NOTES AND UNIQUE PHENOMENA

EVALUATION OF DAIRY MANURE NITROGEN-15 ENRICHMENT METHODS ON SHORT-TERM CROP AND SOIL NITROGEN BUDGETS

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Abstract

Indirect estimates of manure N availability to crops are highly variable. We developed two methods that label dairy manure N components with the stable isotope ^{15}N for direct measurement of manure N availability to crops. The *forage method* involved the labeling then feeding of ^{15}N-enriched forage to dairy cows (*Bos taurus*) to label urine N, fecal endogenous N, and fecal undigested feed N. The *urea method* involved the direct feeding of ^{15}N-enriched urea to label urine N and fecal endogenous N. Manure from each enrichment method was applied to a Plano silt loam (fine-silty, mixed, mesic, Typic Argiudolls) using field plots in 1999 and 2000; corn (*Zea mays* L.) was grown for 2 yr after each application. No significant differences were observed in manure ^{15}N recoveries in corn, soil inorganic N, or soil total N due to manure application year or manure enrichment method. Corn took up 14 to 16% of manure ^{15}N the first year and 4 to 8% the second year after application. Most ^{15}N recovery in soil inorganic and total N was found in the upper 30 cm of soil, indicating little downward movement of applied manure ^{15}N. On average, 68% of applied manure ^{15}N was accounted for, either in crop uptake (21%) or in the soil (47%). The less laborious and less costly urea enrichment method may be adequate for short-term (2 yr or less, the range of this study) manure–soil–crop–N cycling studies. Longer-term studies may need to include fecal undigested feed ^{15}N derived from the forage enrichment method.

Manure *N credits*, or the amount of applied manure N available to succeeding crops, are usually derived from indirect measurements that can vary greatly. For example, the *difference method* and the *fertilizer equivalent approach* estimated that −31 to 63% of dairy manure N was taken up by corn during the first growing season after application (Motavalli et al., 1989; Klausner et al., 1994). Muñoz et al. (2004) found that ^{15}N-enriched manure provided much less variable field estimates of first-year dairy manure N uptake by corn than either the difference method or the fertilizer equivalence approach. Manure N availability during the second and subsequent years can be more difficult to predict. More accurate estimates of manure N credits are needed to improve N management on dairy farms.

Most (70–80%) of the N consumed by a dairy cow is excreted about equally in urine and feces. Fecal N can be divided into two components: (i) endogenous N consisting of microbial products and microorganisms from the rumen, intestine, and hind gut, and N originating from the digestive tract itself; and (ii) undigested feed N. Urine N mineralizes rapidly in soil, followed by the less rapid mineralization of fecal endogenous N and fecal undigested feed N (Sørensen et al., 1994). The undigested feed N component of feces does not make a significant contribution to crop N requirements during the year following its application (Sørensen et al., 1994). However, this manure N component could be a significant contributor to soil organic matter and crop N requirements over the long-term and repeated applications.

Two methods were developed to enrich dairy manure N components with ^{15}N (Powell et al., 2004). The *forage method* involves the labeling and then feeding of ^{15}N-enriched forage to dairy cows to label urine N, fecal endogenous N, and fecal undigested feed N. This method is very labor intensive, expensive, and must be planned for well in advance, since the ^{15}N-enriched crops must be grown before they can be fed to livestock and the ^{15}N-enriched manure produced. The *urea method* involves feeding ^{15}N-enriched urea directly to dairy cows to label urine N and fecal endogenous N. No fecal undigested feed N is labeled using the urea method since no ^{15}N-enriched forage is fed. If urinary N and fecal endogenous N are the only manure components to make a significant short-term contribution to crop N requirements, then it may be possible to label only these two components for short-term N cycling studies. Also, the urea labeling method is much less laborious, less costly, and the labeled manure can be produced in a much more timely manner.

The objective of this note is to compare corn ^{15}N uptake, and the amount and forms of soil ^{15}N remaining in field plots amended with manure derived from the forage or urea labeling methods developed by Powell et al. (2004). Results of this field experiment may allow researchers to choose which method of manure labeling would be necessary for their trials.

Materials and Methods

A field trial was conducted from 1999 to 2001 at the West Madison Agricultural Research Station in Madison, WI (45°05′ N lat, 89°31′ W long). Manure N components were enriched in ^{15}N following the procedures outlined by Powell et al. (2004). In brief, "forage manure" was fabricated by feeding ^{15}N-enriched alfalfa hay (*Medicago sativa* L.) and corn

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Abbreviations: FM, forage manure; MEM, manure enrichment method; UM, urea manure.

silage to nonlactating Holstein dairy cows for 3 to 4 d and collecting all of the urine and feces excreted; and "urea manure" was made by periodically dosing the rumen (through fistulas) with ^{15}N-enriched urea of the same cow type fed unlabeled alfalfa hay and corn silage. *Forage manure* (FM) consisted of ^{15}N-enriched urine N, ^{15}N-enriched fecal endogenous N, ^{15}N-enriched undigested feed N, and unlabeled straw bedding. *Urea manure* (UM) consisted of ^{15}N-enriched urine, ^{15}N-enriched fecal endogenous N, unlabeled fecal undigested feed N, and unlabeled straw bedding. Feces and urine were collected separately for a period of 4 to 6 d and then recombined in the approximate weight ratios they were excreted, mixed with wheat (*Triticum sativa* L.) straw bedding, and stored in covered, 121-L plastic trash cans for 3 to 5 d before field application. The amount of bedding was determined by weighing and sampling the wheat straw a herdsman would add daily to four stanchions over a 5-d period (Powell, unpublished data, 1998). An average feces wet weight/straw air-dry weight ratio of 1.00:0.18 was used for all manure mixes. The amount of feces and urine applied each year was the same for each labeling method, but varied in total N and ^{15}N content (Table 1). Highest manure ^{15}N enrichments were applied as FM due to the greater ^{15}N content of the forage compared with the amount of ^{15}N-enriched urea fed to the dairy cows (Powell et al., 2004). Urea manure in 1999 had lower ^{15}N enrichment than 2000 due to less ^{15}N enriched urea fed the first study year.

Manure derived from the two enrichment methods was surface applied to 1.5 m wide by 2.3 m long micro plots containing three corn rows, as suggested by Jokela and Randall (1987). For the year before this experiment started, plots did not receive any manure and were planted to corn, which was removed as silage. Micro plots were established in 1999 and 2000 within eight row 6.0 by 10.6 m main plots. Main plots received unlabeled manure of approximately the same composition and rate (about 90 kg ammonical N ha^{-1}). Six replications of each manure type were used in 1999 and four in 2000. Manure was applied uniformly by hand over the surface of each micro plot, followed by two diskings within 3 to 4 h after application. Corn (cv. Lemke 6063) was planted immediately thereafter. Starter fertilizer (N–P_2O_5–K_2O composition of 9–23–30, 224 kg ha^{-1} in 1999; and 168 kg ha^{-1} in 2000) was band-applied to all plots. Whole corn plants (grain plus stover, cut approximately 5 cm above soil surface) were harvested at physiological maturity (110–120 d after planting) from the main and micro plots. In the main plots, 10 adjacent plants from one row were harvested in 1999, and 15 plants from three rows (five from each) were harvested in 2000 and 2001. Three plants were cut from the middle row of each of the ^{15}N microplots. After cutting, harvested plants were weighed, chopped in a stationary silage chopper (approximately 1 cm lengths), and 700 to 800 g (wet wt.) taken as a subsample. The subsamples were oven-dried (55°C, 7–10 d) to determine dry matter content. Samples from the untreated control plot and the ^{15}N microplots were ground in a stainless steel Wiley mill to pass a 2-mm screen, further ground in a Udy mill to pass a 1-mm screen, and analyzed for total N and ^{15}N. After sampling, the remaining plants were removed from the field. The site was chisel plowed each fall.

The effects of manure enrichment method on soil inorganic N and total N levels 1 or 2 yr after manure application were gleaned from analyses of soil samples taken from the untreated controls and the ^{15}N micro plots after the harvest of 2000. Soil samples were taken from the ^{15}N micro plots by combining cores systematically taken 25 cm from the midpoint of the plot in all four directions. In this way, two cores were taken from within the row and two cores were taken from between the rows. Four cores (two in row and two between rows) were also taken from the untreated controls. Soil samples were taken using a stainless steel auger to 90-cm depth in 30-cm increments. Subsamples were oven-dried (60°C), ground to pass a 2-mm sieve, analyzed for NH_4–N and NO_3–N, hand-ground in a ceramic mortar, and sieved to pass a 100-μm mesh for total N and ^{15}N analysis.

Table 1. Quantities of manure N applied using the forage and urea manure ^{15}N enrichment methods.

	Manure enrichment method			
	Forage†		Urea†	
	Manure N component‡			
Manure application year	Total N	Atom % ^{15}N	Total N	Atom % ^{15}N
	g m^2		g m^2	
1999	28.4	1.199	29.3	0.640
2000	23.8	1.637	30.4	1.096

† Forage manure contained ^{15}N labeled urine N, fecal endogenous N and fecal undigested feed N. Urea manure contained ^{15}N labeled urine N, fecal endogenous N, and unlabeled fecal undigested feed N.

‡ Unlabeled wheat straw bedding applied (3.6 g N m^{-2}) to each plot not included in this table.

Chemical Analyses

Total N and ^{15}N concentrations in applied urine, oven-dried (60°C, 48 h) feces, and soil and corn samples from the untreated control plots and the ^{15}N micro plots were determined using a Carlo Erba elemental analyzer coupled with a Europa 20/20 isotope ratio mass spectrometer. Samples were combusted at 1700°C and then swept through the analyzer using He gas. Ammonium–N and NO_3–N were determined according to a modification of the procedure described by Liegel et al. (1980). Soil KCl extracts were filtered through Whatman no. 2 paper and analyzed for NH_4–N in an automated colorimeter using the QuikChem Method 13-107-06-2-D (Lachat Instruments, Mequon, WI) with sodium phenate and 5.2% sodium hypochlorite, and for NO_3–N using the QuikChem Method 12-107-04-1-B (Lachat Instruments, Mequon, WI). Soil KCl extracts were treated following the micro diffusion technique described by Stark and Hart (1996) and analyzed for ^{15}N enrichment using the Carlo Erba elemental analyzer coupled with the Europa 20/20 mass spectrometer.

Calculations and Statistical Analyses

Manure ^{15}N additions, total corn ^{15}N uptake, and total soil ^{15}N to a depth of 90 cm were used to compute N balances for each manure enrichment method. Main plot corn yields and corn total N and ^{15}N concentrations from the three plants harvested from the microplots were used to calculate recovery of manure ^{15}N in corn using the following equation:

$$\text{Corn } ^{15}\text{N recov } \% = \frac{P\,(c - d)}{f\,(a - b)} \times 100 \qquad [1]$$

where P = total corn N, f = total applied manure N, a = atom % ^{15}N of applied manure, b = atom % ^{15}N in unlabeled manure, c = atom % ^{15}N in corn, and d = atom % ^{15}N in control corn.

Similarly, recovery of manure ^{15}N in total soil N was calculated as:

$$\text{Soil } ^{15}\text{N recov } \% = \frac{Q\,(e - g)}{f\,(a - b)} \times 100 \qquad [2]$$

where Q = total soil N, e = atom % ^{15}N of total soil N in treatment plots, g = atom % ^{15}N in control plots, and the other terms are the same as above.

Total manure N recovery was calculated as the sum of recoveries in soil and crop components as follows:

$$\text{Total N recovery }\% = \%\text{ N recov}_{\text{harv corn}} + \%\text{ N recov}_{\text{soil}} \quad [3]$$

Percentage ^{15}N recovery in NH_4– and NO_3–N (inorganic soil N) was calculated as:

$$^{15}IN\text{ recov }\% = \frac{IN_f\,(e - g)}{f\,(a - b)} \times 100 \quad [4]$$

where IN_f are soil NH_4– or NO_3–N concentrations measured in the fall of 2000, e = atom % ^{15}N of soil NH_4– or NO_3–N in treatment plots, g = atom % ^{15}N of soil NH_4– or NO_3–N in control plots, and the other terms are the same as above.

Statistical analyses were performed using SAS software (SAS Inst., Cary, NC) to test for significant differences in corn ^{15}N uptake, and ^{15}N in soil NH_4–N, NO_3–N, and total N at various depths due to year of manure application, manure enrichment method, and possible interactive effects of these two treatments.

Results and Discussion

Manure Nitrogen-15 Uptake by Corn

Corn whole-plant dry matter yields (Mg ha^{-1}) in the main plots that received unlabeled manures were 20.5, 20.2 (Muñoz et al., 2004), and 19.3 (unpublished, 2001) in 1999, 2000, and 2001, respectively. In the ^{15}N-treated micro plots, corn ^{15}N uptake during the first year after manure application was not significantly affected by either year or manure ^{15}N enrichment method (Table 2). Of the total manure ^{15}N applied, 14 to 16% was accounted for in corn harvested the cropping season after manure application. Average residual ^{15}N uptake by corn in 2001 (8%) was significantly greater than residual ^{15}N uptake in 2000 (4%). Total (first year plus residual year) corn ^{15}N uptake ranged from 18 to 23% with no significant differences due to year of application or manure ^{15}N enrichment method.

In other measurements from experimental plots during 1998, 1999, and 2000 (Muñoz et al., 2004), average 1st year corn recovery of applied ^{15}N-labeled FM was 10 to 22% and of unlabeled manure was 15 to 18% (apparent recovery). Although average manure N uptake estimates for each manure type were similar, the variability associated with ^{15}N-labeled FM (range 4–42%) was much less than with unlabeled manure (range −31 to 62%). Our estimates of corn ^{15}N uptake in FM- and UM-amended plots also corresponded well to crop ^{15}N uptake after sheep (*Ovis aries*) manure ^{15}N application (Sørensen et al., 1994; Thomsen et al., 1997). Higher ^{15}N uptake (56%) was found by Jensen et al. (1999) when barley (*Hordeum vulgare* L.) was undersown with ryegrass (the soil was continuously cropped) in small lysimeters where manure was immediately covered with soil, minimizing NH_3 volatilization losses.

The relatively low uptake of manure N by corn in this study was likely due to the initial high available soil N of the experimental site. At the onset of this study, our micro plots contained an equivalent of approximately 7900 and 32 kg ha^{-1} of total N and NO_3–N, respectively, in the upper 30 cm of soil (Muñoz et al., 2004), which is 20 to 30% higher than the typical averages for this soil.

Soil Nitrogen

Soil samples were taken from each ^{15}N micro plot only at the end of the 2000 cropping season. Therefore, soil inorganic and total N levels in plots amended with ^{15}N manure in 1999 reflect soil ^{15}N recovery two cropping seasons after application, and soil inorganic and total N levels in plots amended with ^{15}N manure in 2000 reflect soil ^{15}N recovery one cropping season after application (Table 3).

Inorganic Soil Nitrogen

No significant differences were observed in $^{15}NH_4$–N across soil depths (average ^{15}N recoveries of 0.69, 0.06, and 0.19% from 0- to 30-, 30- to 60-, and 60- to 90-cm soil depths, respectively) and manure enrichment methods (average recoveries of 0.34 and 0.31% from applied FM and UM, respectively) from either manure application year. Except for plots amended with UM in 2000, most (69–99%) ^{15}N in inorganic soil N (NH_4– plus NO_3–N) was found in the upper 30 cm (Table 3). Approximately two-thirds of the inorganic ^{15}N recovered in plots amended with UM in 2000 was found in the lower soil depths. This may have been due to the higher manure ^{15}N applied in the UM-amended (30 g m^2) than in the FM-amended plots (24 g m^2) in 2000 (Table 1), which may have caused some of this additional N to leach to depths below 30-cm. Plots amended with manure in 2000 had significantly greater ($P < 0.001$) soil inorganic ^{15}N than plots amended in 1999. This was primarily due to the time

Table 2. Nitrogen-15 uptake by corn the first and second year after application of ^{15}N- labeled dairy manure derived from the forage or urea enrichment methods.

Manure application year	Manure enrichment method	1st year ^{15}N uptake	2nd year ^{15}N uptake	Total ^{15}N uptake
		% of manure ^{15}N applied		
1999	forage	(6)† 14.0	4.3	18.3
	urea	(6) 15.9	3.8	19.8
2000	forage	(4) 14.8	8.4	23.2
	urea	(4) 15.9	7.3	23.3
Mean		15.1	5.6	20.7
Statistical difference ($P_r > F$)				
Year		0.899	0.022	0.299
Manure enrichment method (MEM)		0.640	0.667	0.808
Year × MEM		0.897	0.871	0.862

† Number in parentheses refers to the number of microplots used in calculation. For example, the six forage manure and urea manure plots used in 1999 to calculate 1st year uptake were the same six plots used in 2000 to calculate residual uptake.

Table 3. Effects of manure application year and enrichment methods on ^{15}N recoveries in soil inorganic and total N.

Manure application year	Soil N component	Manure enrichment method	Soil depth 0–30 cm	30–60 cm	60–90 cm	0–90 cm
			% of manure ^{15}N applied			
1999	inorganic†	forage	0.9 (0.18)‡	0.1 (0.04)	0.3 (0.20)	1.3 (0.17)
		urea	0.7 (0.17)	0.0 (0.01)	0.0 (0.03)	0.7 (0.15)
	total	forage	38.9 (8.58)	5.8 (0.78)	1.6 (0.58)	46.3 (7.32)
		urea	46.6 (9.28)	2.9 (1.07)	1.2 (1.26)	48.8 (9.05)
2000	inorganic	forage	1.7 (0.30)	0.2 (0.07)	0.1 (0.01)	2.0 (0.26)
		urea	1.8 (0.56)	1.7 (1.53)	2.1 (1.54)	5.6 (1.22)
	total	forage	34.9 (3.56)	6.9 (1.74)	3.2 (0.70)	45.0 (3.08)
		urea	35.5 (9.72)	5.7 (1.13)	3.3 (0.25)	44.5 (7.90)

† Ammonium-N plus nitrate-N.
‡ Standard errors are given in parentheses.

elapsed between manure application and soil sampling, and the number of corn crops grown. Whereas plots amended with manure in 1999 had two corn harvests, plots amended in the spring of 2000 had only one corn harvest before soil sampling.

Total Soil Nitrogen

No significant differences were observed in ^{15}N recoveries in total soil N (0–90 cm) due to year of application or manure enrichment method (Table 3). Average ^{15}N recoveries in plots amended with FM and UM were 46 and 47%, respectively. Averaged across years, the relative amount of applied ^{15}N recovered in the 0- to 30-, 30- to 60-, and 60- to 90-cm soil depths were not significantly different between the two manure enrichment methods, averaging 37, 6, and 2% in the FM-amended plots and 42, 4, and 2% in the UM-amended plots, respectively. Depth differences in ^{15}N recovery were statistically significant ($P < 0.001$), with highest recoveries obtained from the top 0- to 30-cm depth. No differences in ^{15}N recovery were observed between the 30- to 60- and 60- to 90-cm depths, suggesting little downward movement of applied manure N, or that leached N may have moved out of the 0- to 90-cm layer. Previous research with dairy manure (Comfort et al., 1988) and sewage sludge (Kelling et al., 1977) on similar soils in central Wisconsin showed little leaching during the corn growing season.

Soil Nitrogen-15 Balance

Manure ^{15}N recoveries (Table 2) in corn and total soil N (Table 3) were not significantly affected by either year of application or manure ^{15}N enrichment method. On average, 68% of applied manure ^{15}N was accounted for, either in crop uptake (21%) or in the soil (47%). The high recoveries of ^{15}N in total soil N were unexpected. We hypothesized that most of the applied urinary and fecal endogenous N (the only labeled components applied in UM and approximately 80% of the N applied as FM; Powell et al., 2004) would be readily available for corn uptake during the first year after application, and that N from these forms not taken up by corn would be lost via leaching, denitrification, or other processes. Our measurements do not indicate very much nitrate leaching since relatively little of the label was found in the lower two increments of the profile (Table 3), although some losses via this pathway may have occurred. Most of the ^{15}N unaccounted for (approximately 32%) was likely lost via ammonia volatilization, and to a lesser extent via denitrification. Although we incorporated manure within 3 to 4 h after surface application, Meisinger and Jokela (2000) reported that N volatilization from land-applied dairy manure can be up to 40% of total applied ammonical N during the first 4 h after surface application. Urine N, which represented 50 to 60% of total applied manure N, may have partially hydrolyzed to ammonium N and lost as ammonia between land application and incorporation. Denitrification losses range from 0.2 to 7.1% of incorporated dairy manure N with usually higher losses (up to 26%) for slurries (Dittert et al., 1998).

Conclusions

First year and residual second year corn N uptake, soil inorganic N, and soil total N levels were similar in plots amended with manure from either the forage or urea enrichment method. This suggests that the less laborious and less costly urea method of labeling only the labile dairy manure N components (urine and fecal endogenous N) may be adequate for evaluating short-term N dynamics (2 yr or less) in the soil–crop continuum. The contribution of fecal undigested feed N to long-term crop N requirements and soil N dynamics is uncertain and would likely need to be labeled using the forage method to produce manure for use in longer-term N cycling studies.

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