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 15N for direct measurement of manure N availability to crops. The forage method involved the labeling then feeding of 15N-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 15N-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 Argudolls) 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 15N 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 15N the first year and 4 to 8% the second year after application. Most 15N recovery in soil inorganic and total N was found in the upper 30 cm of soil, indicating little downward movement of applied manure 15N. On average, 68% of applied manure 15N 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 15N 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 15N-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 15N (Powell et al., 2004). The forage method involves the labeling and then feeding of 15N-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 15N-enriched crops must be grown before they can be fed to livestock and the 15N-enriched manure produced. The urea method involves feeding 15N-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 15N-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 15N uptake, and the amount and forms of soil 15N 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 15N following the procedures outlined by Powell et al. (2004). In brief, “forage manure” was fabricated by feeding 15N-enriched alfalfa hay (Medicago sativa L.) and corn

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 15N-enriched urea of the same cow type fed unlabeled alfalfa hay and corn silage. Forage manure (FM) consisted of 15N-enriched urinary N, 15N-enriched fecal endogenous N, 15N-enriched undigested feed N, and unlabeled straw bedding. Urea manure (UM) consisted of 15N-enriched urinary N, 15N-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 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 15N content (Table 1). Highest manure 15N enrichments were applied as FM due to the greater 15N content of the forage compared with the amount of 15N-enriched urea fed to the dairy cows (Powell et al., 2004). Urea manure in 1999 had lower 15N enrichment than 2000 due to less 15N 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-P₂O₅-K₂O 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 15N 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 15N 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 15N. 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 15N micro plots after the harvest of 2000. Soil samples were taken from the 15N 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 NH₄–N and NO₃–N, handground in a ceramic mortar, and sieved to pass a 100-μm mesh for total N and 15N analysis.

Chemical Analyses

Total N and 15N concentrations in applied urine, oven-dried (60°C, 48 h) feces, and soil and corn samples from the untreated control plots and the 15N 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 NO₃–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 NH₄–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 NO₃–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 15N enrichment using the Carlo Erba elemental analyzer coupled with the Europa 20/20 mass spectrometer.

Calculations and Statistical Analyses

Manure 15N additions, total corn 15N uptake, and total soil 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 15N concentrations from the three plants harvested from the microplots were used to calculate recovery of manure 15N in corn using the following equation:

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>Manure application year</th>
<th>Total N Atom % 15N Total N Atom % 15N</th>
<th>Manure N component</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
<td>FM</td>
</tr>
<tr>
<td></td>
<td>Forage manure contained 15N labeled urine N, fecal endogenous N and fecal undigested feed N. Urea manure contained 15N labeled urine N, fecal endogenous N, and unlabeled fecal undigested feed N.</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>28.4</td>
<td>1.199</td>
</tr>
<tr>
<td></td>
<td>23.8</td>
<td>1.637</td>
</tr>
<tr>
<td>2000</td>
<td>29.3</td>
<td>0.640</td>
</tr>
<tr>
<td></td>
<td>30.4</td>
<td>1.096</td>
</tr>
</tbody>
</table>

1 FM Forage manure contained 15N labeled urine N, fecal endogenous N and fecal undigested feed N. Urea manure contained 15N labeled urine N, fecal endogenous N, and unlabeled fecal undigested feed N.
2 Unlabeled wheat straw bedding applied (3.6 g N m⁻²) to each plot not included in this table.
Total manure N recovery was calculated as the sum of recoveries in soil and crop components as follows:

\[ \text{Total N recovery} \% = \frac{\% \text{ N recoy}_{\text{soil}} + \text{\% N recoy}_{\text{corn}}}{3} \]

Percentage \(^{15}\text{N} \) recovery in NH\(_4\)- and NO\(_3\)-N (inorganic soil N) was calculated as:

\[ ^{15}\text{N recoy \%} = \frac{IN_i (e - g)}{f (a - b)} \times 100 \]

where \( IN_i \) are soil NH\(_4\)- or NO\(_3\)-N concentrations measured in the fall of 2000, \( e = \text{atom} \% ^{15}\text{N} \) of soil NH\(_4\)- or NO\(_3\)-N in treatment plots, \( g = \text{atom} \% ^{15}\text{N} \) 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 \(^{15}\text{N} \) uptake, and \(^{15}\text{N} \) 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 \(^{15}\text{N} \)-treated micro plots, corn \(^{15}\text{N} \) uptake during the first year after manure application was not significantly affected by either year or manure \(^{15}\text{N} \) enrichment method (Table 2). Of the total manure \(^{15}\text{N} \) applied, 14 to 16% was accounted for in corn harvested the cropping season after manure application. Average residual \(^{15}\text{N} \) uptake by corn in 2001 (8%) was significantly greater than residual \(^{15}\text{N} \) uptake in 1998, 1999, and 2000 (Muñoz et al., 2004), average recoveries of 0.34 and 0.31% from applied FM and UM, 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 \(^{15}\text{N} \) micro plot only at the end of the 2000 cropping season. Therefore, soil inorganic and total N levels in plots amended with \(^{15}\text{N} \) manure in 1999 reflect soil \(^{15}\text{N} \) recovery two cropping seasons after application, and soil inorganic and total N levels in plots amended with \(^{15}\text{N} \) manure in 2000 reflect soil \(^{15}\text{N} \) recovery one cropping season after application (Table 3).

**Inorganic Soil Nitrogen**

No significant differences were observed in \(^{15}\text{NH}_4\)-N across soil depths (average \(^{15}\text{N} \) 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%) \(^{15}\text{N} \) 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 \(^{15}\text{N} \) recovered in plots amended with UM in 2000 was found in the lower soil depths. This may have been due to the higher manure \(^{15}\text{N} \) 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 \(^{15}\text{N} \) than plots amended in 1999. This was primarily due to the time...
Table 3. Effects of manure application year and enrichment methods on $^{15}$N recoveries in soil inorganic and total N.

<table>
<thead>
<tr>
<th>Manure application year</th>
<th>Soil N component</th>
<th>Manure enrichment method</th>
<th>Soil depth</th>
<th>% of manure $^{15}$N applied (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0–30 cm</td>
<td>30–60 cm</td>
</tr>
<tr>
<td>1999</td>
<td>inorganic†</td>
<td>forage</td>
<td>0.9 (0.18)</td>
<td>0.1 (0.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>urea</td>
<td>0.7 (0.17)</td>
<td>0.0 (0.01)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>forage</td>
<td>38.9 (8.58)</td>
<td>5.8 (0.78)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>urea</td>
<td>46.0 (9.28)</td>
<td>2.9 (1.07)</td>
</tr>
<tr>
<td>2000</td>
<td>inorganic</td>
<td>forage</td>
<td>1.7 (0.30)</td>
<td>0.2 (0.07)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>urea</td>
<td>1.8 (0.56)</td>
<td>1.7 (1.53)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>forage</td>
<td>34.9 (3.56)</td>
<td>6.9 (1.74)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>urea</td>
<td>35.5 (9.72)</td>
<td>5.7 (1.13)</td>
</tr>
</tbody>
</table>

† 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 U.M 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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