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Complete List of Authors:	Bélanger, Gilles; Agriculture and Agri-Food Canada, Tremblay, Gaëtan; Agriculture and Agri-Food Canada, Quebec Research and Development Centre Papadopoulos, Yousef; Agriculture and Agri-Food Canada, Duynisveld, John; AAFC, Nappan Research Farm Lajeunesse, Julie; Agriculture and Agri-Food Canada, Lafrenière, Carole; Université du Québec en Abitibi-Témiscamingue Station de Recherche Agroalimentaire, Fillmore, Sherry ; Agriculture Agri-food Canada, Kentville RDC
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Yield and nutritive value of grazed complex legume-grass mixtures under increasing N application rates

Gilles Bélanger¹, Gaëtan F. Tremblay¹, Yousef A. Papadopoulos², John Duynisveld³,
Julie Lajeunesse⁴, Carole Lafrenière⁵, and Sherry A.E. Fillmore⁶

¹Agriculture and Agri-Food Canada, Quebec Research and Development Centre, 2560 Hochelaga Blvd., Québec, QC, G1V 2J3 Canada; ²Agriculture and Agri-Food Canada, Faculty of Agriculture, Dalhousie University, PO Box 550, Truro, NS, B2N 5E3 Canada; ³Agriculture and Agri-Food Canada, Kentville Research and Development Centre, Nappan, NS, B0L 1C0 Canada; ⁴Agriculture and Agri-Food Canada, Normandin Research Farm, 1468 Saint-Cyrille St., Normandin, QC, G8M 4K3 Canada; ⁵Université du Québec en Abitibi-Témiscamingue, 79 Côté St., Notre-Dame-du-Nord, QC, J0Z 3B0 Canada; ⁶Agriculture and Agri-Food Canada, Kentville Research and Development Centre, 32 Main St., Kentville, NS, B4N 1J5 Canada.

Corresponding author: gilles.belanger@canada.ca

Abstract

Complex pasture mixtures are advantageous but little information exists on the best forage species and N management in eastern Canada. We compared under mob stocking four complex mixtures of one of two legume species [alfalfa (*Medicago sativa* L.); birdsfoot trefoil (*Lotus corniculatus* L.)] plus one of two grass mixes [#1 – timothy (*Phleum pratense* L.), meadow fescue (*Schedonorus pratensis* (Huds.) P. Beauv.), reed canarygrass (*Phalaris arundinacea* L.), Kentucky bluegrass (*Poa pratensis* L.); #2 – tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.), meadow brome (*Bromus biebersteinii* Roem. & Schult.), reed canarygrass, Kentucky bluegrass] under three N application rates at three sites over the first three post-seeding years. Legume species had little effect on most forage attributes mainly because of their low contribution to forage DM yield (< 30%) in second and third years. The grass mix with tall fescue and meadow brome (#2) yielded similarly or slightly better than the one with timothy and meadow fescue (#1) but tended to have a greater neutral detergent fibre concentration, and lower N and total digestible nutrient concentrations. Nitrogen fertilization increased forage DM yield only in second and third years when the legume contribution to forage DM yield was < 30%, while it increased N concentration and decreased non-structural carbohydrate concentration. These results highlight the challenge of maintaining legume species in rotationally-grazed pastures of eastern Canada and confirm recommendations of applying no or little N fertilizer on grazed legume-grass mixtures when the legume contribution to forage DM yield is > 30%.

Key words: alfalfa, trefoil, grass, forage, nitrogen, grazing

Introduction

Ecological research suggests that plant biodiversity benefits ecosystem functions including primary productivity, stability of production, and resistance of weed invasions in natural grasslands (Sanderson 2005). Similar benefits have been observed in managed grasslands (Frankow-Linberg et al. 2009; Picasso et al. 2011; Finn et al. 2013; Sturludóttir et al. 2013). Consequently, pastures with increased plant species diversity could improve forage yield and yield stability. In studies conducted in Atlantic Canada, more complex mixtures were shown to result in more resilient pastures that were ultimately more productive (McKenzie et al. 2005; McElroy et al. 2012; Papadopoulos et al. 2012).

Recommended forage mixtures in eastern Canada usually contain a maximum of three species, including both grasses and legumes. Designing forage mixtures, particularly complex mixtures, is challenging because several grass and legume species are recommended in eastern Canada. New grass species are now recommended in eastern Canada but their performance when grown with other grass species and a legume species under grazing has not yet been determined. Meadow fescue is not widely used in eastern Canada but it is often seeded with timothy in Scandinavian countries (Virkajärvi 2004). Both timothy and meadow fescue are well adapted to growing conditions in eastern Canada but they are not tolerant to frequent defoliation (Kunelius et al. 2003; Drapeau and Bélanger 2009) and timothy has poor regrowth potential. Tall fescue and meadow brome grass have excellent regrowth potential and good tolerance to frequent cutting (Bélanger et al. 2018).

Alfalfa is a fast growing legume species with a high yield potential but it is sensitive to poor drainage, soil acidity, and winter conditions (Bélanger et al. 2006). As compared with alfalfa, birdsfoot trefoil is a slow growing species with a lower yield potential but with a greater tolerance to poor growing conditions (e.g. soil acidity, poor drainage). Both birdsfoot trefoil and a grazing-type alfalfa ‘AAC Trueman’ (previously referred to as CRS1001) performed well in binary mixtures with a grass species under grazing or frequent cutting (Bélanger et al. 2018).

Very few studies have looked at the fertilizer N requirements of complex mixtures including at least one legume species. In a study conducted in Switzerland, complex legume–grass mixtures fertilized with 50 kg N ha⁻¹ year⁻¹ produced yields comparable to grass monocultures fertilized with 450 kg N ha⁻¹ year⁻¹ when the legume proportion was maintained between 50 to 70% (Nyfeler et al. 2009). The benefits of having several species were reduced at the highest N fertilization rate of 450 kg N ha⁻¹ year⁻¹ with a general trend towards a grass-dominated sward. Current recommendations for N fertilization of legume–grass mixtures in eastern Canada consider the proportion of legumes in the sward and no or little N fertilizer is recommended on grazed legume–grass mixtures as long as the legume contribution to forage DM yield is greater than between 30 and 50%. More specifically, in Ontario, 60 kg N ha⁻¹ year⁻¹ are recommended when the sward has between 33 and 50% legumes but no N fertilizer is recommended for swards containing more than 50% legumes (OMAFRA 2017). In Québec, rates of 75 to 160 kg N ha⁻¹ year⁻¹ are recommended when the sward has less than 40% legumes whereas 0 to 75 kg N ha⁻¹ year⁻¹ are recommended when legumes make up more than 40% of the sward (CRAAQ 2010). In Nova Scotia, only 20 kg N ha⁻¹ year⁻¹ are

recommended in the spring when the sward has more than 30% legumes (PERENNIA 2018).

Legume–grass mixtures and N fertilization can also affect the forage nutritive value. The high concentration of crude protein (CP) in forage legumes and the fast degradation of proteins compared to the amount of fermentable organic matter available in the rumen may lead to inefficient utilization of N by ruminants, resulting in high N losses to the environment mainly under grazing (Kleen et al. 2011). Enhanced levels of readily available energy from forages with a high concentration of water soluble carbohydrates (WSC) and/or non–structural carbohydrates (NSC) improved microbial N synthesis in the rumen (Berthiaume et al. 2010) and the efficiency of N utilization by dairy cows (Miller et al. 2001; Brito et al. 2009). As compared with legume monocultures, mixtures of legume species with grasses have been shown to increase the WSC concentration of forages (Bélanger et al. 2014).

This study focused on four complex mixtures that had previously performed well in a study with grazing or frequent cutting in eastern Canada (unpublished data). The four complex mixtures included grass species with either slow regrowth/poor tolerance to frequent defoliation or fast regrowth/good tolerance to frequent defoliation along with legume species with either low yield potential/good tolerance to poor growing conditions or high yield potential/poor tolerance to poor growing conditions. The four complex mixtures combining two grass mixes and two legume species were studied under different rates of N fertilization with the assumption that the presence of the legume species will reduce N fertilizer requirements. The objective was to compare the forage

yield and nutritive value of four complex pasture mixtures of one legume and four grasses under varying N fertilization during the first three post-seeding years.

Materials and Methods

This study was conducted at three sites of eastern Canada (Nappan, NS; Normandin, QC; New Liskeard, ON). The initial soil characteristics for the three sites are presented in Table 1. The soils at the three sites differed by their texture, pH, and available soil P and K.

Four complex mixtures were evaluated under three N rates (0, 60, and 120 kg N ha⁻¹ year⁻¹) with half of the N applied in the spring and the second half applied after the second grazing. The N application rates were chosen to represent a wide range of N nutrition, while ensuring that, in some treatments, the benefits of the presence of legumes were maximized. Ammonium nitrate was used at Nappan and New Liskeard, while calcium ammonium nitrate was used at Normandin; the N availability of the two forms of fertilizer is similar.

The four complex forage mixtures were a combination of a grazing-type alfalfa (cv. AAC Trueman; 6 kg seeds ha⁻¹) or birdsfoot trefoil (cv. AC Langille; 6 kg seeds ha⁻¹) with two four-grass species mixes based on either timothy (Tm, cv. Express; 4 kg seeds ha⁻¹) and meadow fescue (Mf, cv. Pradel; 7 kg seeds ha⁻¹) or tall fescue (Tf, cv. Courtenay; 6 kg seeds ha⁻¹) and meadow brome (Mb, cv. Fleet; 5 kg seeds ha⁻¹). AAC Trueman is characterized by its unique rhizomatous growth habit and is late-flowering, winterhardy, and tolerant to mid-summer drought, spring and fall water-logging, and frequent grazing. Reed canarygrass (Rc, cv. Venture; 2 kg seeds ha⁻¹) and

Kentucky bluegrass (Kb, cv. Troy; 3 kg seeds ha⁻¹) were included in both grass mixes. Their roles to enhance sward productivity were clearly demonstrated in a previous study (Papadopoulos et al. 2012). The two grass mixes were referred to in the tables as TmMfRcKb and TfMbRcKb. The four species of each grass mix were seeded in equal rates with an overall seeding rate of 1000 seeds m⁻².

The 12 treatments, within a factorial arrangement of 3 × 4, were replicated three times at each site within a split-plot design with three N application rates as main plots and four complex mixtures combining two legume species with two grass mixes as subplots. The N application rates were latinised so that that not all N application rates appear in one end of all blocks. Phosphorus and potassium were applied at the beginning of each growing season based on soil test and local recommendations. Plots were seeded in the spring of 2013. Dates of N fertilizer application, soil sampling, grazing events, and botanical separation are presented in Table 2.

Mob stocking, that is, grazing of plots with animals in 1 or 2 days was used at the three sites. Mob stocking is a useful technique for evaluating in small experimental plots new species and germplasms intended for grazing (McCartney and Bittman 1994; Papadopoulos et al. 2012). The number of animals varied as a function of the forage DM yield at the time of grazing with the objective of grazing the plots in 1 or 2 days. Growing beef steers, beef cows with their calves, and heifers were used at Nappan, Normandin, and New Liskeard, respectively. Due to the plot size, fencing of the subplots was not practical and all mixtures were grazed at the same time at each site when timothy reached about 33 cm in height. Grazing events lasted a maximum of two days. The exit grazing height was 4-5 cm in the initial rotation grazing and 10 cm in subsequent grazing

cycles. Mob stocking at a high stocking rate was used to help achieve quickly a uniform exit grazing height.

At each grazing event, DM yield was measured prior to cattle entering to graze by sampling every plot at a 5-cm height with two quadrats of 0.25 m² in area. Forage samples were dried at 55°C for at least 72 hours prior to recording the sample dry weight and then ground for the analysis of nutritive value. Seeded grasses, seeded legumes, and weeds were manually separated twice during the season generally at the first and third grazing, except at Nappan in 2016 when it was done once at the second grazing and at New Liskeard in 2015 when it was done at three grazing events (Table 2). One forage sample was taken at a 5-cm height in each plot from a 0.25-m² quadrat; each component was dried at 55°C in a force-draft oven for 72 hours and weighed to determine their proportion assessed as their contribution to DM yield.

All forage samples collected from the three years at the three sites (n = 991) were scanned by visible near infrared reflectance spectroscopy (VNIRS) using a Foss NIR System 6500 monochromator instrument (Foss NIRSystems Inc., Silver Spring, MD). From VNIRS sample scans, a set of 356 forage samples (285 for calibration and 71 for validation) was selected by the WinISI software version 4.5.0.1407 (Infrasoft International, LLC, Silver Spring, MD). This set of forage samples were chemically analyzed for the concentrations of dry matter (DM), ash, acid detergent fibre (ADF), neutral detergent fibre assayed with a heat-stable α -amylase (aNDF), nitrogen (N), neutral detergent insoluble crude protein (NDICP), ether extract, WSC, and starch, as well as for the *in vitro* neutral detergent fibre digestibility (NDFd).

Dry matter and ash concentrations were determined by thermogravimetry (Leco Corporation, 2009) using an auto-analyser (model TGA701, Leco Corporation, St. Joseph, MI, USA). Nitrogen 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% H₂O₂. 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 N with the method 13-107-06-2-D and P with the method 13-115-01-2-A (Lachat, 2013, Zellweger Analytics, Lachat Instruments, Milwaukee, WI). The ADF concentration was determined according to AOAC (1990). The 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, NY, USA). The concentration of NDICP was determined according to Licitra et al. (1996). The NDFd 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, NY, USA). 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 10200 kg milk yr⁻¹. The NDFd (g kg⁻¹ aNDF) was calculated as below:

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

Crude fat (ether extract) was determined using Ankom xt15 Extractor Technology Method (AOCS, 2003). Concentrations of WSC and starch were measured according to dos Passos Bernardes et al. (2015) and the concentration of NSC was calculated as the sum of WSC and starch concentrations. Using concentrations of ash, N, ADF, aNDF, NDFd, NDICP, and EE, concentration of total digestible nutrients (TDN) was calculated based on NRC (2001) using the Excel spreadsheet Milk2013 of the University of Wisconsin Alfalfa/Grass Evaluation System (Undersander et al. 2013) for all chemically analyzed forage samples.

Results from chemical analyses and calculations for the calibration set of samples were then used to develop calibration equations using the WinISI 4 software for the following nutritive attributes: N, aNDF, NDFd, NSC, and TDN. Calibration equations were validated using the same software by comparing predicted against reference laboratory values obtained for the 71 validation samples. The VNIRS predictions were considered excellent with the ratio of prediction to deviation (RPD = ratio of standard deviation of the reference data used in the validation set to standard error of prediction corrected for bias) values greater than 3, ranging from 3.03 to 6.63 among all nutritive attributes (Table 3). Using the modified PLSR method of the WinISI 4 software, predicted values of each nutritive attribute were then generated for all forage samples.

Soil samples (0 – 15 cm) were taken in each plot prior to the N application in the first, second, and third post-seeding years except at Nappan in the first post-seeding year when soil sampling was done 13 d after the N application (Table 2). A second set of soil samples were taken in late summer or early fall in the three years. The soil N-NH₄ and N-NO₃ concentrations were determined by extraction with 1 mol L⁻¹ KCl using a 1:25

material/extractant by weight ratio. Soil samples taken prior to the N application in the spring of the first post-seeding year were used to determine the initial values of soil mineral N (N-NH_4 and N-NO_3) at each site. In the second and third post-seeding years, however, soil sampling prior to the N application in spring reflected the residual soil mineral N ($\text{N-NH}_4 + \text{N-NO}_3$) in each of the plots following the fertilization treatments in the previous year.

Data were assessed by analyses of variance (ANOVA) using the GENSTAT 17 statistical software (VSN International 2013). The data were analyzed for each site and post-seeding year with blocks, main plots, and sub plots considered random effects, while N application rates, legume species, and grass mixes were considered fixed effects. To provide more general information, the data were also analyzed with combining the three sites in each post-seeding year with sites as a random factor, and with combining both sites and post-seeding years with sites as a random factor and post-seeding years as a repeated measure. Differences were considered significant when $P < 0.05$. Seasonal values of DM yield were calculated as the sum of the DM yield at each grazing. Seasonal values of the nutritive attributes were calculated as the average of their values at each grazing. Seasonal values were reported with the objective of presenting and discussing the overall response over the three years of the study.

Results and Discussion

There were very few significant interactions between factors (N application rate, legume species, and grass mix) for seasonal DM yield and nutritive attributes (Tables 4 and Tables 6–11). When they occurred, the interactions were more related to the size of

the differences among treatments than the direction of the differences. Consequently, the main effects of any of the three factors were considered stable across the other factors at all sites and post-seeding years.

Seasonal forage DM yield

Increasing N application rates did not significantly increase seasonal forage DM yield at any of the three sites in the first post-seeding year but significantly increased the forage DM yield at New Liskeard in the second post-seeding year and at Normandin and New Liskeard in the third post-seeding year (Table 4). This positive response to N fertilization was quite limited in the second post-seeding year but was more important in the third post-seeding year. In the second post-seeding year at New Liskeard, forage DM yield with no applied N was 89% of the maximum forage DM yield that was obtained with 60 kg N ha⁻¹. With 120 kg ha⁻¹, N was in excess of the crop N demand as there was no increase in yield compared to 60 kg N ha⁻¹. In the third post-seeding year, forage DM yield with no applied N was 69% of the maximum forage DM yield obtained with 120 kg N ha⁻¹ at Normandin and 79% at New Liskeard. Although the response was not statistically significant at Nappan, forage DM yield with no applied N was around 85% of the maximum forage DM yield obtained with 60 kg N ha⁻¹ in the second and third post-seeding years. Furthermore, there was no interaction between N application rate and any of the other two factors (legume species and grass mix), except at New Liskeard in the third post-seeding year where the response to N application rate was greater for the tall fescue-meadow brome-grass-based mixtures (7.4 to 10.0 Mg DM ha⁻¹) than for the timothy-meadow fescue-based mixtures (6.3 to 7.1 Mg DM ha⁻¹). Averaged across the

three sites, applying 60 kg N ha⁻¹ compared with no applied N significantly increased forage DM yield from 6.9 to 7.7 Mg ha⁻¹ in the second post-seeding year and from 5.3 to 5.8 Mg ha⁻¹ in the third post-seeding year.

The lack of N response in the first post-seeding year could be attributed to the significant contribution of the legume species to forage DM yield. In the first post-seeding year, alfalfa contributed 38–49% to forage DM yield, while birdsfoot trefoil contributed 18–45% to forage DM yield (Table 5). In the second and third post-seeding years, the contribution of the legume species to forage DM yield was less than 30%. The absence of a response to N fertilization in the first post-seeding year suggests that sufficient N was available to the forage mixtures, either from legume N fixation or soil mineral N. Legume N fixation was not measured and, although soil mineral N was measured in the spring of each growing season, it is a poor indicator of N availability for the growing season of forage grasses under the humid conditions of eastern Canada (Ziadi et al. 2000). Our results, however, confirm our hypothesis and results from other studies (Nyfeler et al. 2009) that the presence of legume species reduces N fertilizer requirements of complex forage mixtures.

The legume species did not affect seasonal forage DM yield in any of the post-seeding years at the three sites (Table 4). The grass mix did not significantly affect forage DM yield in the first post-seeding year at Normandin and Nappan but, at New Liskeard, the tall fescue-meadow brome grass-based mixtures with either alfalfa or birdsfoot trefoil had a greater forage DM yield than the two timothy-meadow fescue-based mixtures (Table 4). The grass mix did not significantly affect forage DM yield at any of the sites in the second post-seeding year. In the third post-seeding year, the two

tall fescue–meadow brome–based mixtures had a greater seasonal forage DM yield than the two timothy–meadow fescue–based mixtures at New Liskeard but there was no significant difference at Nappan and Normandin. Averaged across sites and years, forage DM yield of the two tall fescue–meadow brome–based mixtures tended ($P = 0.07$) to be greater than that of the two timothy–meadow fescue–based mixtures (6.8 vs. 6.5 Mg ha⁻¹).

The choice of grass mixes had a very limited effect on seasonal forage DM yield even though grass species are known to differ in their tolerance to frequent defoliations (Cullen et al. 2006; Drapeau and Bélanger 2009). The grass mix containing timothy and meadow fescue, known to be sensitive to frequent defoliations, performed nearly as well in terms of seasonal forage DM yield as the grass mix with tall fescue and meadow brome grass, known for their greater tolerance to frequent defoliations, over three post-seeding years. In a previous study conducted in eastern Canada, timothy, meadow fescue, tall fescue, and meadow brome grass grown in binary mixtures with a legume species and grazed or frequently cut persisted well over five post-seeding years (Bélanger et al. 2018). Our results confirm that four recommended forage grass species in eastern Canada, including timothy and meadow fescue, perform well under the type of grazing management used in our study and the study of Bélanger et al. (2018), whether they are in binary or complex legume–grass mixtures. The legume species had little effect on forage DM yield.

Contribution of the seeded species to forage DM yield

Legume species

The contribution of the legume species to forage DM yield across sites and grass mixes ranged from 18 to 49% in the first post-seeding year, 1 to 17% in the second post-seeding year, and 2 to 29% in the third post-seeding year (Table 5). The poor contribution of both legume species to forage DM yield in the second and third post-seeding years could be attributed to poor grazing tolerance, poor winter survival (Bélanger et al. 2006), a strong competition from the forage grasses, and/or selective grazing in the first post-seeding year observations (Bélanger et al. 2018). In a recent study conducted in Nova Scotia with the grazing of binary legume–grass mixtures, the low proportion of alfalfa and birdsfoot trefoil after the second post-seeding year was attributed to selective grazing by cattle based on visual observations (Bélanger et al. 2018). Our measurements, however, did not allow for the determination of the exact cause of this reduction in the contribution of the legume species to forage DM yield.

Grass mixes

The contribution of timothy to forage DM yield varied with sites and grass mixes from 14 to 40% in the first post-seeding year, 35 to 70% in the second post-seeding year, and 13 to 59% in the third post-seeding year (Table 5). The contribution of meadow fescue to forage DM yield ranged from 6 to 17%, 5 to 19%, and 10 to 39% in the first, second, and third post-seeding years, respectively. The contribution of meadow fescue to forage DM yield in the timothy–meadow fescue–based mixtures increased in the third post-seeding year at the expense of timothy. Changes in the botanical composition of the sward and the identity of the species with the largest contribution to forage DM yield have previously been reported (Picasso et al. 2011).

The contribution of tall fescue to forage DM yield varied from 14 to 41% in the first post-seeding year, 8 to 72% in the second post-seeding year, and 24 to 49% in the third post-seeding year (Table 5). The contribution of meadow brome grass to forage DM yield ranged from 5 to 30%, 0 to 70%, and 1 to 32% in the first, second, and third post-seeding years, respectively. The contribution of meadow brome grass was low ($< 2\%$) in both second and third post-seeding years at Nappan. Meadow fescue and tall fescue seem to have performed better at New Liskeard than at Normandin, while timothy and meadow brome grass seem to have performed better at Normandin.

Reed canarygrass and Kentucky bluegrass, the two other grasses seeded in the two grass mixes, did not contribute much to forage DM yield in all three post-seeding years at Normandin and New Liskeard but their contributions were greater at Nappan but still below 15% in all three years (Table 5). The contribution of weeds to forage DM yield averaged across mixtures, sites, and years was 11% and was always less than 23%.

Forage nutritive value

Nitrogen concentration

Increasing N application rates significantly increased forage N concentration in all post-seeding years at the three sites (Table 6). Averaged across the three sites, forage N concentration with no N applied divided by that with 120 kg N ha^{-1} was 0.94 in the first post-seeding year, 0.79 in the second post-seeding year, and 0.81 in the third post-seeding year. Averaged across sites and years, forage N concentration increased from 23.9 g kg^{-1} DM with no applied N to 28.2 g kg^{-1} DM with 120 kg N ha^{-1} . This result confirms the greater response of forage N concentration to N application rates in the

second and third post-seeding years than in the first post-seeding year, and the greater benefits of applying N when the contribution of the legume species to forage DM yield is less than 30%. Increases in N concentration of forage grasses with increasing N fertilization have often been reported in eastern Canada (Tremblay et al. 2005; Pelletier et al. 2009; Bélanger et al. 2016).

Forage N concentration was significantly greater with the alfalfa-based mixtures than with the birdsfoot trefoil-based mixtures in the first post-seeding year at New Liskeard (Table 6) and in the second post-seeding year at Nappan. This result could possibly be explained by the greater contribution of alfalfa than birdsfoot trefoil to forage DM yield in the first post-seeding year at New Liskeard (41 vs. 26%; Table 5) and in the second post-seeding year at Nappan (16 vs. 3%; Table 5). Averaged across sites and years, however, forage N concentration of the alfalfa-based mixtures (26.2 g kg⁻¹ DM) and the birdsfoot trefoil-based mixtures (25.9 g kg⁻¹ DM) did not differ significantly.

Forage N concentration of the timothy-meadow fescue-based mixtures was significantly greater than that of the tall fescue-meadow brome grass-based mixtures in the second and third post-seeding years at New Liskeard, and in the second post-seeding year at Normandin, but was significantly less in the first and second post-seeding years at Nappan. Averaged across sites and years, forage N concentration of the tall fescue-meadow brome grass-based mixtures (26.0 g kg⁻¹ DM) and the timothy-meadow fescue-based mixtures (26.2 g kg⁻¹ DM) did not differ significantly.

Neutral detergent fibre (aNDF) concentration

Forage aNDF concentration was not significantly affected by N application rates at any of the sites in the three years of the study (Table 7). The legume species did not significantly affect forage aNDF concentration in the second and third post-seeding years. In the first-post-seeding year, however, the alfalfa-based mixtures had a greater forage aNDF concentration than the birdsfoot trefoil-based mixtures at Normandin but a lower forage aNDF concentration at New Liskeard. These differences in forage aNDF concentration could be due to legume species differences in aNDF concentration or to their relative contribution to forage DM yield. Alfalfa is known to have a greater aNDF concentration than birdsfoot trefoil (Cassida et al. 2000) and this might explain the result at Normandin where the contribution to forage DM yield of the two legume species was similar. Because forage legume species are known to have a lower aNDF concentration than forage grasses, the greater contribution of alfalfa to forage DM yield might explain the lower forage aNDF concentration of the alfalfa-based mixtures at New Liskeard.

The effect of the grass mix on forage aNDF concentration varied with sites and years (Table 7). The timothy-meadow fescue-based mixtures had a greater forage aNDF concentration than the tall fescue-meadow brome grass-based mixtures in the first two post-seeding years at Nappan and in the first post-seeding year at Normandin, but a lower forage aNDF concentration in the three post-seeding years at New Liskeard and in the second post-seeding year at Normandin. Averaged across the three sites, the timothy-meadow fescue-based mixtures had a lower forage aNDF concentration than the tall fescue-meadow brome grass-based mixtures in the second (526 vs. 541 g kg⁻¹ DM) and third (516 vs. 538 g kg⁻¹ DM) post-seeding years.

In vitro neutral detergent fibre digestibility (NDFd)

Increasing N application rates significantly increased forage NDFd at Normandin and New Liskeard in the second post-seeding year and at Normandin in the third post-seeding year (Table 8) but this increase was less than $24 \text{ g kg}^{-1} \text{ aNDF}$. At Nappan in the first post-seeding year, forage NDFd decreased when the N application rate increased from 0 to 60 kg N ha^{-1} but then increased when the N application rate went from 60 to 120 kg N ha^{-1} . Averaged across sites and years, forage NDFd increased from $712 \text{ g kg}^{-1} \text{ NDF}$ with no applied N to $724 \text{ g kg}^{-1} \text{ NDF}$ with an application of 120 kg N ha^{-1} .

The legume species did not significantly affect forage NDFd in any of the three years at the three sites, except in the first post-seeding year at New Liskeard where the alfalfa-based mixtures had a lower forage NDFd than the birdsfoot trefoil-based mixtures (Table 8). The timothy-meadow fescue-based mixtures had lower forage NDFd than the tall fescue-meadow brome-grass-based mixtures in the first post-seeding year at Normandin and in the first and second post-seeding years at New Liskeard, but a greater forage NDFd in the third post-seeding year at New Liskeard. Differences in forage NDFd, however, were less than $22 \text{ g kg}^{-1} \text{ aNDF}$. Across sites and years, forage NDFd of the tall fescue-meadow brome-grass-based mixtures ($721 \text{ g kg}^{-1} \text{ aNDF}$) and the timothy-meadow fescue-based mixtures ($719 \text{ g kg}^{-1} \text{ aNDF}$) did not differ significantly.

Non-structural carbohydrate (NSC) concentration

Increasing N application rates significantly decreased forage NSC concentration in all post-seeding years at the three sites (Table 9), except at Nappan in the first post-seeding year. Differences in forage NSC concentration between no applied N and 120 kg N ha^{-1}

varied across sites from 5.6 to 8.3 g kg⁻¹ DM in the first post-seeding year, 15.4 to 23.5 g kg⁻¹ DM in the second post-seeding year, and 17.7 to 26.3 g kg⁻¹ DM in the third post-seeding year. Averaged across sites and years, the forage NSC concentration decreased from 84.0 g kg⁻¹ DM with no applied N to 68.5 g kg⁻¹ DM with 120 kg N ha⁻¹. This result confirms the greater negative response of forage NSC concentration to N application rates in the second and third than in the first post-seeding year. Both negative effect (Tremblay et al. 2005; Bélanger et al. 2016) or no effect (Pelletier et al. 2009) of increasing N fertilization on NSC or WSC concentration of forage grasses have been reported.

The legume species only significantly affected forage NSC concentration in the first post-seeding year at Normandin where forage NSC concentration was greater in the birdsfoot trefoil-based mixtures than in the alfalfa-based mixtures (Table 9). Averaged across sites and years, however, forage NSC concentration of the alfalfa-based mixtures (75.1 g kg⁻¹ DM) and the birdsfoot trefoil-based mixtures (76.4 g kg⁻¹ DM) did not differ significantly.

The grass mix did not significantly affect forage NSC concentration in the first post-seeding year at any of the sites (Table 9). The timothy-meadow fescue-based mixtures had a significantly greater NSC concentration than the tall fescue-meadow brome-grass-based mixtures in the second post-seeding year at Normandin and in the third post-seeding year at New Liskeard, but a lower NSC concentration the third post-seeding year at Nappan. Across sites and years, forage NSC concentration of the timothy-meadow fescue-based mixtures (76.8 g kg⁻¹ DM) tended ($P = 0.068$) to be greater than that of the tall fescue-meadow brome-grass-based mixtures (74.7 g kg⁻¹ DM).

Total digestible nutrient (TDN) concentration

Increasing N application rates significantly decreased forage TDN concentration at Nappan in the first post-seeding year but increased it at New Liskeard in the second post-seeding year (Table 10). Across sites and years, N application rates did not significantly affect forage TDN concentration. Nitrogen fertilization generally did not affect the nutritive attributes associated with forage digestibility such as TDN concentration and NDFd. The effect of N fertilization on forage DM digestibility is the subject of conflicting reports (Bélanger et al. 2001).

The legume species did not significantly affect forage TDN concentration at most sites and post-seeding years (Table 10). The significant effect of the legume species at Normandin in the first post-seeding year was quite small. The timothy-meadow fescue-based mixtures had a significantly greater TDN concentration than that of the tall fescue-meadow brome-grass-based mixtures in the three post-seeding years at New Liskeard (Table 10) and in the second and third post-seeding years at Normandin, but a lower TDN concentration in the first post-seeding year at Nappan. Across sites, the TDN concentration of the timothy-meadow fescue-based mixtures was significantly greater than that of the tall fescue-meadow brome-grass-based mixtures in the second (606 vs. 590 g kg⁻¹ DM) and third (613 vs. 586 g kg⁻¹ DM) post-seeding years.

The timothy-meadow fescue-based mixtures generally had a lower forage aNDF concentration, and greater forage N and TDN concentrations than the tall fescue-meadow brome-grass-based mixtures in the second and third post-seeding years. This result can be explained by differences in DM yield and the negative relationship between forage

nutritive value and DM yield (Bélanger et al. 2001), to differences in the proportion of grasses and legumes, or to differences in the aNDF concentration of timothy and meadow fescue compared to tall fescue and meadow brome grass. Because the aNDF, N, and TDN concentrations of the individual species in the mixtures were not measured, we cannot conclude with certainty on the reasons for the observed effects.

Soil mineral N

Increasing N fertilization significantly increased the soil mineral N concentration measured in late summer or early fall at all sites in the first and second post-seeding years but only at Normandin in the third post-seeding year (Table 11). This increase was particularly important ($> 39 \text{ mg kg}^{-1}$) at New Liskeard in the first two post-seeding years and at Nappan in the second post-seeding year. Averaged across the three sites, the soil mineral N concentration increased from 10.9 to 21.0 mg kg^{-1} in the first post-seeding year, from 8.8 to 41.2 mg kg^{-1} in the second post-seeding year, and from 13.0 to 23.4 mg kg^{-1} in the third post-seeding year when the N application rate was increased from 0 to 120 kg N ha^{-1} . The soil mineral N concentration in the spring of the second and third post-seeding years was not affected significantly by the N fertilization, and it was less than 20 mg kg^{-1} at Normandin and Nappan and less than 28 mg kg^{-1} at New Liskeard (data not shown). Differences in soil mineral N concentration observed in the late summer or early fall disappeared over the winter either due to crop uptake in late fall or early spring or to N losses to the environment.

The legume species and the grass mix did not significantly affect the soil mineral N concentration in late summer or early fall of the three post-seeding years. Although the

choice of forage species, particularly the presence of legumes with grasses, has been reported to affect soil N mineralizable N (Dhakal and Anowarul Islam 2018) and soil mineral N (Enriquez-Hidalgo et al. 2016; Crème et al. 2016), the differences in the proportion of legumes and grasses in the different mixtures were probably too small to affect the soil mineral N concentration.

Agronomic applications

No or little N fertilization was required to reach maximum forage DM yield when 30% or more legumes were present in the legume–grass mixtures in the second and third post-seeding years. The need for N fertilization in legume–based complex mixtures therefore increases with time as the contribution of the legumes to forage DM yield decreases. The current recommendations in eastern Canada of not applying or applying very little fertilizer N when the contribution of the legumes to forage DM yield is greater than between 30 and 50% was therefore confirmed. Nitrogen fertilization of legume–grass mixtures, when a significant proportion of legumes is present, could increase soil mineral N in late summer or early fall with a corresponding greater risk of N losses to the environment.

Our results also indicate that increasing N fertilization of complex legume-grass mixtures decreases the readily available energy in the form of NSC and increases N concentration of grazed forages with possible negative consequences for N use efficiency by ruminants. Recent studies conducted in eastern Canada have shown that a difference of 10 g NSC kg⁻¹ DM is sufficient to affect milk production and N use efficiency by dairy cows (Brito et al. 2008, 2009). This decrease in forage NSC concentration with

increasing N fertilization combined with an increase in forage N concentration has therefore the potential to reduce animal production and N use efficiency.

The legume species (alfalfa and birdsfoot trefoil) affected seasonal forage DM yield and some nutritive attributes only in the first post-seeding year. This effect of the legume species in the first post-seeding year, however, varied with sites and no general conclusion can be drawn. The absence of an effect of the legume species in the second and third post-seeding years on forage DM yield, most nutritive attributes, and the soil mineral N concentration is not surprising considering the low contribution of the legume species to forage DM yield (< 30%). The low contribution of the two legume species to forage DM yield in the second and third post-seeding years (< 30%) at all sites highlights the challenge of maintaining legume species in the rotationally-grazed pastures of eastern Canada.

The two grass mixes in the complex legume-grass mixtures affected the forage DM yield and some nutritive attributes but mostly at one site. Averaged across the three sites, the tall fescue-meadow brome-grass-based mixtures had a greater seasonal DM yield than the timothy-meadow fescue-based mixtures. This superior performance of the tall fescue-meadow brome-grass-based mixtures, however, was mostly seen at New Liskeard where the contribution of tall fescue to DM yield was greater than at the other sites (32–72% vs. 8–36%). The performance of grass mixes in complex legume-grass mixtures might vary with the soil and climate conditions of different locations.

Our results suggest that forage DM yield and nutritive value are not greatly influenced by the composition of complex grass-legumes mixtures compared to other factors such as N fertilization, sites, and post-seeding years. Consequently, the species composition of

complex forage mixtures might not be a critical factor in ensuring adequate forage productivity under grazing. Our results, however, are based on four forage mixtures made of species that are relatively well adapted to conditions of eastern Canada. Further research is needed to consider a broader range of forage mixtures.

Conclusions

The low contribution of alfalfa and birdsfoot trefoil to forage DM yield of the legume–grass mixtures in the second and third post-seeding years (< 30%) at all sites resulted in very little effect of the legume species on most forage attributes and this highlights the challenge of maintaining legume species in the rotationally-grazed pastures of eastern Canada. Regardless of legume species, the legume–grass mixtures based on tall fescue and meadow brome grass performed similarly or slightly better than the legume–grass mixtures based on timothy and meadow fescue in terms of seasonal forage DM yield but they tended to have a greater forage aNDF concentration, and lower forage N and TDN concentrations. Our results confirm the current recommendations in eastern Canada of not applying or applying very little N fertilizer on grazed legume–grass complex mixtures as long as the contribution of the legumes to forage DM yield is greater than 30%. Increasing the N application rates increased forage N concentration, decreased forage NSC concentration, but had no effect of forage TDN concentration and aNDF digestibility.

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References

- AOAC. 1990. Method 973.18: Determination of acid detergent fiber by refluxing. Official Method of Analysis, 15th edition. AOAC International, Gaithersburg, MC, USA.
- Bélanger, G., Michaud, R., Jefferson, P.G., Tremblay, G.F., and Brégar, A. 2001. Improving the nutritive value of timothy through management and breeding. *Can. J. Plant Sci.* **81**: 577–585.
- Bélanger, G., Castonguay, Y., Bertrand, A., Dhont, C., Rochette, P., Couture, L., Drapeau, R., Mongrain, D., Chalifour, F.-P., and Michaud, R. 2006. Winter damages to perennial forage crops in eastern Canada: Causes, mitigation, and prediction. *Can. J. Plant Sci.* **86**: 33–47.
- Bélanger, G., Castonguay, Y., and Lajeunesse, J. 2014. Benefits of mixing timothy with alfalfa for forage yield, nutritive value, and weed suppression in northern environments. *Can. J. Plant Sci.* **94**: 51–60.

- Bélanger, G., Tremblay, G.F., Bertrand, A., Mongrain, D., Parent, G., Savoie, P., Massé, D., Gilbert, Y., and Babineau, D. 2016. Reed canarygrass crop biomass and silage as affected by harvest date and nitrogen fertilization. *Can. J. Plant sci.* **96**: 413–422.
- Bélanger, G., Tremblay, G.F., Papadopoulos, Y.A., Duynisveld, J., Lajeunesse, J., Lafrenière, C., and Fillmore, S.A.E. 2018. Yield and nutritive value of binary legume-grass mixtures under grazing or frequent grazing. *Can. J. Plant Sci.* **98**: 395–407.
- Berthiaume, R., Benchaar, C., Chaves, A.V., Tremblay, G.F., Castonguay, Y., Bertrand, A., Bélanger, G., Michaud, R., Lafrenière, C., McAllister, T.A., and Brito, A.F. 2010. Effects of non-structural carbohydrate concentration in alfalfa on fermentation and microbial synthesis in continuous culture. *J. Dairy Sci.* **93**: 693–700.
- Bruto, A.F., Tremblay, G.F., Bertrand, A., Castonguay, Y., Bélanger, G., Michaud, R., Lapierre, H., Benchaar, C., Petit, H., Ouellet, D.R., and Berthiaume, R. 2008. Alfalfa cut at sundown and harvested as baleage improves milk yield of late-lactation dairy cows. *J. Dairy Sci.* **91**: 3968–3982.
- Bruto, A.F., Tremblay, G.F., Lapierre, H., Bertrand, A., Castonguay, Y., Bélanger, G., Michaud, R., Benchaar, C., Ouellet, D.R., and Berthiaume, R. 2009. Alfalfa cut at sundown and harvested as baleage increases bacterial protein synthesis in late-lactation dairy cows. *J. Dairy Sci.* **92**: 1092–1107.
- Cassida, K.A., Griffin, T.S., Rodriguez, J., Patching, S.C., Hesterman, O.B., and Rust, S.R. 2000. Protein degradability and forage quality in maturing alfalfa, red clover, and birdsfoot trefoil. *Crop Sci.* **40**: 405–412.
- CRAAQ. 2010. Guide de référence en fertilisation. 2nd ed. (In French.) Centre de Référence en Agriculture et Agroalimentaire du Québec, Québec City, QC.

- Crème, A., Rumpel, C., Gastal, F., de la Luz Mora Gil, M., and Chabbi, A. 2016. Effects of grasses and a legume grown in monoculture or mixture on soil organic matter and phosphorus forms. *Plant Soil* **402**: 117–128.
- Cullen, B.R., Chapman, D.F., and Quigley, P.E. 2006. Comparative defoliation tolerance of temperate perennial grasses. *Grass Forage Sci.* **61**: 405–412.
- dos Passos Bernardes, A., Tremblay, G.F., Bélanger, G., Brégar, A., Seguin, P., and Vanasse, A. 2015. Sugar yield of sweet pearl millet and sweet sorghum as influenced by harvest dates and delays between biomass chopping and pressing. *Bioenerg. Res.* **8**: 100–8.
- Dhakal, D., and Anowarul Islam, M. 2018. Grass-legume mixtures for improved soil health in cultivated agroecosystem. *Sustainability* **10**: 2718.
- Drapeau, R., and Bélanger, G. 2009. Comparison of meadow fescue and meadow brome grass in monoculture and in association with white clover. *Can. J. Plant Sci.* **89**: 1059–1063.
- Enriquez-Hidalgo, D., Gilliland, T.J., and Hennessy, D. 2016. Herbage and nitrogen yields, fixation and transfer by white clover to companion grasses in grazed swards under different rates of nitrogen fertilization. *Grass Forage Sci.* **71**: 559–574.
- Finn, J.A., Kirwan, L., Connolly, J., Sebastià, M.T., Helgadóttir, Á., Baadshaug, O.H., Bélanger, G., Black, A., Brophy, C., Collins, R.P., Čop, J., Dalmanndóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B.E., Ghesquiere, A., Golinska, B., Golinski, P., Grieu, P., Gustavsson, A.-M., Höglind, M., Huguenin-Elie, O., Jørgensen, M., Kadziulienė, Z., Kurki, P., Llurba, R., Lunnan, T., Porqueddu, C., Suter, M., Thumm, U., and Lüscher, A. 2013. Ecosystem function enhanced by

- combining four functional types of plant species in intensively managed grassland mixtures: a 3-yr continental scale field experiments. *J. Appl. Ecol.* **50**: 365–375.
- Frankow-Lindberg, B.E., Brophy, C., Collins, R.P., and Connolly, J. 2009. Biodiversity effects on yield and unsown species invasion in a temperate forage ecosystem. *Ann. Bot.* **103**: 913–921.
- Goering, H.K., and Van Soest, P.J. 1970. Forage Fiber Analysis (apparatus, reagents, procedures and some applications). USDA Agriculture Handbook 379. U.S. Gov. Print Office, Washington, D.C.
- Isaac, R.A., and Johnson, W.C. 1976. Determination of total nitrogen in plant tissue, using a block digester. *J. Assoc. Off. Anal. Chem.* **59**: 98–100.
- Kleen, J., Taube, F. and Gierus, M. 2011. Agronomic performance and nutritive value of forage legumes in binary mixtures with perennial ryegrass under different defoliation systems. *J. Agric. Sci.* **149**: 73–84.
- Kunelius, H.T., Dürr, G.H., McRae, K.B., Fillmore, S.A.E., Bélanger, G., and Papadopoulos, Y.A. 2003. Yield, herbage composition, and tillering of timothy cultivars under grazing. *Can. J. Plant Sci.* **83**: 57-63.
- Leco Corporation, 2009. Moisture and ash determination in flour. Organic application note. <http://www.leco.com/index.php/component/edocman/?view=document&id=193> (accessed 16 May 2017, available after joining the mailing list). Leco Corporation, St. Joseph, MI.
- Licitra, G., Hernandez, T.M., and Van Soest, P.J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.* **57**: 347–358.

- McCartney, D.H., and Bittman, S. 1994. Persistence of cool-season grasses under grazing using the mob-grazing technique. *Can. J. Plant Sci.* **74**: 723–728.
- McElroy, M.S., Papadopoulos, Y.A., and Adl, S. 2012. Effect of plant species diversity and composition on soil organism functional diversity in an establishing pasture. *Canadian Journal of Plant Science*. *Can. J. Plant Sci.* **92**: 687–697.
- McKenzie, D.B., Papadopoulos, Y.A., McRae, K.B., and Butt, E. 2005. Compositional changes over four years for binary mixtures of grass species grown with white clover. *Can. J. Plant Sci.* **85**: 351–360.
- Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: collaborative study. *J. Assoc. Chem. Int.* **85**: 1217–1240.
- Miller, L.A., Moorby, J.M., Davies, D.R., Humphreys, N.D., Scollan, J.C., MacRae, J.C., and Theodorou, M.K. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): Milk production from late-lactation dairy cows. *Grass Forage Sci.* **56**: 383–394.
- NRC. 2001. Nutrient requirements of dairy cattle, 7th rev. ed. National Academic Press, Washington, DC.
- Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., and Lüscher, A. 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *J. Appl. Ecol.* **46**: 683–691.
- OMAFRA. 2017. Agronomy guide for field crops, Publication 811. Ministry of Agriculture, Food and Rural Affairs. Queen's Printer for Ontario. Toronto, ON.

- PERENNIA. 2018. Pasture manual. [Online]. Available from <http://www.perennia.ca/portfolio-items/forages/> [22 November 2018].
- Papadopoulos, Y.A., McKenzie, D.B., McRae, K.B., Clark, E.A., and Charmley, E. 2005. Evaluating the performance of alfalfa cultivars in rotationally grazed pastures. *Can. J. Plant Sci.* **85**: 147–150.
- Papadopoulos, Y.A., McElroy, M.S., Fillmore, S.A.E., McRae, K.B., Duyinsveld, J.L., and Fredeen, A.H. 2012. Sward complexity and grass species composition affects the performance of grass-clover pasture mixtures. *Can. J. Plant Sci.* **92**: 1159–1205.
- Pelletier, S., Tremblay, G.F., Lafrenière, C., Bertrand, A., Bélanger, G., Castonguay, Y., and Roswell, J. 2009. Nonstructural carbohydrate concentrations in timothy as affected by N fertilization, stage of development, and time of cutting. 2009. *Agron. J.* **101**: 1372–1380.
- Picasso, V.D., Brummer, E.C., Liebman, M., Dixon, P.M., and Wilsey, B.J. 2011. Diverse perennial crop mixtures sustain higher productivity over time based on ecological complementarity. *Renew. Agr. Food Syst.* **26**:317–327.
- Sanderson, M.A. 2005. Management of mixed swards in temperate regions. In *Sward dynamics, N-flows and forage utilization in legume-based systems*, eds. M. Wachendorf, A. Helgadottir, G. Parente. Proc. 2nd COST 852 workshop held in Grado, Italy, 10–12 November. p. 95–101.
- Sturludóttir, E., Brophy, C., Bélanger, G., Gustavsson, A.-M., Jørgensen, M., Lunnan, T., and Helgadóttir, Á. 2013. Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. *Grass Forage Sci.* **69**: 229–240.

- Tremblay, G.F., Bélanger, G., and Drapeau, R. 2005. Nitrogen fertilizer application and developmental stage affect silage quality of timothy (*Phleum pratense* L.). Grass Forage Sci. **60**: 337–355.
- Undersander, D., Combs, D., Shaver, R., and Hoffman, P. 2013. University of Wisconsin alfalfa/grass evaluation system – MILK 2013. UW Extension. <http://www.uwex.edu/ces/forage/articles.htm#milk2000> (accessed 2017–05–12).
- Virkajärvi, P. 2004. Growth and utilization of timothy-meadow fescue pastures. Academic dissertation. Department of Applied Biology Publications 19. University of Helsinki, Helsinki.
- VSN International. 2013. GenStat for Windows 17th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk.
- Ziadi, N., Simard, R.R., Allard, G, and Parent, G. 2000. Yield response of forage grasses to N fertilizer as related to spring soil nitrate sorbed on anionic exchange membranes. Can J. Soil Sci. **80**: 203–212

Table 1. Site characteristics.

Information	Nappan	Normandin	New Liskeard
Latitude	45°46'N	48°51'N	47°51'N
Longitude	64°15'W	72°32'W	79°67'W
Elevation (above sea level)	20	137	348
Annual rainfall ^a , mm	916	612	686
Annual temperature ^a , °C	5.8	0.9	2.8
Cumulative growing degree-days (5°C basis) ^a	1718	1359	1618
Soil texture	Silt Loam	Clay Loam	Clay
Soil pH (water) ^{b,c}	5.6	6.2	6.0
Soil organic matter ^{b,c} (%)	3.5	3.8	5.5
Soil N–NO ₃ ^{b,c} , mg kg ^{–1}	7.8	1.7	9.9
Soil N–NH ₄ ^{b,c} , mg kg ^{–1}	6.7	5.6	5.7
Soil available P ^{b,c} , mg kg ^{–1}	79	29	22
Soil available K ^{b,c} , mg kg ^{–1}	147	207	668
Subplot size, m ²	30	15	18

^a30-yr average (1971–2000); http://climate.weather.gc.ca/climate_normals/index_e.html.

^bValues at the start of the experiment in the spring of the first post-seeding year prior to N fertilization (0–15 cm).

^cThe soil pH was determined following the method of McKeague (1978) in a 1:1 soil:water ratio solution. Potassium and P were extracted following the Mehlich III method (Tran and Simard, 1993) and determined by Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES, Optical emission spectrometer, model Optima 4300 DV, Perkin Elmer, Woodbridge, ON, Canada).

Table 2. Dates of N fertilizer applications, soil sampling, grazing events, and botanical separation at the three sites.

Event	Year	Occurrence	Nappan	Normandin	New Liskeard
N application	2014	First	13 June	12 May	17 June
		Second	31 July	29 July	18 July
	2015	First	12 June	4 May	9 June
		Second	8 Aug.	22 July	21 July
	2016	First	11 May	20 May	19 May
		Second	1 Aug.	27 July	13 July
Soil sampling	2014	First	26 June	12 May	21 May
		Second	18 Sept.	26 Aug.	20 Aug.
	2015	First	7 May	4 May	3 June
		Second	29 Sept.	25 Aug.	31 Aug.
	2016	First	n.a.	20 May	10 May
		Second	n.a.	29 Aug.	9 Oct.
Grazing	2014	First	6 June	6 June	6 June
		Second	24 July	25 June	11 July
		Third	16 Sept.	28 July	25 Aug.
		Fourth	-	26 Aug.	-
	2015	First	24 June	16 June	3 June
		Second	30 July	20 July	13 July
		Third	17 Sept.	25 Aug.	17 Aug.
		Fourth	-	-	23 Sept.
	2016	First	15 June	17 June	10 June
		Second	31 Aug.	28 July	12 July
		Third	-	9 Sept.	30 Sept.
		Fourth	-	-	-
Botanical separation	2014	First	24 July	6 June	11 July
		Second	16 Sept.	26 Aug.	25 Aug.
	2015	First	30 July	16 June	3 June
		Second	17 Sept.	25 Aug.	13 July
		Third	-	-	17 Aug.
	2016	First	31 Aug.	16 June	9 June
		Second	-	29 Aug.	15 Sept.

Note: n.a., information not available.

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

Attribute	<i>n</i>	Slope	Mean	SD	SEP	RSQ	SEP(C)	RPD
aNDF, g kg ⁻¹ DM	71	0.99	508	87.2	13.1	0.98	13.2	6.6
N, g kg ⁻¹ DM	71	1.01	23.2	6.08	1.13	0.97	1.13	5.4
NSC, g kg ⁻¹ DM	71	1.03	85	29.5	6.3	0.96	6.3	4.7
NDFd, g kg ⁻¹ aNDF	71	0.94	694	93.8	30.7	0.90	30.9	3.0
TDN, g kg ⁻¹ DM	71	0.99	584	77.5	21.6	0.92	21.7	3.6

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)]; aNDF, neutral detergent fiber assayed with a heat-stable α -amylase; NSC, non-structural carbohydrates (water soluble carbohydrates plus starch); NDFd, *in vitro* aNDF digestibility; TDN, total digestible nutrients.

Table 4. Forage dry matter (DM) yield of complex legume–grass mixtures as influenced by N application rate, legume species, and grass mix in three post-seeding years at three sites. Probability values for the effects of N application rate, legume species, and grass mix along with their interactions are also presented.

Site Year	Forage DM yield (Mg ha ⁻¹)								
	Nappan			Normandin			New Liskeard		
	1	2	3	1	2	3	1	2	3
N application rate									
0	6.9	8.6	4.8	5.1	2.5	2.7	8.4	9.4	6.8
60	7.7	10.2	5.6	5.0	2.6	3.4	8.5	10.5	7.5
120	7.3	9.4	5.4	5.0	2.7	3.9	8.5	10.3	8.6
Legume									
Alfalfa	7.6	9.5	5.4	5.0	2.7	3.4	8.3	10.0	7.8
Birdsfoot trefoil	7.1	9.3	5.2	5.2	2.5	3.2	8.6	10.1	7.4
Grass mix									
TmMfRcKb	7.7	9.5	5.2	5.2	2.4	3.4	7.8	9.9	6.6
TfMbRcKb	7.0	9.3	5.3	4.9	2.7	3.3	9.1	10.2	8.6
						<i>P</i> values			
N application rate (N)	na ^a	ns	ns	ns	ns	0.027	ns	0.002	<0.001
Legume (L)	ns	ns	ns	ns	ns	ns	ns	ns	ns
Grass mix (G)	ns	ns	ns	ns	ns	ns	<0.001	ns	<0.001
N × L	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × G	ns	ns	ns	ns	ns	ns	ns	ns	0.017
L × G	ns	ns	ns	ns	ns	ns	0.020	ns	0.028
N × L × G	ns	ns	ns	ns	ns	ns	ns	ns	0.026

Note: Tm, Timothy; Mf, Meadow fescue; Kb, Kentucky bluegrass; Tf, Tall fescue; Rc, Reed canarygrass; Mb, Meadow brome grass.

^ans, not significant at $P \leq 0.05$.

Table 5. Proportion (%) of the seeded species assessed as their contribution to forage dry matter yield of the complex legume–grass mixtures in the first three post–seeding years at three sites.

		Alfalfa/Birdsfoot trefoil			Timothy/Tall fescue			Meadow fescue/Meadow brome grass			Reed canarygrass			Kentucky bluegrass		
Year		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Legume	Grass	Nappan														
Alfalfa	TmMfRcKb	nd	17	29	nd	43	18	nd	6	10	nd	4	5	nd	3	6
Alfalfa	TfMbRcKb	nd	14	22	nd	18	36	nd	1	2	nd	9	4	nd	15	10
Birdsfoot trefoil	TmMfRcKb	nd	3	5	nd	44	13	nd	5	39	nd	15	2	nd	4	8
Birdsfoot trefoil	TfMbRcKb	nd	3	3	nd	31	35	nd	0	1	nd	10	12	nd	14	7
Normandin																
Alfalfa	TmMfRcKb	44	6	3	39	70	59	6	7	16	2	0	3	0	3	0
Alfalfa	TfMbRcKb	38	4	2	21	8	30	30	70	40	1	1	4	1	1	1
Birdsfoot trefoil	TmMfRcKb	40	4	4	40	63	54	7	8	18	2	1	1	1	2	1
Birdsfoot trefoil	TfMbRcKb	45	3	5	14	8	24	30	69	41	1	1	1	0	2	2
New Liskeard																
Alfalfa	TmMfRcKb	49	6	27	14	35	18	6	17	23	0	0	0	0	6	0
Alfalfa	TfMbRcKb	43	3	15	32	72	40	5	11	12	0	0	0	0	0	0
Birdsfoot trefoil	TmMfRcKb	33	6	12	22	38	25	17	19	32	0	0	0	0	10	0
Birdsfoot trefoil	TfMbRcKb	18	1	2	42	65	49	20	16	22	0	0	0	0	0	0

Note: Tm, Timothy; Mf, Meadow fescue; Kb, Kentucky bluegrass; Tf, Tall fescue; Rg, Reed canarygrass; Mb, Meadow brome grass; nd, not determined.

Table 6. Forage N concentration of complex legume–grass mixtures as influenced by N application rate, legume species, and grass mix in the first three post–seeding years at three sites. Probability values for the effects of N application rate, legume species, and grass mix along with their interactions are also presented.

Site Year	Forage N concentration (g kg ⁻¹ DM)								
	Nappan			Normandin			New Liskeard		
	1	2	3	1	2	3	1	2	3
N application rate									
0	26.6	20.6	18.4	32.8	19.4	20.2	29.5	22.5	24.6
60	25.3	25.0	22.8	32.2	23.4	22.8	31.2	25.2	27.6
120	27.8	28.5	23.7	34.8	26.1	25.3	32.0	27.5	28.6
Legume									
Alfalfa	26.3	25.4	22.1	33.8	22.7	22.4	31.9	25.0	27.1
Birdsfoot trefoil	26.9	24.0	21.2	32.7	23.2	23.2	30.0	25.1	26.9
Grass mix									
TmMfRcKb ^a	25.9	23.6	21.9	33.0	23.4	23.1	31.3	25.5	28.1
TfMbRcKb ^a	27.2	25.8	21.4	33.5	22.4	22.5	30.6	24.6	25.8
	<i>P</i> values								
N application rate (N)	0.001	<0.001	<0.001	0.018	<0.001	<0.001	<0.001	<0.001	<0.001
Legume (L)	ns	0.001	ns	ns	ns	ns	<0.001	ns	ns
Grass mix (G)	0.011	<0.001	ns	ns	0.021	ns	ns	0.023	<0.001
N × L	ns ^a	ns	ns	ns	ns	ns	ns	ns	ns
N × G	ns	ns	ns	ns	ns	ns	ns	ns	ns
L × G	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × L × G	ns	0.001	ns	ns	ns	ns	ns	ns	ns

Note: Tm, Timothy; Mf, Meadow fescue; Kb, Kentucky bluegrass; Tf, Tall fescue; Rc, Reed canarygrass; Mb, Meadow brome grass.

^ans, not significant at $P \leq 0.05$.

Table 7. Forage neutral detergent fiber (aNDF) concentration of complex legume–grass mixtures as influenced by N application rate, legume species, and grass mix in the first three post–seeding years at three sites. Probability values for the effects of N application rate, legume species, and grass mix along with their interactions are also presented.

Site Year	Forage aNDF concentration (g kg ⁻¹ DM)								
	Nappan			Normandin			New Liskeard		
	1	2	3	1	2	3	1	2	3
N application rate									
0	421	568	583	375	523	475	439	516	493
60	445	560	591	388	518	491	434	509	502
120	452	571	600	379	528	495	438	509	503
Legume									
Alfalfa	442	562	584	390	522	494	428	512	504
Birdsfoot trefoil	436	571	598	371	523	480	447	510	501
Grass mix									
TmMfRcKb ^a	456	577	591	386	506	479	423	495	477
TfMbRcKb ^a	423	556	592	374	540	495	451	527	528
	<i>P</i> values								
N application rate (N)	ns ^a	ns	ns	ns	ns	ns	ns	ns	ns
Legume (L)	ns	ns	ns	0.013	ns	ns	0.012	ns	ns
Grass mix (G)	0.002	0.013	ns	<0.001	<0.001	ns	<0.001	<0.001	<0.001
N × L	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × G	ns	ns	0.039	ns	ns	ns	ns	ns	ns
L × G	ns	ns	ns	ns	ns	ns	0.016	ns	ns
N × L × G	ns	ns	ns	ns	ns	ns	ns	0.041	0.017

Note: Tm, Timothy; Mf, Meadow fescue; Kb, Kentucky bluegrass; Tf, Tall fescue; Rc, Reed canarygrass; Mb, Meadow brome grass.

^ans, not significant at $P \leq 0.05$.

Table 8. Forage *in vitro* neutral detergent fiber digestibility (NDFd) of complex legume–grass mixtures as influenced by N application rate, legume species, and grass mix in the first three post–seeding years at three sites. Probability values for the effects of N application rate, legume species, and grass mix along with their interactions are also presented.

Site Year	Forage NDFd (g kg ⁻¹ aNDF)								
	Nappan			Normandin			New Liskeard		
	1	2	3	1	2	3	1	2	3
N application rate									
0	689	706	671	719	760	756	671	737	708
60	665	709	698	727	767	763	685	760	720
120	691	715	667	726	774	780	681	757	708
Legume									
Alfalfa	681	704	671	722	763	766	665	754	708
Birdsfoot trefoil	682	717	686	726	771	766	694	749	717
Grass mix									
TmMfRcKb ^a	676	704	680	719	766	768	677	742	723
TfMbRcKb ^a	687	716	677	729	768	764	682	761	702
	<i>P</i> values								
N application rate (N)	0.025	ns	ns	ns	0.037	0.003	ns	<0.001	ns
Legume (L)	ns ^a	ns	ns	ns	ns	ns	0.007	ns	ns
Grass mix (G)	ns	ns	ns	0.044	ns	ns	0.040	<0.001	0.002
N × L	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × G	ns	ns	ns	ns	ns	ns	ns	ns	ns
L × G	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × L × G	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Tm, Timothy; Mf, Meadow fescue; Kb, Kentucky bluegrass; Tf, Tall fescue; Rc, Reed canarygrass; Mb, Meadow brome grass.

^ans, not significant at $P \leq 0.05$.

Table 9. Forage non-structural carbohydrate (NSC) concentration of complex legume-grass mixtures as influenced by N application rate, legume species, and grass mix in the first three post-seeding years at three sites. Probability values for the effects of N application rate, legume species, and grass mix along with their interactions are also presented.

Site	Forage NSC concentration (g kg ⁻¹ DM)								
	Nappan			Normandin			New Liskeard		
Year	1	2	3	1	2	3	1	2	3
N application rate									
0	79.8	90.6	71.6	72.7	97.8	118.6	58.3	88.9	77.5
60	78.2	79.3	57.5	72.8	81.3	96.7	56.2	81.8	69.7
120	74.2	70.5	52.4	64.4	74.3	92.3	51.5	73.5	59.8
Legume									
Alfalfa	76.3	78.0	58.5	67.4	85.5	101.7	53.5	83.0	69.1
Birdsfoot trefoil	78.6	82.3	62.5	72.5	83.1	103.3	57.1	79.8	68.9
Grass mix (G)									
TmMfRcKb ^a	76.1	79.4	57.1	69.9	93.3	105.3	55.0	82.2	73.1
TfMbRcKb ^a	78.8	80.9	63.8	70.0	75.6	99.7	55.6	80.6	64.8
	<i>P</i> values								
N application rate (N)	ns ^a	<0.001	<0.001	0.012	<0.001	<0.001	0.018	<0.001	<0.001
Legume (L)	ns	ns	ns	0.043	ns	ns	ns	ns	ns
Grass mix (G)	ns	ns	0.024	ns	<0.001	ns	ns	ns	<0.001
N × L	ns	ns	ns	ns	ns	ns	ns	ns	0.046
N × G	ns	ns	ns	ns	ns	ns	ns	ns	ns
L × G	ns	ns	ns	ns	ns	ns	ns	ns	0.007
N × L × G	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Tm, Timothy; Mf, Meadow fescue; Kb, Kentucky bluegrass; Tf, Tall fescue; Rc, Reed canarygrass; Mb, Meadow brome grass.

^ans, not significant at $P \leq 0.05$.

Table 10. Forage total digestible nutrient (TDN) concentration of complex legume–grass mixtures as influenced by N application rate, legume species, and grass mix in the first three post–seeding years at three sites. Probability values for the effects of N application rate, legume species, and grass mix along with their interactions are also presented.

Site Year	Forage TDN concentration (g kg ⁻¹ DM)								
	Nappan			Normandin			New Liskeard		
	1	2	3	1	2	3	1	2	3
N application rate									
0	643	601	578	669	633	633	607	578	599
60	618	603	586	665	624	624	614	588	599
120	630	601	567	667	627	627	612	590	586
Legume									
Alfalfa	627	600	578	663	606	625	614	587	592
Birdsfoot trefoil	633	603	575	671	606	631	608	584	598
Grass mix									
TmMfRcKb ^a	623	596	578	666	622	640	623	599	622
TfMbRcKb ^a	634	607	576	668	591	616	599	572	567
	<i>P</i> values								
N application rate (N)	0.003	ns	ns	ns	ns	ns	ns	0.035	ns
Legume (L)	ns ^a	ns	ns	0.022	ns	ns	ns	ns	ns
Grass mix (G)	0.032	ns	ns	ns	<0.001	0.004	<0.001	<0.001	<0.001
N × L	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × G	ns	ns	ns	ns	ns	ns	ns	ns	ns
L × G	ns	ns	ns	ns	ns	ns	0.018	ns	ns
N × L × G	ns	0.050	ns	ns	ns	ns	ns	ns	0.004

Note: Tm, Timothy; Mf, Meadow fescue; Kb, Kentucky bluegrass; Tf, Tall fescue; Rc, Reed canarygrass; Mb, Meadow brome grass.

^ans, not significant at $P \leq 0.05$.

Table 11. Residual soil mineral N^a in late summer or early fall as affected by N application rate, legume species, and grass mixes in the first three post-seeding years at three sites. Probability values for the effects of N application rate, legume species, and grass mix along with their interactions are also presented.

Site Year	Soil mineral N (N-NO ₃ + N-NH ₄) (mg kg ⁻¹)								
	Nappan			Normandin			New Liskeard		
	1	2	3	1	2	3	1	2	3
N application rate									
0	5.9	7.3	10.4	8.3	6.7	10.1	18.5	12.4	18.6
60	6.5	20.8	10.0	9.6	7.2	11.1	37.2	20.6	17.0
120	8.2	54.0	10.8	11.3	9.1	13.3	54.7	58.5	18.4
Legume									
Alfalfa	7.1	27.1	10.7	9.5	7.8	14.0	35.6	31.8	17.6
Birdsfoot trefoil	6.6	27.6	10.2	10.0	7.5	12.5	38.1	29.1	18.4
Grass mix									
TmMfRcKb ^b	6.7	27.9	10.2	9.1	7.3	13.1	42.9	33.1	16.3
TfMbRcKb ^b	7.0	26.8	10.7	10.4	8.0	13.5	30.7	27.9	19.6
	<i>P</i> values								
N application rate (N)	0.001	<0.001	ns	0.010	0.006	<0.001	0.006	<0.001	ns
Legume (L)	ns ^c	ns	ns	ns	ns	ns	ns	ns	ns
Grass mix (G)	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × L	ns	ns	ns	ns	ns	ns	0.049	ns	ns
N × G	ns	ns	ns	ns	ns	ns	ns	ns	ns
L × G	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × L × G	0.022	0.040	ns	0.042	ns	ns	ns	ns	ns

^aSoil sampling was done to a depth of 20 cm at Nappan and Normandin, and to a depth of 15 cm at New Liskeard.

^bTm: Timothy, Mf: Meadow fescue, Kb: Kentucky bluegrass, Tf: Tall fescue, Rg: Reed canarygrass, Mb: Meadow brome grass.

^cns, not significant at $P \leq 0.05$.