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ARTICLE

Combining perennial grass–legume forages and liquid dairy manure contributes to nitrogen accumulation in a clayey soil

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Abstract: Repeated applications of liquid dairy manure (LDM) and perennial crops generally favor nitrogen (N) stocks in soils, but in ways that may differ with soil type and other management practices. The objective of this study was to assess the long-term (21 yr) changes in soil N stocks (0–50 cm) of a silty clay soil, in a cool humid climate, in response to mineral fertilization (MIN) or LDM, combined with two tillage practices [chisel plow (CP), or moldboard plow (MP)], and two crop rotations [cereal monoculture (monoculture) or cereal–perennial forage rotation (forage-based rotation)]. The forage-based rotation favoured a greater accumulation of N in the first 20 cm of soil (+50 kg N·ha^{-1}·yr^{-1}) when compared with the monoculture. Tillage practices did not impact N stocks in the whole soil profile, but influenced its vertical distribution, with greater accumulation at the surface with CP, and at depth with MP. Annual input of LDM increased N stocks at the surface (0–20 cm) compared with MIN, especially when combined with the forage-based rotation. After 21 yr, soil N stocks (0–50 cm) with LDM were 32% (+2 t N·ha^{-1}) higher in the forage-based rotation than in the monoculture, suggesting better retention and more efficient use of manure-N with perennial forages than cereals. Comparisons between the N mass balance computed for each cropping system, and the changes in soil N stocks indicated that accumulation of N under the forage-based rotation was largely due to symbiotic fixation by legumes in the forage mixture.

Key words: soil nitrogen stock, liquid dairy manure, mineral fertilization, tillage, crop rotation, grass–legume forage mixture.

Résumé : L'apport réitéré de lisier de bovins laitiers (LBL) et la culture de vivaces augmentent généralement les réserves d'azote (N) dans le sol, mais de manières différentes, selon la nature du sol et les autres pratiques agricoles. L'étude devait évaluer la fluctuation à long terme (21 ans) des stocks de N (0–50 cm) dans un sol limono-argileux, en climat frais et humide, consécutivement à l'application d'un engrais minéral (MIN) ou de LBL, combiné à deux modes de travail du sol (labour au chisel (CH) ou avec une charrue à versoirs (LA)) et à deux assolements (monoculture de céréales ou assolement céréale-vivace fourragère). Comparativement à la monoculture, l'assolement fourrager favorise une plus forte accumulation de N dans les 20 premiers cm de sol (+50 kg de N par hectare annuellement). Le travail du sol n'a eu aucune incidence sur les réserves de N dans le profil, mais a influé sur la répartition verticale de l'élément, celui-ci étant plus concentré en surface sous le régime CH et en profondeur sous le régime LA. L'application annuelle de LBL a accru les réserves de N en surface (0–20 cm) comparativement à celle de MIN, surtout avec l'assolement fourrager. Au terme de 21 années, les réserves de N (0–50 cm) après application de LBL étaient de 32 % (+2 t de N par hectare) plus élevées avec l'assolement fourrager qu'avec la monoculture, signe que les vivaces fourragères entraînent une meilleure rétention et une utilisation plus efficace du N du lisier que les céréales. Quand on compare le bilan azoté de chaque système agricole et la fluctuation des stocks de N, on constate que l'accumulation de N dans l'assolement fourrager découle en bonne partie de la fixation symbiotique de l'azote par les légumineuses. [Traduit par la Rédaction]

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Mots-clés : réserve d'azote du sol, lisier de bovins laitiers, engrais minéral, travail du sol, assolement, mélange fourrager graminée-légumineuse.

Introduction

Approximately 180 million tonnes of livestock manure are applied to Canada's agricultural land each year. This represents more than 1 million tonnes of N per year, with 36% of it coming from dairy production (Statistics Canada 2006). Application of livestock manure to crops provides a source of N for plants, reducing the need for mineral fertilizer, and a source of organic matter (Haynes and Naidu 1998; Chantigny et al. 2008; Nyiraneza et al. 2009) that can improve soil physical and biological properties (Bissonnette et al. 2001; Nyiraneza et al. 2010; Blanchet et al. 2016). Although the general impact of repeated applications of livestock manure on the long-term accumulation of organic matter in soil is well documented (Ladha et al. 2011; Maillard and Angers 2014; Triberti et al. 2016), the influence of factors such as soil type and crop management practices on the response to manure application is still poorly documented (Maillard and Angers 2014). In addition, although most studies have examined the impact of management practices on soil carbon (C) stocks, the impact of these practices on soil N stocks has generally been overlooked.

The source and rate of applied N have variable effects on soil C and N stocks, which may depend on the specific soil and climate conditions of each study (Wuest and Gollany 2013; Maillard and Angers 2014; Zhang et al. 2016). Nevertheless, several studies have reported that N stocks increased more in soils receiving N with manure than with mineral fertilizers (e.g., Bittman et al. 2007; Jagadamma et al. 2008; Nyiraneza et al. 2009; Ladha et al. 2011) under a range of soil and climate conditions. A portion of the accumulated N can potentially be released and taken up by subsequent crops (Nyiraneza et al. 2010; Blanchet et al. 2016).

The inclusion of perennial forage species in crop rotations generally has a positive impact on soil C stocks (Aziz et al. 2013; Maillard et al. 2016; Triberti et al. 2016) and may contribute to better N retention in soil (Kaisi et al. 2005*a*; Sainju et al. 2017) due to greater root biomass and more efficient N recovery than annual crops (Fuller et al. 2010). Furthermore, the presence of legumes in perennial forage crop mixtures provides 12–25 million tonnes N annually to agricultural systems at the global scale (Herridge et al. 2008). A portion of N fixed in legume nodules can be transferred to the soil reserve through root exudation (Fustec et al. 2010) and turnover (Rasmussen et al. 2007; Frankow-Lindberg and Dahlin 2013), and thus can promote the accumulation of N in soil (Li et al. 2016; Wu et al. 2017).

Although some studies have shown that no-till and reduced tillage increase soil C and N stocks relative to conventional tillage (Soon and Clayton 2003; Liang et al. 2004; Diekow et al. 2005; Kaisi et al. 2005*b*), other studies have reported that under cool, humid conditions, tillage practices have an effect on the distribution of C and N in the soil profile but not on total stocks (e.g., Angers et al. 1997; Dimassi et al. 2013; Maillard et al. 2016).

The majority of studies available in the literature have examined the effect of isolated management practices, and information on the net effect of combined practices is scarce. Multifactorial studies are required to better understand and disentangle the combined effects of varying practices in different cropping systems. For example, a long-term study comparing various crop rotations [a 9-yr rotation (corn–wheat–corn–wheat–corn–wheat–alfalfa–alfalfa–alfalfa), two 2-yr rotations (corn–wheat and sugar beet–wheat), continuous corn, and continuous wheat] receiving mineral N or dairy manure N did not report any significant interactions between the effects of crop rotations and fertilization on soil C stocks (Triberti et al. 2016). Other studies have shown that there is no interaction between the nutrient source (organic or mineral) and different primary tillage practices (Viaud et al. 2011; Sainju et al. 2017) on organic matter accumulation in soils. Although some studies have shown a beneficial effect on crop yields and N uptake when the inclusion of perennial forage crops into the rotation is coupled with the use of livestock manure (Nyiraneza et al. 2010; Lafond et al. 2017), studies regarding the net effects of combined management practices on soil N stocks are lacking. Access to long-term experimental sites is necessary to clarify the interactive effects of various management practices on soil organic matter stocks (Janzen 1995; Ladha et al. 2011).

The main objective of this project was to compare the total N stocks and their relative long-term changes (after 21 yr) in the soil profile (0–50 cm) for eight cropping systems consisting of two crop rotations (cereal monoculture vs. perennial forage-based rotation), combined with two fall tillage practices [chisel plow (CP) vs. moldboard plow (MP)] and two nutrient sources [mineral fertilizer vs. liquid dairy manure (LDM)]. We also computed a N mass balance (defined as the difference between N inputs and outputs) to explore the sources of variation in soil N stocks under the different cropping systems. Our main hypotheses were (*i*) that accumulation of N in soil under the cropping systems combining forage-based rotation with LDM fertilization would be greater than in any other system based on cereal monoculture, and (*ii*) that soil tillage would not influence soil N stocks when considering the whole-soil profile.

Materials and Methods

Description of site and cropping systems

Crop management and the experimental site are presented in detail by Lafond et al. (2017). The experiment was established in 1989 at the Agriculture and Agri-Food Canada Experimental Farm in Normandin (48°50′N, 73°33′E), in the province of Québec. This region is characterized by a cold and humid continental climate, and the surface soil (0–20 cm) is usually frozen from December to April. From 1990 to 2009, the average precipitation for the growing season (May to October) was 479 mm, and the average temperature was 12.1 °C (Environment Canada 2016). The site is located on a silty clay (Labarre serie), classified as a Humic Gleysol (Soil Classification Working Group 1998). At the time of plot establishment, the 0–20 cm soil layer had the following characteristics: pH (1:1, soil:water ratio) of 5.6, 26.1 $g{\cdot}kg^{-1}$ total C, 1.7 $g{\cdot}kg^{-1}$ total N, 490 $g{\cdot}kg^{-1}$ clay, and 80 $g{\cdot}kg^{-1}$ sand.

Before initiation of this research, the site was under a rotation of spring barley (*Hordeum vulgare* L. ‘Chapais’) and alfalfa (*Medicago sativa* L.). Following a glyphosate burn-off of the alfalfa in October 1989, the soil was tilled using a MP to establish a factorial experiment including eight cropping systems according to a split-split-plot design comparing two crop rotations as the main factor, two primary tillage practices as the sub-factor, and two sources of nutrients as the sub-sub-factor.

The two crop rotations were a monoculture of spring barley (*Hordeum vulgare* L. ‘Chapais’), and a 3 yr rotation where spring barley was underseeded with a mixture of perennial forage crops including timothy (*Phleum pratense* L. ‘Champ’) and red clover (*Trifolium pratense* L. ‘Prosper’) from 1990 to 1999. From 2000 to date, timothy was replaced by orchard grass (*Dactylis glomerata* L. ‘Okay’) in the perennial forage mixture. Forage production is continued on the second and third years of rotation with three cuts per year. Both tillage practices were carried out in the fall and consisted of inversion tillage with a MP at a depth of 20 cm, or reduced tillage with a CP at a depth of 15 cm. The two crop rotations had different tillage frequencies: once a year for the barley monoculture, and every third year in the forage-based rotation (at the end of the forage phase).

The nutrient source was either a complete mineral fertilization (MIN) or LDM applied according to local recommendations and crop and soil analyses. On barley years, MIN and LDM were broadcast in the spring, 2 d before seeding, and incorporated immediately with a harrow to prepare the seedbed. On forage years, MIN and LDM were broadcast and left on the soil surface. For the duration of the study (1990–2010), plots under MIN fertilization received 70 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ as ammonium nitrate (34–0–0), 40 kg $P_2O_5{\cdot}ha^{-1}{\cdot}yr^{-1}$ as triple superphosphate (0–46–0) and 70 kg $K_2O{\cdot}ha^{-1}{\cdot}yr^{-1}$ as potassium chloride (0–0–60) on barley years. On forage years, MIN was applied in the spring with 74 kg $N{\cdot}ha^{-1}$ (65–80 kg $N{\cdot}ha^{-1}$ depending on year) as ammonium nitrate (34–0–0), 40 kg $P_2O_5{\cdot}ha^{-1}{\cdot}yr^{-1}$ as triple superphosphate (0–46–0), and 140 kg $K_2O{\cdot}ha^{-1}{\cdot}yr^{-1}$ as potassium chloride (0–0–60). A second application of 46 kg $N{\cdot}ha^{-1}$ (25–60 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ depending on year) in the form of ammonium nitrate (34–0–0) was applied after the first cut.

The LDM was obtained from a local dairy farm and was applied in the spring at a rate of 50 $m^3{\cdot}ha^{-1}{\cdot}yr^{-1}$ on the barley and forage stands from 1990 to 2010 (based on local recommendations in 1989). From 1996 to 2010, the plots under perennial forages received a second LDM application of 30 $m^3{\cdot}ha^{-1}$ after the first cut based on updated local recommendations (a third application at a rate of 30 $m^3{\cdot}ha^{-1}$ was made in 2000 only, after the second cut). The LDM rates were adjusted to fulfill crop N requirements and provided an average of 152 kg total $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ (73–242 kg total $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ depending on year) to the forage crop, and 102 kg total $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ (72–153 kg total $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ depending on year) to barley.

Soil sampling

In the fall of 2010, the soil bulk density was determined in three trenches (30 cm × 60 cm) within each plot using stainless steel cylinders (5 cm height × 5 cm diameter) inserted horizontally at five depths (0–10, 10–20, 20–30, 30–40, and 40–50 cm). The soil cores were weighed before and after drying at 105 °C to determine porosity, and bulk density was calculated assuming a density of 2.65 $g{\cdot}cm^{-3}$ for the mineral phase. A composite soil sample was taken at each depth by inserting horizontally a stainless-steel auger (2 cm diameter) and mixing the cores from all trenches at a given depth. The composite samples were then sieved to 6 mm in the field. Half of the soil volume was kept at 4 °C, whereas the other half was air-dried at 20 °C and ground to 0.5 mm using a ball mill (MM 400 model, Retsch, Germany).

Soil N analyses and calculation of total N stocks

The total N concentration ($g{\cdot}kg^{-1}$) of the dried and ground soil samples was obtained by dry combustion (950 °C) using an automated analyzer (TruSpec CN model, Leco Corp., St. Joseph, MI, USA). To correct for differences in soil bulk density between experimental treatments, total N stocks ($t{\cdot}ha^{-1}$) were calculated using the equivalent soil mass approach (Ellert and Bettany 1995; Ellert et al. 2006) as used by Maillard et al. (2016) for carbon stocks on the same experimental design. Soil N stocks were calculated for increasing depths (0–10, 0–20, 0–30, 0–40, and 0–50 cm) using the following equivalent soil masses: 130, 260, 390, 520, and 650 kg, respectively. The N stocks based on equivalent soil mass were calculated by subtracting the excess soil mass for depths with a heavier soil mass than the equivalent mass (Ellert et al. 2006). For depths with a lighter soil mass, the N stocks were calculated by summing the N stocks of the depth under consideration and the N stocks of an additional thickness taken from the underlying depth to obtain an equivalent mass (Ellert and Bettany 1995). The N stocks by depth (0–10, 10–20, 20–30, 30–40, and 40–50 cm) were obtained by subtracting the corresponding cumulative N stocks. For example, to obtain the value of N stocks for the 10–20 cm depth, we subtracted the value of N stocks calculated for the 0–10 cm depth from the value obtained for the 0–20 cm layer.

Changes in N stocks and N mass balances

Changes in soil N stocks after 21 yr (Δ stock) were assessed in a relative manner by assuming that (*i*) total N stocks (0–50 cm) did not change over 21 yr in the reference treatment (Δ stock = 0), and (*ii*) that the study site had a uniform N content when the plots were established. The cropping system combining the cereal monoculture with MP and MIN was chosen as the reference system (rs) because of its presumed limited potential to increase soil N stocks, and as this system may limit crop productivity and stimulate the mineralization of soil organic matter (Soon and Clayton 2003). Consequently, relative changes in N stocks (Δ stock; $\text{t}\cdot\text{ha}^{-1}$) were calculated for each alternative system (as) according to eq. 1:

$$(1)\qquad \Delta\,\text{stock}_{\text{as}} = \text{N stock}_{\text{as: 0–50cm}} - \text{N stock}_{\text{rs: 0–50cm}}$$

where $\text{N stock}_{\text{as: 0–50cm}}$ and $\text{N stock}_{\text{rs: 0–50cm}}$ represent the N stocks in the 0–50 cm soil profile in the alternative system and in the reference system, respectively. The N stock values used to calculate the Δ stock for each cropping system are presented in Supplementary Table S1[1]. A positive $\Delta\,\text{stock}_{\text{as}}$ indicates an accumulation of soil N over 21 yr, whereas a negative value indicates a depletion.

To identify potential sources of variation in soil N stocks under the different cropping systems, a N mass balance (defined as the difference between N inputs and N outputs) was calculated by recording or estimating the N inputs and outputs for each cropping system and was compared with the $\Delta\,\text{stock}_{\text{as}}$. The N inputs associated with fertilization as well as N exports associated with crop harvest were measured. In each forage crop phase, we estimated that 85% of the aboveground biomass was harvested in the first production year, whereas 75% of the aboveground biomass was harvested in the second year (Bolinder et al. 2007). For barley, we considered that 100% of the grains were exported, whereas 100% of the straw was left in the field. The N input associated with LDM was calculated by multiplying the volume applied by the total N content of LDM (Chantigny et al. 2007).

In the forage-based rotation, the N input from symbiotic fixation by red clover was estimated using two different methods:

Method 1

The European empirical model proposed by Høgh-Jensen et al. (2004) estimates symbiotic N fixation during the whole growing season using legume aboveground dry matter and its N concentration, and considers that the proportion of total plant N derived from N fixation is constant. This method considers (1) the amount of N fixed in the belowground parts and in the stubble, (2) the amount of fixed N transferred to the other crops, and (3) the amount of fixed N immobilized in decomposing soil organic matter.

For years under perennial forage, legume aboveground dry matter and its N concentration were measured (Lafond et al. 2017), and we assumed a proportion of 50% legumes in forage harvested in the first production year (second year of the forage-based rotation), and 25% legume in the second production year (third year of the forage-based rotation). The detailed equation proposed by Høgh-Jensen et al. (2004), and the coefficients used for calculations are presented in the Supplementary Materials[1].

Method 2

The SOILN module of the North American *Integrated Farm System Model* (*IFSM*; Rotz et al. 2018) was used to calculate the N input by symbiotic fixation in soils during the forage phase. This method provides the maximum atmospheric N_2 that can be fixed by the legume crop. This value is modulated by three coefficients according to (1) the concentration of nitrates in the 0–10 cm soil layer, (2) the average daily soil temperature, and (3) the soil moisture level (see Supplementary Materials[1] for more details about these parameters; Figs. S1 and S2[1]). We used the same proportions of legumes in harvested forages as in method 1.

For the eight cropping systems, other inputs such as free N fixation, atmospheric deposition (dry and wet), and environmental losses were estimated based on the scientific literature, government databases, and agro-environmental indicator data. In brief, a value of $4.55\ \text{kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ was used for atmospheric N deposition (Zhang et al. 2009; Canadian Council of Ministers of the Environment 2010), and a value of $4\ \text{kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ was used for non-symbiotic fixation of atmospheric N_2 by free-living soil microorganisms (Yang et al. 2010; Clair et al. 2014). Gaseous N emissions in the form of nitrous oxide (N_2O) were estimated to be 1.25% of the N input with fertilization, crop residues, and symbiotic fixation (Rochette and Janzen 2005; Styles et al. 2015). In addition, we assumed a N_2O-to-N_2 emission ratio of 1, as proposed by The Canadian Agricultural Nitrogen Budget model (Yang et al. 2007) because of the clayey texture of the soil under study, and its generally high moisture and C concentration. Losses through ammonia (NH_3) volatilization were estimated using the global NH_3-N loss coefficients presented by Styles et al. (2015), and results obtained under climate conditions similar to the present study (Chantigny et al. 2007; Rochette

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[1] Supplementary data are available with the article at https://doi.org/10.1139/cjss-2020-0132.

et al. 2008; Sheppard and Bittman 2013). We used a coefficient of 1.8% of the total amount of N applied with mineral fertilizers for all cropping systems, 8% for LDM in monoculture systems, as manure spreading was carried out with immediate incorporation, and 27% for LDM on forage years in forage-based rotation systems because the manure was left on the soil surface. Nitrate leaching was estimated to average 14% of applied N under barley, and 5% under perennial forages in eastern Canada (Fuller et al. 2010; Styles et al. 2015). The N inputs (from fertilization, free and symbiotic fixation, and atmospheric deposition) and the N outputs (from harvest and environmental losses) were summed to calculate the N mass balance of each cropping system for the 21 yr of the study. Detailed N input and output estimates are presented in Supplementary Figs. S3 and S4[1].

Statistical analyses

A three-way analysis of variance (ANOVA) was used to establish the effects of crop rotation, tillage practice, nutrient source, and their interactions on soil N stocks using the SAS MIXED procedure (©2012–2015, University Edition, SAS Institute Inc., Cary, NC, USA). The ANOVA was performed for each soil depth separately and on the total N stocks for the 0–20 and 0–50 cm profiles. Residual normality and variance homogeneity were verified using the UNIVARIATE procedure. The Shapiro–Wilk's and Kolmogorov–Smirnov's tests were used to verify the normality of the data distribution. Logarithmic transformation was necessary in some cases to ensure homogeneity of variance. The tables and figures present the least square means (LSMEANS) and standard errors of the original untransformed data. A Tukey's test was used to compare the means of the different treatments. The main effects and interactions were considered significant at $P \leq 0.05$.

Results and Discussion

Crop rotation influences soil N stocks and modulates the tillage effect

Overall, the presence of perennial forages in the rotation favoured the accumulation of soil N. After 21 yr, the average soil N stock was 25% higher (+1.05 t N·ha^{-1}) under the rotation than the monoculture in the 0–20 cm of soil, and 21% higher (+1.36 t N·ha^{-1}) under the rotation than the monoculture in the 0–50 cm profile (Table 1). In the 0–50 cm profile, however, the difference was statistically significant only for the forage-based rotation receiving LDM (Table 1; significant crop rotation × nutrient source interaction). These results are consistent with previous work on soil C stocks at this site (Maillard et al. 2016) and confirm the close relationship between soil C and N as influenced by long-term crop management. Compared with the cereal monoculture, the greater accumulation of N measured in the forage-based rotation after 21 yr (+1.05 t N·ha^{-1} in the 0–20 cm profile; +1.36 t N·ha^{-1} in the 0–50 cm profile) represented an average annual gain of 50 and 65 kg N·ha^{-1}, respectively. These values are within the range of values reported by Huss-Danell et al. (2007) who estimated that the amount of N accumulated in the 0–60 cm soil profile under perennial forages ranges between 44 and 73 kg·ha^{-1}·yr^{-1}.

Table 1. Soil nitrogen stocks (t·ha^{-1}) in the 0–20 and the 0–50 cm soil profiles as influenced by management practices, and summary of analysis of variance (ANOVA).

	Soil depth (cm)	
Factor	0–20	0–50
Crop rotation (CR)		
Rotation (R)	**5.19**	**7.80**
Monoculture (M)	**4.14**	**6.44**
Tillage practice (TP)		
CP	**5.00**	7.19
MP	**4.34**	7.05
Nutrient source (NS)		
LDM	**4.97**	**7.39**
MIN	**4.37**	**6.85**
CR × NS		
R-LDM	**5.65a**	**8.41a**
R-MIN	**4.74b**	**7.19b**
M-LDM	**4.29bc**	**6.37b**
M-MIN	**4.00c**	**6.52b**

	Probability values	
ANOVA	0–20	0–50
Crop rotation (CR)	**0.012**	**0.051**
Tillage practice (TP)	**0.010**	0.757
Nutrient source (NS)	**<0.001**	**0.003**
CR × TP	0.075	0.757
CR × NS	**0.004**	**0.001**
TP × NS	0.969	0.653
CR × TP × NS	0.208	0.095

Note: Rotation, cereal–perennial forage rotation; Monoculture, cereal monoculture; CP, chisel plowing at 15 cm; MP, moldboard plowing at 20 cm; LDM, liquid dairy manure; MIN, mineral fertilization. Different letters in a column under a specific interaction indicate significant difference (Tukey's honestly significant difference comparison test). Treatment means are only presented for significant factors ($P < 0.05$). Probability values in bold indicate significant differences.

In the 0–10 cm soil layer, the crop rotation modulated the effect of tillage on soil N stocks (Table 2; significant crop rotation × tillage practice interaction). Indeed, in the forage-based rotation, soil N stocks were 36% higher with CP than with MP, whereas there was no significant effect of tillage in the cereal monoculture (Table 2). The combination of a reduced tillage frequency in the

Table 2. Soil nitrogen stocks ($t{\cdot}ha^{-1}$) at different soil depths as influenced by management practices, and summary of analysis of variance (ANOVA).

	Soil depth (cm)				
Factor	0–10	10–20	20–30	30–40	40–50
Crop rotation (CR)					
Rotation (R)	**2.93**	**2.26**	1.43	0.66	0.49
Monoculture (M)	**2.22**	**1.92**	1.26	0.61	0.45
Tillage practice (TP)					
CP	**2.88**	2.12	**1.14**	0.59	0.46
MP	**2.27**	2.07	**1.55**	0.67	0.47
Nutrient source (NS)					
LDM	**2.76**	**2.20**	1.33	0.63	0.44
MIN	**2.39**	**1.98**	1.35	0.64	0.49
CR × TP					
R-CP	**3.37a**	2.35	1.28	0.67	0.52
R-MP	**2.48b**	2.18	1.57	0.65	0.45
M-CP	**2.39b**	1.89	0.99	0.51	0.41
M-MP	**2.05b**	1.95	1.52	0.70	0.49
CR × NS					
R-LDM	**3.22a**	2.42	**1.50a**	0.69	**0.50a**
R-MIN	**2.64b**	2.10	**1.35ab**	0.63	**0.47a**
M-LDM	**2.31bc**	1.98	**1.16b**	0.56	**0.38b**
M-MIN	**2.14c**	1.86	**1.35ab**	0.65	**0.52a**
	Probability values				
ANOVA	0–10	10–20	20–30	30–40	40–50
Crop rotation (CR)	**0.009**	**0.047**	0.237	0.456	0.599
Tillage practice (TP)	<**0.001**	0.643	**0.012**	0.349	0.940
Nutrient source (NS)	<**0.001**	**0.001**	0.818	0.623	0.088
CR × TP	**0.017**	0.300	0.328	0.233	0.281
CR × NS	<**0.001**	0.065	**0.028**	0.075	**0.014**
TP × NS	0.315	0.365	0.193	0.674	0.125
CR × TP × NS	0.428	0.176	0.185	0.877	0.873

Note: Rotation, cereal-perennial forage rotation; Monoculture, cereal monoculture; CP, chisel plowing at 15 cm; MP, moldboard plowing at 20 cm; LDM, liquid dairy manure; MIN, mineral fertilization. Different letters in a column under a specific interaction indicate significant difference (Tukey's honestly significant difference comparison test). Treatment means are only presented for significant factors ($P < 0.05$). Probability values in bold indicate significant differences.

forage-based rotation (1 of 3 yr) may have fostered this accumulation of N in the soil surface (Soon and Clayton 2003). The difference in N stocks in the 10–20 cm layer between CP and MP in the forage-based rotation was not statistically significant, but the trend was similar to the 0–10 cm layer (Table 2). When the 0–20 cm soil layer was considered, the soil N stock was higher with CP than MP (Table 1).

In contrast with the top soil layers, the soil N stocks were greater with MP than with CP in the 20–30 cm layer (Table 2). Previous work at our study site (Maillard et al. 2016) and at other sites in eastern Canada (Angers et al. 1997; Poirier et al. 2009; Fuller et al. 2010) reported that MP resulted in a greater accumulation of organic matter at depth as compared with reduced tillage or no-till. The cool, humid conditions at these sites limit the mineralization of organic matter buried in the soil (MacDonald et al. 2010), which would explain the accumulation of N observed with MP in our study in the 20–30 cm layer. This accumulation of N at depth under MP compensated for the greater accumulation of N at the surface under CP. As a consequence, soil N stocks in the entire soil profile (0–50 cm) were similar with both tillage practices (Table 1). This highlights the importance of measuring C and N stocks at depth to draw an accurate conclusion about the impact of tillage. Our results show that under cool and wet conditions, tillage has little effect on the accumulation of N in the soil but has an impact on its distribution within the soil profile.

Crop rotation modulates the effect of nutrient source on soil N stocks

After 21 yr, the soil N stock in the 0–10 cm depth was 22% higher with LDM than MIN under the forage-based rotation, whereas the difference was only 8% (not significant) under the cereal monoculture (Table 2; significant crop rotation × nutrient source interaction). A similar trend ($P = 0.065$) was found in the 10–20 cm soil layer. As a result, this interaction was also significant when considering the 0–20 cm soil profile (Table 1). Previous studies have shown a positive effect of livestock effluents on surface soil N stocks (Triberti et al. 2016; Nath and Lal 2017), total N concentration (Bittman et al. 2007), and organic N concentration (Ladha et al. 2011), when compared with MIN. The relative increase in soil N stocks with LDM under the forage-based rotation might be due to a greater input of N with LDM (average of 152 kg total $N{\cdot}ha^{-1}{\cdot}yr^{-1}$) than with MIN (average of 126 kg total $N{\cdot}ha^{-1}{\cdot}yr^{-1}$) (Supplementary Fig. S3[1]). In addition, the interaction with the crop rotation found in the present study could be partly explained by an increase in forage aboveground and root biomass induced by the addition of LDM (Nyiraneza et al. 2010; Triberti et al. 2016). In our field experiment, Lafond et al. (2017) reported greater forage yields with LDM as compared with MIN. One other explanation could be that the higher tillage frequency in the monoculture (each year) than in the rotation system (once every third year) accelerated the mineralization of soil organic N, thus offsetting the accumulation of N caused by LDM application.

An interaction between crop rotation and nutrient source was found at the 20–30 and 40–50 cm depths, where soil N stocks were greater in the forage-based rotation than in the cereal monoculture when fertilized with LDM, whereas there were no differences between the crop rotations fertilized with MIN (Table 2). As a result of the interactions found at 0–10, 20–30, and 40–50 cm depths, soil N stocks for the whole soil profile (0–50 cm) were 17% higher (+1.22 $t\ N{\cdot}ha^{-1}$) with LDM than with MIN when combined with the forage-based rotation, whereas there was no significant difference between nutrient sources under the cereal monoculture (Table 1). Our results show that after 21 yr, a cropping system combining LDM with a crop rotation including perennial crops favours a greater accumulation of N in the soil than if perennial crops are fertilized with MIN. This beneficial effect of LDM was not present in the monoculture. A positive effect of the combination of perennial crops with LDM was also observed for soil C stocks at the same site (Maillard et al. 2016). The authors showed that, over 21 yr, 38% of the C contributed by LDM was retained in the soil under the rotation, whereas LDM-C retention was negligible under the monoculture. Previous studies have shown that the inclusion of perennial forage crops in cropping systems fertilized with livestock effluents increases crop yields and N uptake (Nyiraneza et al. 2010; Lafond et al. 2017), reduces N losses through leaching (Bittman et al. 2007; Fuller et al. 2010), improves soil quality (Bissonnette et al. 2001; Nyiraneza et al. 2009), and fosters the build up of C in the soil profile (Maillard et al. 2015, 2016). To our knowledge, the present study is the first to report on the long-term impacts of a combination of livestock effluent with perennial forage crops on soil N stocks at various depths.

Relative changes in soil N stocks and N mass balance of the different cropping systems

In all cropping systems with the forage-based rotation, except rotation-MP-MIN, the ΔN stocks were positive (+0.87 to +1.78 $t{\cdot}ha^{-1}$), indicating a beneficial effect of reduced tillage intensity and (or) organic fertilization on soil N stocks when combined with perennial forage crops (Fig. 1). The calculated N balances were consistent with the positive ΔN stocks observed in the forage-based rotation, except for the rotation-MP-MIN system (Fig. 1). However, large discrepancies are observed between estimated N inputs (Table 3) and associated N balances (Fig. 1) depending on the method used to estimate symbiotic N fixation. Using method 1, the estimated symbiotic N fixation ranged from 45 to 131 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ on years under established forage stand, whereas with method 2, symbiotic N fixation ranged between 89 and 267 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ (Supplementary Fig. S3[1]); N fixation was considered negligible during the forage establishment year (barley year). That discrepancy could be partly explained by the modulating factor associated with soil nitrate concentration included in method 2 but not considered in method 1. Despite the considerable differences between the two methods used to estimate symbiotic N fixation, the range of estimates calculated using either methods (45–267 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$) are within the broad range of values reported for forage legumes in Canada (27–300 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$; Yang et al. 2010). Such large differences in estimates have often been noticed (Watson et al. 2002; Herridge et al. 2008; Clair et al. 2014; Lüscher et al. 2014; Anglade et al. 2015), pointing to the need to accurately measure in situ symbiotic fixation to decrease uncertainty on this potentially very important source of N for soils and crops. In any case, our results show that symbiotic fixation was a major component contributing to N inputs in the cropping systems including red clover.

The ΔN stock was slightly negative in the rotation-MP-MIN system and contrasted with the estimated positive N balance (Fig. 1). In that cropping system, the more intensive tillage and MIN may have triggered soil N mineralization. This likely increased soil mineral N availability, which may have either inhibited nodulation in

Fig. 1. Nitrogen (N) balances and relative changes in soil N stocks (ΔN stocks, 0–50 cm; Supplementary Table S1[1]) over 21 yr for eight cropping systems composed of three factors: (*i*) crop rotation: R, cereal–perennial forage rotation; M, cereal monoculture; (*ii*) tillage practice: CP, chisel plowing; MP, moldboard plowing; (*iii*) nutrient source: LDM, liquid dairy manure; MIN, mineral fertilization. The ΔN stock (black columns) is the relative change in soil N for the seven "alternative" cropping systems as compared with the reference treatment (M-MP-MIN). The N balance was calculated using two methods to estimate N fixation by red clover: method 1 (light grey columns) = N inputs − N outputs (Table 3) including N inputs from symbiotic fixation estimated by the Empirical method of Høgh-Jensen et al. (2004); method 2 (dark grey columns) = N inputs − N outputs (Table 3) including N inputs from symbiotic fixation estimated using the SOILN module of the *Integrated Farm System Model* (Rotz et al. 2018). Details on the two methods are given in the Supplementary Materials[1].

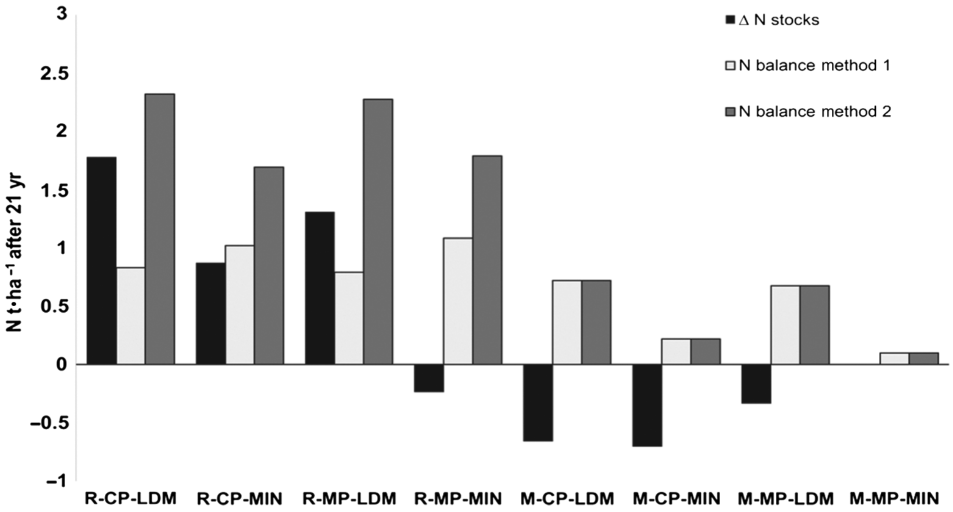

Table 3. Nitrogen (N) inputs and outputs over 21 yr for the different cropping systems.

Cropping system	N inputs without symbiotic N fixation^a (t·ha^{-1})	N inputs by symbiotic N fixation method 1^b (t·ha^{-1})	N inputs by symbiotic N fixation method 2^c (t·ha^{-1})	N outputs by harvest^d (t·ha^{-1})	N outputs by environmental loss^e (t·ha^{-1})
R-CP-LDM	3.104	1.309	2.800	2.479	1.103
R-CP-MIN	2.435	1.197	1.869	2.328	0.282
R-MP-LDM	3.104	1.316	2.800	2.525	1.102
R-MP-MIN	2.435	1.162	1.869	2.239	0.274
M-CP-LDM	2.325	0	0	1.050	0.554
M-CP-MIN	1.650	0	0	1.190	0.239
M-MP-LDM	2.325	0	0	1.097	0.552
M-MP-MIN	1.650	0	0	1.310	0.240

Note: R, cereal–perennial forage rotation; M, cereal monoculture; CP, chisel plowing at 15 cm; MP, moldboard plowing at 20 cm; LDM, liquid dairy manure; MIN, mineral fertilization.

[a] N inputs without symbiotic N fixation = measured N from nutrient source + estimated atmospheric N deposition and free N fixation.

[b] N inputs by symbiotic N fixation method 1, estimated N inputs from symbiotic N fixation in legumes (forage phase) by the Empirical method of Høgh-Jensen et al. (2004).

[c] N inputs by symbiotic N fixation method 2, estimated N inputs from symbiotic N fixation in legumes (forage phase) using the SOILN module of the *Integrated Farm System Model* (Rotz et al. 2018).

[d] N outputs by harvest, measured N exported with harvests.

[e] N outputs by environmental loss, estimated N losses through gaseous emissions and leaching.

red clover, thereby reducing symbiotic fixation (Macduff et al. 1996), or may have increased environmental losses, thereby counterbalancing N inputs through symbiotic fixation.

In the cropping systems including the cereal monoculture, N inputs over 21 yr were greater than N outputs (Table 3), resulting in positive N balances (Fig. 1). This suggests that N may have accumulated in the soil over time. However, compared with the reference system (monoculture-MP-MIN), the ΔN stocks of the three other monoculture-based cropping systems were negative (−0.33 to −0.70 t·ha^{-1}), indicating a relative depletion in soil N over the 21 yr period (Fig. 1). This depletion indicates that in monoculture-based cropping systems, reducing tillage intensity (chisel vs. moldboard) and using organic rather than mineral nutrient sources (LDM vs. MIN) were not sufficient to increase soil N content. This finding corroborates the results of Maillard et al. (2016) who reported no beneficial effects of CP and LDM on soil C stocks under the same monoculture systems. The estimated environmental losses (Supplementary Fig. S4[1]) might thus have been largely underestimated for these cropping systems. Indeed, large additional environmental losses would be necessary to reconcile the N balance with the ΔN stocks (Fig. 1).

In summary over the 21 yr study, the inclusion of a perennial grass–legume forage mixture in rotation with barley resulted in significantly greater soil N stocks in the 0–50 cm profile (average gain of 50–65 kg $N \cdot ha^{-1} \cdot yr^{-1}$) compared with the cereal monoculture. The use of LDM in combination with this forage mixture maximized the accumulation of N, indicating a more efficient use and retention of LDM-N than with an annual crop such as barley. However, it is also possible that the reduced frequency of tillage in the forage-based rotation favoured the accumulation of N in the soil. Calculation of the N mass balance for each cropping system over the study period suggested that the inclusion of red clover in the perennial forage mixture contributed substantially to the increase in soil N stocks through symbiotic N fixation. However, estimates of symbiotic N fixation were highly variable depending on the method of calculation used, and in situ measurement of N fixation is, therefore, required to more accurately determine its contribution to soil N storage. The large soil N stocks and positive N balances found in cropping systems where LDM was repeatedly applied to a forage-based crop rotation suggest that the soil N supply capacity was substantially increased over time in these cropping systems (e.g., Nyiraneza et al. 2010). The possible legacy N effect must be evaluated in these soils and taken into account in estimating crop N needs to avoid over fertilization and associated environmental threats.

Competing Interests

The authors declare there are no competing interests.

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