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REGULAR ARTICLE

Nitrogen dynamics after low-emission applications of dairy slurry or fertilizer on perennial grass: a long term field study employing natural abundance of δ^{15}N

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Abstract

Background Defining the long-term effects of liquid dairy manure (LDM) applied by a recommended low emission method is important to ensure sustainable grass production and dairy operations. We used natural abundance δ^{15}N and conventional measurements in a long term field experiment to better understand the long-term fate of N applied as LDM, mineral fertilizer (MIN) and both (ALT).

Methods We investigated the effects of long-term applications of LDM (with low-emission trailing shoe), MIN, and alternating LDM and MIN (ALT) on grass N uptake, soil N stocks, N losses and δ^{15}N natural abundance in grass, soil and fine heavy fraction (silt + clay size heavy soil). Nominal annual rates of total N were 200 and 400 kg ha^{-1} for MIN and 400 and 800 kg ha^{-1} for LDM.

Results MIN and LDM (at 400 kg N ha^{-1}) had similar NUE but LDM accumulated more total soil N (13% of applied) with less losses than MIN; ALT had high yields and losses. Herbage δ^{15}N of MIN declined to near MIN levels after 10–15 years, indicating influence of pre trial management. Herbage δ^{15}N of LDM was consistently lower than applied LDM, implying uptake of depleted urine N. High rates of enriched LDM had little effect on soil δ^{15}N suggesting soil N was quite stable and enriched N was lost by non

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Core Ideas
- Long term NUE of 65% for both low-emission LDM and MIN (at 400 kg N ha^{-1}), but LDM accumulated more soil N (13% of applied) and MIN had greater N losses.
- Using natural abundance δ^{15}N, effect of pre-trial management on MIN and Control herbage was evident for 10-15 years.
- Weak effect of enriched LDM on herbage δ^{15}N suggests substantial plant uptake of depleted urine N which was likely helped by low-emission application.
- Weak effect of large amounts of enriched LDM on soil δ^{15}N suggests stable soil N and loss of added enriched N by non-discriminating processes like leaching.
- Most soil N was in physically protected fine heavy fraction.

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discriminating pathways like leaching. The physically protected fine heavy fraction contained most soil N.
Conclusion Clearer understanding of long-term N dynamics can improve sustainability of heavily fertilized perennial grasses on dairy farms.

Keywords Δ^{15}N natural abundance · Nitrogen use efficiency · Mineral fertilizer · Liquid dairy manure · Soil N · Crop N uptake · Fine heavy fraction · Grass herbage

Abbreviations

LDM	Liquid dairy manure
MIN	Mineral fertilizer
TAN	Total ammoniacal nitrogen
TN	Total nitrogen

Introduction

Slurry manure (hereafter referred to as slurry) is an important N source for crops on dairy farms in Canada and elsewhere (Sheppard et al. 2011). Although slurry can increase crop yields and improve soil properties (Bittman et al. 2005; Schlegel et al. 2015), inefficient use of slurry N may result in N losses through ammonia volatilization, leaching and denitrification (Bittman et al. 2011, 2007; Paul and Zebarth 1997).

Efficiency of N uptake by crops receiving regular applications of slurry or commercial fertilizer may be best studied in long-term field trials in which crop N uptake would include both recent and legacy doses. In short-term trials, efficacy of total slurry-N may be lower than fertilizer N due to volatilization losses from the ammoniacal N portion of the slurry and to relative unavailability of the organic N component of the slurry (Bittman et al. 1999). Typically approximately half of applied cattle slurry-N is in the ammoniacal (ammonia and ammonium) form, referred to as total ammoniacal N (TAN) which largely behaves like fertilizer N (Zhang et al. 2020). Much of the organic N fraction (in faeces) is gradually mineralized to ammonium (60% in 3 years; Schröder 2005) and subject to all of the losses mentioned above as well as immobilization by microbes (Hoekstra et al. 2011).

Artificial isotopic labelling of N is an important tool for examining the short or medium term fate of applied N from fertilizer, and less commonly from manure, the latter requiring complex preparation (Chantigny et al. 2019) and rarely in long term manure application trials. The fate of applied slurry-N can also be examined by assessing the δ^{15}N natural abundance. The δ^{15}N can be expressed per mil (‰) (Coplen 2011): $\delta^{15}N=((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{std})-1$ where $(^{15}N/^{14}N)_{\text{sample}}$ is the N isotopic composition of the sample, and $(^{15}N/^{14}N)_{\text{std}}$ is the N isotopic composition of standard material (0‰). The δ^{15}N can be used as an indicator of N sources and processes in soil (Sørensen et al. 1994). The principle is that mineral and slurry N have contrasting δ^{15}N natural abundance signatures that can be used to infer long-term contributions of N to soils by these N sources (Kendall 1998). Typically, synthetic nitrogen fertilizer has a δ^{15}N value of approximately 0‰ while slurry manure is much higher, generally between 5 and 15‰ (Kendall 1998). Slurry becomes proportionally enriched upon storage and application because ^{14}N is preferentially lost by ammonia volatilization and denitrification (Hristov et al. 2009).

Fractionation associated with N transformations in soil leads to enriched δ^{15}N natural abundance in the remaining substrate because the lighter ^{14}N isotope reacts more rapidly than heavier ^{15}N due to lower bond strength (Kriszan et al. 2009). Many N forms (NH_3, N_2O, NO, N_2 and NO_3^-) emitted after soil N processes such as ammonia volatilization, nitrification and denitrification may result in δ^{15}N enrichment of the remaining natural abundance in soil N (Högberg 1997; Robinson 2001). In contrast, the processes of N mineralization or N uptake by plants generally discriminate less between ^{14}N and ^{15}N and thus plant uptake and mineralization are not thought to strongly affect the δ^{15}N natural abundance (Handley and Raven 1992).

Long-term manure application on cereals and clover/grass in Europe increased δ^{15}N natural abundance

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in plant and soil, suggesting both plant uptake and soil retention of the enriched N from manure (Bol et al. 2005; Bogaard et al. 2007; Senbayram et al. 2008). Kriszan et al. (2009) showed that the δ^{15}N natural abundance values of pasture soil and plants increased with increasing slurry-N rate after 20-yr of slurry applications. In contrast, long-term applications of mineral fertilizer increased, decreased, or had no effects on plant and soil δ^{15}N (Meints et al. 1975; Lobe et al. 2005). Variation of δ ^{15} N natural abundance in plants and soils after long-term fertilization may identify differences in amended N sources, N turnover and transformation process of amended N (Choi et al. 2006; Senbayram et al. 2008; Kriszan et al. 2009). Therefore, assessing δ^{15}N natural abundance concentrations in soil and plant may help to reveal the relative importance of soil N sources (slurry vs. mineral-N) and to trace back the N use efficiency of long-term fertilizer.

Soil organic matter plays a significant role in nutrient accumulation and C and N accumulate in organo-mineral complexes after long-term slurry application (Gerzabek et al. 2001; Maillard et al. 2015). However, few studies have investigated the long-term effects of manure vs. fertilizer amendments with different δ^{15}N natural abundance on N distribution and δ^{15}N natural abundance in plant, soil layers and various soil fractions (Gerzabek et al. 2001; Kriszan et al. 2009). Among studies analyzing the effects of animal manure on soil organic matter fractions, few focused on stable fractions, such as mineral-associated organic matter, although some of these studies showed a significant effect of manure on mineral-associated C and N (Hai et al. 2010; Huang et al. 2010).

The changes in δ^{15}N natural abundance of soil and grass can be used to study the N sources of slurry-N and mineral fertilizer N that is contained in soil and harvested grass. Our study focused on the effects of long-term, low-emission, applications of liquid dairy manure (LDM) vs. ammonium nitrate fertilizer (MIN) on grass N uptake, soil N stock, δ^{15}N natural abundance in grass herbage, bulk soil and its silt + clay size heavy fraction (referred to as fine heavy fraction). We hypothesized that the majority of applied N sources with low δ ^{15} N natural abundance such as mineral fertilizer N is used by plants, whereas more enriching δ^{15}N natural abundance of slurry-N could be preserved in whole soil and in the fine heavy fraction and grass (enriching δ^{15}N natural abundance due to a heavier source).

Material and methods

Site description

The field study was conducted from 1995 to 2011 in southwestern BC, Canada (49°17’ N, 121°45’ W) under moderate maritime conditions averaging 1690 mm of precipitation annually with mean daily air temperature ranging from 2.5 °C in January to 18.2 °C in July. The soil is a moderately-well to well-drained silt-loam soil which belongs to the Monroe series and is classified as an Eluviated Eutric Brunisol (Luttmerding 1981). The soil sampled in March 2011, contained 97 g kg^{-1} sand, 767 g kg^{-1} silt and 166 g kg^{-1} clay at 0–30 cm and the soil pH at 0–15 cm was 5.6 in water (1:1). The 0–15 cm soil layer contained 35.0 g kg^{-1} total-C and 3.0 g kg^{-1} total-N, and had a bulk density of 1.15 g cm^{-3}.

The long-term nitrogen study was initiated in 1994 on a perennial stand of tall fescue (*Festuca arundinacea* Schreb.) established in 1993. Background samples were taken randomly in untreated areas in 1995. Previously the field had been used for over 20 years to produce orchardgrass forage (*Dactylis glo*merata L.) and silage corn (*Zea mays* L.) with modest rates of dairy slurry or mineral fertilizer (estimated 100 kg total N ha^{-1} yr^{-1}). The stand was reseeded in 2003 after tilling (moldboard plowing, 25 cm depth). There was no N application carried out in 2003 and the light crop of juvenile grass was not sampled.

Ten N treatments, applied to plots measuring 3 × 90 m, were randomized in four complete blocks. Six treatments reported in this study are: unfertilized Control; LDM applied at nominally 200 (LDM-low) and 400 kg (LDM-high) TAN ha^{-1} per yr^{-1}; ammonium nitrate fertilizer applied at 200 (MIN-low) and 400 kg N ha^{-1} (MIN-high) per yr^{-1}; and an alternating LDM and MIN treatment (ALT) each at 100 kg mineral-N ha^{-1} per application (400 kg ha^{-1} TAN per annum). The nominal total N rates were 400 and 800 kg ha^{-1} yr^{-1}. Actual application rates are summarized in Table 1. These treatments were applied in four equal doses each year from 1997 to 2010, except 2003, in early spring (early April) and after each cut (May,

Table 1 Characteristics and application rates of manure slurry from year 1995 to 2010

Year	Total-N application rate						Property			
	MIN-low	MIN-high	LDM-low	LDM-high	ALT	pH	DM	Tot-N	NH_4^+-N	$\delta^{15}N$
	kg N ha^{-1}						g kg^{-1}			‰
1995	120	240	267	528	426	7.1	82.6	2.24	1.53	9.09
1996	158	316	321	611	466	7.1	51.4	1.88	1.20	11.38
1997	220	440	369	739	589	7.4	86.3	2.11	1.50	10.52
1998	210	420	418	759	589	6.7	83.2	2.11	1.58	14.50
1999	230	410	456	897	678	6.8	82.3	1.83	1.48	15.54
2000	196	392	313	581	302	7.2	69.6	2.41	1.28	13.05
2001	218	436	347	641	538	7.4	49.8	2.35	0.83	11.46
2002	215	430	398	730	580	7.2	45.4	2.34	1.05	9.21
2004	200	400	444	844	618	6.9	69.8	2.24	1.05	11.80
2005	200	400	428	844	614	7.1	46.4	1.87	1.20	14.96
2006	200	400	408	812	576	7.2	71.3	2.07	1.38	9.74
2007	200	400	396	740	578	7.1	72.2	1.84	1.61	12.26
2008	200	400	400	752	562	7.2	71.2	2.15	1.63	13.31
2009	200	400	376	740	592	7.1	72.2	2.22	1.53	9.00
2010	200	400	348	668	532	6.3	63.9	2.12	1.70	12.59
Mean	198	392	379	726	549	7.0	67.8	2.11	1.36	11.89

MIN: ammonium nitrate; LDM: liquid dairy manure; ALT: alternate MIN-high and LDM-high. Note that mean of $\delta^{15}N$ for fertilizer was 0.43‰

July, and August), except for the final cut in the fall (Sept-Oct). The treated plots were also fertilized twice in 1994 and three times in 1995 and 1996. The MIN treatments received other nutrients (P, K and S) and lime according to local soil test recommendations,

The slurry, which was obtained from local high production dairy farms (using wood shaving bedding), was applied by a sliding-shoe applicator to reduce ammonia emissions (up to 60% of ammoniacal N) and ensure uniform application across the plots (Bittman et al. 1999; Webb et al. 2010). The manure tank was weighed before and after every application pass. The actual N application rates of the slurry are based on samples taken at the time of spreading and are shown in Table 1. The nitrogen was determined by Kjeldahl (total N) and steam distillation (ammonium N) methods. Note that MIN-high and LDM-low received similar average rates of total N (392 and 379 kg ha^{-1} yr^{-1}, respectively).

Soil sampling

Soil was sampled with a soil corer annually at 8 random locations within the crop sampling area in each plot to 60 cm depth after final harvest each autumn. These samples were pooled by depth and air dried at room temperature and ground. In addition, three pits were dug to 40 cm depth in random locations in each experimental plot in March 2011. One soil sample was collected from the sides of each pit at each of 5 soil layers: 0–5, 5–10, 10–20, 20–30 and 30–40 cm. Soil bulk density was determined for each layer by collecting three samples at each depth using stainless steel cylinders (5-cm diameter; 5-cm high). The samples at each depth were combined and passed through a 6-mm sieve in the field, then air-dried at room temperature for analysis.

Soil silt and clay heavy fraction

A soil silt- and clay-size heavy fraction was collected after a density and particle size fractionation of soil organic matter adapted from Sohi et al. (2001). This was meant to separate organic matter into fractions with different levels of physical protection (Maillard et al. 2015). Briefly, the soil heavy fraction was recovered after separation of light fraction in sodium iodide (NaI) aqueous solution (1.8 g NaI mL^{-1}). Then, the heavy fraction was washed with distilled water through a sieve (53 µm) to obtain the fine heavy fraction (heavy fraction associated with silt- and clay-sized particles). All the details of the fractionation are described in Maillard et al. (2015). For use in the present study, the fine heavy fraction was collected for two sampling depths (0–5 and 20–30 cm) with contrasting soil N contents. Here we focused on the fine heavy fraction because it was expected to be stable with a high level of physical protection. Also we had determined that the fine heavy fraction dominated the N stocks in our soil: 75% of the surface (0–5 cm) and 84% of the subsurface soils (20–30 cm).

Calculation of soil N stocks

Total N stocks (Mg N ha^{-1}) in the soil (0–40 cm) for each layer was calculated by multiplying soil N concentration (kg N Mg $soil^{-1}$) × soil layer depth (m) × soil bulk density (Mg m^{-3}) × 10,000 m^2 ha^{-1}. Nitrogen stock (Mg N ha^{-1}) of the fine heavy fraction for each soil layer was determined by N concentration of fine heavy fraction (g N kg^{-1} fraction) × mass of the fine heavy fraction (g kg^{-1} soil) × mass of soil (Mg m^{-3}) × 10,000 m^2 ha^{-1}. The soil N stock was calculated using a fixed depth approach as there was no significant treatment effect on soil bulk density in the lower depth.

Grass sampling and measurement

Historical grass sampling and N uptake measurements were described in Bittman et al. (2007). Grass tissue N concentration and $\delta^{15}N$ natural abundance were analyzed on dried ground composite samples for all cuts for year 1996, 1999, 2005 and 2010. Plant N concentration was determined using the Dumas dry ash method (LECO FP-428 analyzer, LECO Corp, USA). Above-ground N uptake was calculated as N concentration × above-ground whole plant yield. Annual samples were created by combining cuts in proportion to their individual yields.

Total N and $\delta^{15}N$ natural abundance measurements

Contents of total N and $\delta^{15}N$ natural abundance in manure, fertilizer, whole soil, fine heavy fraction and grass were determined at Lethbridge Research and Development Centre (Isotope lab) by dry combustion on a Flash2000 analyzer coupled with DeltaV continuous-flow isotope ratio mass spectrometer (ThermoFinnigan, Bremen, Germany). The accuracy of the isotopic data was evaluated by the analysis of four types of the certified standards ($-4.43 \pm 0.21‰$, $4.09 \pm 0.27‰$, $6.99 \pm 0.30‰$, and $8.48 \pm 0.14‰$). The analytical precision of measurements was from 0.14 to 0.30‰ for N.

Statistical analysis

Soil and plant data for each year were analyzed as a randomized complete block design (4 blocks) using PROC MIXED procedure of SAS 9.2 software considering N treatments as fixed effects and blocks as random effects. The data were tested to ensure normality using the Shapiro–Wilk test. Soil $\delta^{15}N$ natural abundance values (0–15 cm) in pre-trial and current treatments were compared by Dunnett's test. Differences among treatment means were tested for significance by the Fisher Protected LSD test. Critical level for significance was set a priori at $p < 0.05$.

Results and discussion

Manure and fertilizer application

The LDM used in this study was sourced from uncovered storage tanks of dairy farms with high producing cows and using wood chips bedding. The physical and chemical characteristics of the LDM over the 15 application years were similar to manure of commercial dairy farms in south coastal British Columbia in previous studies (Bittman et al. 2007). The Tot-N of LDM averaged 2.1 g kg^{-1}, ranging from 1.8 g kg^{-1} in 1999 to 2.4 g kg^{-1} in 2000 while the ratio of NH_4^+-N: Tot-N averaged 0.64 (Table 1). The LDM averaged 68 g dry matter (DM) kg^{-1}, 433 g C kg^{-1} DM (not shown) and a pH of 7.0. Between 1995 and 2010, cumulative rates of applied Total-N were 5685 kg ha^{-1} for LDM-low and 10,890 kg ha^{-1} for LDM-high, and 2970 and 5880 kg ha^{-1} for Min-low and MIN-high, respectively, and 8235 kg ha^{-1} for the ALT treatment (Table 2). LDM-low and MIN-high received similar annual rates of Tot-N (379 and 392 kg ha^{-1}, respectively). The $\delta^{15}N$ natural abundance of LDM averaged 11.9‰ and ranged from 9.0 in 2009 to 15.5‰ in 1999 which is typical for LDM (Högberg 1997), with no statistical trend over the study years (Table 1). The variation is possibly due

Table 2 Summary of background (pre-treatment) soil N, N inputs and effects of N inputs on N removal and soil N

	Applied Tot-N Annual	Applied Tot-N 15 yr	Total soil-N (0-15 cm) Pre-treatment	Total soil-N in 0–40 cm (2011)	Total soil-N over control (2011)	Increase over control as % of applied (2011)	Applied N: total soil N (0–40 cm) (2011)	Tot.-N removed by crop over 15 yr	Net Tot.-N (applied—removed by crop) over 15 yr	Net applied Tot.-N as % of 2011 total soil N over 15 yr	Ratio of net applied total N to 2011 total soil N over 15 yr	Mean annual yields	N Losses Annual £
	kg N ha^{-1}	kg N ha^{-1}	g N kg^{-1}	kg N ha^{-1}	kg N ha^{-1}	%	ratio	kg N ha^{-1}	kg N ha^{-1}	ratio	ratio	kg ha^{-1} yr^{-1}	kg N ha^{-1}
Control	0	0	2.9	9376^{c} [§]	0	0	0.000	1530	-1530	-0.163	0.000	5891	-102
MIN-low	198	2970	2.9	9321^{c}	-55	-1.9	0.319	3390	-420	-0.045	0.131	11,363	-24
MIN-high [¥]	392	5880	2.9	9799^{c}	423	8.2	0.600	4380	1500	0.153	0.282	11,108	72
LDM-low	379	5685	2.9	10,115^{b}	739	13.0	0.562	4410	1275	0.126	0.580	13,898	36
LDM-high	726	10,890	2.9	11,039^{a}	1663	15.2	0.987	5295	5595	0.507	0.297	14,342	262
ALT	549	8235	2.9	9645^{c}	269	3.2	0.854	5565	2670	0.277	0.101	14,966	160

MIN: ammonium nitrate; LDM: liquid dairy manure; ALT: alternate MIN-high and LDM-high

[§] Values within the column followed by the same letter are not significantly different at the $P < 0.05$

[¥] Bold Min high and LDM low treatments have similar amounts of applied Tot-N

[£] These loss values do not include inputs from atmospheric deposition, estimated as 40 kg N ha^{-1} yr^{-1}(see text) to different manure handling practices, such as frequency and method of barn cleaning and amount of bedding, that affect processes such as ammonia volatilization and denitrification which enrich δ^{15}N. We found no clear relationship between δ^{15}N and other manure properties such as DM or pH. As expected, δ^{15}N abundance in ammonium nitrate fertilizer (MIN) was much lower (0.43‰) than in LDM. Previous local reports show slightly lower (negative) values of δ^{15}N for synthetic N fertilizers (Suchy et al. 2018).

Long-term grass N uptake and nitrogen use efficiency

The grass N concentrations for the MIN, LDM and ALT treatments were significantly greater than for Control (22–31 vs. 18–20 g kg^{-1}) in each sampling year (except year 2010 for MIN-low) (data not shown). At similar total-N inputs, MIN-high had significantly greater N concentrations than LDM-low (25–31 vs. 22–24 g kg^{-1}) with similar N uptake but lower DM yield (Table 2),

Grass N uptake (removal) in the Control herbage decreased from 102 kg N ha^{-1} in 1996 to 74 kg N ha^{-1} in 2010 (Table 3) likely due to depletion of residual labile N from pre-study N applications; the slight bumps in 1999 and 2005 could be due high precipitation in the summer of 1999 and to increased mineralization stimulated by tillage in 2003. Over the course of the study 1530 kg N ha^{-1} was taken up by the unfertilized Control herbage with perhaps about 450 kg N ha^{-1} (30 kg N ha^{-1} per annum) returned by atmospheric wet deposition (Lin et al. 2020) and additional N may have come from dry deposition of ammonia emitted from nearby dairy facilities and manure spreading on nearby fields, from biological N fixation by small amounts of volunteer wild white clover plants (*Trifolium repens* L.) that escaped control, and perhaps small amount from mineralization after cultivation in 2003. Uptake of 74 kg N ha^{-1} in 2010 can be substantially explained by wet and dry deposition, suggesting that the control soil had a tightened N cycle and was approaching soil N equilibrium, which means it was neither gaining nor losing significant amounts of N. The grass sward density remained very high in the Control plots throughout the study. Few studies have reported on sources of N in control plots, especially the roles of legacy N and deposition, which is generally important for a full understanding effects of N application.

Grass N uptake was greatest for the LDM-high and ALT treatments (averaging 353 and 371 kg N ha^{-1} yr^{-1}, respectively), even though applied N was almost 180 kg N ha^{-1} yr^{-1} greater for LDM-high than for ALT treatment (Table 2). The

Table 3 N uptake (kg N ha^{-1}) by tall fescue and adjusted and unadjusted values of nitrogen use efficiency (NUE) as affected by applications of N as ammonium nitrate (MIN) and liquid dairy manure (LDM). All years from 1995–2010 were similarly treated except 2003 when the stand was renovated

Treatment	Applied Tot.-N	Crop N Uptake				Unadjust.mean NUE ¶	Adjusted means NUE for 40 kg N ha^{-1} dep
		1996	1999	2005	2010		
	kg N ha^{-1} yr^{-1}					%	
Control	0	102 ^{d§}	117 ^{f}	116 ^{d}	74 ^{d}	–	–
MIN-low	198	200 ^{c}	287 ^{e}	197 ^{c}	220 ^{c}	114.1	93.9
MIN-high ¥	392	279 ^{a}	271 ^{c}	267 ^{b}	250 ^{bc}	74.5	64.3
LDM-low	379	219 ^{b}	335 ^{d}	261 ^{b}	266 ^{b}	77.6	67.0
LDM-high	726	291 ^{a}	428 ^{b}	345 ^{a}	349 ^{a}	48.6	43.1
ALT	549	289 ^{a}	466 ^{a}	366 ^{a}	362 ^{a}	67.6	60.3
LSD	–	15.2	25.6	39.1	33.3	12.4	11.1

MIN: ammonium nitrate; LDM: liquid dairy manure; ALT: alternate MIN-high and LDM-high

§ Values within the column followed by the same letter are not significantly different at the $P < 0.05$

¥ Bold Min high and LDM low treatments have similar amounts of applied Tot.-N

¶ Unadjusted mean NUE: (crop N uptake/input of tot.-N) × 100; Adjusted means NUE for 40 kg N ha^{-1} dep. includes N input of 40 kg ha^{-1} as atmospheric deposition (30 wet and 10 dry) (based on Lin et al., 2020)

ALT treatment received slightly less mineral N than LDM-high (434 and 468 kg N ha^{-1}, respectively) but LDM-high received substantially greater doses than ALT of organic N (258 vs. 129 kg N ha^{-1}, respectively). This demonstrates that higher N use efficiency (NUE) (see below) can be achieved with a combination of manure and commercial fertilizer N than with manure alone, and suggests the possibility substituting commercial fertilizer with separated liquid fraction which behaves similarly (Zhang et al. 2020) in order to reduce farm N surplus.

Nitrogen Use Efficiency, defined as crop N Uptake (i.e. N removed by harvest) divided by total applied N, averaged from 49% for LDM-high to 114% for Min-low (Table 3). NUE exceeding 100% could be due to soil N mining but there is no evidence of loss of soil N hence is more likely due to significant N input by atmospheric deposition (see below). NUE remained fairly constant over years in this trial with only the MIN-low treatment showing some decline due to possible benefit from historical N in 1996 and 2000 (Fig. 1) and there is little indication of increasing benefit from legacy N during the trial. Turning off ammonia emissions (assuming 30% of TAN for LDM, Søgaard et al. 2002; Webb et al. 2010) increases NUE estimates for LDM-high and ALT to about 60 and 76%, respectively, not accounting for N stored in soil (see below). Adjusting for likely wet and dry N deposition (40 kg N ha^{-1}, see above) the NUE declined from 49 to 43% for LDM-high and from 114 to 94% for Min-low. The deposition adjusted NUE for the intermediate 400 kg N ha^{-1} (nominal) total N rates typical of local farm practices was 64% and 67% for Min-high and LDM-low, respectively. Although the N application rates are higher than proposed, these values are between the 50–90% NUE limits suggested by the EU Nitrogen Expert Panel (n.d.). The surplus (lost N) N, 36 kg N ha^{-1} for LDM-low and 72 kg N ha^{-1} for MIN-high (Table 2; surplus is 76 and 112, respectively, when including atmospheric deposition), challenges the EU recommendation of 80 kg N ha^{-1} but the N uptake of 292–294 kg N ha^{-1} exceeded the maximum level of around 275 kg N ha^{-1} in the EU recommendations. The values in our study reflect the possibility for sustainable intensification, especially if more ammonia can be retained and used, which may be important for food security in land limited agricultural regions. The relatively high NUE for LDM-low reflects the use of relatively low emission trailing shoe applicator, a tool rarely used in long term experiments started in 1995, which reduces ammonia emissions and, importantly, applies manure uniformly. The grass NUE in this trial also reflects favourable soil physical, chemical and biochemical properties

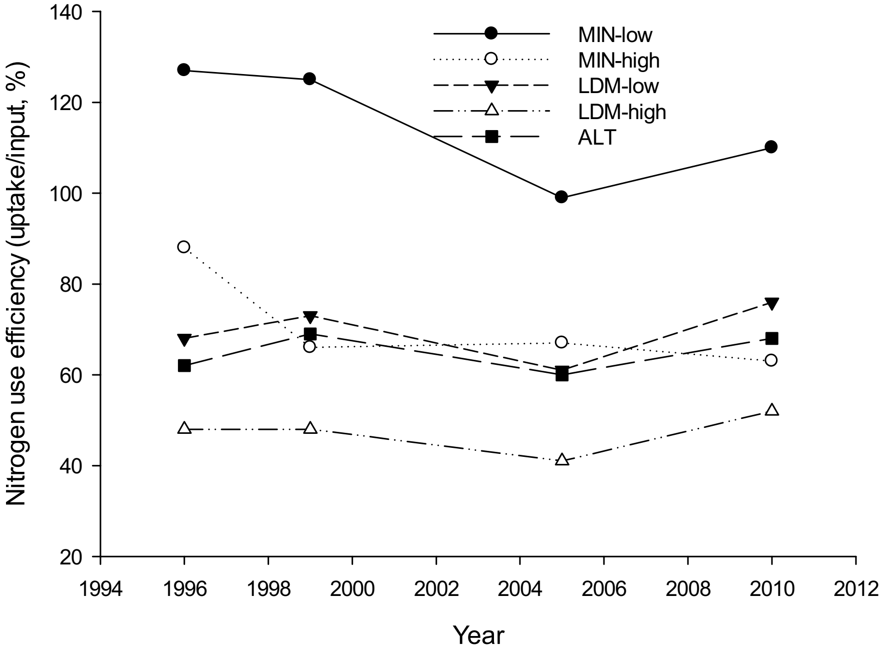


Fig. 1 Nitrogen use efficiency of grass from 1996 to 2010 based on total N uptake / total N application with no adjustment for N uptake of the control or for wet and dry N deposition (see text for explanation). Note MIN: ammonium nitrate; LDM: liquid dairy manure; ALT: alternate MIN-high and LDM-high

and crop productivity which is favoured by manure application (Neufeld et al. 2017). It is expected that NUE at the 400 kg N ha^{-1} rate might be somewhat improved by combining higher LDM applications in spring with lower MIN (or liquid fraction separated LDM) applications in summer to further reduce NH_3 and N_2O losses, and permit more in-season time for LDM-N mineralization and crop uptake although this needs to be tested (Hunt et al. 2019).

The δ^{15}N natural abundance in grass

The δ^{15}N natural abundance in Control grass decreased from 4.32‰ in 1999 to 2.01‰ in 2010 (Table 4). This result is related to the decreased grass N uptake from soil due to deficiency of available N, consistent with a tightening of the N cycle over time (Garten 1993; Compton et al. 2007; Craine et al. 2009). This also is consistent with the assumption that N input into control plots in later years is increasingly from deposition of depleted atmospheric N and some biologically fixed N from volunteer clover plants (Elliott et al. 2019; Liz Elmstgrom, University of WA, personal communication, 2020; Jana Compton, personal communication, 2020). The data suggests the multi-year effects of pre-trial management on the Control treatments.

Table 4 Grass δ^{15}N (‰) natural abundance affected by historical treatments in year 1996, 1999, 2005 and 2010

Treatment	Applied Tot.-N (kg N ha^{-1} yr^{-1})	Grass δ^{15}N (‰) by year sampled			
		1996	1999	2005	2010
		δ^{15}N (‰)			
Control	0	4.32 $^{\mathrm{b}}$[§]	3.75 $^{\mathrm{de}}$	2.05 $^{\mathrm{e}}$	2.01 $^{\mathrm{c}}$
MIN-low	198	3.62 $^{\mathrm{c}}$	3.42 $^{\mathrm{e}}$	2.16 $^{\mathrm{e}}$	1.10 cd
MIN-high [¥]	392	3.09 $^{\mathrm{d}}$	4.81 cd	2.61 $^{\mathrm{d}}$	0.77 $^{\mathrm{d}}$
LDM-low	379	5.99 $^{\mathrm{a}}$	6.10 $^{\mathrm{ab}}$	6.28 $^{\mathrm{b}}$	5.20 $^{\mathrm{b}}$
LDM-high	726	6.39 $^{\mathrm{a}}$	7.14 $^{\mathrm{a}}$	7.49 $^{\mathrm{a}}$	7.40 $^{\mathrm{a}}$
ALT	549	4.72 $^{\mathrm{b}}$	5.66 $^{\mathrm{bc}}$	5.05 $^{\mathrm{c}}$	4.27 $^{\mathrm{b}}$
LSD	–	0.47	1.06	0.21	1.21

MIN: ammonium nitrate; LDM: liquid dairy manure; ALT: alternate MIN-high and LDM-high

[§] Values within the column followed by the same letter are not significantly different at the $P < 0.05$

[¥] Bold Min high and LDM low treatments have similar amounts of applied Tot.-N

The δ^{15}N natural abundance in grass herbage of MIN-low and MIN-high treatments decreased over years, from 3.6 and 3.1‰, respectively, in 1996 to 1.1 and 0.77‰ in 2010, with the N depleted N source shift likely explaining this change (Table 4). In 2010 the δ^{15}N level in MIN-high herbage was approaching that in the fertilizer (0.43‰). The relatively similar decline in δ^{15}N of Control and MIN treatments from 1996 to 2005 suggests a similar proportion depleted N taken up from either deposition (Control) or fertilizer plus deposition (MIN). The δ^{15}N values suggest that by 2010 there was little legacy N in the herbage and that most of the N uptake was from the fertilizer and deposition. These results show that legacy N probably originating from pre-study applications of LDM was available to the crop in diminishing amounts for about 10–15 years after applications ceased, and that at 15 years there were only trace amounts used by the crop, especially in the MIN-high plots. This is an important inference for studies considering pre-trial management effects which is rarely reported or quantified in the literature.

In contrast to Control and MIN, LDM-low herbage maintained δ^{15}N of 5.20 to 6.28‰ in all sampling years despite annual slurry inputs of 379 kg N ha^{-1} with δ^{15}N averaging 11.89‰ (Table 4). This suggests that herbage N consisted substantially of urinary N which is known to be more depleted than faecal N (Balter et al., 2006). Since urinary N is mostly ammoniacal, some enrichment of LDM would be expected from ammonia volatilization after LDM application. The preferential volatilization and uptake of urine N in slurry is often not considered in N budgets; in this study availability of urine N was enhanced by the low emission applicator, a measure recommended for slurry on grass by the United Nations Economic Commission for Europe (UNECE) and is becoming increasingly mandated in countries. Atmospheric deposition also contributed depleted N. The herbage δ^{15}N from the LDM-high plots had plateaued (7.14 to 7.49 ‰) between 1999 and 2010. The greater δ^{15}N in the herbage of LDM-high than LDM-low (difference of 0.4 in 1996 to 2.2 in 2010) indicates that the LDM-high herbage contained a greater proportion of faecal and soil N and less urinary and deposited N compared to LDM-low. This shift in N source over time is important for understanding long term applications of manure so that somewhat less urinary N might be supplied with high manure rates over time, perhaps via solid liquid separation. The δ^{15}N of ALT herbage averaged 1.0‰ lower than LDM-low (significant in 2 of 4 years) and 2.2‰ lower than LDM-high (significant every year). The relatively low δ^{15}N values in the LDM herbage compared to the applied LDM might also suggest that mineralized legacy N from long-term manure applications is somewhat depleted in δ^{15}N or that plant uptake discriminates against ^{15}N but these possible factors have not been reported and need further study (Handley and Raven 1992).

Soil N stocks and δ^{15}N

Background concentration of total soil-N at the start of the study averaged 2.89 g kg^{-1} for the 0–15 cm depth (Table 2). Comparing total soil-N at 0–40 cm depth in 2011 shows that 15 years of LDM treatments significantly increased these values relative to Control; the cumulative increase by applications of LDM-low was 739 kg N ha^{-1}, averaging 49.3 kg N ha^{-1} year^{-1}, and representing 13.0% of applied total-N. Applications of LDM-high increased total soil N relative to Control by 1663 kg N ha^{-1} or 110.9 kg N ha^{-1} year^{-1}, which is 15.3% of applied total-N. This is slightly lower than reported for a shorter period (Bittman et al. 2007) and may indicate that the rate of N and C sequestration is declining so longer testing is needed.

In comparison to Control, there was no significant change in total soil N at 0–40 cm due to any of the treatments containing MIN, including the ALT treatment which also contained LDM. However, at the 0–5 cm depth (Table 5) there were significant increases in soil N associated with MIN containing treatments: 147 kg N ha^{-1} for MIN-low, 339 kg N ha^{-1} for MIN-High, and 281 kg N ha^{-1} for ALT, compared to increases of 287 and 504 kg N ha^{-1} for LDM-low and LDM-high, respectively. Of note, the values for treatments MIN-High and LDM-Low, which received similar rates total N, were similar (339 vs 287 kg ha^{-1}, respectively). Treatment differences were progressively less pronounced with increasing soil depth, particularly for the MIN treatments, so that the increases which were significant on the shallow layers were not significant at the 0–40 cm soil layer (269 for ALT and 423 kg N ha^{-1} for MIN-high) perhaps due to a weaker signal to noise ratio (see below). Our data that MIN at both rates increased soil N at 0–5 cm and that there was a non-significant increase at the 0–40 cm depth for MIN-high raises the possibility that MIN application to perennial grass will eventually increase total soil N and that longer study might be needed to clarify this important question. While MIN applications on perennial grass did not significantly increase stored soil N, the significant increase of total-N in the shallow layer would likely be beneficial to activity of soil macro- and micro-organisms (Neufeld et al. 2017). Enhanced shallow soil N would tend to benefit fibrous rooted plants like grasses but would also

Table 5 Whole-soil total N stocks (kg N ha^{-1}) and total N stocks in the heavy fraction in both topsoil and subsoil layers as affected by historical treatments in 2011. The comparison is based on fixed depth

Treatment	Applied Tot.-N (kg N ha^{-1} yr^{-1})	Soil depth (cm) Whole soil						Heavy fraction Soil depth (cm)	
		0–5	5–10	10–20	20–30	30–40	0–40	0–5	20–30
		kg N ha^{-1}							
Control	0	1330 ^{d}[§]	1457 ^{d}	2640 ^{c}	2683 ^{ab}	1265	9376 ^{c}	992	2271
MIN-low	198	1477 ^{c}	1419 ^{d}	2769 ^{bc}	2496 ^{bc}	1160	9321 ^{c}	1000	2031
MIN-high [¥]	392	1669 ^{b}	1520 cd	2741 ^{bc}	2581 ^{bc}	1289	9799 ^{bc}	1110	2131
LDM-low	379	1617 ^{b}	1629 ^{ab}	2850 ^{b}	2669 ^{ab}	1351	10,115 ^{b}	1028	2079
LDM-high	726	1834 ^{a}	1700 ^{a}	3213 ^{a}	2863 ^{a}	1429	11,039 ^{a}	1039	2138
ALT	549	1611 ^{b}	1582 ^{bc}	2654 ^{c}	2410 ^{c}	1389	9645 ^{bc}	1121	2002
LSD	–	102	105	184	245	438	707	108	210

MIN: ammonium nitrate; LDM: liquid dairy manure; ALT: alternate MIN-high and LDM-high

[§] Values within the column followed by the same letter are not significantly different at the $P < 0.05$

[¥] Bold Min high and LDM low treatments have similar amounts of applied Tot.-N enhance emissions of N_2O which occur near the soil surface (Thompson et al. 2016). The enhanced N in the upper layers of MIN plots probably originated from decomposing plant stubble, residue and roots and soil fauna near the surface.

Mineralized N in this site is prone to leaching especially during the rainy months of Oct. through March, although leaching loss from the MIN-low treatment is likely small in view of the high NUE (94% or 114% depending on inclusion of atmospheric depositions) which suggests a surprisingly low-loss tight N cycle (Table 2). Applied N may prime mineralization of organic N, but this was not evident from the soil N balance. Given that most N applied to MIN-low plots was harvested, it is not surprising that this treatment did not increase soil Tot-N. The increase in total soil N was not apparent in the fine heavy soil fraction at either 0–5 or 20–30 cm depths (Table 5) so the additional Tot.-N at the 0–5 cm layer is probably more prevalent in the lighter fractions, suggesting it is more labile.

Cumulative total N inputs minus crop N removal (i.e. net applied N) over 15 years ranged from -420 kg N ha^{-1} for MIN-low (i.e. total N removed exceeded total N applied) to 5595 for LDM-high (Table 2). Like MIN-low, the Control treatment had a negative crop N removal of -1530 kg ha^{-1}. As mentioned above, net N removed on the Control plots was likely returned in part by wet and dry deposition and N fixation so that if any soil N loss occurred in the Control plots it was likely increasingly small as the labile N declined. While rate of wet deposition would be similar for all treatments, rates of both N fixation and dry deposition may be favoured on low N plots, the former by greater encroachment by invasive legumes and the latter by steeper atmospheric and to plant-apoplast to atmosphere N gradients.

The total soil N (0-40 cm depth) in 2011 was positively related to cumulative net applied soil N (applied minus crop uptake) over 15 years regardless of source (Fig. 2). This means that of the applied N not taken up by the crop, from 10 to 58% accumulated in the soil, while the remainder, 90 to 42%, was lost from the plots, likely by NH_3 volatilization (especially LDM), NO_3 leaching (especially MIN, since little ammonia is lost from ammonium nitrate) and smaller rates of NO_3 denitrification (both LDM and MIN) (Paul and Zebarth 1997).

Table 6 shows soil $\delta^{15}N$ in spring of 2011 after 15 years of applications of MIN and LDM at contrasting rates. For the 0-15 cm soil depth, the $\delta^{15}N$ values for LDM were significantly higher than the initial (background) $\delta^{15}N$ of 6.48‰ using the Dunnet Test. The decline in the $\delta^{15}N$ of the Control treatment is unlikely to be caused by gaseous emissions as these would tend to enrich the soil $\delta^{15}N$. Also, there is no clear evidence that

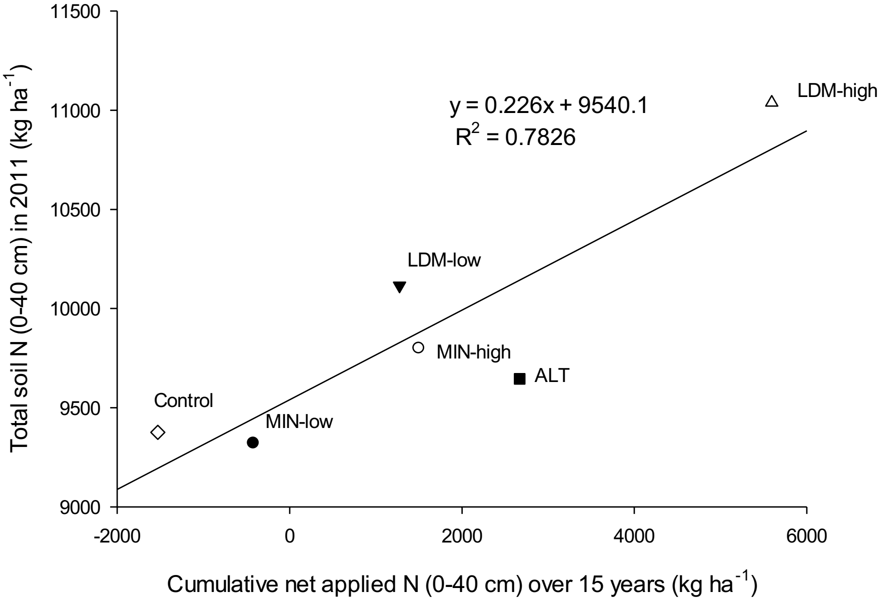


Fig. 2 Effect of net applied N (N applied—crop N uptake) over 15 years and total soil N in the 0–40 cm depth. Note MIN: ammonium nitrate; LDM: liquid dairy manure; ALT: alternate MIN-high and LDM-high

Table 6 The δ^{15}N (‰) natural abundance for whole soil and the heavy fraction in both topsoil and subsoil layers as affected by historical treatments in 2011

Treatment	Applied Tot.-N	Prior to exp. (cm)[£]	2011 (cm)[¶]	Whole soil depth (cm)					Heavy fraction soil depth (cm)	
		0–15	0–15	0–5	5–10	10–20	20–30	30–40	0–5	20–30
	(kg N ha^{-1} yr^{-1})	δ^{15}N (‰)								
Control	0	6.48	*5.82* ^{d§}	5.37 ^{d}	5.85 ^{d}	6.21 ^{b}	6.34 ^{b}	6.60 ^{bc}	5.77 ^{d}	6.47 ^{b}
MIN-low	198	6.48	*5.63* ^{e}	4.95 ^{e}	5.80 ^{d}	6.16 ^{b}	6.10 ^{c}	6.52 ^{bc}	5.53 ^{e}	6.21 ^{c}
MIN-high [¥]	**392**	6.48	*5.50* ^{e}	4.81 ^{e}	5.65 ^{d}	6.02 ^{b}	6.22 ^{c}	6.40 ^{c}	5.19 ^{e}	6.22 ^{c}
LDM-low	**379**	6.48	*6.81* ^{b}	6.87 ^{b}	6.81 ^{b}	6.73 ^{a}	6.81 ^{a}	6.77 ^{ab}	7.25 ^{b}	6.73 ^{a}
LDM-high	726	6.48	*7.35* ^{a}	7.58 ^{a}	7.37 ^{a}	7.09 ^{a}	6.86 ^{a}	6.92 ^{a}	7.91 ^{a}	6.92 ^{a}
ALT	549	6.48	6.49 ^{c}	6.48 ^{c}	6.48 ^{c}	6.54 ^{a}	6.57 ^{a}	6.72 ^{ab}	6.77 ^{c}	6.75 ^{a}
LSD	–	–	0.17	0.24	0.21	0.22	0.34	0.27	0.29	0.21

MIN: ammonium nitrate; LDM: liquid dairy manure; ALT: alternate MIN-high and LDM-high

[£] δ^{15}N in background soil (0–15 cm) prior to experiment: 6.48‰

[¶] Values in italic in this column are significantly different from pre-trial value of 6.48

[§] Values within the column followed by the same letter are not significantly different at the $P < 0.05$

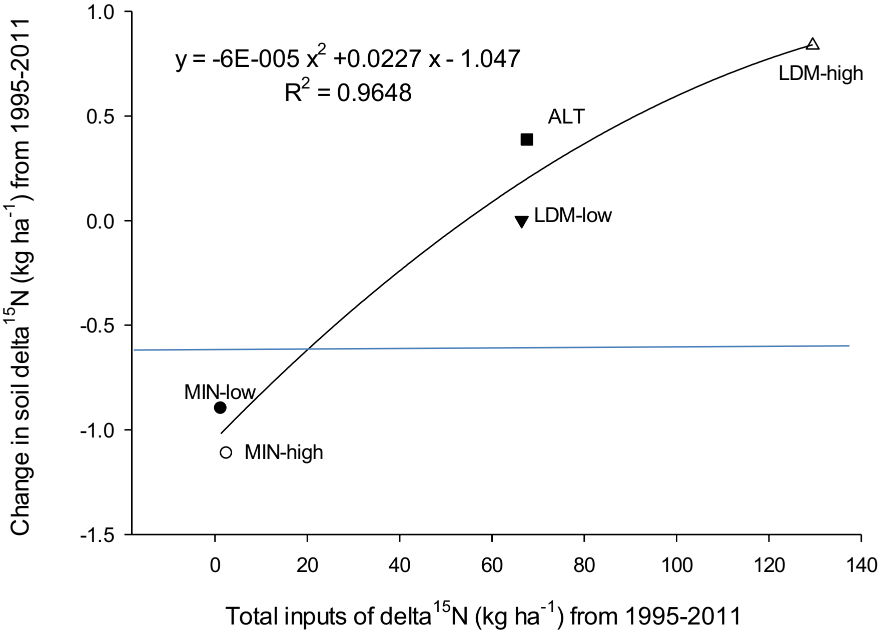
[¥] Bold Min high and LDM low treatments have similar amounts of applied Tot.-N

plant uptake depletes soil δ^{15}N (Evans 2001) while nitrate leaching is by mass flow processes which tends to not discriminate between isotopes (Ostrom et al. 1998). It is more likely that the decline in the Control treatment reflects inputs of N from atmosphere wet deposition of ammonium and NOx (~30 kg N ha^{-1} Lin et al. 2020) and dry deposition of depleted ammonia emitted by nearby dairy facilities and manure spreading (esp. due to the low ammonia compensation point of the control plants), and some N fixation by sparse volunteer white clover plants that escaped control, all N sources with depleted δ^{15}N (Compton et al. 2007). The decline in δ^{15}N in MIN plots was greater than in the Control plots likely due to addition of large amounts of depleted ammonium nitrate fertilizer but the depletion in the δ^{15}N of MIN-high was only slightly greater than that of MIN-low plots (5.50 vs 5.63‰, respectively) despite input difference of 200 kg N ha^{-1} yr^{-1}. This suggests that most of the soil N is non labile and that the labile N was taken up by the crop so that the soil δ^{15}N depletion may have happened soon after the start of the experiment when the labile N was enriched from historic (pre-trial) manure. That plant uptake better reflects added N than soil N is evidence that labile added N is the main source of N uptake for the plants.

Soil enrichment from addition of LDM-low at δ^{15}N of 11.89‰ was modest (6.81 vs 6.48‰ background), especially since additional enrichment is expected from depleting gaseous ammonia emissions, further suggesting that the soil N is quite stable. The lack of change in the ALT treatment was expected as this treatment contained both enriched LDM and depleted MIN. The enrichment due to LDM-high relative to background (0.87 ‰) was more pronounced and reflected the rather large increase in soil N in the LDM-high treatment (1663 kg N ha^{-1} or 15.3% of applied, Table 2). The relationship between 15 years of additions of contrasting δ^{15}N rates on soil δ^{15}N can be seen in Fig. 3. Addition of about 120 kg ha^{-1} of enriched N over the trial duration enriched the soil by almost 1.0‰ compared to the background whereas the addition of low δ^{15}N N diminished the soil by about 1.0‰. From the change in soil δ^{15}N values we estimate that the Control plots received about 18 kg of enriched N but the sources are not known although limited gaseous emissions might have contributed slightly to enrichment. Changes in δ^{15}N below 20 cm is probably due to tillage (1993 and 2003) and invertebrate activity (Raworth et al. 2004).

The concentration of δ^{15}N in the fine heavy fraction was consistently higher than the bulk soil which probably reflects less depleted N (from urine or MIN) in this fraction than the bulk soil

Fig. 3 Effect of total δ^{15}N (kg ha^{-1}) inputs over 15 years on change of soil δ^{15}N from background levels. Intersection of horizontal line with curve is assumed values for the Control. Note MIN: ammonium nitrate; LDM: liquid dairy manure; ALT: alternate MIN-high and LDM-high



(Table 6). Significant treatment effects on δ^{15}N in fine heavy fraction reflected the δ^{15}N levels in the MIN and LDM inputs. However, treatment effects on the fraction and bulk soil were similar at both sampling depths examined. The observations of substantial change in δ^{15}N but not in total N at the 0–5 cm layer, taken together, suggest that while the size of the fine heavy fraction N pool is relatively stable, it is subject to molecular N exchange with the N sources. This exchange may occur through decomposition of soil OM fraction by microorganisms or through immobilization of decomposing crop biomass which increasingly expressed differences in δ^{15}N (Table 4). The total inputs of N in MIN and LDM plots (except LDM-low) were approximately 50–100% (5685 to 10,890 kg N ha^{-1}) of total soil N to (40 cm depth) and several times greater than soil N at the 0–5 cm depth. Nevertheless these inputs with strikingly different δ^{15}N signatures from the soil (0.43 and 11.9 for MIN and LDM, respectively, compared to 6.5 for soil) had a relatively weak effect on δ^{15}N of bulk soil or the fine heavy fraction indicating that these fractions were quite stable despite moist and mild conditions and an active soil biota (Neufeld et al. 2017; Bittman et al. 2005; Raworth et al. 2004). The δ^{15}N values for LDM in current study (5.2 -7.9‰) were slightly lower than reported (8.7) for both control and long term application (1875 kg N ha^{-1}) of composted animal manure (Gerzabek et al. 2001). These authors reported that most of the soil N was divided between the silt and the clay fractions but applications of composted manure did not alter the silt δ^{15}N and only slight increased the clay δ^{15}N.

Conclusions

This study is one of the first to show the long term effects off low-emission application of LDM on crop N uptake, soil N accumulation and soil N losses in perennial grass sward. At equal long term rates of N, low-emission LDM and MIN had similar N recovery and NUE, but LDM produced a greater increase in soil N (13% of applied) whereas MIN had no significant effect on soil N (0–40 cm) but had greater soil N losses. Our study suggests that it is difficult to significantly build up soil N with LDM or MIN without incurring N losses. Alternating MIN and LDM produced high yield and NUE but also high N losses and no gain in soil N; future work should test reducing ALT losses with lower N rates, modifying timing of products and possibly replacing MIN with separated dairy liquids which tends to behave similarly. The increase in soil N at 0–5 cm depth with MIN may be indicative of gradually increasing soil N that might take longer to validate, but in itself may be important agronomically for fibrous-rooted grasses and for N_2O emissions.

Our study showed that $\delta^{15}N$ in MIN herbage declined through the trial and approached fertilizer $\delta^{15}N$ levels after 10–15 years, indicating the longevity of pre-trial management effects and the need for long term trials to clarify the fate of N. Unlike MIN, the $\delta^{15}N$ of LDM herbage stayed relatively constant and much lower than the LDM itself suggesting that crop N was largely obtained from the more depleted and more labile urine N, which was partially conserved by the low emission application technique. LDM-high had higher herbage $\delta^{15}N$ than soil $\delta^{15}N$ values compared to LDM-low treatment suggesting a greater role for the organic LDM fraction. The declining $\delta^{15}N$ in the control herbage suggest a labile, low $\delta^{15}N$ N source, which was probably atmospheric deposition and natural N fixation. Differences in the soil $\delta^{15}N$ signature of treatments were generally consistent with the applied inputs. However, very high quantities of applied N (approximately 50–100% of soil N at 0–40 cm depth, except MIN-low) with contrasting levels $\delta^{15}N$ of 0.43 for MIN and 11.9 for LDM vs. 6.5 for soil, the effect on the soil over 15 years were relatively weak suggesting a stable soil organic N pool despite moderate environmental conditions and active soil biota. It also suggests that some of the enriched organic fraction in LDM was lost probably by non discriminatory processes such as leaching. Even at the 0–5 cm depth which was physically close to the applications, the effects of the comparatively massive N inputs were significant but small. This may be attributed to high proportion of physically-protected fine heavy fraction.

More information is needed on long term N dynamics including soil N and C storage and crop growth to improve strategic management of manure and mineral fertilizer. Our study shows that despite limitations, natural N isotopes have a role in understanding N in long term trials, probably in conjunction with short term isotope spiking.

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Declarations

Competing interests None.

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