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ARTICLE

Yield and nutritive value of binary legume–grass mixtures under grazing or frequent cutting

Gilles Bélanger, Gaëtan F. Tremblay, Yousef A. Papadopoulos, John Duynisveld, Julie Lajeunesse, Carole Lafrenière, and Sherry A.E. Fillmore

Abstract: Although most forage production in eastern Canada is comprised of species mixtures, little research has identified the best species to include in forage mixtures. Our objective was to identify binary legume–grass mixtures with high forage yield and nutritive value under either simulated grazing with frequent cutting or cattle grazing. The experiment was conducted at three sites in eastern Canada with 18 binary legume–grass mixtures of one of six grass species {Kentucky bluegrass (*Poa pratensis* L.), meadow fescue (*Festuca pratensis* Huds.), orchardgrass (*Dactylis glomerata* L.), tall fescue [*Schedonorus phoenix* (Scob.) Holub], timothy (*Phleum pratense* L.), and meadow bromegrass (*Bromus biebersteinii* Roem. & Schult.)} seeded in 2010 with a grazing-type alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.), or birdsfoot trefoil (*Lotus corniculatus* L.). The six grass species grown in mixture with alfalfa, birdsfoot trefoil, or white clover persisted well under frequent cutting or rotational grazing at the three sites. White clover grown in a binary mixture with a grass species did not perform well under frequent cutting or rotational grazing. Meadow bromegrass based binary mixtures were overall the best performing in terms of dry matter yield; although their nutritive value was average, meadow bromegrass combined with alfalfa or birdsfoot trefoil were among the best legume–grass mixtures for estimated milk production per hectare. The greatest estimated milk production per hectare was obtained with birdsfoot trefoil mixed with meadow bromegrass followed by the alfalfa–timothy and the alfalfa–meadow bromegrass mixtures.

Key words: alfalfa, clover, forage, grass, grazing.

Résumé : Bien que la majeure partie des fourrages récoltés dans l'est du Canada viennent d'un mélange d'espèces, on a relativement peu effectué de recherches pour déterminer quelles espèces constitueraient le meilleur mélange. Les auteurs voulaient établir les mélanges binaires légumineuse-graminée dont le rendement fourrager est le plus élevé et qui ont la plus grande valeur nutritive en simulant la paissance avec des coupes fréquentes ou sous pâturage avec des animaux. L'expérience, réalisée à trois endroits dans l'est du Canada, portait sur 18 mélanges binaires de légumineuses et de graminées. Les six graminées employées étaient le pâturin des prés (*Poa pratensis* L.), la fétuque des prés (*Festuca pratensis* Huds.), le dactyle pelotonné (*Dactylis glomerata* L), la fétuque élevée [*Schedonorus phoenix* (Scob.) Holub], la fléole (*Phleum pratense* L.) et le brome des prés (*Bromus biebersteinii* Roem. & Schult.). En 2010, ils ont semé les graminées avec de la luzerne adaptée à la paissance (*Medicago sativa* L.), du trèfle blanc (*Trifolium repens* L.) ou du lotier corniculé (*Lotus corniculatus* L.). Les six graminées cultivées avec la luzerne, le lotier corniculé ou le trèfle blanc ont bien résisté aux coupes fréquentes et aux pâturages tournants, aux trois sites. Mélangé à une graminée, le trèfle blanc n'a pas bien performé avec des coupes fréquentes ni comme pâturage tournant. De manière générale, les mélanges à base de brome des prés s'avèrent les plus performants au niveau du rendement en matière sèche, mais leur valeur nutritive reste moyenne. Le brome des prés

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combiné à la luzerne ou au lotier corniculé figure parmi les meilleurs mélanges légumineuse-graminée pour ce qui est du volume estimatif de lait obtenu par hectare. La quantité estimative de lait produite par hectare la plus élevée a été obtenue avec le lotier corniculé combiné au brome des prés. Suivent les mélanges luzerne–fléole et luzerne–brome des prés. [Traduit par la Rédaction]

Mots-clés : luzerne, trèfle, fourrage, graminée, paissance.

Introduction

Legume–grass mixtures generally provide more consistent forage yield across a wide range of environments than grass or legume monocultures (Sleugh et al. 2000; Bélanger et al. 2014). Legume–grass mixtures have also been shown to reduce weed invasion compared with monocultures (Tracy and Sanderson 2004; Picasso et al. 2008; Frankow-Lindberg et al. 2009; Sanderson et al. 2012; Finn et al. 2013; Bélanger et al. 2014).

Nutritive value should also be considered because of its impact on animal productivity and meat and milk quality. Results of a pan-European experiment, which included a Canadian site, have demonstrated that mixing grasses and legumes increases dry matter (DM) yield (Finn et al. 2013) with no negative effects on nutritive value (Sturludóttir et al. 2013). Adding a legume into a grass sward has been shown to increase forage DM yield and crude protein concentration (Barnett and Posler 1983) and improve forage nutritive value (Papadopoulos et al. 2001). Furthermore, mixing timothy (*Phleum pratense* L.) with alfalfa (*Medicago sativa* L.) has been shown to increase the non-structural carbohydrate concentration of forages (Bélanger et al. 2014), potentially resulting in a more efficient use of nitrogen (N) by ruminants (Brito et al. 2009).

Kentucky bluegrass (*Poa pratensis* L.), meadow fescue (*Festuca pratensis* Huds.), orchardgrass (*Dactylis glomerata* L.), tall fescue [*Schedonorus phoenix* (Scob.) Holub], timothy, and meadow bromegrass (*Bromus biebersteinii* Roem. & Schult.) are forage grass species that are well adapted to cool-season growing regions and recommended in eastern Canada. Alfalfa, white clover (*Trifolium repens* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) are perennial legume species also recommended in eastern Canada but their performance and nutritive value in mixtures with grasses and under grazing are not well documented.

Although most forage production in eastern Canada is comprised of species mixtures, there is limited research on identifying the best species to include within forage mixtures. Our objective was to identify persistent binary legume–grass mixtures with high forage yield and nutritive value to be used under both simulated grazing with frequent cutting or cattle grazing in eastern Canada.

Materials and Methods

The experiment was conducted at three sites: (*i*) Chapais Research Farm of Agriculture and Agri-Food Canada (AAFC), Lévis, QC, (*ii*) Normandin Research Farm, AAFC, Normandin, QC, and (*iii*) Nappan Research Farm, AAFC, Nappan, NS. Site characteristics are presented in Table 1. Binary legume–grass mixtures (18) of one of six grass species (orchardgrass 'Killarney', Kentucky bluegrass 'Troy', meadow bromegrass 'Fleet', meadow fescue 'Pradel', tall fescue 'Courtenay', and timothy 'Express') were seeded in 2010 with a grazing-type alfalfa 'CRS1001', birdsfoot trefoil 'AC Langille', or white clover 'Milkanova'. Seeding rates for each species are presented in Simili da Silva et al. (2013). At each site, binary mixtures were replicated three times in a split-plot layout with legume species as main plots set out as a Latin square and grass species randomized to the subplots. Phosphorus (P) and potassium (K) fertilizers were applied before seeding and each year if needed based on provincial recommendations in Québec for the sites at Lévis and Normandin (CRAAQ 2003) and in Nova Scotia for the site at Nappan (AACCPCFC 1991). Lime was applied at Lévis in 2011 at a rate of 3.8 Mg ha^{-1} after the first and second cutting and in the spring of 2012 at a rate of 3.2 Mg ha^{-1}. Nitrogen was applied at seeding at rates of 30 kg N ha^{-1} at Lévis, 25 kg N ha^{-1} at Normandin, and 24 kg N ha^{-1} at Nappan. No N was applied in the post-seeding years except for 40 kg N ha^{-1} after the second cutting at Lévis and Normandin in 2014 and 2015 and 34 kg N ha^{-1} after the first grazing from 2013 to 2015 at Nappan. Our intent was to rely entirely on legume N_2 fixation to provide N to the legume–grass mixtures. Towards the end of the study, we applied a low rate of fertilizer N to help the forage grasses because the legume component of the mixture was much decreased.

For logistics reasons, all mixtures were grazed or cut at the same time and the timing of those events was based on timothy, the main forage grass species in eastern Canada, reaching 33 cm in height (Table 2). Because of the lack of grazing facilities and cattle at Lévis and Normandin, grazing was simulated by frequent cutting of all plots to a 5-cm height. Dry matter yield was determined by cutting an area of 7.3 m^2 at Lévis using a self-propelled flail-type Carter™ forage harvester (Carter MGF Co., Inc., Brookston, IN) and an area of 6.0 m^2 at Normandin using a walk-behind flail harvester (Swift Machine and Welding, Swift Current, SK). A fresh forage sample of approximately 500 g was taken from each plot, weighed, dried at 55 °C in a force-draft oven to determine DM concentration, and then ground using

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Table 1. Site characteristics.

Soil/crop information	Lévis (QC)	Normandin (QC)	Nappan (NS)
Latitude	46°48′ N	48°51′ N	45°46′ N
Longitude	71°23′ W	72°32′ W	64°15′ W
Elevation (m above sea level)	43	137	20
Annual rainfall[a] (mm)	924	612	916
Annual temperature[a] (°C)	4.0	0.9	5.8
Growing degree days (5 °C basis)[a]	1713	1359	1718
Soil texture	Fine sandy loam	Silty clay	Loam
Soil pH (water)[b]	5.2	5.9	7.1
Soil-available P[b] (mg kg^{-1})	86	143	133
Soil-available K[b] (mg kg^{-1})	199	284	118
Plot size (m^2)	12	15	10

[a]30-yr average (1971–2000); http://climate.weather.gc.ca/climate_normals/index_e.html.
[b]Values at the start of the experiment (0–20 cm).

Table 2. Cutting or grazing dates in the five post-seeding years at the three sites.

Year	Cutting/ grazing no.	Lévis	Normandin	Nappan
2011	1	3 June	6 June	15–20 June
	2	22 June	27 June	9 Aug.
	3	13 July	28 July	7 Oct.
	4	17 Aug.	31 Aug.	—
	5	6 Oct.	—	—
2012	1	24 May	7 June	22–31 May
	2	18 June	12 July	4–10 July
	3	9 July	23 Aug.	—
	4	21 Aug.	—	—
	5	3 Oct.	—	—
2013	1	28 May	12 June	27–31 May
	2	14 June	16 July	4–10 July
	3	5 July	10 Sept.	19–23 Aug.
	4	21 Aug.	—	10–11 Oct.
	5	19 Sept.	—	—
2014	1	29 May	9 June	16 June
	2	16 June	3 July	4–10 July
	3	8 July	19 Aug.	19–23 Aug.
	4	8 Aug.	—	—
	5	10 Sept.	—	—
2015	1	29 May	15 June	5–8 June
	2	17 June	15 July	4–10 July
	3	9 July	18 Aug.	19–23 Aug.
	4	13 Aug.	—	—
	5	16 Sept.	—	—

a Wiley mill (standard model 4, Arthur H. Thomas Co., Philadelphia, PA) to pass through a 1-mm screen. At Nappan, rotational grazing with beef steers was initiated in the spring and concluded in late summer or fall. A block of 8–16 growing beef steers, weighing 500 kg on average, was allocated to this trial and animals started on the first grazing paddock (replicate 1) and were moved to the next paddock at the required sward height. Grazing of all four replicates, each 0.3 ha in size, was completed in a maximum of 6 d from initiation, which was set when timothy reached about 33 cm in height. Sward height was monitored to ensure that cattle would exit the paddocks at the appropriate sward exit height (6–8 cm). Forage samples were taken just prior to the beginning of each grazing cycle using a 0.25-m^2 quadrat randomly placed within each plot. Samples were dried in an oven at 55 °C and weighed to estimate DM yield. All samples from each grazing cycle were then ground with a Wiley mill to pass through a 1-mm screen.

In the first 2 yr, the frequency grid technique (Vogel and Masters 2001) was used approximately 2 wk after the first cutting or grazing to determine the presence of each seeded species in each plot. Two grids of 25 squares (5 cm × 5 cm) were placed in each plot. The presence of at least one seeded plant species and one other species was noted for each square. This information is an estimate of the minimum plant density. In the last 3 yr, seeded grasses, seeded legumes, and weeds were manually separated twice during the season (first and third cutting or grazing) from one sample taken in each plot from a 0.25-m^2 quadrat, and then each component was dried at 55 °C in a force-draft oven and weighed to determine their proportion assessed as their contribution to DM yield.

Dried and ground forage samples were scanned by visible near-infrared reflectance spectrometry (VNIRS) using a NIRSystem 6500 monochromator (Foss, Silver Spring, MD) in the range of 400 to 2500 nm intervals. Out of approximately 1100 forage samples per post-seeding year, WinISI IV (version 4.5.0.14017) software (Infrasoft International LLC, State College, PA) was used to select approximately 75 samples per year from 2011 to 2014 with spectra that contributed the most to the variability within all samples. Of these 75 samples per post-seeding year, approximately 60 and 15 were randomly selected for calibration and validation sets, respectively. Samples selected for the calibration and validation sets (≈75 samples per post-seeding

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year × 4 post-seeding years ≈ 300 samples) were chemically analyzed in duplicates that were averaged prior to the development of the calibration equations. The DM and ash concentrations (Leco Corporation 2009) were determined using a thermogravimetric analyser (model TGA701, Leco Corp., St. Joseph, MI). Crude fat (ether extract) was determined using Ankom xt15 Extractor Technology Method (AOCS 2003). Concentrations of water-soluble carbohydrates (WSC) and starch were measured according to dos Passos Bernardes et al. (2015) and the concentration of non-structural carbohydrates (NSC) was calculated as the sum of WSC and starch concentrations. Total N concentration was measured using a method adapted from Isaac and Johnson (1976). Ground samples (100 mg) were digested for 60 min at 380 °C in a 1.5 mL mixture of selenious and sulfuric acid plus 2 mL of 30% H_2O_2. After cooling, the mixture was diluted to 75 mL with deionized water. An auto analyzer (QuikChem 8000 Lachat, Zellweger Analytics Inc., Lachat Instruments, Milwaukee, WI) was used to measure total N with the method 13-107-06-2-D and P with the method 13-115-01-2-A (Lachat, 2013, Zellweger Analytics Inc.). The acid detergent fiber (ADF) was determined according to AOAC (1990). The neutral detergent fiber (aNDF) was analyzed following Mertens (2002) with addition of a heat-stable α-amylase and sodium sulfite. These fiber extractions were done using the Ankom filter bag technique (ANKOM Technology Corp., Macedon, NY). The in vitro true digestibility (IVTD) was measured using the method of Goering and Van Soest (1970) based on a 48-h incubation with buffered rumen fluid followed by an aNDF determination of the post-digestion residues. The rumen fluid incubation was performed with Ankom F57 filter bags and an Ankom Daisy II incubator, using the bath incubation procedures outlined by Ankom Technology Corp. (ANKOM Technology Corp.). Rumen fluid was obtained from a ruminally fistulated dairy cow that was offered a diet of 37% grass silage, 15% corn silage, 8% hay, 30% corn grain, and 10% concentrate mix formulated to meet the nutritional requirements of a lactating dairy cow expected to produce 10 200 kg milk yr^{-1}. The IVTD of DM (g kg^{-1} DM) and the in vitro aNDF digestibility (NDFd; g kg^{-1} aNDF) were calculated as below:

$$\text{IVTD} = [1 - (\text{post-digestion dry weight following aNDF wash/predigestion dry weight})] \times 1000.$$

$$\text{NDFd} = [1 - (\text{post-digestion dry weight following aNDF wash/predigestion dry weight of aNDF})] \times 1000.$$

The total N, ADF, aNDF, neutral detergent insoluble crude protein (NDICP; Licitra et al. 1996), ash, and ether extract concentrations, along with NDFd, were used to calculate the total digestible nutrients (TDN; NRC 2001) using the University of Wisconsin Alfalfa/Grass Evaluation System and milk production per hectare with Milk2013 (Undersander et al. 2013).

The VNIRS calibration equations were developed using a modified least squares regression method of the WinISI IV software. Depending on the nutritive attribute and the calibration set of approximately 240 forage samples (≈60 samples per post-seeding year × 4 post-seeding years ≈ 240), the number of calibration samples used to develop the final calibration equations varied between 223 and 237. Calibration equations were selected based on Martens and Naes (2001) as follows: Reference data = f(spectral data) + SEC, where f() means "function of" and SEC is the standard error of calibration. The best VNIRS calibration equations were the ones that minimize SEC. Cross-validation was performed by using four subgroups from the calibration set to choose the optimal number of terms and to avoid over-fitting the calibration model (Shenk and Westerhaus 1991). Calibration equations were validated using WinISI IV software by comparing predicted against reference values. Statistics on the VNIRS performance to predict nutritive attributes in the validation set (n = 61) are presented in Table 3. The ratio of standard error of prediction to standard deviation {[RPD = standard deviation (SD) of the reference data used in the validation set divided by the standard error of prediction corrected for bias [SEP(C)]} was greater than 3 and the VNIRS predictions were, therefore, considered successful for all nutritive attributes.

Data were assessed by analysis of variance (ANOVA) using GENSTAT 17 statistical software (VSN International 2013). Sites, legume species, and grass species were considered fixed effects. Differences were considered significant when $p < 0.05$. Seasonal values of DM yield were calculated as the sum of the DM yield at each cutting or grazing. Seasonal values of the nutritive attributes were calculated as the average of their values at each cutting or grazing. Seasonal values were reported with the objective of presenting and discussing the overall response over the 5 yr of the study. The question of seasonal distribution of DM yield and nutritive attributes will be addressed in future manuscripts. For each variate, extreme high or low values were identified after calculating an upper [overall mean + (2.81 × SEM/2)] and a lower [overall mean − (2.81 × SEM/2)] limit centered about the overall mean. A principal component analysis (PCA) was used to assess the relationships among variates (DM yield and nutritive attributes) and how variations in these variates were related to legume–grass mixtures. The PCA was performed on the least squares means of the treatments using the correlation matrix method to give equal weight to all variates.

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Table 3. Statistics of the performance of near-infrared spectroscopy to predict the nutritive attributes of the validation set of forage samples.

Attribute	*n*	Slope	Mean	SD	SEP	RSQ	SEP(C)	RPD
ADF (g kg^{-1} DM)	61	1.03	338	50.4	10.8	0.96	10.8	4.7
aNDF (g kg^{-1} DM)	61	0.98	509	90.7	14.5	0.97	14.6	6.2
IVTD (g kg^{-1} DM)	61	1.02	837	61.5	15.8	0.94	15.4	4.0
NDFd (g kg^{-1} aNDF)	61	0.96	676	93.5	29.8	0.90	30.1	3.1
TDN (g kg^{-1} DM)	61	0.96	573	77.6	20.0	0.94	19.7	3.9
NSC (g kg^{-1} DM)	61	0.97	77	24.7	5.9	0.94	5.9	4.2
Total N (g kg^{-1} DM)	61	0.96	24.4	7.21	1.01	0.98	1.01	7.2

Note: *n*, number of samples in the validation set; SD, standard deviation; SEP, standard error of prediction; SEP(C), standard error of prediction corrected for the bias; RSQ, coefficient of determination for the prediction; RPD, ratio of prediction to deviation [SD/SEP(C)]; ADF, acid detergent fiber; aNDF, neutral detergent fiber assayed with a heat-stable α-amylase; IVTD, in vitro true digestibility of dry matter; NDFd, in vitro aNDF digestibility; TDN, total digestible nutrients; NSC, non-structural carbohydrates (water-soluble carbohydrates plus starch).

Table 4. Analysis of variance (probability values) for the effects of sites, legume species, and grass species in the binary mixtures on forage dry matter yield and several nutritive attributes.

Sources of variation	DM yield	ADF	aNDF	IVTD	NDFd	TDN	NSC	Total N
Site (S)	0.002	0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
Nappan vs. (Lévis + Normandin)	0.001	ns	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
Lévis vs. Normandin	0.037	<0.001	0.10	0.048	<0.001	ns	<0.001	<0.001
Legume (L)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C vs. (A + B)[3]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
A vs. B	<0.001	0.011	ns	0.014	<0.001	<0.001	ns	ns
Grass (G)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Kb vs. (Ti + Tf + Or + Mf + Mb)	ns	0.064	ns	<0.001	<0.001	<0.001	0.002	<0.001
Ti vs. (Tf + Or + Mf + Mb)	ns	<0.001	<0.001	<0.001	<0.001	<0.001	0.007	<0.001
Or vs. (Tf + Mf + Mb)	0.002	ns	0.028	ns	<0.001	<0.001	<0.001	<0.001
Mb vs. (Tf + Mf)	<0.001	<0.001	ns	<0.001	<0.001	<0.001	<0.001	<0.001
Tf vs. Mf	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	ns
S × L	<0.001	<0.001	<0.001	0.006	0.057	<0.001	<0.001	<0.001
S × G	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L × G	<0.008	ns	0.043	ns	0.001	ns	0.005	0.013
S × L × G	ns	ns	ns	ns	0.083	ns	<0.001	0.003

Note: Not significant at $p < 0.10$. ADF, acid detergent fiber; aNDF, neutral detergent fiber assayed with a heat-stable α-amylase; IVTD, in vitro true digestibility of dry matter; NDFd, in vitro aNDF digestibility; TDN, total digestible nutrients; NSC, non-structural carbohydrates (water-soluble carbohydrates plus starch). C, white clover; A, alfalfa; B, birdsfoot trefoil; Kb, Kentucky bluegrass; Ti, timothy; Tf, tall fescue; Or, orchardgrass; Mf, meadow fescue; Mb, meadow bromegrass.

Results and Discussion

Main effects of sites, legume species, and grass species

The three sites differed significantly for most variates [contrasts: Nappan vs. Lévis and Normandin, Lévis vs. Normandin; Table 4]. Average forage DM yields across mixtures and years were 6.67, 5.21, and 4.15 Mg ha^{-1} at Nappan, Lévis, and Normandin, respectively. The forage nutritive value was lower at Nappan than at Lévis and Normandin [contrast: Nappan vs. Lévis and Normandin; Table 4] with greater ADF (345 vs. 300 and 316 g kg^{-1} DM) and aNDF (538 vs. 440 and 453 g kg^{-1} DM) concentrations along with lower total N concentration (21.8 vs. 26.5 and 25.5 g kg^{-1} DM), IVTD (826 vs. 895 and 888 g kg^{-1} DM), and NDFd (676 vs. 761 and 739 g kg^{-1} aNDF). Site differences in forage DM yield and nutritive value are expected and can be explained by differences in soil and climatic conditions and the different management practices (grazing vs. frequent cutting) used at the three sites.

Sites also differed in the composition of the mixtures in the last three post-seeding years. The average proportion of the legume species in each of the last three post-seeding years (2013, 2014, and 2015) was greater at Lévis (23%, 20%, and 4%) and Normandin (30%, 27%, and 5%) than at Nappan (4%, 3%, and 2%) (Table 5). Our visual observations in the first post-seeding year suggest that

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Table 5. Proportion (%) of each seeded species assessed as their contribution to forage DM yield of 18 binary legume–grass mixtures in years 3, 4, and 5 after seeding.

	Lévis			Normandin			Nappan		
Main effects	Year 3	Year 4	Year 5	Year 3	Year 4	Year 5	Year 3	Year 4	Year 5
Legume									
White clover	<u>2</u>	<u>8</u>	<u>2</u>	<u>15</u>	<u>7</u>	<u>0</u>	4	3	1
Birdsfoot trefoil	**38**	**29**	4	**43**	**43**	5	<u>1</u>	<u>1</u>	1
Alfalfa	**30**	23	**6**	33	**31**	**11**	**8**	**6**	**4**
Mean	23	20	4	31	27	6	4	3	2
SEM	2.4	2.9	0.8	1.5	1.9	0.9	0.7	1.0	0.7
Upper limit[a]	27	24	5	33	30	7	5	5	3
Lower limit	20	16	3	28	25	4	3	2	1
Grass									
Timothy	<u>28</u>	<u>21</u>	<u>23</u>	<u>35</u>	<u>36</u>	<u>50</u>	<u>48</u>	<u>41</u>	<u>44</u>
Kentucky bluegrass	59	<u>35</u>	<u>43</u>	<u>49</u>	<u>47</u>	<u>51</u>	57	<u>39</u>	49
Tall fescue	**74**	**71**	**82**	**64**	**58**	61	**86**	**69**	**78**
Orchardgrass	58	50	62	58	**57**	60	60	51	49
Meadow fescue	**72**	**72**	**78**	**65**	<u>47</u>	**68**	**70**	**64**	**65**
Meadow bromegrass	56	52	**65**	**63**	**55**	**74**	<u>39</u>	<u>21</u>	<u>23</u>
Mean	58	50	59	56	50	61	60	48	51
SEM	4.8	4.4	3.4	1.9	2.2	2.8	7.6	3.8	4.2
Upper limit[a]	63	54	62	58	52	63	68	51	56
Lower limit	53	45	55	54	48	58	52	44	47

Note: Values are the average of two measurements in each post-seeding year; SEM, standard error of the mean.
[a]Mixture values that are greater by more than one-half of the least significant difference of the grand mean are in bold type while those less the same amount are underlined.

selective grazing of birdsfoot trefoil and alfalfa might explain the lack of legume persistence under grazing at Nappan. This difference in the proportion of the legume species might explain in part the lower concentrations of ADF and aNDF and the greater N concentration in forages at Lévis and Normandin than at Nappan because of the known lower ADF and aNDF concentrations of legume species and their greater N concentration compared with grass species (Pelletier et al. 2010). Along with their differences in species composition, lower forage DM yields at Lévis and Normandin could explain the greater forage TDN concentration and IVTD at those two sites because of the known negative relationship between forage DM yield and digestibility (Bélanger et al. 2001).

The three legume species in binary mixtures with grasses differed significantly for most variates (contrast: C vs. A + B, A vs. B; Table 4). The average seasonal forage DM yield across sites, years, and grass species in the mixtures was the least with the white clover based mixtures and the greatest with the birdsfoot trefoil based mixtures (Table 6). The white clover based mixtures also had the greatest ADF and aNDF concentrations, the greatest NDFd, and the lowest total N concentration (Table 6). Although statistically significant, differences in TDN concentration and IVTD were small. The proportion of white clover was much less than that of birdsfoot trefoil and alfalfa at Lévis and Normandin in the last three post-seeding years (Table 5). This lower proportion of white clover could explain the greater ADF and aNDF concentrations, the greater NDFd, and the lower total N concentrations of the white clover based mixtures.

White clover was nearly absent in the last three post-seeding years at all three sites (Table 5). The decline of white clover in mixtures with grasses under frequent cutting has been reported previously. In a study conducted in Québec where white clover was grown in a mixture with either meadow fescue or meadow bromegrass under frequent cutting (Drapeau and Bélanger 2009), the proportion of white clover decreased from the first to the third post-seeding year, reaching values below 10%. In a study of white clover in mixtures with grasses conducted in Newfoundland, the proportion of white clover also decreased from 40% in the first post-seeding year to 27% in the third post-seeding year (McKenzie et al. 2005). White clover in mixtures with forage grasses is, therefore, not well adapted to frequent cutting or rotational grazing under the conditions of eastern Canada.

The proportion of alfalfa and birdsfoot trefoil was also poor in the last three post-seeding years of the study at Nappan where cattle grazing was used (Table 5). At Lévis and Normandin, two sites with frequent cutting, the proportion of alfalfa and birdsfoot trefoil ranged between 23% and 43% in the third and fourth post-seeding years. As mentioned above, selective grazing in

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Table 6. Means of all nutritive attributes and dry matter (DM) yield across five post-seeding years and three sites for the main effects of one of three legume species and one of six grass species present in binary legume–grass mixtures.

Main effects	DM yield (Mg ha^{-1})	ADF (g kg^{-1} DM)	aNDF (g kg^{-1} DM)	IVTD (g kg^{-1} DM)	NDFd (g kg^{-1} aNDF)	TDN (g kg^{-1} DM)	NSC (g kg^{-1} DM)	Total N (g kg^{-1} DM)
Legume								
White clover	4.86	**311**	**483**	**878**	**750**	610	**99**	23.3
Birdsfoot trefoil	**5.76**	303	464	877	734	**620**	94	**25.2**
Alfalfa	5.45	305	464	874	725	613	93	**25.4**
SEM	0.059	0.7	1.8	0.7	0.9	1.1	0.6	0.11
Upper limit[a]	5.45	307	474	877	737	617	96	24.9
Lower limit	5.27	305	468	875	735	613	94	24.5
Grass								
Timothy	5.38	290	439	**884**	734	**643**	**98**	**25.7**
Kentucky bluegrass	5.33	**308**	472	856	701	605	93	**25.2**
Tall fescue	**5.52**	**311**	**495**	875	**750**	595	**99**	23.3
Orchardgrass	5.12	**308**	473	**879**	737	**619**	90	24.7
Meadow fescue	4.92	304	467	**888**	**758**	614	**107**	23.3
Meadow bromegrass	**5.86**	**316**	**476**	876	737	612	85	**25.6**
SEM	0.083	1.0	2.5	0.9	1.3	1.6	0.8	0.15
Upper limit[a]	5.48	307	475	877	738	617	96	24.9
Lower limit	5.24	305	467	875	734	613	94	24.5
Overall mean	5.36	306	471	876	736	615	95	24.7

Note: DM, dry matter; ADF, acid detergent fiber; aNDF, neutral detergent fiber assayed with a heat-stable α-amylase; IVTD, in vitro true digestibility of dry matter; NDFd, in vitro aNDF digestibility; TDN, total digestible nutrients; NSC, non-structural carbohydrates (water-soluble carbohydrates plus starch); SEM, standard error of the mean.

[a]Legume or grass values that are greater by more than one-half of the least significant difference of the overall mean are in bold type while those less the same amount are underlined.

the first two post-seeding years might explain the poor persistence of alfalfa and birdsfoot trefoil under grazing at Nappan. In the first post-seeding year, all seeded legume species were present at the three sites. Measurements with the frequency grid technique indicated that at least 27 plants m^{-2} of each species were present (data not shown).

The six grass species in binary mixtures with a legume species also differed significantly for most variates (Table 4). Average forage DM yields of Kentucky bluegrass- and timothy-based mixtures across sites, years, and legume species in the mixture did not differ from those of the other grass-based mixtures [contrast: Kb vs. (Ti + Tf + Or + Mf + Mb), Ti vs. (Tf + Or + Mf + Mb); Table 4]. Forage DM yields of other grass-based mixtures, however, differed with average DM yields being the lowest for the meadow fescue- and orchardgrass-based mixtures and the greatest with the meadow bromegrass- and tall fescue-based mixtures (Table 6). The timothy-based mixtures had lower average ADF and aNDF concentrations and greater TDN concentration than the average of all grass species-based mixtures (Table 6). The Kentucky bluegrass based mixtures had lower IVTD, NDFd, and TDN concentrations than the five other grass species-based mixtures. The meadow fescue-based mixtures had lower concentrations of ADF and aNDF, greater IVTD and NDFd, and greater concentrations of TDN and NSC than the tall fescue based mixtures.

All grass species persisted well at the three sites. Their proportion ranged between 21% and 86% and this proportion was relatively stable in the last three post-seeding years (Table 5). The proportion of timothy and Kentucky bluegrass, although significant, tended to be less than that of other forage grasses in the last three post-seeding years, while that of tall fescue and meadow fescue tended to be greater. All six grass species in mixtures with a legume species can, therefore, tolerate frequent cutting or grazing under the conditions of eastern Canada.

Comparison of the 18 binary legume–grass mixtures

The effects of both legume and grass species in the mixtures were affected by the sites, as indicated by the significant site × legume and site × grass interactions for all variates (Table 4). There was also a significant interaction between grass species and legume species in the mixtures for DM yield, NDFd, and concentrations of aNDF, NSC, and N. The 18 binary legume–grass mixtures were, therefore, compared at each site using PCA with the overall objective of determining the best binary mixture for several forage nutritive attributes and DM yield.

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The first principal component explained 55%, 58%, and 50%, whereas the second component explained 30%, 20%, and 29% of the total variation at Nappan, Lévis, and Normandin, respectively (Fig. 1). The first two principal components, therefore, explained at least 78% of the total variation. At Nappan, the first component was defined mostly by the forage ADF concentration, DM yield, aNDF concentration, and total N concentration on the positive side and by IVTD, NDFd, and concentrations of NSC and TDN on the negative side. At Lévis and Normandin, the first component was mostly defined by concentrations of total N and TDN, DM yield, and IVTD on the positive side and by ADF and aNDF concentrations and NDFd on the negative side. Attributes within the same group on each side were positively correlated, while attributes in opposing groups were negatively correlated.

The first component of the PCA mostly defined differences among grass species in the mixtures at Nappan and differences among legume species in the mixtures at Lévis and Normandin (Fig. 1). At Nappan, Kentucky bluegrass-based mixtures with high ADF concentration along with low IVTD, NDFd, and concentrations of NSC and TDN were opposed to mixtures with timothy and meadow fescue with high IVTD, NDFd, concentrations of NSC and TDN, and low ADF concentration. At Lévis and Normandin, mixtures with alfalfa and birdsfoot trefoil with high concentrations of total N and TDN, and low ADF and aNDF concentrations were opposed to white clover-based mixtures with low concentrations of total N and TDN, and high concentrations of ADF and aNDF.

The PCA confirmed differences among sites in their response to the 18 binary legume–grass mixtures. The legume component in the last three post-seeding years at Nappan was very low, which might explain why the grass species was the main driver of the first component of the PCA at that site. At Lévis and Normandin, the legume proportion was greater than at Nappan and the legume species were the main driver of the first component of the PCA. The main drivers of the second component of the PCA also differed among sites. At Nappan, forage aNDF concentration was opposed to total N concentration while forage DM yield was opposed to IVTD and NSC concentration at Lévis and Normandin.

Forage DM yield was one of the main drivers of the relationship among variates on the first component at Nappan and on the first and second component at Lévis and Normandin. It is an important attribute for ensuring farm profitability and it should be considered along with nutritive attributes in the selection of legume–grass mixtures. At Nappan, the timothy- and meadow fescue-based mixtures had above average nutritive value but they tended to have below average DM yield (Table 7). Conversely, Kentucky bluegrass based mixtures had below average nutritive value and

Fig. 1. Diagram of the first two principal components (PC) of a principal component analysis to illustrate the relationship among forage nutritive attributes [ADF, acid detergent fiber; aNDF, neutral detergent fiber assayed with a heat-stable α-amylase; IVTD, in vitro true digestibility of dry matter; N; NDFd, in vitro aNDF digestibility; NSC, non-structural carbohydrates (water-soluble carbohydrates plus starch); TDN, total digestible nutrients] and dry matter yield averaged across five post-seeding years for 18 legume–grass binary mixtures (A, alfalfa; B, birdsfoot trefoil; C, white clover; Kb, Kentucky bluegrass; Mb, meadow bromegrass; Mf, meadow fescue; Or, orchardgrass; Tf, tall fescue; and Ti, timothy). λ_1 and λ_2 are the contribution of the first and second principal components to the total variation. [Colour online.]

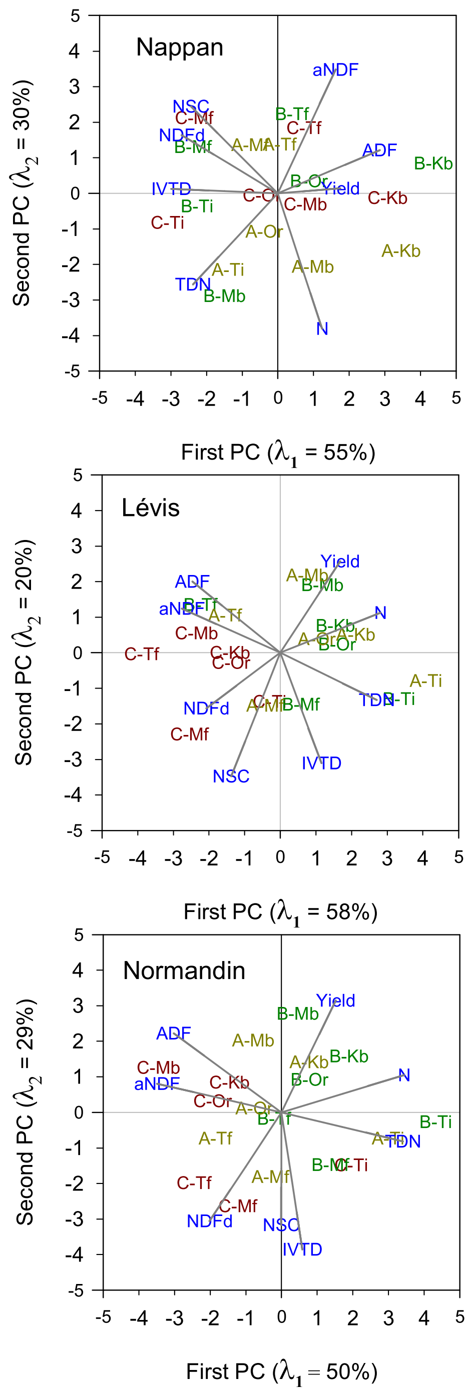

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Table 7. The first two component scores and variate means[a] for the 18 binary legume–grass mixtures sorted according to the first principal component (PC) score and averaged across five post-seeding years (2011–2015) at Nappan.

Mixture	PC 1 (λ_1 = 55%)	PC 2 (λ_2 = 30%)	IVTD (g kg^{-1} DM)	ADF (g kg^{-1} DM)	NDFd (g kg^{-1} aNDF)	NSC (g kg^{-1} DM)	TDN (g kg^{-1} DM)	DM yield (Mg ha^{-1})	aNDF (g kg^{-1} DM)	Total N (g kg^{-1} DM)
B–Kb	**4.36**	0.85	790	**363**	616	76.2	538	7.00	**562**	21.8
A–Kb	**3.45**	−1.61	795	**354**	608	73.8	556	7.07	531	**24.2**
C–Kb	**3.08**	−0.12	797	**356**	630	79.8	548	6.69	550	**23.2**
A–Mb	0.98	−2.06	825	348	658	75.8	580	6.90	525	**24.6**
B–Or	0.87	0.34	820	351	673	82.8	572	6.66	548	21.5
C–Mb	0.77	−0.31	830	**354**	681	82.6	574	7.11	537	22.6
C–Tf	0.73	**1.84**	830	348	**701**	91.2	552	**7.78**	**559**	20.8
B–Tf	0.39	**2.23**	827	349	**699**	91.7	553	7.02	**562**	19.9
A–Tf	0.04	**1.38**	828	344	**691**	93.3	562	7.08	**555**	20.9
A–Or	−0.38	−1.08	830	342	673	82.9	**585**	6.37	532	22.8
C–Or	−0.45	−0.06	827	346	681	86.9	577	5.99	536	21.6
A–Mf	−0.79	**1.36**	834	345	**696**	**95.5**	568	6.59	547	20.2
A–Ti	−1.40	−2.14	**839**	332	679	86.8	**602**	**7.28**	514	**23.4**
B–Mb	−1.50	−2.88	**837**	336	680	84.3	**595**	6.41	499	**24.5**
B–Ti	−2.28	−0.36	**837**	335	**690**	**94.0**	**596**	5.90	528	20.9
C–Mf	−2.36	**2.13**	**841**	341	**708**	**109.4**	570	6.23	539	18.8
B–Mf	−2.39	**1.31**	**840**	340	**706**	**102.7**	571	5.70	532	19.6
W–Ti	−3.10	−0.82	**845**	331	**706**	**96.2**	**605**	6.30	520	21.4
Mean	(0.00)	(0.00)	826	345	676	88.1	572	6.67	538	21.8
SEM	(1.00)	(1.00)	6.4	5.1	8.2	3.63	8.1	0.329	9.8	0.72
LSD (5%)										
Upper limit[b]	1.20	1.21	835	353	688	93.3	584	7.14	552	22.8
Lower limit	−1.21	−1.21	817	338	665	82.9	561	6.20	524	20.8

Note: IVTD, in vitro true digestibility of dry matter; DM, dry matter; ADF, acid detergent fiber; NDFd, in vitro digestibility of NDF; NSC, non-structural carbohydrates (water-soluble carbohydrates plus starch); TDN, total digestible nutrients; aNDF, neutral detergent fiber assayed with a heat-stable α-amylase; B, birdsfoot trefoil; Kb, Kentucky bluegrass; A, alfalfa; C, white clover; Mb, meadow bromegrass; Or, orchardgrass; Tf, tall fescue; Mf, meadow fescue; Ti, timothy; LSD, least significant difference.

[a]Variates were arranged according to their correlation with the first PC score. λ_1 = the contribution of the first principal component to the total variation; λ_2 = the contribution of the second principal component to the total variation.

[b]Mixture values that are greater by more than one-half of the LSD of the overall mean are in bold type while those less the same amount are underlined.

had an average DM yield. Of the 18 binary legume–grass mixtures at Nappan, the alfalfa–timothy mixture is the only one that combined above average DM yield along with above average IVTD, concentrations of TDN and total N, and below average ADF and aNDF concentrations. At Lévis and Normandin, white clover based mixtures had above average ADF and aNDF concentrations and below average DM yield (Fig. 1). Conversely, alfalfa- and birdsfoot-based mixtures had below average ADF and aNDF concentrations and above average total N and TDN concentrations along with greater DM yield. At these two sites, timothy-based mixtures with either alfalfa or birdsfoot trefoil tended to have above average DM yield, IVTD, and concentrations of total N and TDN along with below average ADF and aNDF concentrations (Tables 8 and 9).

The potential milk production per hectare was estimated for the 18 legume–grass mixtures in an effort to integrate both forage DM yield and nutritive value into one variate. Averaged across the three sites and 5 yr, birdsfoot trefoil mixed with either timothy, Kentucky bluegrass, tall fescue, orchardgrass, or meadow bromegrass or alfalfa mixed with either timothy or meadow bromegrass resulted in above average estimated milk production (Table 10). The highest estimated milk production was obtained with birdsfoot trefoil mixed with meadow bromegrass followed by the alfalfa–timothy mixture.

Meadow bromegrass based mixtures were overall the best performing in terms of DM yield (data not shown). The meadow bromegrass based mixtures, however, had above average ADF and aNDF concentrations, average IVTD and NDFd, and below average TDN concentration. Although the nutritive value of meadow bromegrass based mixtures was average, it provided one of the best combinations with alfalfa and birdsfoot

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Table 8. The first two component scores and variate means[a] for the 18 binary legume–grass mixtures sorted according to the first principal component (PC) score and averaged across five post-seeding years (2001–2015) at Lévis.

Mixture	PC 1 (λ_1 = 58%)	PC 2 (λ_2 = 20%)	Total N (g kg^{-1} DM)	aNDF (g kg^{-1} DM)	TDN (g kg^{-1} NDF)	ADF (g kg^{-1} DM)	NDFd (g kg^{-1} aNDF)	DM yield (Mg ha^{-1})	NSC (g kg^{-1} DM)	IVTD (g kg^{-1} DM)
A–Ti	**4.08**	−0.78	**30.5**	382	**663**	278	740	**5.68**	94	**903**
B–Ti	**3.32**	−1.30	**29.3**	401	**664**	278	757	**6.19**	98	**905**
A Kb	**2.11**	0.51	**29.6**	408	**637**	289	732	5.21	97	888
B Or	**1.59**	0.24	**28.3**	427	**642**	295	760	**5.99**	92	**899**
B–Kb	**1.54**	0.77	**28.3**	422	**637**	290	735	5.43	97	885
B–Mb	1.17	**1.91**	**28.5**	442	**633**	**306**	765	**7.01**	84	897
A–Or	1.02	0.39	**27.8**	426	**634**	299	751	5.03	91	896
A–Mb	0.75	2.19	**28.4**	441	621	308	753	**6.34**	85	892
B–Mf	0.57	−1.45	26.6	428	**633**	292	**774**	**5.77**	**104**	**904**
C–Ti	−0.30	−1.37	25.2	425	628	297	759	3.93	**103**	896
A–Mf	−0.43	−1.48	25.1	435	623	296	**770**	4.88	**104**	**902**
C–Or	−1.39	−0.27	25.0	**461**	624	**309**	**778**	4.32	95	897
C–Kb	−1.42	0.01	24.9	**453**	622	**305**	754	3.77	**102**	884
A–Tf	−1.56	1.06	25.1	**465**	601	**305**	758	5.32	100	884
B–Tf	−2.24	**1.35**	24.1	**477**	596	**309**	764	**5.93**	**102**	883
C–Mb	−2.35	0.56	24.6	**477**	611	**320**	**787**	4.76	92	897
C–Mf	−2.55	−2.29	23.1	**461**	614	**305**	**788**	3.93	**110**	**902**
C–Tf	−3.90	−0.03	22.3	**492**	592	**312**	**780**	4.35	**107**	886
Mean	(0.00)	(0.00)	26.5	440	626	300	761	5.21	98	895
SEM	(1.00)	(1.00)	0.29	5.1	3.3	2.4	3.6	0.216	2.1	1.8
LSD (5%)										
Upper limit[b]	1.20	1.21	26.9	447	631	303	767	5.52	101	897
Lower limit	−1.21	−1.21	26.1	433	621	296	756	4.90	95	892

Note: DM, dry matter; aNDF, neutral detergent fiber assayed with a heat-stable α-amylase; TDN, total digestible nutrients; ADF, acid detergent fiber; NDFd, in vitro digestibility of NDF; NSC, non-structural carbohydrates (water-soluble carbohydrates plus starch); IVTD, in vitro true digestibility of dry matter; A, alfalfa; Ti, timothy; B, birdsfoot trefoil; Kb, Kentucky bluegrass; Or, orchardgrass; Mb, meadow bromegrass; Mf, meadow fescue; C, white clover; Tf, tall fescue, SEM, standard error of the mean; LSD, least significant difference.

[a]Variates were arranged according to their correlation with the first PC score. λ_1 = the contribution of the first principal component to the total variation; λ_2 = the contribution of the second principal component to the total variation.

[b]Mixture values that are greater by more than one-half of the LSD of the overall mean are in bold type while those less the same amount are underlined.

trefoil for the estimated milk production. There is limited information in eastern Canada on the alfalfa–meadow bromegrass mixture. In a study with frequent cutting, meadow bromegrass yielded more than meadow fescue in the second and third post-seeding years when they were grown with white clover (Drapeau and Bélanger 2009).

Tall fescue based mixtures also performed well in terms of DM yield but had above average ADF and aNDF concentrations and below average TDN concentration. Meadow fescue based mixtures had below average DM yield over the 5 yr of the study but lower than average ADF concentration and above average IVTD, NDFd, and NSC concentration. Alfalfa and timothy are known to be not well adapted to grazing. Under the conditions of our study, however, the grazing-type alfalfa cultivar performed very well in mixture with timothy.

Limitations and perspectives

This study was not specifically designed to compare the effect of cattle grazing and simulated grazing with frequent cutting but our results indicate that the performance of binary legume–grass mixtures managed under frequent cutting over five post-seeding years seem to differ with cattle grazing. Although it was not measured, our visual observations in the first post-seeding year at Nappan suggest that selective grazing of birdsfoot trefoil and alfalfa might have reduced their persistence and their contribution to forage DM yield in subsequent years. Selective grazing of birdsfoot trefoil over tall fescue has been reported previously (Wen et al. 2004). Our results confirm the importance of evaluating forage mixtures under cattle grazing if those mixtures are to be used mainly for grazing.

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Table 9. The first two component scores and variate means[a] for the 18 legume–grass mixtures sorted according to the first principal component (PC) score and averaged across five post-seeding years (2011–2015) at Normandin.

Mixture	PC 1 (λ_1 = 50%)	PC 2 (λ_2 = 29%)	NDF (g kg^{-1} DM)	N (g kg^{-1} DM)	TDN (g kg^{-1} DM)	ADF (g kg^{-1} DM)	NDFd (g kg^{-1} aNDF)	DM yield (Mg DM ha^{-1})	IVTD (g kg^{-1} DM)	NSC (g kg^{-1} DM)
B–Ti	**4.32**	−0.26	396	**29.0**	**663**	292	726	**4.81**	**895**	76
A–Ti	**2.99**	−0.73	405	**27.5**	**649**	298	719	4.07	**894**	81
C–Ti	**1.96**	−1.48	420	**26.9**	**651**	303	**745**	3.61	**897**	77
B–Kb	**1.89**	1.58	429	**27.0**	**631**	308	710	**4.89**	874	**83**
B–Mf	**1.37**	−1.48	429	**26.6**	**634**	305	**752**	4.23	**899**	**84**
B–Or	0.78	0.92	450	**26.3**	**629**	315	733	**4.66**	888	72
A–Kb	0.77	**1.42**	438	26.1	618	315	699	4.13	871	**86**
B–Mb	0.45	**2.78**	458	**27.0**	620	**329**	730	**5.42**	881	66
B–Tf	−0.21	−0.17	460	25.3	614	311	**755**	**4.64**	890	79
A–Mf	−0.32	−1.81	447	**24.9**	617	312	**746**	3.54	**895**	**91**
A–Or	−0.77	0.12	**461**	25.0	615	**321**	733	3.53	886	76
A–Mb	−0.80	**2.03**	**468**	25.8	607	**333**	727	**4.61**	881	71
C–Mf	1.24	−2.63	460	24.1	617	316	**771**	3.45	**901**	**89**
C–Kb	−1.47	0.85	**477**	24.3	608	**324**	727	3.79	873	**84**
A–Tf	−1.86	−0.73	**475**	23.7	599	318	**753**	3.80	886	**86**
C–Or	−1.94	0.32	**482**	24.2	611	**328**	**750**	3.56	885	70
C–Tf	−2.46	−1.98	**484**	23.0	601	319	**777**	3.79	**895**	**89**
C–Mb	−3.46	**1.26**	**508**	23.3	591	**342**	**755**	4.14	886	68
Mean	(0.00)	(0.00)	453	25.5	621	316	739	4.15	888	79
SEM	(1.00)	(1.00)	5.5	0.37	3.6	2.7	1.8	0.136	2.3	1.3
LSD (5%)										
Upper limit[b]	1.20	1.21	460	26.1	626	320	742	4.34	891	81
Lower limit	−1.21	−1.21	445	25.0	616	312	737	3.95	884	78

Note: NDF, neutral detergent fiber assayed with a heat-stable α-amylase; DM, dry matter; TDN, total digestible nutrients; ADF, acid detergent fiber; NDFd, in vitro digestibility of NDF; IVTD, in vitro true digestibility of dry matter; NSC, non-structural carbohydrates (water-soluble carbohydrates plus starch); B, birdsfoot trefoil; Ti, timothy; A, alfalfa; C, white clover; Kb, Kentucky bluegrass; Mf, meadow fescue; Or, orchardgrass; Mb, meadow bromegrass; Tf, tall fescue; SEM, standard error of the mean; LSD, least significant difference.

[a]Variates were arranged according to their correlation with the first PC score. λ_1 = the contribution of the first principal component to the total variation; λ_2 = the contribution of the second principal component to the total variation.

[b]Mixture values that are greater by more than one-half of the LSD above the overall mean are in bold type while those less the same amount are underlined.

Birdsfoot trefoil and alfalfa persisted well for four post-seeding years under frequent cutting while white clover did not perform well under frequent cutting or rotational grazing. The six grass species persisted well under frequent cutting or rotational grazing. Although the persistence of most legume and grass species was acceptable except for white clover, the productivity and nutritive value of the binary mixtures varied. Meadow bromegrass based binary mixtures were overall the best performing in terms of DM yield. Although the nutritive value of meadow bromegrass based binary mixtures was average, they provided one of the best combinations with alfalfa or birdsfoot trefoil for estimated milk production per hectare. The greatest estimated milk production per hectare was obtained with birdsfoot trefoil mixed with meadow bromegrass (11.23 Mg ha^{-1}) followed by the alfalfa–timothy (10.56 Mg ha^{-1}) and the alfalfa–meadow bromegrass (10.39 Mg ha^{-1}) mixtures (Table 10). The performance of birdsfoot trefoil, grazing-type alfalfa, meadow bromegrass, and timothy in this experiment conducted over five post-seeding years confirms their potential for grazing and frequent cutting.

Although no or little N fertilizer was applied in this study, the forage mixtures performed well and the forage grasses were relatively productive over the 5 yr of the study. The grasses in the binary legume–grass mixtures depended mostly on soil N and on the transfer of N from the legume species. The importance of N fertilization for binary legume–grass mixtures remains, however, poorly understood, primarily when the legume component is declining with time.

This study, conducted at three sites and over five post-seeding years, provides valuable information on the performance of binary mixtures of legume and grass species that are adapted to the cool and humid

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Table 10. Estimated milk production per hectare from the 18 legume–grass mixtures averaged across five post-seeding years (2011–2015) at the three sites.

		Milk production (Mg ha^{-1})			
Legume	Grass	Lévis	Normandin	Nappan	Mean
White clover	Timothy	<u>7.26</u>	<u>6.79</u>	11.15	<u>8.52</u>
White clover	Kentucky bluegrass	<u>6.70</u>	<u>6.55</u>	10.57	<u>8.12</u>
White clover	Tall fescue	<u>7.09</u>	<u>6.35</u>	**12.20**	<u>8.79</u>
White clover	Orchardgrass	<u>7.67</u>	<u>6.19</u>	<u>9.94</u>	<u>8.12</u>
White clover	Meadow fescue	<u>6.79</u>	<u>5.95</u>	<u>10.10</u>	<u>7.91</u>
White clover	Meadow bromegrass	<u>8.23</u>	7.07	**11.83**	9.28
Birdsfoot trefoil	Timothy	**11.87**	**9.28**	<u>10.22</u>	**10.30**
Birdsfoot trefoil	Kentucky bluegrass	**9.94**	**8.84**	10.90	**10.05**
Birdsfoot trefoil	Tall fescue	9.72	**8.02**	10.97	**9.76**
Birdsfoot trefoil	Orchardgrass	**11.15**	**8.41**	10.99	**10.35**
Birdsfoot trefoil	Meadow fescue	**10.39**	7.59	<u>9.25</u>	9.22
Birdsfoot trefoil	Meadow bromegrass	**12.72**	**9.66**	10.90	**11.23**
Alfalfa	Timothy	**10.87**	7.61	**12.75**	**10.56**
Alfalfa	Kentucky bluegrass	9.50	7.24	11.39	9.51
Alfalfa	Tall fescue	8.85	<u>6.37</u>	11.34	<u>9.01</u>
Alfalfa	Orchardgrass	9.14	<u>6.22</u>	10.70	<u>8.85</u>
Alfalfa	Meadow fescue	<u>8.62</u>	<u>6.16</u>	10.72	<u>8.65</u>
Alfalfa	Meadow bromegrass	**11.17**	**7.95**	11.55	**10.39**
Mean		9.32	7.35	10.97	9.37
SEM		0.384	0.244	0.499	0.245
LSD (5%)					
Upper limit[a]		9.87	7.70	11.69	9.72
Lower limit		8.76	7.00	10.25	9.02

Note: SEM, standard error of the mean; LSD, least significant difference.
[a]Mixture values that are greater by more than one-half of the LSD above the overall mean are in bold type while those less the same amount are underlined.

climate conditions of eastern Canada. For logistics reasons, all mixtures were grazed or cut at the same time and the timing of those events was based on timothy reaching a certain height. This approach may have introduced a bias in favor of timothy, the main forage grass species in eastern Canada. More research is required to assess some of those binary mixtures with cutting or grazing based on the development and growth of each mixture.

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