing of the fiber residue to provide results corrected for the ash content of the fiber residue. From the chemically-determined ADF, aNDF, CP, ash, crude fat, and NDIN concentrations, along with the NDFd value, the total digestible nutrient (TDN) concentration and the estimated milk production per kg of forage were calculated for the calibration and validation sets of forage samples using the Excel spreadsheet Milk2013 (Undersander et al., 2013).

The nutritive attributes described above were then predicted for all forage samples using VNIRS (WinISI IV ver. 4.0 software, Infrasoft International, LLC, Silver Spring, MD). The VNIRS predictions were considered excellent when the ratio of prediction to deviation (RPD), which is calculated by dividing the standard deviation (SD) of the reference data used in the validation set by the standard error of prediction corrected for bias [SEP(C)]

[RPD = SD/ SEP(C)], was greater than 4, successful when the RPD was between 3 and 4, and moderately successful when it was between 2.25 and 3 (Nie et al., 2009). All attributes obtained excellent or successful predictions (RPD > 3.0) except NDFd (RPD = 2.86), for which the prediction was moderately successful.

3.4 Statistical Analysis

A two-way analysis of variance (ANOVA) was performed on DM yields and nutritive attributes using PROC mixed of the SAS software (version 9.4; SAS Institute, Inc., 2013). Data were analyzed by experimental site. Replicates at each site were considered a random effect and the production years, mixtures, and harvest stages were considered fixed effects. Because the treatment × year interactions were often significant, data were further analyzed for each production year. A multiple comparisons adjustment using the simulation method on the least squares means was included in our analysis to account for our large number of treatments. Differences between treatment means were considered significant at $P \leq 0.05$; only such significant effects are later discussed.

A principal component analysis (PCA) was also performed on the least squares means of the 12 mixture × harvest stage treatment combinations using the correlation matrix method of the SAS software (version 9.4; SAS Institute, Inc., 2013); equal weight was given to all variables. This PCA allowed us to characterize the relationship between DM yields, NDFd, IVTD, concentrations of CP, aNDF, and TDN, and the estimated milk production per hectare, and to observe how treatments related to these variables.

3.5 Tables and Figures

Table 3.1. Harvest dates and growing degree-days between harvests of binary alfalfa-grass mixtures for two harvest regimes based on the developmental stage of alfalfa, at three study sites in eastern Canada, and for three production years.

	2015		2016		2017	
	Early bud	**Early flower**	**Early bud**	**Early flower**	**Early bud**	**Early flower**
Normandin						
Harvest 1	June 9	June 22	June 7	June 27	June 13	June 22
GDD5†	255	372	236	428	281	374
Harvest 2	July 10	July 28	July 13	Aug. 1	July 12	July 26
GDD5	323	424	384	420	329	358
Harvest 3	Aug- 13	-	Aug. 11	-	Aug. 17	-
GDD5	418	-	358	-	369	-
Saint-Augustin-de-Desmaures						
Harvest 1	June 3	June 15	June 10	June 21	June 12	June 22
GDD5	336	461	356	487	341	476
Harvest 2	July 6	July 20	July 8	July 18	July 12	July 20
GDD5	376	420	363	397	391	379
Harvest 3	Aug. 7	Aug. 26	Aug. 2	Aug. 22	Aug. 7	Sept. 1
GDD5	434	561	385	532	341	503
Harvest 4	Sept. 3	-	Sept. 6	-	-	-
GDD5	411	-	527	-	-	-
Sainte-Anne-de-Bellevue						
Harvest 1	May 28	June 12	May 30	June 13	June 1	June 19
GDD5	375	535	313	468	340	559
Harvest 2	July 2	July 16	June 28	July 18	July 7	July 24
GDD5	419	480	391	554	450	530
Harvest 3	July 29	Aug. 24	July 26	Aug. 18	July 31	Sept. 6
GDD5	423	605	452	528	390	509
Harvest 4	Sept. 1	-	Aug. 22	-	Sept. 6	-
GDD5	517	-	456	-	419	-

†GDD5, cumulated growing degree-days calculated using a 5°C basis from 1 April to harvest 1, and between harvests.

Chapter IV Results and Discussion

The analyses of variance indicated that there were generally no significant interactions between the mixtures and alfalfa stages at harvest for seasonal DM yields and nutritive attributes (Tables 2-7), thus the emphasis in the presentation and discussion of the results is on the main effects of mixtures and alfalfa developmental stages at harvest.

4.1 Alfalfa-Grass Mixtures

4.1.1 Seasonal Dry Matter Yield

At Normandin, alfalfa-grass mixtures differed in total, alfalfa, and grass DM yields in at least one production year (Table 4.1). In the first production year (2015), mixtures with tall fescue, meadow fescue, or meadow bromegrass had similar total, alfalfa, and grass DM yields to the alfalfa-timothy mixture. Mixtures with festulolium and perennial ryegrass, however, had lower total DM yields due to lower grass DM yields. Indeed, the grass DM yields of the alfalfa-festulolium and alfalfa-perennial ryegrass mixtures ranged from 0.0 to 0.1 Mg ha^{-1} in all production years (2015-2017); festulolium and perennial ryegrass were nearly absent from their respective mixtures because of their poor survival in the first winter following seeding. In the second and third production years (2016, 2017), the alfalfa DM yields of the alfalfa-festulolium and alfalfa-perennial ryegrass mixtures were comparable to or greater than those of other mixtures, including the alfalfa-timothy mixture. Therefore, despite the absence or near absence of festulolium and perennial ryegrass, the numerically greater alfalfa DM yields in these two mixtures allowed for similar total DM yields of all mixtures in the second and third production years (2016, 2017). In addition, the grass DM yield of the alfalfa-timothy mixture in the third production year (0.3 Mg ha^{-1}) was less than that of the alfalfa-tall fescue mixture (1.5 Mg ha^{-1}), indicating a better persistence of tall fescue over the three production years, perhaps due to its greater competitive ability.

At Saint-Augustin-de-Desmaures, total DM yields of all six mixtures did not differ in the three production years (2015-2017; Table 4.2), although differences in alfalfa and grass DM yields were observed. On average, across the three production years, mixtures containing meadow fescue, festulolium, or perennial ryegrass had lower alfalfa DM yields and greater grass DM yields than mixtures with timothy or meadow bromegrass. Furthermore, meadow bromegrass generally had a lower grass DM yield than all grasses except timothy (2015-2017). At this site, the cumulative precipitation for March and April, when water infiltration in the frozen soil and evaporation are still limited, was well above 200 mm in the establishment year (2014), and in 2016 and 2017 (Table 4.4); the expected precipitation for this period based on the monthly 30-yr average (1971-2000) is 168 mm at Saint-Augustin-de-Desmaures (Government of Canada, 2018). This excess water early in the growing season may have negatively affected the emergence and spring regrowth of meadow bromegrass, a forage species reportedly sensitive to flooding (Ogle et al., 2006), resulting in lower grass seasonal DM yields of the alfalfa-meadow bromegrass mixture.

At Sainte-Anne-de-Bellevue, the mixtures differed in total, alfalfa, and grass DM yields averaged across the three production years (Table 4.3). Mixtures containing tall fescue, meadow fescue, or meadow bromegrass had similar total DM yields to the alfalfa-timothy mixture. Mixtures containing festulolium or perennial ryegrass, however, consistently had lower total DM yields (2015-2017) due to lower alfalfa DM yields in 2015, and to lower grass DM yields in 2016 and 2017. This result probably reflects a poor survival of festulolium and perennial ryegrass during the second winter after seeding.

Winter conditions at Normandin and Sainte-Anne-de-Bellevue may have contributed to the poor performance of festulolium and perennial ryegrass at these two sites, two species known to be prone to winterkill (Cosgrove, 2009). At Normandin, festulolium and perennial ryegrass had initially established properly according to visual observations in the fall of 2014. However, conditions in the first winter after seeding, which included a shallow snow cover (avg. Dec.-March: 5.8 cm) combined with particularly low sub-freezing temperatures for extended periods of time (Fig. 4.1), could have been lethal to festulolium and perennial ryegrass. The historical average snow cover at this site is 35 cm (1971-2000; Government of Canada, 2018). These two grasses were, indeed, already nearly absent from their respective mixtures in the first harvest of the first production year (2015; Fig. 4.4). At Sainte-Anne-de-Bellevue, these two grasses had similar DM yields to grasses of other mixtures in the first production year (2015), but their DM yields decreased from 2015 to 2016 (Table 4.3), and this trend was already observed at the first harvest of 2016 (Fig. 4.6). During the 2015-2016 winter, multiple periods of above zero temperatures (Fig. 4.3) possibly dehardened plants (Sakai and Larcher, 1987), reduced the insulating snow cover, and led to the formation of ice sheets above the plants. Indeed, the snow cover recorded at this site (Avg. Dec.-Apr.: 2.6 cm) was less than half what is usually expected for this region (10 cm; Government of Canada, 2018). This site also experienced prolonged periods with no snow on the ground during which the air temperature reached values below -15°C (Fig. 4.3). These combined detrimental climatic conditions could have resulted in the mortality of winter-sensitive forage species (Bélanger et al., 2006) and, consequently, in inferior DM yields of the alfalfa-festulolium and alfalfa-perennial ryegrass mixtures at Normandin (Table 4.1) and Sainte-Anne-de-Bellevue (Table 4.3).

Our results thus suggest the winter susceptibility of the cultivars of festulolium (cv. Spring Green) and perennial ryegrass (cv. Remington) when grown in eastern Canada. Perennial ryegrass generally has poor winter hardiness (Ogle et al., 2008; Cosgrove, 2009), yet different cultivars may vary in performance and competitivity at given locations (Jung et al., 1996). The cultivar Remington used in our experiment is a tetraploid ryegrass with improved winter hardiness and drought tolerance relative to traditional tetraploid perennial ryegrass cultivars. Festulolium, a hybrid grass between meadow fescue or tall fescue, and annual ryegrass or perennial ryegrass, has been developed for an enhanced nutritive value and winter hardiness relative to its parental species (DLF International Seeds, 2013). However, the specific genetic background of festulolium cultivars strongly affects their DM productivity, winter hardiness, and persistence, as demonstrated by Boberfeld and Banzhaf (2006). The festulolium cultivar Spring Green used in our experiment is a selection from hybrids of meadow fescue with perennial ryegrass or Italian ryegrass (Casler et al, 2001). The festulolium 'Spring Green', like the perennial ryegrass 'Remington', was developed for enhanced winter hardiness. However, it appeared to be insufficient for them to thrive at Normandin and Sainte-Anne-de-Bellevue. While other cultivars of festulolium and perennial ryegrass could have potentially performed better under current eastern Canadian climatic conditions, the two cultivars used in this project may be, in some years, risky alternatives to timothy when grown in mixture with alfalfa in eastern Canada.

At Normandin and Saint-Augustin-de-Desmaures, starting in the first production year (2015), the grass contribution to the DM yield of all mixtures was less than expected (Tables 4.1 and 4.2; Fig. 4.4 and 4.5). Indeed, the grass contribution averaged 18% (ranging between 0 and 32%), which is below optimal values typically expected for alfalfa-grass mixtures. An adequate grass contribution to alfalfa-based mixtures has been associated with a better persistence of all seeded species (Thomas, 1992), while reducing bloat risk, a potentially fatal condition that may occur when ruminants pasture a legume-rich diet (Mouriño et al., 2003; Burggraaf et al., 2008). Because all six grass species had poor DM yield contributions at these two sites, external factors may have contributed to giving an advantage to alfalfa over the grass in the mixtures. There was a prolonged period with limited rainfall in June and July of the establishment year (2014) at Normandin and Saint-Augustin-de-Desmaures. Rainfall was about 12 mm over a period of 23 days at Normandin, and 22 mm over 31 days at Saint-Augustin-de-Desmaures, while the 30-yr monthly rainfall averages (June-July) at these two sites are 95 and 115 mm, respectively. These dry periods in the establishment year could have negatively affected the growth and survival of forage grasses, and potentially have given alfalfa, a relatively drought-tolerant species, a competitive advantage over the seeded grasses in the mixtures, even in subsequent years.

At all three sites and three production years, no difference in total DM yields was observed among alfalfa-timothy, alfalfa-tall fescue, alfalfa-meadow fescue, and alfalfa-meadow bromegrass mixtures (Tables 4.1-4.3). Some differences in total DM yields between these four mixtures were reported by Bélanger et al. (2018) in an experiment where mixtures were frequently harvested when timothy reached about 33 cm in height to simulate a grazing scenario. The proportion of the seeded grasses in the last three production years of that 5-yr experiment averaged 55% while, in our experiment, grass DM yield contributions to the total DM yield averaged only 24% for the three production years (2015-2017). If the grass species had contributed to a greater proportion of total DM yields, differences in yields between these four mixtures might have been more pronounced.

Although one of the main concerns with timothy is its limited regrowth under warm and dry conditions after the first harvest (Jing et al., 2013; Piva et al., 2013), in the present experiment, tall fescue, meadow fescue, and meadow bromegrass had similar regrowth patterns to timothy over the season at the three sites and the two alfalfa developmental stages at harvest (Fig. 4.4-4.6). Festulolium and perennial ryegrass also had similar regrowth patterns to other grasses at Saint-Augustin-de-Desmaures. The regrowth of these two grasses was difficult to evaluate at Normandin and Sainte-Anne-de-Bellevue due to their relatively poor DM yields at the first harvest (Fig. 4.4 and 4.6). Therefore, despite the poor regrowth potential and drought susceptibility of timothy (Cosgrove, 2009), its regrowth does not seem to differ significantly from that of tall fescue, meadow fescue, and meadow bromegrass when grown in mixture with alfalfa under the current climatic conditions prevailing in eastern Canada.

Differences in DM yields of non-seeded species (i.e., weeds) were observed between the six alfalfa-grass mixtures at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Tables 4.2 and 4.3), but these differences were not consistent from one production year to the other, and between the two sites. More importantly, at the three sites, the DM yield contribution of non-seeded species ranged only between 2 and 6% of the total DM yield of mixtures, with an average of 3%. Our result are consistent with other experiments reporting that mixing legumes with one or many grasses reduces weed invasion compared to monocultures (e.g., Sanderson et al., 2012; Sturludóttir et al., 2013; Bélanger et al., 2014).

Although differences were observed between the alfalfa-grass mixtures for most nutritive attributes measured at all sites and in most years (Tables 4.4-4.6), none of the mixtures had a consistently superior or inferior nutritive value relative to other treatments. The CP, aNDF, and TDN concentrations generally appeared to be correlated to the grass and alfalfa DM yields; grasses typically have a greater fiber concentration, and lower CP and TDN concentrations, than legumes (Ball et al., 2001). Indeed, binary mixtures with greater alfalfa DM yields, or with lower grass DM yields, mostly had lower aNDF, and greater CP and TDN concentrations. Such trends were observed with the alfalfa-festulolium and alfalfa-perennial ryegrass mixtures at Normandin and Sainte-Anne-de-Bellevue (Tables 4.4 and 4.6), and for the alfalfa-timothy and alfalfa-meadow bromegrass mixtures at Saint-Augustin-de-Desmaures (Table 4.5). In addition, across sites and years, and regardless of the botanical compositions of mixtures, the alfalfa-tall fescue mixture was often associated with greater aNDF and lower CP and TDN concentrations than the alfalfa-timothy mixture (Tables 4.4-4.6), which is consistent with observations reported by Bélanger et al. (2018). The alfalfa-meadow fescue and alfalfa-meadow bromegrass mixtures generally had a nutritive value comparable to that of the alfalfa-timothy mixture, and this, at the three sites (Tables 4.4-4.6). The grass species mostly had negligible effects on the IVTD and NDFd (Tables 4.4-4.6), and on nutritive value variations between harvests of a growing season (data not presented) of the binary alfalfa-grass mixtures.

Previous field studies in eastern Canada (Pelletier et al., 2010) and Finland (Virkajärvi et al., 2012b) have reported that the NDF concentration of tall fescue tended to be lower than that of timothy when grown in pure stands, yet our study demonstrated that alfalfa-tall fescue mixtures generally had greater NDF concentrations than alfalfa-timothy. However, our alfalfa-tall fescue mixture had numerically or statistically superior grass seasonal DM yields that the alfalfa-timothy mixture for most production years, and at the three sites (Tables 4.1-4.3). In addition, in a study by Bélanger et al. (2018) reporting greater NDF concentrations for legume-grass binary mixtures with tall fescue than with timothy, tall fescue had a greater contribution to DM yield than timothy. Therefore, since grasses have greater NDF concentrations than legumes (Ball et al., 2001), the greater NDF concentrations observed with the alfalfa-tall fescue mixture could have been caused by the superior grass seasonal DM yields of this mixture relative to alfalfa-timothy, rather than by the grass species themselves.

Even though the alfalfa-tall fescue mixture tended to have a lower nutritive value, this grass was not more mature than other grass species when mixtures were harvested, for the two alfalfa developmental stages at harvest, and at the three sites (data not presented). When considering the nutritive value of binary alfalfa-grass mixtures at a given alfalfa developmental stage, both the grass species and the maturity of this grass have to be taken into account. Indeed, the nutritive value of forage grasses is negatively correlated to their maturity; as forages mature, fiber concentrations increase, and CP and TDN concentrations, as well as the NDFd and IVTD, decrease (Bélanger et al., 2001; Yu et al., 2003). Grass species also differ in the regrowth upon the first defoliation of the season; in this experiment, only timothy will produce reproductive stem after this first defoliation, while the five other grass species will have vegetative growth only. Yet, none of the six studied grasses was repeatedly more mature when mixtures were harvested, and tall fescue even tended to be slightly less mature than other grasses (data not presented). Therefore, the lower nutritive value of the alfalfa-tall fescue observed in our experiment cannot be explained by a difference in the relative maturity of these species, and difference between mixtures was most likely related to the grass contribution to the total forage DM yield.

The estimated milk production per hectare of forage crop for the six mixtures followed a similar pattern as their seasonal DM yields (Tables 4.4-4.6). In all years, at Normandin and Sainte-Anne-de-Bellevue, alfalfa-festulolium and alfalfa-perennial ryegrass mixtures were indeed associated with lower estimated milk production per hectare compared to the alfalfa-timothy mixture and these two mixtures also had lower seasonal total forage DM yields (Tables 4.1 and 4.3). At Saint-Augustin-de-Desmaures, for the three production years, the estimated milk production per hectare was similar for the six binary forage mixtures (Table 4.5). Johansen et al. (2017) presented similar milk yields when cows were fed pure timothy, tall fescue, meadow fescue, festulolium, or perennial ryegrass (average forage : concentrate ratio of 83 : 17), demonstrating that these five grass species, when given in similar quantities, have the potential to produce comparable milk yields. These results, along with the fact that minimal differences in term of forage nutritive value have been observed between mixtures in our experiment, hint that differences in estimated milk production per hectare between alfalfa-grass binary mixtures are due more to differences in DM yields rather than to the grass species in the mixtures.

4.1.2 Nutritive Attributes

Alfalfa-grass mixtures differed for most nutritive attributes at all sites and in most years (Tables 4.7-4.9). Averaged across the three production years, the TDN and CP concentrations of the alfalfa-tall fescue mixture were less and the aNDF concentration was greater than that of the alfalfa-timothy mixture at the three sites, while there was no or little difference in NDFd and IVTD. Similar results were reported by Bélanger et al. (2018) from a study of 18 legume-grass binary mixtures under grazing or frequent cutting in eastern Canada. Previous field studies in eastern Canada (Pelletier et al., 2010) and Finland (Virkajärvi et al., 2012a) reported that the NDF concentration of tall fescue tended to be lower than that of timothy when grown in pure stands, yet our study demonstrated that the alfalfa-tall fescue mixture generally had a greater aNDF concentration than the alfalfa-timothy mixture. The alfalfa-tall fescue mixture had a greater proportion of grasses than the alfalfa-timothy mixture at the three sites (16 vs. 11% at Normandin; 49 vs. 39% at Sainte-Anne-de-Bellevue; 29 vs. 13% at Normandin). Because grasses have greater NDF concentrations than legumes (Ball et al., 2001), the greater aNDF concentrations observed with the alfalfa-tall fescue mixture could to a large extent be explained by its greater grass proportion relative to the alfalfa-timothy mixture, rather than by the grass species themselves. When considering the nutritive value of alfalfa-grass mixtures at a given alfalfa developmental stage, the grass stage of development should be taken into account. Tall fescue was not more advanced in phenological development than the other grass species when mixtures were harvested at either of the two alfalfa developmental stages and at the three sites (data not presented). Therefore, the lower nutritive value of the alfalfa-tall fescue observed in our experiment cannot be explained by a difference in the relative stages of development of tall fescue and timothy, and differences between mixtures was most likely related to the grass contribution to the total forage DM yield.

The alfalfa-festulolium and alfalfa-perennial ryegrass mixtures tended to have a greater TDN concentration and a lower aNDF concentration than that of the alfalfa-timothy mixture at Normandin and Sainte-Anne-de-Bellevue, while there was no or little difference in NDFd and IVTD, and CP concentration for values averaged across the three production years. Because grasses typically have a greater fiber concentration and a lower TDN concentration than legumes (Ball et al., 2001), differences in aNDF and TDN concentration can be partly explained by the lower proportion of the grasses in the alfalfa-festulolium and alfalfa-perennial ryegrass mixtures than in the alfalfa-timothy mixture (0 and 0 vs. 11% at Normandin; 33 and 34 vs. 39% at Sainte-Anne-de-Bellevue).

Averaged across the three production years, the alfalfa-meadow bromegrass mixture had a greater aNDF concentration than the alfalfa-timothy mixture at the three sites, and a lower TDN concentration at Normandin and Sainte-Anne-de-Bellevue with no or little difference in CP concentration, and IVTD and NDFd. Bélanger et al. (2018) also observed a greater aNDF concentration and a lower TDN concentration of the alfalfa-meadow bromegrass mixture compared to the alfalfa-timothy mixture. The proportion of grasses in the alfalfa-meadow bromegrass mixture did not differ much from that in the alfalfa-timothy mixture (14 vs. 11% at Normandin; 41 vs. 39% at Sainte-Anne-de-Bellevue; 13 vs. 9% at Saint-Augustin-de-Desmaures). It appears, therefore, that meadow bromegrass might have had a lower TDN concentration and a greater aNDF concentration than timothy.

The estimated milk production per hectare was generally less with the alfalfa-festulolium and alfalfa-perennial ryegrass mixtures than with the alfalfa-timothy mixture at Normandin and Sainte-Anne-de-Bellevue (Tables 4.7-4.9). The lower total seasonal DM yields of the alfalfa-festulolium and alfalfa-perennial ryegrass mixtures had more impact on the estimated milk production per hectare than their TDN concentration. The mixtures with tall fescue, meadow bromegrass, or meadow fescue had similar estimated milk production per hectare to the alfalfa-timothy mixture.

4.2 Alfalfa Developmental Stage at Harvest

At the three sites and three production years, the total seasonal forage and alfalfa DM yields of the six alfalfa-grass mixtures were similar or superior when mixtures were harvested at the early flower stage of alfalfa than at the early bud stage (Tables 4.1-4.3), and this, even if an additional cut was taken when mixtures were harvested at the early bud stage (Normandin and Sainte-Anne-de-Bellevue: 2015-2017; Saint-Augustin-de-Desmaures: 2015 and 2016). The grass DM yields, however, did not differ between the two stages of development of alfalfa at harvest (Tables 4.1-4.3). Grass DM accumulation was, therefore, negligible between the early bud and early flower stages of alfalfa.

Alfalfa-grass mixtures harvested at the early flower stage of alfalfa had an improved persistence over mixtures harvested at the early bud stage (Tables 4.1-4.3). Total DM yields between the first and third production years decreased less or increased more with harvests at the early flower stage than at the early bud stage of alfalfa at Saint-Augustin-de-Desmaures (-19% vs. -43%) and Sainte-Anne-de-Bellevue (+19% vs. -14%), with little difference at Normandin (+17% vs +16%). Similarly, the alfalfa DM yields between the first and third production years increased more or decreased less with harvests at the early flower stage than at the early bud stage of alfalfa at Saint-Augustin-de-Desmaures (-21% vs. -51%), Sainte-Anne-de-Bellevue (+89% vs. +8%), and Normandin (+33% vs. 0%). Consequently, the difference in DM yields between mixtures harvested at the early bud and early flower stages of alfalfa generally became more pronounced in the third production year. The persistence of alfalfa-grass mixtures is, therefore, greater with harvests at the early flower stage than at the early bud stage of alfalfa. Waiting for alfalfa to flower allows carbohydrate reserves to replenish and reduces the occurrence of winterkill (Vignau-Loustau and Huyghe, 2008).

For the three production years and at the three sites, alfalfa-grass mixtures harvested at the early flower stage of alfalfa generally had a greater aNDF concentration, and lower CP and TDN concentrations, as well as lower NDFd and IVTD than mixtures harvested at the early bud stage of alfalfa (Tables 4.7-4.9). Although the year × stage interaction was sometimes significant, similar trends were observed in the three production years. Averaged across the three production years, the aNDF concentration in the alfalfa-grass mixtures was greater (+6 to +15%), and the CP (-7 to -13%) and TDN (-4 to -9%) concentrations along with NDFd (-10 to -12%) and IVTD (-5 to -7%) were lower with harvests taken at the early flower stage compared to the early bud stage of alfalfa. Decreases in nutritive value with advancing stages of development are well known for pure stands of alfalfa and timothy (Bélanger et al., 2001; Yu et al., 2003). In alfalfa-grass mixtures, however, this decrease in nutritive value could be affected or mitigated by the relative contribution of alfalfa and the grass species to DM yield and how this relative contribution changes with advancing stages of development. In our experiment, the relative contribution of alfalfa to total DM was greater when mixtures were harvested at the early flower stage than at the early bud stage of alfalfa (88% vs. 82% at Normandin; 80% vs. 69% at Saint-Augustin-de-Desmaures; 61% vs. 46% at Sainte-Anne-de-Bellevue; Tables 4.1-4.3). Because alfalfa has a lower fiber concentration, and greater CP and TDN concentrations, and IVTD than grasses (Yu et al., 2003), the greater presence of alfalfa when mixtures were harvested at the early flower stage of alfalfa mitigated the benefits of harvesting alfalfa-grass mixtures at an earlier stage of development in terms of nutritive value. It is known from previous experiments that the net energy available for lactation (NE_L) and the TDN concentration in a forage sample is negatively correlated to plant maturity (Yu et al., 2003) and to grass DM yield contributions in the mixtures (Yu et al., 2003; Johansen et al., 2018).

Alfalfa-grass mixtures harvested at the early flower stage of alfalfa generally were associated with a similar or greater estimated milk production per hectare than those harvested at the early bud stage, in the three production years and at the three sites (Tables 4.7-4.9). On average across the three production years, harvesting mixtures at the early flower stage of alfalfa increased the estimated milk production per hectare by 32% at Saint-Augustin-de-Desmaures and 51% at Sainte-Anne-de-Bellevue relative to mixtures harvested at the early bud stage of alfalfa; the total DM yields of mixtures harvested at the early flower stage were also numerically greater by 38% at Saint-Augustin-de-Desmaures and 65% at Sainte-Anne-de-Bellevue (Tables 4.2 and 4.3). At Normandin, the estimated milk production per hectare was comparable for the two alfalfa developmental stages at harvest (Table 4.7), along with their DM yields (Table 4.1).

Therefore, the greater estimated milk production per hectare of forage mixtures harvested at the early flower stage of alfalfa seems to be related primarily to greater total DM yields.

4.3 Relationship among Nutritive Attributes and Dry Matter Yield

Three PCAs, one for each site, were performed to characterize the relationship among seasonal values of DM yield and selected nutritive attributes averaged across the three production years (Fig. 4.7). The first two principal components (PC) accounted for 86% ($\lambda_1 + \lambda_2$) of the total variation at Normandin, 89% at Saint-Augustin-de-Desmaures, and 90% at Sainte-Anne-de-Bellevue. For each component, variables on the same side of the axis were positively correlated, and variables on opposite sides were negatively correlated. At the three sites, the first component was positively correlated to the CP and TDN concentrations, IVTD, and NDFd of the mixtures and the DM yield of non-seeded species. On the negative side, the first component was mostly defined by the total, alfalfa, and grass DM yields, and by the aNDF concentration (Fig. 4.7). This first component was also defined on the negative side by the estimated milk production per hectare at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Fig. 4.7b and 4.7c). The first component at the three sites was primarily driven by the two alfalfa developmental stages at harvest (Fig. 4.7). Our results suggest that forage DM yield and nutritive attributes, and their relationship is more affected by the stage of development at harvest than by the alfalfa-grass mixture.

Harvesting alfalfa-grass mixture at the early flower stage of alfalfa, as compared to the early bud stage, resulted in greater total, alfalfa, and grass DM yields, and estimated milk production per hectare at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Fig. 4.7b and 4.7c; Tables 4.2 and 4.3), while, at Normandin, harvesting at the early flower stage had negligible effects on total and grass DM yields (Fig. 4.7a; Table 4.1), and resulted in lower estimated milk production per hectare (Fig. 4.7a), although not significantly (Table 4.7). At all sites, harvesting the mixtures at the alfalfa early flower stage also resulted in lower forage nutritive value with higher aNDF concentration, and lower CP and TDN concentrations, IVTD, and NDFd than harvesting at the early bud stage of alfalfa (Fig. 4.7; Tables 4.7-4.9). The first component of the PCA, therefore, confirms that harvesting alfalfa-grass mixtures at the early flower stage of alfalfa results in similar or greater DM yields and estimated milk production per hectare, despite a lower nutritive value, than harvesting at the early bud stage of alfalfa (Yu et al., 2003).

The second component of the PCAs was primarily driven by the six alfalfa-grass mixtures, but with different patterns at the three sites (Fig. 4.7). At Normandin, mixtures with festulolium and perennial ryegrass were associated with low grass DM yield, and opposed to the mixtures with other grasses, which had high grass DM yields (Fig. 4.7a). At Saint-Augustin-de-Desmaures, mixtures with meadow bromegrass and timothy were associated with low grass DM yields, and opposed to the mixtures with most other grasses, which had high grass DM yields (Fig. 4.7b). At Sainte-Anne-de-Bellevue, the mixture with tall fescue was associated with a high grass DM yield, and opposed the mixture with festulolium (Fig. 4.7c). This second component confirms the generally poor performance of festulolium and perennial ryegrass at Normandin and Sainte-Anne-de-Bellevue (Fig. 4.7a and 4.7c; Tables 4.1 and 4.3), and a performance of the mixtures with tall fescue, meadow fescue, and meadow bromegrass similar to that of the alfalfa-timothy mixture.

In the first component of the PCAs at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Fig. 4.7b and 4.7c), and in the second component of the PCA at Normandin (Fig. 4.7a), the total DM yield was positively correlated with the grass DM yield, aNDF concentration, and estimated milk production per hectare, while being negatively correlated with the TDN and CP concentrations, the NDFd, and the DM yield from non-seeded species (Fig. 4.7). These correlations confirm that the estimated milk production per hectare associated with our twelve treatments was mostly driven by the yield of alfalfa-grass mixtures rather than their nutritive value. Our results (Fig. 4.7) also confirm those of other studies (e.g., Yu et al., 2003) showing that, regardless of the grass species in the alfalfa-grass mixtures, greater grass DM yields are associated with lower CP and TDN concentrations, but a greater aNDF concentration.

As expected, grass DM yields and alfalfa DM yields were opposed on the second component, a component mostly driven by the alfalfa-grass mixtures (Fig. 4.7). Mixtures with low grass DM yields (e.g. festulolium and perennial ryegrass at Normandin; meadow bromegrass at Saint-Augustin-de-Desmaures) tended to have greater alfalfa DM yields than those with high grass DM yields because of compensation between alfalfa and the seeded grass. The second component also indicates that total DM yield was positively correlated to the alfalfa DM yield at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Fig. 4.7b and 4.7c), but negatively correlated at Normandin (Fig. 4.7a). These correlations demonstrate that greater total DM yields of the alfalfa-grass mixtures were due to greater alfalfa yields at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue. At Normandin, however, all mixtures had similar alfalfa DM yields, yet the alfalfa-festulolium and alfalfa-perennial ryegrass had lower total and grass DM yields

(Table 4.1), resulting in the alfalfa DM yield being negatively correlated to the total and grass DM yields at this site (Fig 4.7a).

4.4 Tables and Figures

Table 4.1. Seasonal forage dry matter yield and yield of individual components (alfalfa, seeded grass, others) for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Normandin (QC) along with the probabilities of fixed effects and their interactions.

		Total				Alfalfa				Seeded Grass				Others			
		2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage		Mg ha^{-1}															
Early bud		5.4	5.2	6.2	5.6	4.6	4.6	4.6	4.6	0.6	0.5	1.0	0.7	0.1	0.2	0.5	0.3
Early flower		4.9	5.7	5.7	5.4	4.0	5.2	5.3	4.8	0.8	0.5	0.2	0.5	0.1	0.0	0.2	0.1
	S.E.M.	0.3	0.1	0.2	0.1	0.5	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.0	0.0	0.1	0.0
Mixture†																	
Alf + Tim		5.6 a‡	5.6	5.7	5.6 Ab	4.4	5.0	5.1 abc	4.8	1.1 ab	0.5 ab	0.3 bc	0.6 b	0.1	0.1	0.4	0.2
Alf + TF		5.2 ab	5.7	6.2	5.7 Ab	4.7	4.7	4.5 bc	4.6	0.4 bc	0.9 a	1.5 a	0.9 ab	0.1	0.1	0.2	0.1
Alf + MF		5.8 a	5.4	6.0	5.7 A	4.5	4.5	4.1 c	4.4	1.3 ab	0.9 a	1.4 ab	1.2 a	0.0	0.1	0.4	0.2
Alf + Fest		4.0 c	5.3	5.7	5.0 C	3.8	5.1	5.2 abc	4.7	0.0 c	0.0 b	0.1 c	0.0 c	0.2	0.2	0.5	0.3
Alf + Rye		4.3 bc	5.4	6.1	5.3 bc	4.2	5.3	5.6 a	5.0	0.1 c	0.0 b	0.0 c	0.0 c	0.1	0.1	0.5	0.2
Alf + Bro		5.9 a	5.4	6.1	5.8 a	4.2	4.9	5.4 ab	4.8	1.6 a	0.4 ab	0.5 abc	0.8 ab	0.1	0.1	0.2	0.1
	S.E.M.	0.3	0.2	0.2	0.2	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.1	0.0	0.1	0.1
ANOVA		**P value**															
Year		-	-	-	***	-	-	-	*	-	-	-	ns§	-	-	-	***
Stage		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mixture		***	ns	ns	***	ns	ns	**	ns	***	***	***	***	ns	ns	ns	ns
Year×stage		-	-	-	***	-	-	-	*	-	-	-	***	-	-	-	***
Year×mixture		-	-	-	***	-	-	-	**	-	-	-	***	-	-	-	ns
Stage×mixture		ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	*	ns	ns	ns
Year×stage×mixture		-	-	-	ns	-	-	-	*	-	-	-	ns	-	-	-	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

‡ Within columns, and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).

§ ns, nonsignificant.

Table 4.2. Seasonal forage dry matter yield and yield of individual components (alfalfa, seeded grass, others) for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Saint-Augustin-de-Desmaures (QC) along with the probabilities of fixed effects and their interactions.

	Total				Alfalfa				Seeded Grass				Others			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage	Mg ha^{-1}															
Early bud	10.2	8.3	5.8b	8.1	7.4	5.8b	3.6	5.6b	2.7	2.0	1.7	2.1	0.1a	0.5	0.5	0.4
Early flower	12.2	11.4	9.8a	11.1	9.9	9.1a	7.8	8.9a	2.3	2.1	1.8	2.1	0.0b	0.2	0.3	0.2
S.E.M.	1.6	1.3	1.1	1.3	1.2	1.2	0.8	1.0	0.4	0.3	0.4	0.3	0.0	0.1	0.1	0.1
Mixture†																
Alf + Tim	11.2	10.2	7.6	9.7	9.7 a‡	8.0 ab	5.8 ab	7.9 ab	1.3 c	1.5 bc	1.1 ab	1.3 b	0.2 a	0.6 ab	0.7 ab	0.5 a
Alf + TF	11.8	10.1	8.3	10.1	8.9 abc	6.5 b	5.6 ab	7.0 bc	2.8 ab	3.3 a	2.5 a	2.9 a	0.0 b	0.2 bc	0.2 ab	0.2 b
Alf + MF	10.6	9.8	7.8	9.4	8.1 bc	7.2 ab	5.4 ab	6.9 c	2.4 abc	2.3 ab	2.1 a	2.3 a	0.1 b	0.3 abc	0.3 ab	0.2 b
Alf + Fest	11.0	9.3	7.8	9.4	7.6 c	7.0 b	5.6 ab	6.7 c	3.4 a	2.1 abc	2.0 a	2.5 a	0.0 b	0.2 c	0.3 ab	0.2 b
Alf + Rye	11.4	9.7	7.6	9.6	8.1 bc	7.4 ab	5.2 b	6.9 c	3.3 a	2.1 bc	2.3 a	2.6 a	0.0 b	0.1 c	0.2 b	0.1 b
Alf + Bro	11.2	10.0	7.8	9.6	9.4 ab	8.6 a	6.6 a	8.2 a	1.6 bc	0.8 c	0.4 b	0.9 b	0.2 a	0.6 a	0.7 a	0.5 a
S.E.M.	1.5	1.3	1.0	1.2	1.1	1.2	0.6	0.9	0.5	0.3	0.5	0.3	0.0	0.1	0.2	0.1
ANOVA	P values															
Year	-	-	-	***	-	-	-	***	-	-	-	***	-	-	-	***
Stage	ns§	ns	*	ns	ns	*	ns	**	ns	ns	ns	ns	*	ns	ns	ns
Mixture	ns	ns	ns	ns	***	***	*	***	***	***	**	***	***	***	*	***
Year×stage	-	-	-	***	-	-	-	***	-	-	-	ns	-	-	-	ns
Year×mixture	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	ns
Stage×mixture	ns	ns	**	ns	ns	***	*	ns	ns	ns	ns	ns	*	ns	ns	ns
Year×stage×mixture	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	ns

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.
‡ Within columns, and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).
§ ns, nonsignificant.

Table 4.3. Seasonal forage dry matter yield and yield of individual components (alfalfa, seeded grass, others) for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Sainte-Anne-de-Bellevue (QC) along with the probabilities of fixed effects and their interactions.

	Total				Alfalfa				Grass				Other			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage	Mg ha^{-1}															
Early bud	6.2	3.3	5.4	5.0b	2.4a	2.1	2.6b	2.3a	3.5	1.2	2.4	2.4	0.3	0.0	0.4	0.3
Early flower	8.4	6.2	10.0	8.2a	3.7b	4.3	7.0a	5.0b	4.3	1.9	2.8	3.0	0.4	0.0	0.2	0.2
S.E.M.	0.9	0.6	0.6	0.7	0.6	0.6	0.4	0.5	0.4	0.2	0.2	0.2	0.0	0.0	0.0	0.0
Mixture†																
Alf + Tim	8.1 a‡	5.2 a	8.4 a	7.2 a	4.0 a	3.7	4.8	4.2 a	3.7 ab	1.5 bc	3.3 ab	2.8 b	0.4 abc	0.0	0.3 bc	0.2 abc
Alf + TF	8.2 a	5.4 a	8.8 a	7.5 a	3.3 a	2.9	4.5	3.6 ab	4.5 a	2.5 a	4.2 a	3.7 a	0.4 abc	0.0	0.1 c	0.2 c
Alf + MF	7.6 a	5.4 a	7.6 ab	6.9 a	3.2 ab	3.3	4.6	3.7 ab	3.9 ab	2.2 ab	2.7 bc	2.9 b	0.5 ab	0.0	0.3 b	0.3 ab
Alf + Fest	6.1 b	3.7 b	6.4 b	5.4 b	1.9 c	3.0	5.0	3.3 b	3.9 ab	0.7 c	0.7 d	1.8 c	0.3 bc	0.1	0.7 a	0.3 a
Alf + Rye	5.8 b	3.4 b	6.7 b	5.3 b	2.2 bc	2.8	5.0	3.3 b	3.4 b	0.6 c	1.4 cd	1.8 c	0.2 c	0.0	0.3 bc	0.2 bc
Alf + Bro	8.2 a	5.5 a	8.3 a	7.3 a	3.7 a	3.6	4.9	4.0 ab	4.0 ab	1.8 ab	3.2 ab	3.0 b	0.5 a	0.0	0.3 bc	0.3 ab
S.E.M.	0.9	0.6	0.6	0.7	0.7	0.6	0.5	0.5	0.3	0.2	0.3	0.2	0.1	0.0	0.1	0.0
ANOVA	**P values**															
Year	-	-	-	***	-	-	-	***	-	-	-	***	-	-	-	***
Stage	ns§	ns	ns	*	*	ns	**	**	ns	ns	ns	ns	ns	ns	ns	ns
Mixture	***	***	***	***	***	ns	ns	**	*	***	***	***	**	ns	***	***
Year×stage	-	-	-	***	-	-	-	***	-	-	-	ns	-	-	-	**
Year×mixture	-	-	-	ns	-	-	-	*	-	-	-	***	-	-	-	***
Stage×mixture	ns	ns	ns	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year×stage×mixture	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	ns

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.
‡ Within columns and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).
§ ns, nonsignificant.

Table 4.4. Monthly precipitations (mm) from 2014 to 2017 along with the 30-year average (1971–2000) at Normandin, QC, Canada.

	2014	2015	2016	2017	Average
	mm				
January	83.3	7.6	49.2	107.0	64.7
February	21.9	58.2	108.0	70.3	48.2
March	56.2	22.3	104.8	62.6	55.5
April	35.2	130.0	77.9	113.5	57.5
May	67.1	50.5	81.0	122.6	84.6
June	78.1	62.0	134.9	136.6	77.9
July	101.1	145.8	125.3	134.5	108.1
August	143.4	101.2	94.1	115.1	94.2
September	85.3	69.0	130.4	90.5	87.2
October	112.5	96.2	190.3	88.8	65.5
November	20.0	60.6	71.6	45.8	60.9
December	73.5	81.7	114.8	28.2	66.4

Table 4.5. Monthly precipitations (mm) from 2014 to 2017 along with the 30-year average (1971–2000) at Saint-Augustin-de-Desmaures, QC, Canada.

	2014	2015	2016	2017	Average
	mm				
January	79.6	92.4	21.3	103.8	94.4
February	76.4	21.1	136.9	81.7	86.5
March	81.4	44.9	178.8	68.8	92.8
April	131.1	118.0	58.7	144.6	74.7
May	75.5	131.0	75.4	118.6	108.2
June	85.0	128.1	103.7	95.7	112.0
July	115.2	127.1	106.5	63.2	119.5
August	141.8	123.4	107.4	29.3	111.0
September	105.0	102.0	88.2	64.8	129.0
October	127.6	176.0	190.8	149.1	92.8
November	32.3	91.7	95.2	141.4	94.3
December	92.9	112.6	127.4	53.8	116.3

Table 4.6. Monthly precipitations (mm) from 2014 to 2017 along with the 30-year average (1971–2000) at Sainte-Anne-de-Bellevue, QC, Canada.

	2014	2015	2016	2017	Average
			mm		
January	45.0	55.1	35.6	64.7	67.8
February	43.1	24.8	123.8	89.5	58.4
March	43.9	27.8	92.4	77.3	71.4
April	135.5	71.6	82.7	143.5	69.6
May	87.8	63.5	28.1	133.6	71.4
June	142.7	130.7	52.6	88.9	88.6
July	77.7	140.4	58.5	104.5	93.6
August	60.9	57.4	168.8	72.2	104.2
September	54.9	90.9	24.3	37.5	96.0
October	82.4	90.7	143.1	113.6	77.2
November	42.0	49.7	53.7	78.8	86.4
December	58.3	93.1	75.4	52.3	78.2

Table 4.7. Nutritive attributes and estimated milk production per hectare for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Normandin (QC) along with the probabilities of fixed effects and their interactions.

		CP†				NDF				NDFd				IVTD				TDN				Milk Production‡			
		2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage		g kg^{-1} DM								g kg^{-1} aNDF				g kg^{-1} DM								Mg ha^{-1} DM			
Early bud		194a §	216	209	206a	355b	356	384	365	660	662a	685a	669a	870a	874a	874a	873a	633a	631a	627	630a	9.7	9.4	11.1	10.1
Early flower		164b	184	192	180b	405a	406	391	400	618	593b	604b	605b	829b	828b	835b	831b	600b	595b	604	600b	8.4	9.7	9.9	9.3
	S.E.M.	4.1	5.7	6.5	5.2	5.1	11.3	10.0	7.2	12.4	6.3	6.3	7.3	6.2	3.9	2.7	1.7	4.6	5.7	4.8	2.2	0.42	0.21	0.37	0.26
Mixture¶																									
Alf + Tim		177 abc	201	207 a	195 ab	396 bc	374 ab	376 bc	382 b	659 a	616 ab	637 ab	637 b	854 a	848	853 ab	851 ab	620 ab	617 ab	624 a	620 ab	9.9 a	9.8	10.3	10.0 a
Alf + TF		182 ab	195	186 b	188 bc	365 cd	403 a	424 a	397 ab	616 b	646 a	670 a	644 b	841 b	854	858 ab	851 ab	616 ab	599 b	592 c	602 c	9.1 ab	9.7	10.4	9.8 ab
Alf + MF		165 bc	196	196 ab	186 c	419 ab	392 ab	397 ab	402 ab	669 a	649 a	669 a	662 a	853 ab	859	865 a	859 a	602 bc	610 ab	617 ab	609 bc	10.0 a	9.4	10.5	10.0 a
Alf + Fest		195 a	204	209 a	203 a	329 d	361 b	358 c	349 c	619 b	612 b	621 b	617 c	854 ab	849	850 ab	851 ab	640 a	622 ab	628 a	630 a	7.3 c	9.3	10.3	9.0 b
Alf + Rye		194 a	202	210 a	202 a	332 d	358 b	356 c	349 c	617 b	605 b	632 b	618 c	853 ab	846	856 ab	852 ab	636 a	624 a	632 a	631 a	8.0 bc	9.6	11.1	9.5 ab
Alf + Bro		161 c	199	196 ab	185 c	439 a	399 a	412 a	417 a	654 a	636 ab	639 ab	643 b	842 ab	851	845 b	846 b	585 c	605 ab	600 bc	597 c	9.9 a	9.3	10.5	9.9 a
	S.E.M.	5.3	5.5	6.8	5.0	8.8	11.2	10.6	6.9	9.8	8.7	8.4	6.4	5.4	4.8	4.3	2.8	6.5	6.2	6.1	3.5	0.44	0.31	0.37	0.29
ANOVA		P values																							
Year		-	-	-	***	-	-	-	ns	-	-	-	***	-	-	-	ns#	-	-	-	ns	-	-	-	***
Stage		*	ns	ns	*	*	ns	ns	ns	ns	**	**	**	*	**	**	**	*	*	ns	**	ns	ns	ns	ns
Mixture		***	ns	***	***	***	**	***	***	***	***	***	***	*	ns	*	ns	***	*	***	***	***	ns	ns	**
Year×stage		-	-	-	***	-	-	-	***	-	-	-	*	-	-	-	ns	-	-	-	ns	-	-	-	***
Year×mixture		-	-	-	***	-	-	-	***	-	-	-	***	-	-	-	ns	-	-	-	**	-	-	-	***
Stage×mixture		ns	ns	**	ns	ns	ns	**	ns	**	ns	ns	*	**	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns
Year×stage×mixture		-	-	-	**	-	-	-	**	-	-	-	ns	-	-	-	ns	-	-	-	*	-	-	-	ns

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† CP, crude protein; aNDF, neutral detergent fiber; NDFd, *in vitro* neutral detergent fiber digestibility; IVTD, *in vitro* true digestibility; TDN, total digestible nutrients. The CP, NDF, and TDN concentrations, as well as the NDFd and IVTD were adjusted for the weight of each harvest on the seasonal DM yield.
‡ Estimated milk production per hectare of forage calculated using the Excel spreadsheet Milk2013 (Undersander et al., 2013).
§ Within columns and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).
¶ Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.
# ns, non significant.

Table 4.8. Nutritive attributes and estimated milk production per hectare for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Saint-Augustin-de-Desmaures (QC) along with the probabilities of fixed effects and their interactions.

	CP†				NDF				NDFd				IVTD				TDN				Milk Production‡			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage	g kg^{-1} DM								g kg^{-1} aNDF				g kg^{-1} DM								Mg ha^{-1} DM			
Early bud	178	183	164	175	367b §	404b	433	401b	678a	682a	671a	677a	883a	864a	843	863a	629a	599a	587	605a	18.1	14.0	9.6b	13.9
Early flower	166	162	163	164	411a	435a	438	428a	602b	603b	604b	603b	834b	818b	814	822b	594b	576b	575	582b	20.4	18.5	16.0a	18.3
S.E.M.	2.6	4.4	3.6	2.3	9.5	5.1	9.7	7.6	6.7	8.4	11.5	8.2	5.4	5.0	8.7	6.1	8.7	7.1	13.2	9.4	2.47	1.96	1.41	1.93
Mixture¶																								
Alf + Tim	179 ab	176 b	167 b	174 b	371 bc	416 b	427 c	405 c	627 b	643 ab	637 b	636 bc	855	839 ab	827 b	840 bcd	623 a	596 ab	591 b	603 ab	19.8	17.2	12.8	16.6
Alf + TF	174 ab	158 c	155 b	162 c	392 b	460 a	467 a	440 a	638 b	641 ab	639 b	639 b	856	830 b	821 b	836 d	606 ab	557 c	556 d	573 d	20.0	15.7	13.0	16.2
Alf + MF	169 bc	164 bc	150 b	161 c	395 ab	430 ab	461 ab	429 a	639 ab	642 ab	637 b	639 b	856	837 ab	820 b	838 dc	609 ab	588 ab	565 cd	587 c	181	16.3	12.3	15.6
Alf + Fest	158 c	168 bc	156 b	161 c	419 a	415 b	441 bc	425 ab	664 a	657 a	645 ab	655 a	862	848 a	831 ab	847 ab	597 b	591 ab	579 bc	589 c	18.3	15.5	12.8	15.5
Alf + Rye	168 bc	174 bc	166 b	169 bc	392 b	419 b	428 c	413 bc	646 ab	643 ab	656 a	648 ab	863	844 a	843 a	850 a	610 ab	584 b	586 b	594 bc	19.6	15.8	12.6	16.0
Alf + Bro	185 a	194 a	188 a	189 a	363 c	378 c	389 d	377 d	627 b	630 b	612 ab	623 c	860	846 a	831 ab	846 abc	625 a	609 a	608 a	614 a	19.7	17.1	13.3	16.7
S.E.M.	3.4	4.9	4.6	2.7	10.7	7.6	10.7	8.2	7.8	8.8	10.0	7.9	6.3	5.5	8.3	6.2	9.6	8.4	13.1	9.7	2.29	1.90	1.27	1.78
ANOVA	P values																							
Year	-	-	-	***	-	-	-	***	-	-	-	ns#	-	-	-	***	-	-	-	***	-	-	-	***
Stage	ns	ns	ns	ns	**	*	ns	*	**	**	*	**	**	**	ns	**	**	*	ns	**	ns	ns	*	ns
Mixture	***	***	***	***	***	***	***	***	**	**	***	***	ns	**	***	***	ns	***	***	***	ns	ns	ns	ns
Year×stage	-	-	-	***	-	-	-	***	-	-	-	ns	-	-	-	***	-	-	-	***	-	-	-	***
Year×mixture	-	-	-	*	-	-	-	***	-	-	-	ns	-	-	-	ns	-	-	-	**	-	-	-	ns
Stage×mixture	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns
Year×stage×mixture	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	***

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† CP, crude protein; aNDF, neutral detergent fiber; NDFd, *in vitro* neutral detergent fiber digestibility; IVTD, *in vitro* true digestibility; TDN, total digestible nutrients. The CP, NDF, and TDN concentrations, as well as the NDFd and IVTD were adjusted for the weight of each harvest on the seasonal DM yield.

‡ Estimated milk production per hectare of forage calculated using the Excel spreadsheet Milk2013 (Undersander et al., 2013).

§ Within columns and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).

¶ Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

ns, non significant.

0

Table 4.9. Nutritive attributes and estimated milk production per hectare for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Sainte-Anne-de-Bellevue (QC) along with the probabilities of fixed effects and their interactions.

	CP†				NDF				NDFd				IVTD				TDN				Milk Production‡			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage	g kg^{-1} DM								g kg^{-1} aNDF				g kg^{-1} DM								Mg ha^{-1} DM			
Early bud	137a §	184a	160a	160a	437b	328b	406b	390b	755a	719a	722a	732a	889a	905a	888a	894a	617a	668a	621a	635a	11.0	6.3	9.5b	8.9
Early flower	115b	159b	146b	140b	498a	411a	465a	458a	697b	624b	603b	641b	842b	833b	812b	829b	573b	603b	561b	579b	13.8	10.7	15.9a	13.5
S.E.M.	5.7	3.4	2.8	3.6	5.9	7.4	4.2	4.8	10.4	12.2	6.2	9.0	3.9	6.7	3.1	4.2	2.9	5.8	3.0	2.8	1.57	1.07	1.00	1.16
Mixture¶																								
Alf + Tim	135 a	177 abc	152 bc	155 abc	454 b	367 b	440 b	420 c	710 cd	669	673	684 ab	857 c	866 b	852 ab	858 b	608 ab	641 b	598 bc	616 b	14.1 a	9.5 a	14.1 a	12.6 a
Alf + TF	123 bc	138 d	135 d	132 d	484 a	454 a	489 a	476 a	729 bc	681	668	693 a	856 c	846 c	837 b	847 c	575 c	579 d	555 d	570 d	13.3 a	8.7 ab	13.6 a	11.9 a
Alf + MF	129 ab	165 c	149 c	148 c	457 b	387 b	442 b	429 c	709 cd	680	669	686 a	862 c	872 ab	850 ab	861 b	595 b	630 bc	590 c	605 b	13.0 a	9.7 a	12.5 ab	11.7 a
Alf + Fest	116 c	186 ab	170 a	157 ab	467 ab	303 c	382 c	384 d	746 ab	676	655	692 a	877 b	889 a	859 a	875 a	598 ab	673 a	623 a	631 a	10.4b	7.0 b	11.2 b	9.5 b
Alf + Rye	123 bc	191 a	164 ab	159 a	452 b	298 c	389 c	380 d	759 a	668	655	694 a	890 a	887 a	860 a	879 a	612 a	675 a	616 ab	634 a	10.1b	6.5 b	11.6b	9.4 b
Alf + Bro	132 ab	171 bc	148 c	150 bc	487 a	410 b	470 ab	456 b	701 d	655	656	671 b	851 c	855 bc	840 b	849 c	580 c	614 c	566 d	587 c	13.5 a	9.5 a	13.3 a	12.1 a
S.E.M.	6.1	4.4	3.6	3.9	7.1	10.3	7.2	5.3	10.7	12.3	7.5	9.2	4.4	7.3	4.2	4.5	3.8	6.7	5.2	3.3	1.52	1.01	1.04	0.11
ANOVA	**P values**																							
Year	-	-	-	***	-	-	-	***	-	-	-	***	-	-	-	***	-	-	-	***	-	-	-	***
Stage	**	*	*	**	**	*	**	**	*	**	**	**	**	**	**	**	**	*	**	**	ns#	ns	*	*
Mixture	***	***	***	***	***	***	***	***	***	ns	ns	***	***	***	**	***	***	***	***	***	***	***	**	***
Year×stage	-	-	-	**	-	-	-	*	-	-	-	***	-	-	-	***	-	-	-	***	-	-	-	***
Year×mixture	-	-	-	***	-	-	-	***	-	-	-	***	-	-	-	*	-	-	-	***	-	-	-	ns
Stage×mixture	ns	ns	**	***	***	ns	**	***	*	ns	ns	*	**	ns	ns	*	**	ns	*	***	ns	ns	ns	ns
Year×stage×mixture	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† CP, crude protein; aNDF, neutral detergent fiber; NDFd, *in vitro* neutral detergent fiber digestibility; IVTD, *in vitro* true digestibility; TDN, total digestible nutrients. The CP, NDF, and TDN concentrations, as well as the NDFd and IVTD were adjusted for the weight of each harvest on the seasonal DM yield.

‡ Estimated milk production per hectare of forage calculated using the Excel spreadsheet Milk2013 (Undersander et al., 2013).

§ Within columns and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).

¶ Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

ns, non significant.

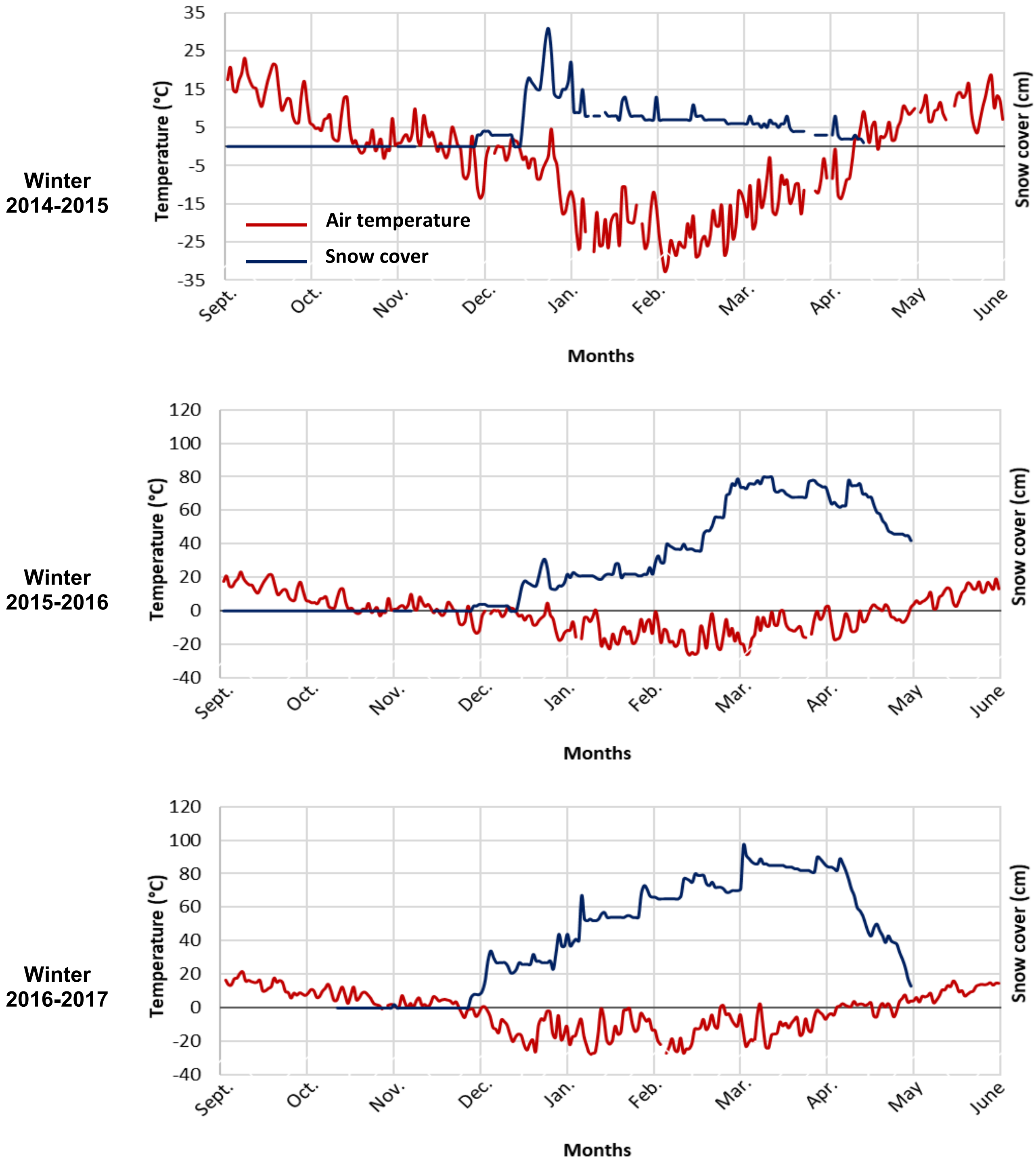


Figure 4.1. Average daily air temperature (red) and snow cover on the ground (blue) from September to June of the winters 2014-2015, 2015-2016, and 2016-2017 at Normandin, QC, Canada.

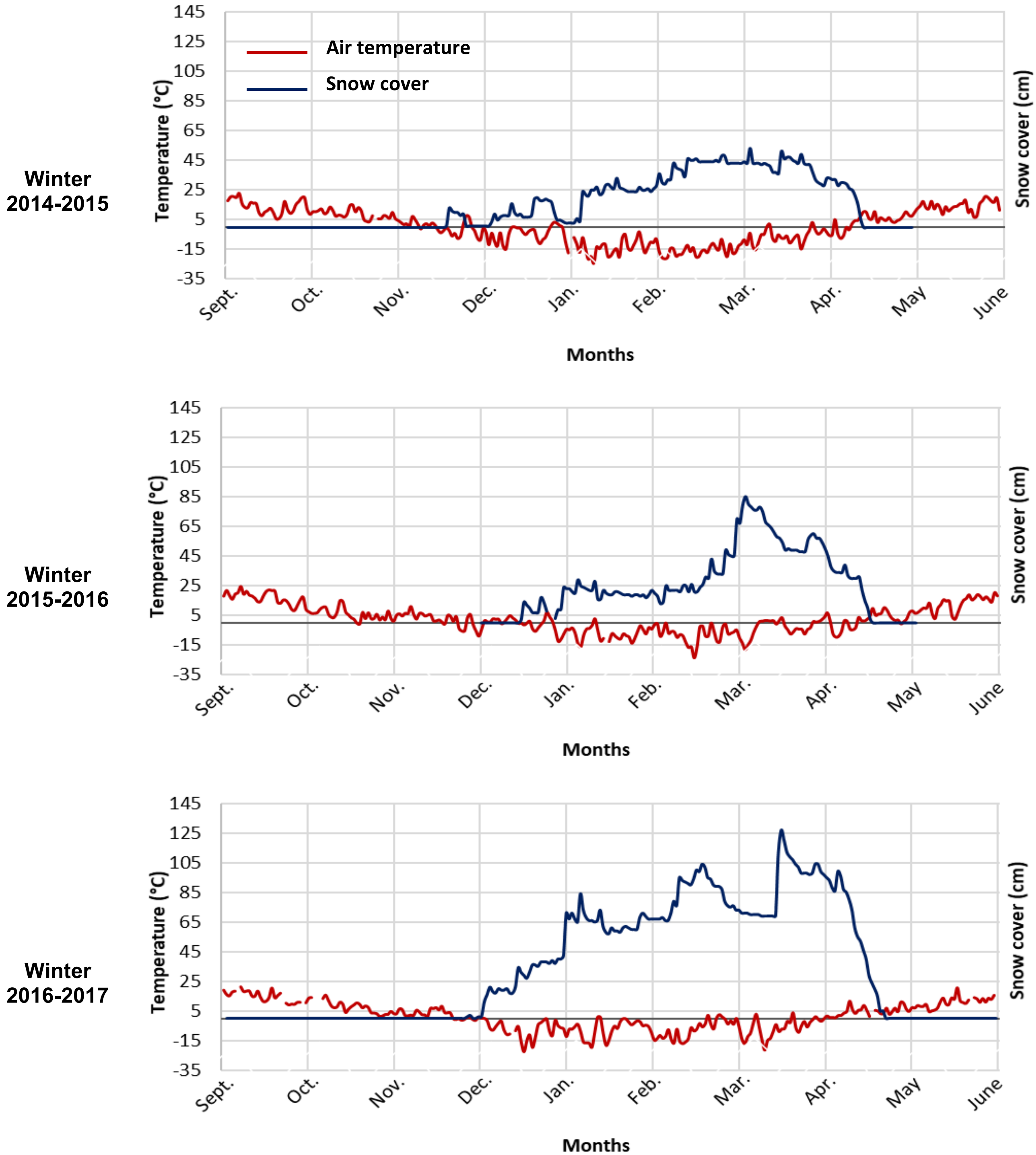


Figure 4.2. Average daily air temperature (red) and snow cover on the ground (blue) from September to June of the winters 2014-2015, 2015-2016, and 2016-2017, at Saint-Augustin-de-Desmaures, QC, Canada.

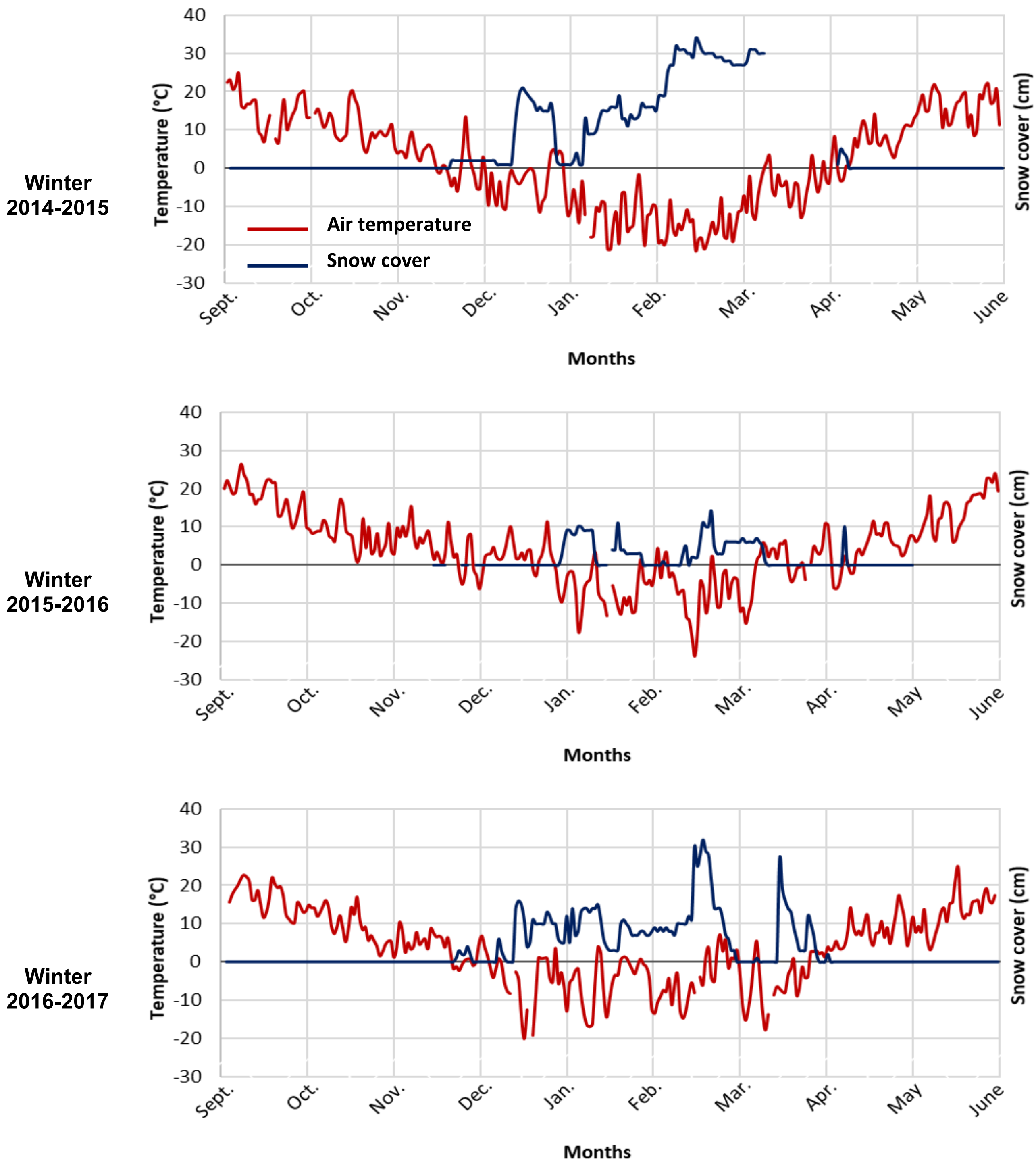


Figure 4.3. Average daily air temperature (red) and snow cover on the ground (blue) from September to June of the winters 2014-2015, 2015-2016, and 2016-2017, at Sainte-Anne-de-Bellevue, QC, Canada.

Figure 4.4. Dry matter yield of alfalfa (full line) and seeded grass (dotted line) of six binary alfalfa-grass mixtures at each harvest, at two alfalfa developmental stages, at a rhythm of two or three cuts per season, in Normandin, QC, Canada. Tim, timothy; T Fes, tall fescue; M Fes, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

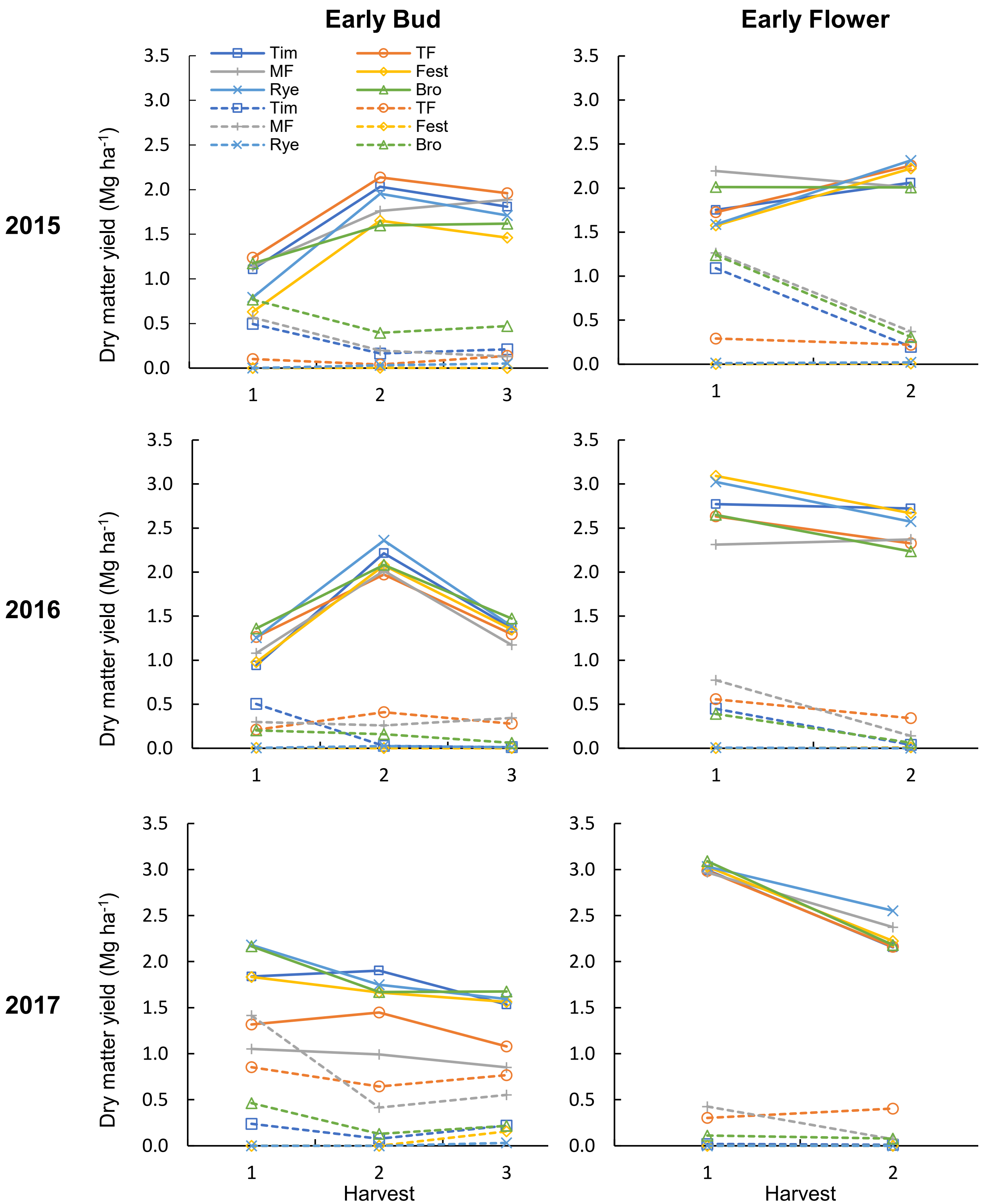


Figure 4.5. Dry matter yield of alfalfa (full line) and seeded grass (dotted line) of six binary alfalfa-grass mixtures at each harvest, at two alfalfa developmental stages, at a rhythm of three or four cuts per season, in Saint-Augustin-de-Desmaures, QC, Canada. Tim, timothy; T Fes, tall fescue; M Fes, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

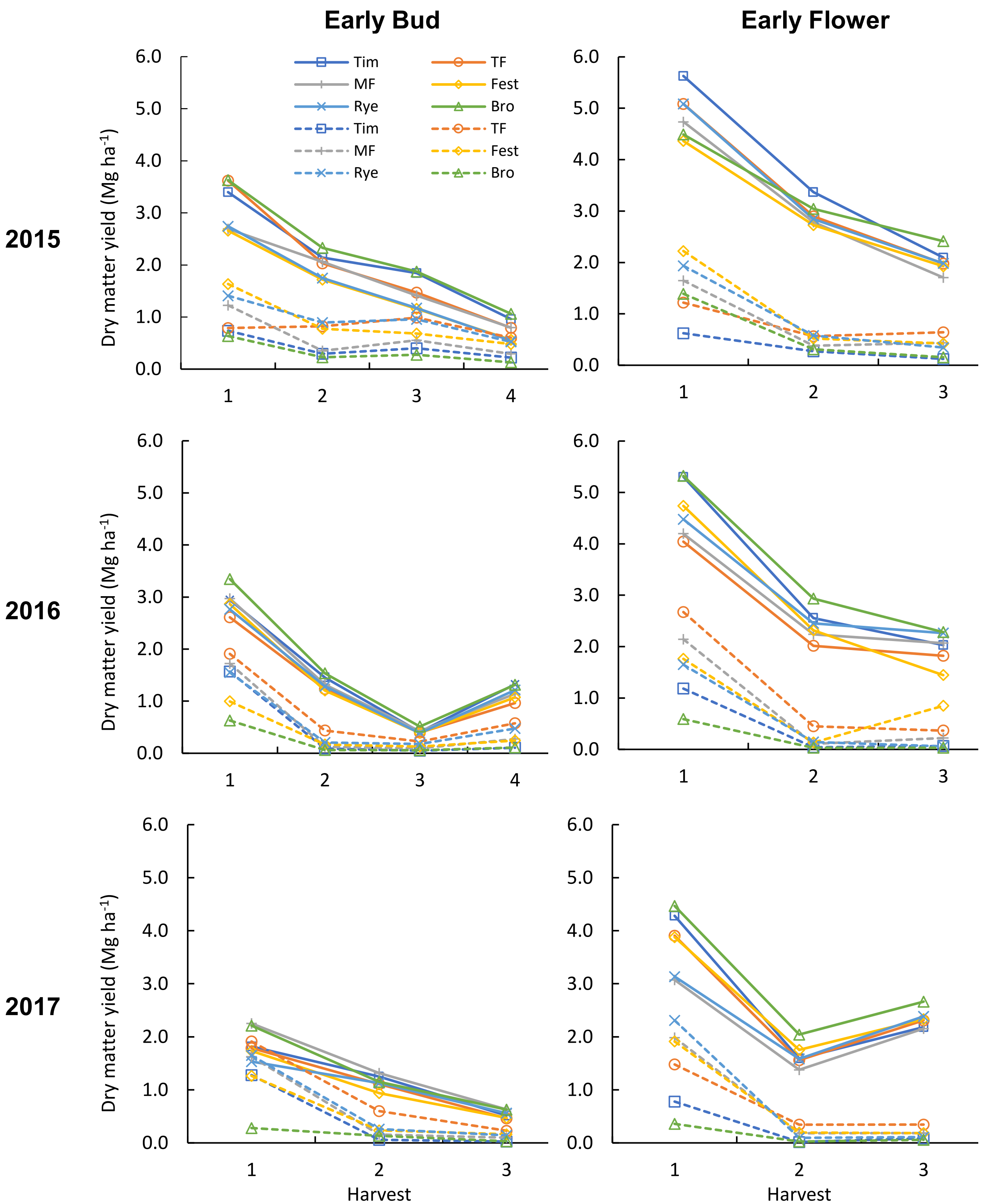


Figure 4.6. Dry matter yield of alfalfa (full line) and seeded grass (dotted line) of six binary alfalfa-grass mixtures at each harvest, at two alfalfa developmental stages, at a rhythm of three or four cuts per season, in Sainte-Anne-de-Bellevue, QC, Canada. Tim, timothy; T Fes, tall fescue; M Fes, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

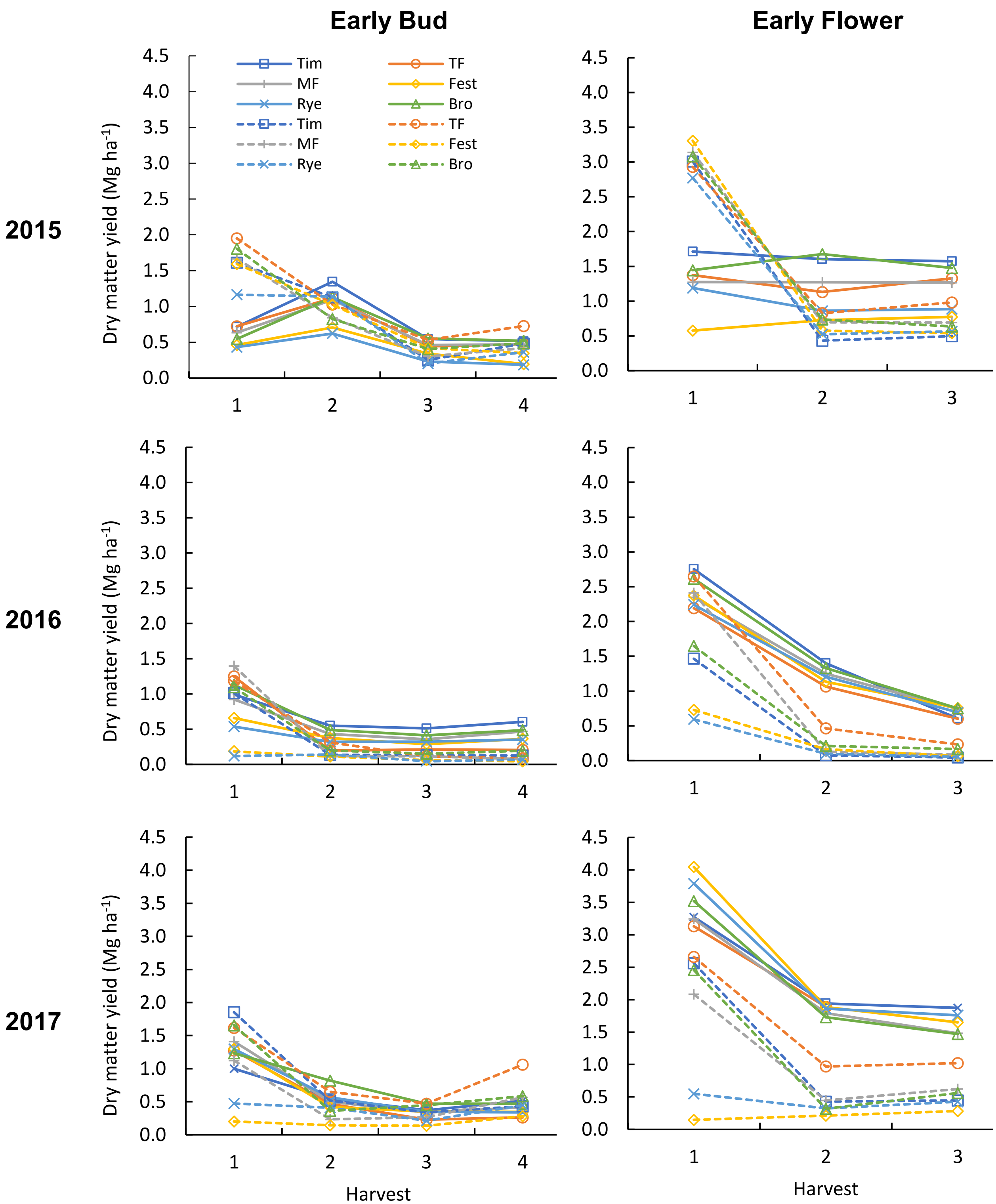
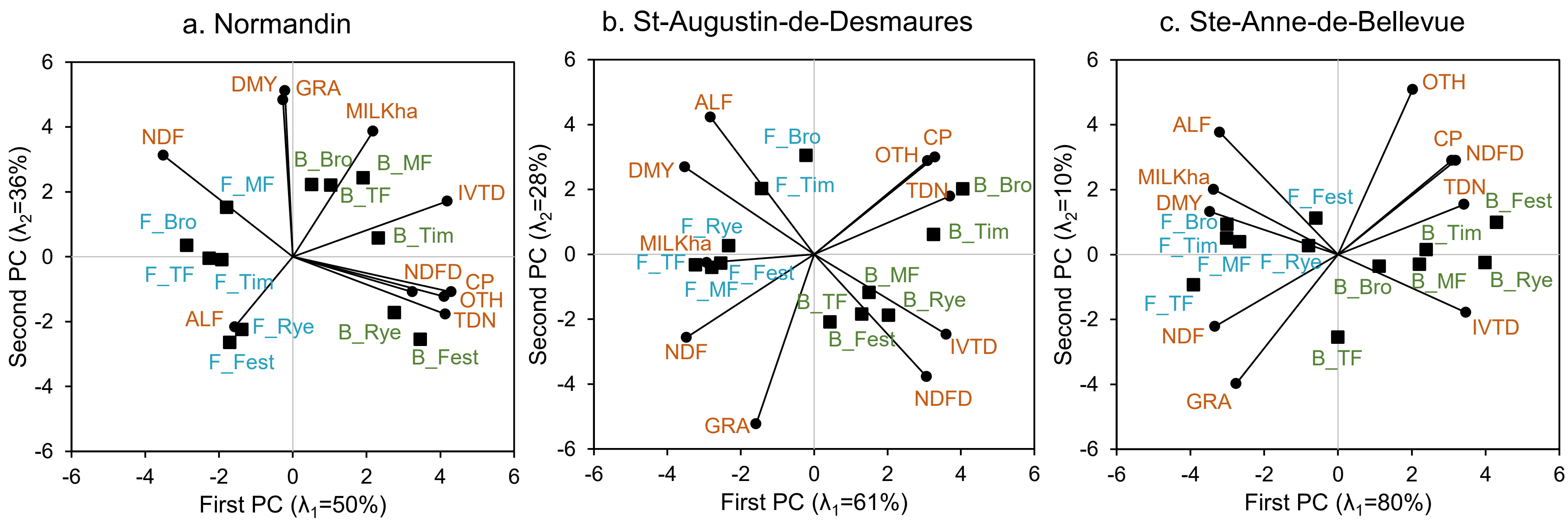


Figure 4.7. Diagram of the first two principal component (PC) scores calculated for each of the twelve combinations of six binary alfalfa and grass mixtures (■) harvested at two alfalfa developmental stages, and for each of the nine attributes (●), on average for three production years, and at three study sites in eastern Canada. The contribution of each PC score to the total covariation (λ) appears in parenthesis on each axis identification. DMY, seasonal dry matter yield; ALF, alfalfa DMY; GRA, grass DMY; OTH, DMY of non-seeded species; CP, crude protein concentration; aNDF, neutral detergent fiber concentration; NDFd; *in vitro* NDF digestibility; IVTD, *in vitro* true digestibility of DM; TDN; total digestible nutrient concentration; MILKha; estimated milk production per hectare of forage; B_, alfalfa early bud stage; F_, alfalfa early flower stage; Tim; alfalfa-timothy mixture; TF, alfalfa-tall fescue mixture; MF, alfalfa-meadow fescue mixture; Fes; alfalfa-festulolium mixture; RG, alfalfa-perennial ryegrass mixture; Bro, alfalfa-meadow bromegrass mixture.



Chapter V Conclusions

The alfalfa-meadow fescue, alfalfa-meadow bromegrass, and alfalfa-tall fescue mixtures generally performed as well as the alfalfa-timothy mixture, and they represent valuable alternatives. The alfalfa-tall fescue mixture generally had a lower nutritive value than the alfalfa-timothy mixture, but it was compensated by a generally greater total DM yield. Timothy, tall fescue, meadow fescue, and meadow bromegrass remained productive over the first three production years when cultivated in mixture with alfalfa. The alfalfa-grass mixtures with the ‘Spring Green’ festulolium or the ‘Remington’ perennial ryegrass had lower total DM yields than the alfalfa-timothy mixture, due most likely to poor winter survival of the two grass species. The cultivars used for these two grass species, therefore, do not seem to be viable alternatives to timothy in mixture with alfalfa grown in eastern Canada. Harvesting alfalfa-grass mixtures at the early flower stage of alfalfa rather than the early bud stage maximizes the persistence of the mixtures, their DM yields, and their estimated milk production per hectare.

Chapter VI Suggestions for Future Research

1. This study evaluated six cultivars of different cool-season forage grasses grown in binary mixture with alfalfa. However, other cultivars could have behaved differently when grown under the same climatic context since every cultivar of a given plant species has specific characteristics. Evaluating more than one cultivar of a forage grass in a single experiment would therefore be interesting.
2. Mixtures in this experiment were grown in a field and mechanically harvested, a procedure used in the making of dry hay or silage. Yet, future research could evaluate the establishment, yield, regrowth, and persistence of the six alfalfa-grass binary mixtures in a pasture experimental design.
3. This project evaluated the potential of six alfalfa-grass mixtures under the current context of climate change in eastern Canada. Yet, many predictive models have produced expected climatic scenarios in the near-future, and future research could be interested in doing growth chamber experiments to evaluate the same binary mixtures under these predicted conditions (i.e., elevated temperature and CO_2 concentration). This would identify the best alternatives to timothy in the predicted context of climate change.
4. Economic implications were not considered in this project. Future research could therefore look at the short and long terms economic implications of growing the different proposed grass species with alfalfa based on their establishment in the first year, their yield, their persistence over many production years, and the resulting quality of the forages produced.

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