Dietary cation–anion difference of Timothy (*Phleum pratense* L.) as influenced by application of chloride and nitrogen fertilizer

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Abstract

The effectiveness of forages to prevent post-calving hypocalcaemia, when used as a feed source for non-lactating dairy cows, can be predicted by the dietary cation–anion difference (DCAD). Three to four weeks before calving, the ration of non-lactating dairy cows should have a DCAD around −50 $mmol_c\ kg^{-1}$ DM. In an experiment, swards, based on Timothy (*Phleum pratense* L.), were used to (i) evaluate the impact of two types ($CaCl_2$ and NH_4Cl) and four application rates of chloride fertilizer per season (0, 80, 160 and 240 kg Cl ha^{-1}) in combination with two N application rates (70 and 140 kg N ha^{-1}) on mineral concentrations and DCAD in the herbage, and (ii) determine the economically optimal rate of chloride fertilizer (Cl_{op}) for DCAD in herbage. Chloride and N fertilizers were applied in the spring and, after the first harvest in 2003 and 2004 at four locations that differed in K content of their soils. Two harvests were taken during each year. Averaged across N-fertilizer application rates, harvests and locations, the highest rate of chloride fertilizer increased chloride concentration in herbage by 8·5 g kg^{-1} dry matter (DM) and decreased DCAD in herbage by 190 $mmol_c\ kg^{-1}$ DM to values as low as −9 $mmol_c\ kg^{-1}$ DM. Both types of chloride fertilizer had the same effect on chloride concentration and DCAD in herbage and had no effect on DM yield. When no chloride fertilizer was applied on soils with a high content of available K, application of N fertilizer increased DCAD in herbage by 47 $mmol_c\ kg^{-1}$ DM at both harvests. Herbage DCAD was lower in summer than in spring by 47–121 $mmol_c\ kg^{-1}$ DM depending on the location. Application of chloride fertilizer can effectively lower the DCAD of Timothy-based herbages; the economically optimal rate of chloride fertilizer in the spring varied from 78 to 123 kg Cl ha^{-1}, depending on soil K and chloride contents and expected DM yield.

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Introduction

Hypocalcaemia, or milk fever, is a metabolic disorder that affects the milk production and reproductive capabilities of dairy cows. It occurs when calcium (Ca) from the intestinal absorption and bone mobilization is not sufficient to meet the high Ca demands at the beginning of lactation. In the USA, 0·05–0·07 of the dairy cow population are affected at a clinical level while up to 0·66 of multiparous cows are affected at a sub-clinical level (Sanchez, 1999). The likelihood of hypocalcaemia occurring in dairy cows is related to the dietary cation–anion difference (DCAD) of the ration served before calving. The DCAD is commonly calculated with an equation that includes potassium (K) and sodium (Na) cations and chloride (Cl) and sulphur (S) anions, all expressed as $mmol_c\ kg^{-1}$ DM, as follows (Ender *et al.*, 1971):

$$\mathrm{DCAD} = (\mathrm{K}^+ + \mathrm{Na}^+) - (\mathrm{Cl}^- + \mathrm{S}^{2-}). \qquad (1)$$

To prevent hypocalcaemia, the DCAD of rations fed to non-lactating dairy cows 3–4 weeks before calving should be around −50 $mmol_c\ kg^{-1}$ dry matter (DM) (Goff and Horst, 2003). Anionic salts can be added to the ration to lower the DCAD but this practice reduces acceptability of the ration and increases production costs (Schauff *et al.*, 2000). It is possible to add anionic salts to herbage without greatly affecting DM intake but the DCAD of the forage should be less than

250 $mmol_c$ kg^{-1} DM (Horst *et al.*, 1997). This type of herbage is difficult to produce on intensive dairy farms because the soils are often rich in K (Kayser and Isselstein, 2005).

Potassium concentration of the ration may be more important than Ca concentration in determining the susceptibility of dairy cows to hypocalcaemia (Horst *et al.*, 1997); a reduction in plant K concentration results in a reduced DCAD. Potassium concentration of herbage varies among species. In an evaluation of five cool-season grasses, Timothy had the lowest plant K concentration and also the lowest DCAD (Tremblay *et al.*, 2006).

Herbage DCAD can be decreased by increasing the concentration of anions, such as Cl^- and SO_4^{2-}. Chloride is more efficient than sulphate in acidifying blood (Horst *et al.*, 1997). It is soluble, weakly retained in soils and readily available to plant roots (Whitehead, 2000). The amount of Cl absorbed by plant roots depends on the soil Cl content and the rate of application of Cl fertilizer which can increase plant Cl concentration (Henning *et al.*, 1997; Pehrson *et al.*, 1999). In an experiment examining the effects of rates of application of Cl and nitrogen (N) fertilizer of reed canarygrass, Thomas *et al.* (1998b) observed that DCAD in herbage was decreased by application of Cl fertilizer but was not affected by application of N fertilizer.

Nitrogen is not included in the DCAD equation. However, plant concentrations of K and Cl can be influenced by the application of N fertilizer. When high amounts of soil K are available, application of N fertilizer can increase the K concentration of grass herbage but, when soil K supplies are low, K concentration of grass herbage can be decreased by the application of N fertilizer (Horst *et al.*, 1997). Uptake of plant Cl may be decreased by an antagonism with N fertilizer if nitrates are predominant (Glass and Siddiqi, 1985). However, if ammonium is present in large amounts, plant Cl uptake may be increased (Britto *et al.*, 2004).

The effect of growth period on DCAD in herbage depends mainly on the application rates of K and Cl. When fertilizers are applied once at the beginning of the season, mineral supplies will decrease between spring and summer growth. Because the K concentration of herbage is the main determinant of DCAD, a concomitant decrease in K and Cl supply is likely to decrease DCAD in herbage (Thomas *et al.*, 1998a; Tremblay *et al.*, 2006). However, K and Cl concentrations in herbage are not reduced when fertilizers are applied after each harvest (Whitehead, 2000). There are no reports on the variation of DCAD between harvests for forages fertilized with Cl only.

This experiment evaluated herbage samples taken from Timothy-based swards grown on soils that differed in K content. The objectives were to: (i) evaluate the impact of two types ($CaCl_2$ and NH_4Cl) and four application rates of Cl fertilizer per season (0, 80, 160 and 240 kg Cl ha^{-1}) on mineral concentrations and DCAD of herbage; (ii) determine the economically optimal application rate of Cl fertilizer (Cl_{op}) for a given DCAD in herbage; and (iii) evaluate the effect of application rate of N fertilizer (70 and 140 kg N ha^{-1}) on mineral concentrations and DCAD in herbage.

Materials and methods

Three fields on research farms and one field on a private farm were chosen based on their soil K content, in the province of Québec, Canada. Timothy (*Phleum pratense* L., cv. Champ) was sown in 2002 at Sainte-Anne-de-Bellevue (45°24′N, 73°57′W), Normandin (48°51′N, 72°32′W) and Saint-Augustin-de-Desmaures (46°44′N, 71°27′W), and in 1998 at Sainte-Perpétue (46°05′N, 72°28′W). Ten fertilizer treatments were applied (0, 80, 160 and 240 kg Cl ha^{-1} as $CaCl_2$; 160 kg Cl ha^{-1} as NH_4Cl; all combined with 70 or 140 kg N ha^{-1} as NH_4NO_3) in a split application: 0·60 prior to the start of spring growth and 0·40 after the first harvest in 2003 and 2004. Thus, rates of application of Cl fertilizer were 0, 48, 96 and 144 kg ha^{-1} at the start of spring growth and 0, 32, 64 and 96 kg ha^{-1} after the first harvest. Individual plot size was 6 m × 1·5 m. A split–split plot design was used with four replicates per treatment for a total of forty plots at each of the four locations; locations were assigned to main plots, fertilizer treatments to subplots and harvests to sub-sub-plots. No K fertilizer was applied. The proportion of Timothy in the sward was almost 1·00 in both years at Sainte-Anne-de-Bellevue (hereafter called Sainte-Anne), Normandin and Saint-Augustin-de-Desmaures (hereafter called Saint-Augustin). At Sainte-Perpétue, the sward was composed of 0·60 Kentucky bluegrass (*Poa pratensis* L.) and 0·40 Timothy.

To determine DM yield in the spring growth and the summer regrowth of 2003 and 2004, all experimental plots were harvested to a 5-cm height when Timothy reached the late-heading stage, according to Pelletier *et al.* (2006). At Normandin and Sainte-Perpétue, a strip of 6 m × 0·9 m was harvested in each plot with a self-propelled flail forage harvester (Carter MGF Co., Inc., Brookston, IN, USA). At Sainte-Anne and Saint-Augustin, strips of 6 m × 0·6 m were harvested with a REM flail forage harvester (Swift Machine & Welding, Swift Current, SK, Canada). A sample of approximately 500 g was taken from each plot, weighed and then dried at 55°C in a forced-draft oven for 3 d to determine the DM content. Samples were then ground using a Wiley mill (Standard model 3; Arthur H. Thomas Co., Philadelphia, PA, USA) to pass through a 1-mm screen.

Before application of fertilizer, four soil samples (one per replication) were collected in May 2003 at each location. Soil samples were taken from each plot at every harvest in 2003 and 2004, and before application of fertilizer in the spring of 2004. Four cores from the top 20 cm of soil were pooled to obtain one representative sample per plot. Soil samples were air-dried and sieved through a 2-mm screen prior to analysis.

Chemical analyses

Herbage

Nitrogen and K were extracted using a method adapted from Isaac and Johnson (1976). Samples (100 mg) were digested for 45 min at 380°C in a 1·5-mL mixture of selenious and sulphuric acid (1:42) plus 2 mL of 30% H_2O_2. After cooling, the mixture was diluted to 75 mL with deionized water. Chloride was extracted using a method adapted from Liu (1998); samples (250 mg) were mixed with 20 mL of H_2SO_4 (7 mmol L^{-1}) for 60 min, centrifuged at 32 570 × ***g*** for 30 min, and the Cl concentration was determined in the supernatant. Extraction of S followed a method adapted from Mills and Jones (1996). Three millilitres of HNO_3 were added to 350-mg samples in digestion tubes that were then covered with perforated aluminium foil, placed on a digester block, heated at 120°C for 30 min, and cooled for 2 min before adding 3 mL of 30% H_2O_2. Tube contents were again digested for 15 min using the same procedure. The previous steps were repeated five times until the solution became colourless. Tubes were cooled and the solution was diluted to 40 mL with deionized water. Finally, Na was extracted by dry ashing (Miller, 1998).

A QuikChem 8000 Lachat autoanalyzer was used to measure N with the method 13-107-06-2-E, and S with the method 13-116-10-1-B (Zellweger Analytics, Inc., Lachat Instruments, Milwaukee, WI, USA). A Perkin-Elmer 3300 atomic absorption spectrometer (Perkin-Elmer, Überlingen, Germany) was used to determine K by flame emission and Na by atomic absorption. Chloride was measured with a Dionex DX 500 chromatograph equipped with a ASII HC column (Dionex Corporation, Sunnyvale, CA, USA). The DCAD was then calculated with equation 1 where each element was expressed in $mmol_c\ kg^{-1}$ DM ($mg\ g^{-1}$ DM × 1000 × valence/atomic weight).

Soils

Chloride and S were extracted on air-dried and sieved (2 mm) soil samples. A volume of 20 mL of deionized water was added to 20 g of soil and shaken for 1 h. The solution was centrifuged at 32 570 × ***g*** for 30 min and filtered through filter papers (Whatman 42) previously washed with deionized water to eliminate contamination. The methods of determination for Cl and S were the same as for plant extracts. Potassium and Na were extracted following Mehlich III (Tran and Simard, 1993) and determined with the same methods as for plant extracts. The soil pH was determined following the method of McKeague (1978) in a 1:1 soil:water ratio solution. Soil organic matter content was estimated from organic C concentration [g organic matter kg^{-1} soil = 3·5 + (1·80 × g organic C kg^{-1} soil)] determined using a dry combustion procedure (Nelson and Sommers, 1982) in a combustion furnace (LECO CNS-1000; LECO Corp., St-Joseph, MI, USA). Soil characteristics for each location prior to the onset of the study (spring 2003) are reported in Table 1.

Statistical analyses

Data were analysed by analysis of variance as a split–split plot design with locations as main plots, fertilizer treatments as sub-plots, and harvests as sub-sub-plots. Production years and replicates within locations were considered to be random effects and harvests within years were considered to be repeated measurements; data were analysed using the Mixed procedure (Littell *et al.*, 1996) with the Repeated option of SAS (SAS, 1999). Sources of variation are presented in Table 2. Statistical significance was postulated at $P \leq 0{\cdot}05$ and least square means and the standard error of the mean were calculated.

Calculation of Cl_{op}, plant chloride uptake and chloride leaching losses

The response of DCAD to applied Cl is described by the following polynomial function:

$$Y = a + bX + cX^2, \tag{2}$$

where Y represents the DCAD in $mmol_c\ kg^{-1}$ DM, X is the application rate of Cl fertilizer in kg Cl ha^{-1}, and a, b and c are parameters estimated by multiple linear regression. The economically optimal Cl fertilizer rate (Cl_{op}) was calculated by setting the first partial derivative of this function equal to CP:

$$\frac{dY}{dX} = b + 2cX = \mathrm{CP}, \tag{3}$$

where CP is the ratio of the cost of Cl fertilizer (1·17 CAD $ kg^{-1} Cl) to the economic benefit of having a forage with a low DCAD. The economic benefit per unit of DM yield (59 CAD $ Mg^{-1} DM) was estimated as the cost savings in anionic salts (12·45 CAD $ per cow) divided by the amount of herbage ingested by a cow in the 3 weeks before calving (0·210 Mg DM per cow). Assuming an expected DCAD reduction of

Table 1 Soil characteristics at the four locations at the onset of the study in spring 2003 ($n = 16$).

Soil variables	Locations			
	Normandin	Sainte-Anne	Saint-Augustin	Sainte-Perpétue
Soil texture	Clay loam	Clay loam	Loam	Sand
Element (kg ha^{-1})				
K*	311^{a}†	290^{a}	199^{b}	124^{b}
P	132^{a}	143^{a}	133^{a}	173^{a}
Ca	3709^{b}	2514^{c}	5368^{a}	1713^{d}
Mg	507^{a}	358^{b}	77^{d}	228^{c}
Na	99·5^{b}	70·7^{c}	37·2^{d}	138·7^{a}
Cl	55·3^{a}	10·0^{a}	56·2^{a}	27·7^{a}
S-SO$_4$	15·1^{a}	18·0^{a}	22·3^{a}	15·9^{a}
pH (water)	5·7^{b}	5·3^{b}	6·4^{a}	5·4^{b}
Organic matter (g kg^{-1})	43^{a}	21^{b}	22^{b}	33^{ab}

*Soils at the four locations were, respectively, classified as rich, rich, medium, and medium in K on the following scale: [low (0–100 kg K ha^{-1}), medium (101–200 kg K ha^{-1}), good (201–250 kg K ha^{-1}), rich (251–500 kg K ha^{-1}) and very rich (more than 500 kg K ha^{-1})] (CRAAQ, 2003).
†In the same line, means followed by different letters are different according to the Duncan test ($P < 0{\cdot}05$).

250 mmol$_c$ kg^{-1} DM with Cl fertilization, and an expected annual forage DM yield of 6 Mg ha^{-1}, the economic benefit per unit of DCAD [1·42 CAD \$ (mmol$_c$ kg^{-1} DM)$^{-1}$] was calculated by dividing the economic benefit per ha (59 CAD \$ Mg^{-1} DM × 6 Mg ha^{-1}) by the expected DCAD reduction.

Equation 2 is solved for X which is set equal to Cl$_{op}$:

$$X = \frac{\mathrm{CP} - b}{2c} = \mathrm{Cl}_{\mathrm{op}}. \qquad (4)$$

Plant Cl uptake (kg Cl ha^{-1}) was calculated by multiplying plant Cl concentration and DM yield. Leaching losses of Cl (kg Cl ha^{-1}) were estimated by adding the soil Cl content at the beginning of the growth period to the Cl applied by fertilization, and then subtracting the soil Cl content at the end of the growth period and plant Cl uptake.

Results and discussion

Application of chloride fertilizer

DCAD in herbage decreased with increasing rate of application of Cl fertilizer at both N application rates in both the spring (Figure 1a and d) and the summer (Figure 2a and d) at all locations. This decrease in DCAD can be explained by an increase in plant Cl concentration with increasing rate of application of Cl fertilizer (Figures 1b,e and 2b,e). Both responses of DCAD and Cl concentration in herbage to rate of application of Cl fertilizer were quadratic (Table 2). The decrease in DCAD in herbage with increasing rate of application of Cl fertilizer varied among locations, as indicated by the significant interaction between fertilizer treatments and locations (Table 2; Figures 1a,d and 2a,d). The smallest decrease in DCAD in herbage with increasing rate of application of Cl fertilizer occurred at Normandin (−108 to −155 mmol$_c$ kg^{-1} DM depending on N application rate and harvest) while the largest decrease occurred at Saint-Augustin (−175 to −266 mmol$_c$ kg^{-1} DM). The increase in Cl concentration of herbage with increasing rate of application of Cl fertilizer was smallest at Normandin (+4·4 to +7·8 mg g^{-1} DM) and highest at Saint-Augustin (+10·2 to +12·0 mg g^{-1} DM). Increasing the rate of application of Cl fertilizer increased the concentration of K in herbage (Table 2), mainly from 0 to 48 kg Cl ha^{-1} in spring (Figure 1c and f) and from 0 to 32 kg Cl ha^{-1} in summer (Figure 2c and f). However, this slight increase in K concentration had a limited effect on DCAD in herbage because the increase in Cl concentration in herbage was much greater.

The overall effect of the NH_4Cl fertilizer on DCAD in herbage was similar to that of the $CaCl_2$ fertilizer for applications of 160 kg Cl ha^{-1}; Cl concentrations in herbage were increased and DCAD in herbage was decreased (Table 2). The type of Cl fertilizer significantly affected Cl concentration in herbage at both N application rates but this effect was not of practical importance. Averaged across all locations and harvests, Cl concentrations of herbage were greater with $CaCl_2$ (10·9 mg Cl g^{-1} DM) than with NH_4Cl (10·8 mg Cl g^{-1} DM) at 70 kg N ha^{-1} but, at 140 kg N ha^{-1}, it was

Table 2 Analysis of variance (*F* values) and levels of significance when comparing four locations, ten fertilizer treatments and two harvests of predominantly Timothy swards, for DCAD and mineral concentrations of herbage, DM yield, plant CL uptake and CL-leaching losses in two years.

Sources of variation	df†	DCAD‡	K	Na	Cl	S	N	DM yield	Plant Cl uptake	Cl-leaching losses
Years	1									
Locations (L)	3	190·26***	98·18***	89·42***	19·68***	21·73***	13·19***	9·19***	11·93***	6·15**
Error a	27									
Fertilizer (F)	9	177·61***	8·66***	2·94**	276·43***	4·31***	39·20***	25·61***	179·16***	405·87***
L × F	27	6·22***	3·56***	0·93	6·62***	1·62*	1·51	2·49***	13·27***	3·07***
Error b	252									
Harvests (H)	1	549·86***	587·59***	0·08	5·58*	20·51***	4·33*	899·79***	628·80***	1·11
L × H	3	18·60***	105·41***	47·51***	47·91***	9·64***	85·64***	91·09***	132·18***	7·43***
F × H	9	1·79	1·98*	0·68	2·95**	5·79***	1·90	2·69**	11·98***	1·67
L × F × H	27	0·95	1·02	0·97	1·67*	0·97	0·40	0·80	3·11***	1·78*
Residual error	280									
Location										
Normandin vs. Sainte-Anne			**		**		***		*	
Sainte-Anne vs. Saint-Augustin		***	*	***		***		**		
Saint-Augustin vs. Sainte-Perpétue		***	***	***	***		***	***	***	
Fertilizer treatment										
N1 vs. N2		*			***		***	***	***	***
$CaCl_2$ linear at N1		***	**		***				***	***
$CaCl_2$ linear at N2		***	**		***				***	**
$CaCl_2$ quadratic at N1		***	***	**	***	***			***	***
$CaCl_2$ quadratic at N2		***	***		***	**			***	***
$CaCl_2$ vs. NH_4Cl at N1				***	*					***
$CaCl_2$ vs. NH_4Cl at N2					***				***	***

$^*P \leqslant 0{\cdot}05$; $^{**}P \leqslant 0{\cdot}01$; $^{***}P \leqslant 0{\cdot}001$.

†Degrees of freedom.

‡DCAD calculated with the equation: $(K^+ + Na^+) - (Cl^- + S^{2-})$ (Ender *et al.*, 1971).

greater with NH_4Cl (11·6 mg Cl g^{-1} DM) than with $CaCl_2$ (11·5 mg Cl g^{-1} DM).

Application of Cl fertilizer in the form of $CaCl_2$ or NH_4Cl did not affect DM yield (Table 2). The critical concentration for Cl toxicity varies greatly among plant species (Xu *et al.*, 2000). Most non-woody species, such as Timothy, can tolerate plant Cl concentrations as high as 15–50 mg g^{-1} DM. The highest Cl concentration found in the herbage samples of this experiment was 19 mg g^{-1} DM.

A Cl_{op} was calculated at all locations for both N application rates but only at the first harvest because it represented more than 0·60 of the yield. The Cl_{op} at Sainte-Perpétue was the lowest at both N application rates (82 kg Cl ha^{-1} at 70 kg N ha^{-1} and 80 kg Cl ha^{-1} at 140 kg N ha^{-1}). Estimated soil Cl-leaching losses were high at this location because of the lower yield and Cl uptake potential (Table 4). The second lowest Cl_{op} was at Normandin (78 kg Cl ha^{-1} at 70 kg N ha^{-1} and 96 kg Cl ha^{-1} at 140 kg N ha^{-1}). This can be explained by the high initial soil Cl content (Table 1); plant Cl uptake was the highest and estimated Cl-leaching losses were small (Table 4). The Cl_{op} at Saint-Augustin was high for both N application rates (123 kg Cl ha^{-1} at 70 kg N ha^{-1} and 102 kg Cl ha^{-1} at 140 kg N ha^{-1}). Soil Cl content before the application of Cl fertilizer (Table 1) and Cl uptake throughout the experiment (Table 4) were high at St-Augustin but, contrary to Normandin, Cl_{op} was also high; this may be explained by the higher estimated Cl-leaching losses at Saint-Augustin. The second highest Cl_{op} was at Sainte-Anne (95 kg Cl ha^{-1} at 70 kg N ha^{-1} and 123 kg Cl ha^{-1} at 140 kg N ha^{-1}). At this location, Cl taken up by plants and estimated soil Cl-leaching losses were similar and intermediate among locations.

The Cl_{op} was greater with the highest N-application rate at Normandin and Sainte-Anne, two locations

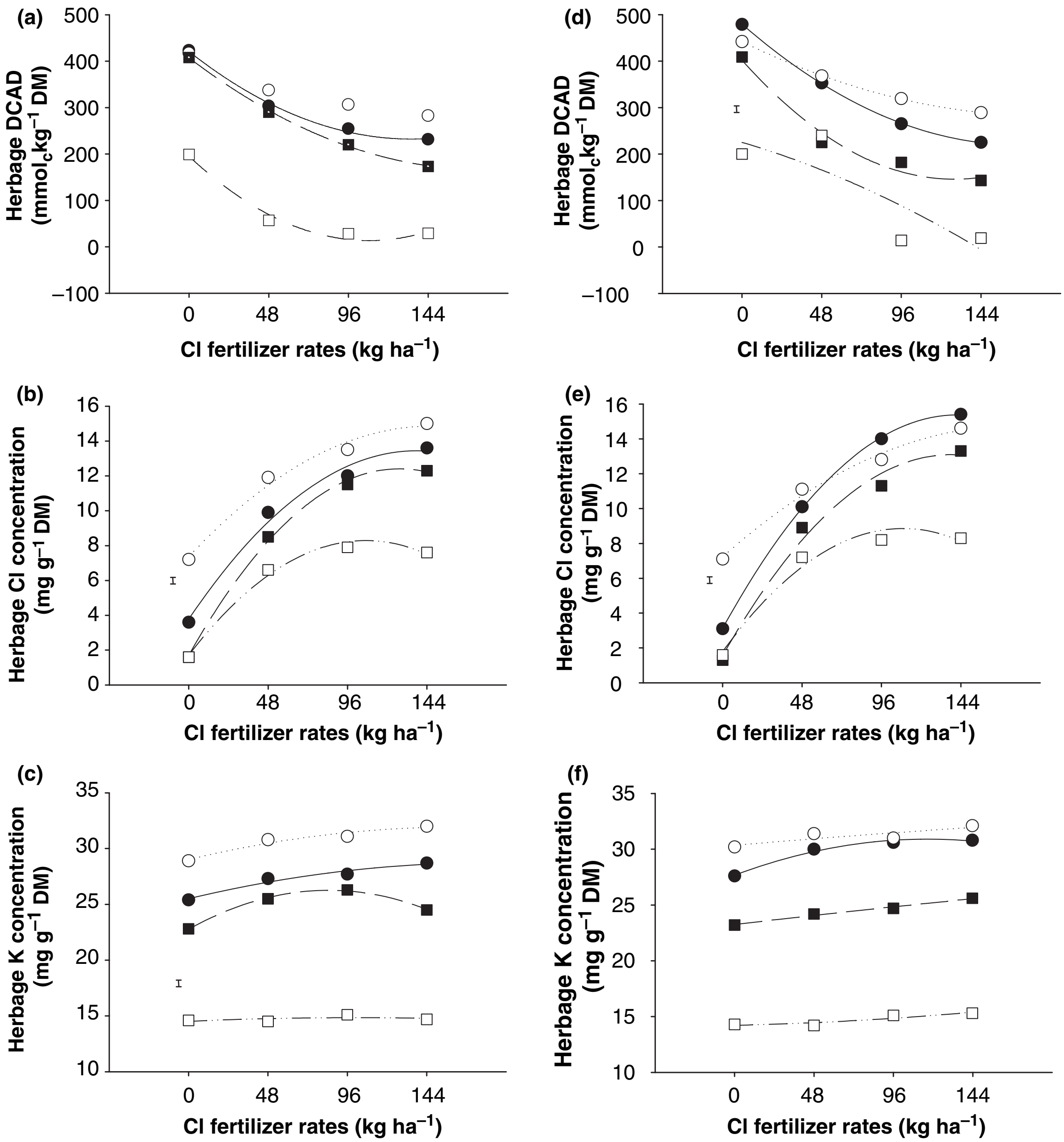

Figure 1 Relationships between dietary cation–anion difference (DCAD) and Cl and K concentrations of herbage of a spring growth of a Timothy-based sward and rate of application of Cl fertilizer applied as $CaCl_2$ at four locations [Normandin (○), soil K content, 311 kg ha^{-1}; Sainte-Anne (●), soil K content, 90 kg ha^{-1}; Saint-Augustin (■), soil K content,199 kg ha^{-1}; and Sainte-Perpétue (□), soil K content, 124 kg ha^{-1}] and with applications of N fertilizer of 70 kg ha^{-1} (a–c) or 140 kg ha^{-1} (d–f). Values are averages of two years, 2003 and 2004. Bars indicate standard error of the mean for the combination of locations and application rates of Cl fertilizer. The fitted quadratic curves have R^2 values of 0·95–0·99, except for the K concentration of herbage at the low (R^2 = 0·30) and high (R^2 = 0·84) application rates of N fertilizer at Sainte-Perpétue, and for the high application rate of N fertilizer at Normandin (R^2 = 0·74).

characterized by high amounts of available soil K; this was primarily related to an increase in K concentration in herbage with higher rates of application of N fertilizer. Thus, under conditions of high available soil K, a higher rate of application of Cl fertilizer might be required to obtain low DCAD values in herbage if a high

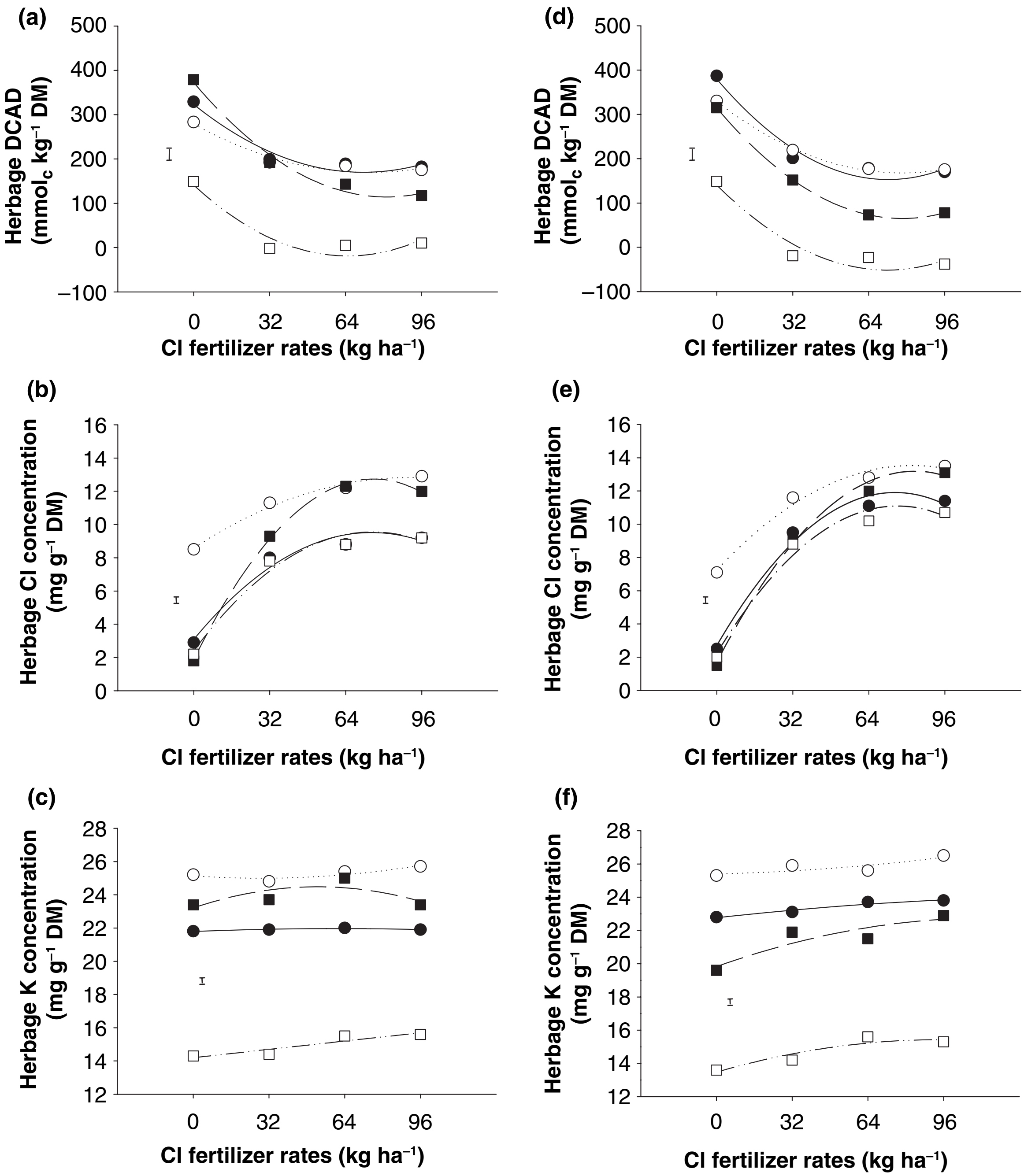

Figure 2 Relationships between dietary cation–anion difference (DCAD) and Cl and K concentrations of herbage of a summer regrowth of a Timothy-based sward and rates of application of Cl fertilizer applied as $CaCl_2$ at four locations [Normandin (○), soil K content, 311 kg ha^{-1}; Sainte-Anne (●), soil K content, 90 kg ha^{-1}; Saint-Augustin (■), soil K content,199 kg ha^{-1}; and Sainte-Perpétue (□), soil K content, 124 kg ha^{-1}] and with applications of N fertilizer of 70 kg ha^{-1} (a–c) or 140 kg ha^{-1} (d–f). Values are averages of two years, 2003 and 2004. Bars indicate standard error of the mean for the combination of locations and application rates of Cl fertilizer. The fitted quadratic curves have R^2 values between 0·80 and 0·99 except for K concentrations of herbage at the low rate of application of N fertilizer at Saint-Augustin ($R^2 = 0{\cdot}56$) and at the high rate of application of N fertilizer at Normandin ($R^2 = 0{\cdot}72$).

Table 3 Concentrations of Na, S and N in herbage from predominantly Timothy swards subjected to ten fertilizer treatments, and harvested at early heading during spring growth and summer regrowth at four locations in Québec, Canada (mean values over two production years, 2003 and 2004).

			Sainte-Anne			Normandin			Saint-Augustin			Sainte-Perpétue		
Harvest	**Cl application (kg Cl ha^{-1})**	**N application (kg N ha^{-1})**	**Na (mg g^{-1} DM)**	**S (mg g^{-1} DM)**	**N (mg g^{-1} DM)**	**Na (mg g^{-1} DM)**	**S (mg g^{-1} DM)**	**N (mg g^{-1} DM)**	**Na (mg g^{-1} DM)**	**S (mg g^{-1} DM)**	**N (mg g^{-1} DM)**	**Na (mg g^{-1} DM)**	**S (mg g^{-1} DM)**	**N (mg g^{-1} DM)**
Spring growth														
NH_4NO_3	0	70	0·01	2·0	15·4	0·03	2·0	23·0	0·03	2·1	17·5	0·16	2·2	21·5
	0	140	0·01	2·2	19·9	0·03	2·1	26·8	0·03	2·4	22·4	0·14	2·1	24·9
$CaCl_2$ +	48	70	0·01	1·9	16·1	0·03	1·9	23·9	0·03	2·1	18·1	0·14	2·1	21·1
NH_4NO_3	96	70	0·01	1·9	15·9	0·03	1·8	23·9	0·04	2·1	18·8	0·20	2·3	20·9
	144	70	0·01	1·9	16·7	0·03	1·9	24·5	0·04	2·0	18·4	0·23	2·3	21·2
	48	140	0·01	2·1	19·7	0·03	2·0	26·3	0·04	2·3	20·3	0·13	2·3	23·2
	96	140	0·01	1·9	19·2	0·03	1·8	26·3	0·04	2·1	20·0	0·17	2·4	23·6
	144	140	0·01	2·1	19·4	0·03	1·9	27·0	0·05	2·2	22·2	0·16	2·3	24·3
NH_4Cl +	96	70	0·01	1·8	16·5	0·03	1·8	24·9	0·03	1·9	19·6	0·12	2·3	20·5
NH_4NO_3	96	140	0·01	2·0	19·2	0·03	2·1	27·2	0·04	2·1	20·4	0·18	2·3	23·6
Summer regrowth														
NH_4NO_3	0	70	0·05	2·4	17·5	0·02	2·0	19·1	0·07	2·7	20·6	0·14	2·6	24·6
	0	140	0·02	2·0	19·3	0·01	1·9	20·5	0·06	2·4	19·8	0·09	2·3	27·7
$CaCl_2$ +	32	70	0·02	2·2	17·9	0·02	2·0	18·6	0·06	2·5	19·0	0·13	2·5	23·5
NH_4NO_3	64	70	0·06	2·1	17·5	0·02	1·9	19·2	0·12	2·4	19·8	0·13	2·4	23·8
	96	70	0·04	1·9	17·2	0·03	1·9	19·3	0·12	2·4	19·1	0·13	2·2	23·9
	32	140	0·03	2·0	20·1	0·02	1·9	20·6	0·09	2·4	21·1	0·09	2·2	27·7
	64	140	0·03	1·9	19·6	0·02	1·9	21·0	0·09	2·3	20·0	0·12	2·2	25·9
	96	140	0·02	1·9	18·8	0·02	1·9	21·0	0·09	2·3	21·1	0·13	2·2	25·8
NH_4Cl +	64	70	0·01	2·0	16·7	0·01	1·8	20·0	0·07	2·3	18·6	0·09	2·5	23·5
NH_4NO_3	64	140	0·01	1·9	20·4	0·01	1·9	21·4	0·07	2·4	20·3	0·11	2·2	26·9
s.e.m.*			0·008	0·06	1·02	0·008	0·06	1·02	0·008	0·06	1·02	0·008	0·06	1·02

*Standard error of the mean allows comparison of values within a column ($n = 8$, df = 280).

Table 4 Soil Cl leaching losses, Cl uptake by plants, and DM yield of a predominantely Timothy sward subjected to ten fertilizer treatments, and harvested at early heading during spring growth and summer regrowth at four locations, Québec, Canada (mean values over two production years, 2003 and 2004).

			Sainte-Anne			Normandin			Saint-Augustin			Sainte-Perpétue		
Harvest	Cl application	N application	Cl losses (kg ha^{-1})	Cl uptake (kg ha^{-1})	DM yield (Mg ha^{-1})	Cl losses (kg ha^{-1})	Cl uptake (kg ha^{-1})	DM yield (Mg ha^{-1})	Cl losses (kg ha^{-1})	Cl uptake (kg ha^{-1})	DM yield (Mg ha^{-1})	Cl losses (kg ha^{-1})	Cl uptake (kg ha^{-1})	DM yield (Mg ha^{-1})
Spring growth														
NH_4NO_3	0	70	−8·6	12·0	3·25	−26·3	31·8	3·87	−18·6	6·7	4·21	1·8	2·7	1·69
	0	140	−8·1	11·8	3·78	−24·5	28·7	3·59	−1·9	6·2	4·77	0·6	2·8	1·77
$CaCl_2$ + NH_4NO_3	48	70	14·4	34·1	3·45	1·0	44·8	3·69	19·8	31·4	3·70	29·2	9·9	1·53
	96	70	51·1	39·1	3·25	14·1	50·3	3·75	32·8	47·0	4·11	52·7	16·0	2·01
	144	70	87·5	43·1	3·17	33·6	55·2	3·66	88·7	50·7	4·21	74·6	12·1	1·65
	48	140	10·9	39·1	3·88	2·2	43·3	3·79	17·1	38·6	4·36	25·2	16·7	2·34
	96	140	36·5	58·9	4·15	23·6	48·8	3·88	48·1	48·3	4·33	49·8	17·5	2·15
	144	140	72·0	63·5	4·13	38·1	54·1	3·78	76·2	54·8	4·25	63·2	20·8	2·70
NH_4Cl + NH_4NO_3	96	70	45·4	49·5	3·71	23·6	53·4	3·88	51·2	45·2	4·07	62·3	12·8	1·83
	96	140	33·2	56·0	4·23	27·5	47·3	3·73	47·7	47·5	4·19	54·5	15·8	1·96
Summer regrowth														
NH_4NO_3	0	70	−1·6	2·7	1·00	−10·1	23·7	2·33	22·3	4·0	2·95	−1·8	2·1	1·07
	0	140	−4·7	4·5	1·90	−10·2	23·5	2·86	4·2	4·9	3·79	−2·1	3·3	1·80
$CaCl_2$ + NH_4NO_3	32	70	15·1	7·9	0·97	9·1	29·4	2·35	11·2	26·8	2·93	25·4	8·6	1·14
	64	70	37·1	9·9	1·07	48·4	33·9	2·47	52·2	33·8	2·80	64·4	10·6	1·28
	96	70	41·8	10·3	1·09	103·2	34·4	2·41	71·1	34·0	3·00	96·5	9·7	1·09
	32	140	5·7	17·8	1·82	5·9	34·6	2·82	−0·7	35·3	3·90	18·4	14·3	1·68
	64	140	19·8	23·2	2·04	40·7	37·0	2·78	27·2	43·6	3·78	51·5	18·8	1·88
	96	140	44·5	20·3	1·72	91·2	37·8	2·68	61·2	48·6	3·86	109·3	21·0	2·02
NH_4Cl + NH_4NO_3	64	70	41·7	10·0	1·05	29·4	31·7	2·40	35·6	30·6	2·81	52·7	8·6	1·10
	64	140	20·9	22·9	1·81	29·1	33·1	2·73	27·1	44·8	3·56	44·2	17·8	1·85
s.e.m.*			8·39	4·11	0·331	9·33	4·11	0·331	8·41	4·11	0·331	8·44	4·11	0·331

*Standard error of the mean allows comparison of values within a column ($n = 8$, df = 280).

N-application rate is used. Differences of Cl_{op} among locations and N-application rates are mainly explained by variations in soil K content and, to a lesser extent, by soil Cl content, yield and uptake potential.

The amount of Cl taken up by plant roots generally reflects the available soil supply (Whitehead, 2000). In the present experiment, plant Cl uptake increased with increasing rate of application of Cl fertilizer but levelled off at the highest application rates of Cl (Table 2). Soil Cl-leaching losses were greatly increased by application of Cl fertilizer (Table 4). The small negative values of estimated leaching losses when no Cl fertilizer was applied are probably due to soil Cl movement or to variations in the laboratory analyses. The movement of Cl within the soil is greatly affected by water fluxes, in particular, the relationship between precipitation and evapo-transpiration (White and Broadley, 2001). Precipitation and evapo-transpiration were not measured in this study but it is reasonable to hypothesize that they partly explain differences in plant Cl uptake and soil Cl-leaching losses across locations.

Although the analysis of variance indicated a significant effect of the rate of application of Cl fertilizer on both Na and S concentrations of herbage with the lower application rate of N, this effect is not of practical importance (Tables 2 and 3). Rate of application of Cl fertilizer had no effect on the N concentration of herbage (Table 2).

Application of nitrogen fertilizer

The effect of rate of application of N fertilizer on DCAD in herbage depended on the soil K content and rate of application of Cl fertilizer (Figures 1a,d and 2a,d). For both harvests, when plots were not fertilized with Cl fertilizer, application of N fertilizer increased the K concentration of herbage where soil K content was high (Normandin, + 0·7 mg K g^{-1} DM; Sainte-Anne, +1·6 mg K g^{-1} DM) and decreased it where soil K content was medium (Saint-Augustin, −2·1 mg K g^{-1} DM; Sainte-Perpétue, −0·5 mg K g^{-1} DM) (Figures 1c,f and 2c,f). Application of N fertilizer, therefore, increased DCAD in herbage at both harvests at locations where soil K content was high (Sainte-Anne: +57 $mmol_c$ kg^{-1} DM; Normandin: +36 $mmol_c$ kg^{-1} DM), decreased it at Saint-Augustin (−31 $mmol_c$ kg^{-1} DM), and had no effect at Sainte-Perpétue. At all locations, DM yield was increased by the application of N fertilizer (Table 4). Thus, without the application of Cl fertilizer and on soils with a medium K content, the application of N fertilizer can increase DM yield and decrease or have no effect on DCAD in herbage. On soils with a high K content, however, there is a risk of increasing DCAD in herbage with increasing rate of application of N fertilizer.

When Cl fertilizer was applied, the application of N fertilizer slightly but significantly increased the Cl concentration of herbage (Table 2; Figures 1b,e and 2b,e) but to a much lesser extent than with the application of Cl fertilizer. An increase in the Cl concentration of herbage with the application of N fertilizer would be expected to cause a decrease in DCAD in herbage but a significant but small increase in DCAD herbage with application of N fertilizer was found. This result can be explained by plant K concentration tending to increase with the application of N fertilizer and, while this effect was not statistically significant, it was sufficient to compensate for the increase in Cl concentration. At all locations, N concentration in herbage increased with application of N fertilizer at both harvests but was not affected by type of Cl fertilizer (Tables 2 and 3).

Locations

For all treatments, the DCAD in herbage was lowest at Sainte-Perpétue and highest at Normandin (Figures 1a,d and 2a,d); this result is explained by the low soil K content at Sainte-Perpétue and the high soil K content at Normandin (Table 1). At Normandin, however, because the soil Cl content was also high, DCAD in herbage was closer to that at Sainte-Anne where the soil K content was lower.

The low soil K content at Sainte-Perpétue was accompanied with a greater soil Na content (Table 1). Some plant species compensate for low soil K availability by absorbing more Na to maintain biophysical functions such as osmoregulation (Peltovuori, 1997). However, the two species grown at Sainte-Perpétue are not known to absorb more Na when K is less available and they usually have a low (Timothy) or low to medium (*Poa pratensis* L.) concentration of Na in herbage (Griffith and Walters, 1966). In the present experiment at Sainte-Perpétue, the concentration of Na was higher and concentration of K in herbage was lower than at the other locations (Table 3; Figures 1c,f and 2c,f). The application of Cl fertilizer at Sainte-Perpétue increased the Na concentration of herbage (Table 3) but did not affect the low K concentration of herbage (Figures 1c,f and 2c,f). This effect was observed at both harvests, although Na concentration in herbage was least at the second harvest (Table 3), probably because soil Na content was depleted by 0·40 (data not shown). The greater Cl uptake would increase the negative ionic charges within the plant and the greater absorption of Na may, in part, be the result of balancing ionic charges. It would be of interest to further investigate the effects of higher Na availability with a low K content in soils on DCAD in herbage and DM yield, especially in combination with application rates of Cl fertilizer.

Harvests

In herbage DCAD values were significantly lower in the second harvest; by 94 $mmol_c\ kg^{-1}$ DM at Sainte-Anne, 121 $mmol_c\ kg^{-1}$ DM at Normandin, 79 $mmol_c\ kg^{-1}$ DM at Saint-Augustin, and 47 $mmol_c\ kg^{-1}$ DM at Sainte-Perpétue. The difference between the two harvests was greater when the DCAD in herbage was high in the first one. This may be due to differences among locations in the rate of depletion in soil K content during the growing season. At Normandin, where the K concentration and DCAD in herbage were highest (Figure 1a, c, d and f) in the first harvest, soil K content was greatly depleted during the summer regrowth (−44 kg K ha^{-1}), and at Sainte-Perpétue, where K concentration and DCAD in herbage was lowest in the first harvest, soil K content was only slightly depleted during the summer regrowth (−6 kg ha^{-1}). At Sainte-Anne and Saint-Augustin, K concentration and DCAD in herbage were intermediate in the first harvest and soil K depletion was also intermediate during the summer regrowth (−14 and −9 kg ha^{-1} respectively). Across all locations, the advantage of a slightly lower DCAD in herbage in the summer regrowth was counterbalanced by a lower DM yield in summer regrowth compared with spring growth (Table 4). Consequently, harvesting the spring growth remains a viable option to produce large amounts of low-DCAD herbage.

Concomitant to the decrease in K concentration of herbage between the first and second harvests, Na concentration in herbage increased at Sainte-Anne and Saint-Augustin (Table 3), indicating that, as previously mentioned, Na may have been absorbed in greater amounts to compensate for the decreasing availability of soil K or to balance ionic charges within plants.

Conclusions

The application of Cl fertilizer increased the Cl concentration and decreased the DCAD of Timothy-based herbage by as much as 266 $mmol_c\ kg^{-1}$ DM with no effect on DM yield. The economically optimal application rate of Cl fertilizer varied among locations (78–123 kg Cl ha^{-1}) and depended primarily on soil K and Cl contents and expected forage DM yield. When applied at an annual application rate of 160 kg Cl ha^{-1}, $CaCl_2$ and NH_4Cl had a similar effect on the Cl concentration and DCAD in herbage. The DCAD values in herbage increased with rate of application of N fertilizer on soils where K was highly available and it was lower in the summer regrowth than in the spring growth.

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