ANIMAL WASTE MANAGEMENT

Nitrogen-15 Labeling of Dairy Feces and Urine for Nutrient Cycling Studies

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ABSTRACT

Estimates of the availability of dairy manure nutrients to crops rely on indirect measurements and can vary greatly. More accurate estimates of manure nutrient availability are needed to improve manure management. The objective of this study was to enrich dairy feces and urine in ^{15}N to study nutrient flow in the feed–animal–manure–soil and crop–environment continuum. Ammonium sulfate (12.3 or 10 atom % ^{15}N) was applied to soil to enrich alfalfa (*Medicago sativa* L.) and corn (*Zea mays* L.) plants during growth. Alfalfa hay contained from 2.386 to 3.980 atom % ^{15}N in three harvests and corn silage contained 8.162 atom % ^{15}N. A feed mixture containing 55% alfalfa hay and 45% corn silage (4.026 atom % ^{15}N) was fed to two mature nonlactating cows (*Bos taurus*) for 36 h. The pattern of ^{15}N excretion in urine and feces was similar for both cows. The ^{15}N appeared in urine by 8 h and in feces by 24 h, and peaked by 30 h in urine (1.642 atom % ^{15}N) and by 54 h in feces (2.341 atom % ^{15}N). Enrichment approached basal levels at 132 h after initial feeding for both urine and feces. Of the total ^{15}N fed, 60% was recovered: 31% from urine and 29% from feces. Approximately 60 to 70% of the total N excreted in dairy feces was endogenous N and 30 to 40% was undigested feed N. Combining feces excreted during the 16- to 122-h period after initial feeding of ^{15}N-enriched feed would produce feces having uniformly labeled N components. The various ^{15}N-enrichment levels of urine and feces collected during different times after feeding offer possibilities for studying differential ^{15}N use in short- and long-term nutrient cycling studies.

THE ECONOMIC VALUE of animal manure depends on its ability to provide nutrients to crops. The timely delivery and application of known amounts of manure nutrients to specific fields form the primary basis for proper manure management. Estimates of manure nutrient availability to crops, otherwise known as *nutrient credits*, are currently single N, P, and K values given for the type of manure applied (solid or liquid) and method of application (incorporated or not). Nutrient credits are adjusted to account for multiple years of manure application, residual nutrient availability, and other such factors.

Although it has been shown that proper manure management can be profitable through reduced fertilizer costs, many farmers do not credit the nutrients contained in manure (Nowak et al., 1997). For example, in areas where manure has been land-spread, many farmers continue to apply fertilizers in sufficient quantities for attaining desired crop yield. The lack of manure nutrient crediting by farmers may be due to a number of factors that make manure an undependable source of plant nutrients, including differences in soil fertility levels where manure application experiments have been conducted, inherent shortcomings of the fertilizer-equivalent approach for estimating nutrient availability (Harmsen and Moraghan, 1988), and other problems. For example, the N and P contents (mean ± 1 SD) of liquid dairy manure (3.4 ± 1.32 and 1.6 ± 1.08 g L^{-1}) and of solid dairy manure (5.0 ± 1.5 and 3.0 ± 1.5 g kg^{-1}) are highly variable (Combs, 1991). Nutrient release from manure is also highly variable. Using the fertilizer equivalent approach, from 12 to 63% of dairy manure N and from 12 to 89% of dairy manure P have been estimated to be taken up by corn during the first growing season after application (Motavalli et al., 1989; Klausner et al., 1994). Nutrient availability in the second and subsequent years can be even more difficult to predict.

The stable isotope ^{15}N has been used extensively to evaluate the availability of fertilizer N to crops (Hauck and Bremner, 1976; Menzel and Smith, 1984), but its use in nutrient cycling studies involving animal manure has been limited (Sørensen et al., 1994). This has been due in part to the high cost of ^{15}N and the large quantities needed to enrich sufficient forage for feeding and labeling feces and urine. Also, the homogeneous ^{15}N labeling of manure N components must be assured. Ruminant fecal N consists of both endogenous N and N remaining in undigested feed. Endogenous N is composed of N contained in microbial products and microorganisms from the rumen, the intestine, and the hind gut, as well as the N originating from the digestive tract itself. A disproportionate labeling of fecal N components may lead to great error in determining the rate and extent of manure ^{15}N mineralization in soils (Sørensen et al., 1994). Our objective in this experiment was to study the ^{15}N-enrichment pattern of feces and urine from nonlactating dairy cows that were fed ^{15}N-enriched alfalfa hay and corn silage. The relative ^{15}N enrichment of undigested feed N and endogenous N in feces was also studied.

MATERIALS AND METHODS

Corn (NK hybrid N1500) and alfalfa (Cenex cv. Trailblazer) plants were enriched in ^{15}N at the University of Wisconsin Hancock Research Station (44°7′ N, 89°32′ W) on a Plainfield sand (mixed, mesic Typic Udipsamments) during the 1997 cropping season. The plot for corn (32 700 plants ha^{-1}) was fertilized with N, P and K at rates of approximately 6, 5, and

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Abbreviations: FNUE, fertilizer N use efficiency; NDF, neutral-detergent fiber; NDIN, neutral-detergent insoluble N; NDSN, neutral-detergent soluble N; PVC, polyvinyl chloride.

110 kg ha^{-1} prior to planting in early June. Ammonium sulfate, 12.3 atom % ^{15}N, was dissolved in water and applied using a watering can at an equivalent rate of 75 kg N ha^{-1} during the growth period to two adjoining corn rows 5 m in length (5 m^2) in each of three applications (total application of 225 kg N ha^{-1}). The corn plants were harvested at one-third milkline (700 g kg^{-1} moisture), chopped to 2- to 3-cm lengths, and ensiled in PVC silos. A 20-m^2 area of a second-year alfalfa stand was fertilized with 10 atom % ^{15}N in the same manner at an equivalent rate of 100 kg N ha^{-1} in each of two applications (total application of 200 kg N ha^{-1}). The first application was made in mid-June, the same day after cutting alfalfa growth to an aboveground height of 2 cm. The first alfalfa harvest occurred 27 d thereafter; all harvests involved cutting total aboveground biomass to a 2-cm height. The second fertilizer application was made immediately after the first harvest. The second alfalfa harvest occurred 35 d after the first harvest. No further fertilizer applications were made. A third alfalfa harvest was taken 42 d after the second harvest. Three alfalfa plants were selected randomly from each harvest and separated into leaf and stem components. All other alfalfa was dried to make hay.

The percent recovery of ^{15}N fertilizer in alfalfa and corn silage, and the percent recovery of ^{15}N feed in feces and urine were calculated according to Hauck and Bremner (1976) as follows:

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

where P is the total N amount in the forage, feces, or urine; f is the amount of ^{15}N fertilizer applied or ^{15}N fed as forage; a is the atom % ^{15}N concentration in the labeled fertilizer (12.3 atom % for alfalfa and 10 atom % for corn silage) or in the forage (4.026%); b is the natural abundance of ^{15}N (assumed to be 0.366 atom %; Hauck and Bremner, 1976) in fertilizer or forage; and c is the % ^{15}N in alfalfa and corn harvested from plots receiving ^{15}N-enriched fertilizer or in feces and urine after feeding ^{15}N-enriched forage.

Two ruminally fistulated nonlactating dairy cows weighing approximately 420 kg were used in the feeding trial. The animals were adapted to a diet consisting of 55% alfalfa hay and 45% corn silage on a dry matter basis (atom % ^{15}N at natural abundance) for 7 d. On the last day of the adaptation period, indwelling catheters were inserted into the bladders for urine collection. For 36 h thereafter, ^{15}N-enriched alfalfa hay and corn silage were fed at the 55:45% ratio used during the adaptation period. The diet consisted of alfalfa and corn harvested from the ^{15}N treated plots as well as alfalfa and corn harvested from border areas of the ^{15}N-treated plots. Border areas included the outer 15-cm perimeter of ^{15}N-treated alfalfa plots and plants within 15 cm of the end of each ^{15}N-treated corn row. Border-area forage was used for two reasons: (i) the amount of forage harvested from the ^{15}N treated plots was inadequate for feeding and subsequent enrichment of sufficient feces and urine for a field trial and (ii) the forage from the border plots was enriched in ^{15}N so its use would result in a greater ^{15}N enrichment of feces and urine than if forages containing ^{15}N at natural abundance were used. Border-area alfalfa from Harvests 1 and 2 contained 1.189 and 1.828 atom % ^{15}N, and border-area corn silage contained 3.522 atom % ^{15}N. Alfalfa hay from each harvest and corn silage were divided into six equal parts on a weight basis. The six hay–silage mixtures were each mixed carefully by hand. One mixture was offered to each animal every 12 h. At 36 h, the small amount of unconsumed feed was put into the rumen through the cannulus and cows began to be fed the same forage as during adaptation. Cows were kept in two adjoining stanchions and bedded with rubber mats. Total feces and urine were collected at 4-, 8-, or 12-h intervals after initial offer of ^{15}N-enriched forage, up to a total of 192 h (Fig. 1 and 2). Feces were hand-scraped from heavy metal catching containers fitted into the gutters and from the rubber mats used for bedding. Urine was collected from the catheter tubes draining into plastic containers embedded in ice. Feces and urine from each collection were frozen immediately.

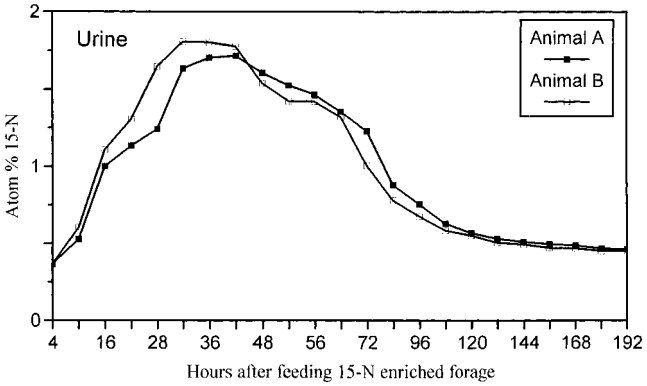

Fig. 1. Concentration of ^{15}N in urine after feeding ^{15}N-enriched forage.

Samples of ^{15}N-enriched feeds and feces were oven-dried (55°C) for dry matter determination. Dried samples were ground to pass a 1-mm screen. Total N and ^{15}N concentrations in feeds, feces and liquid urine were determined using a Carlo Erba (Milan, Italy) elemental analyzer coupled with a Europa 20/20 tracermass. Samples were flash-combusted at 1700°C and then swept through the analyzer using He gas. Cell-wall components of feeds and feces were determined using the detergent system (Goering and Van Soest, 1970) as neutral-detergent fiber (NDF). Total N and ^{15}N contained in cell walls of feeds and feces were determined as neutral-detergent insoluble N (NDIN). The NDF-soluble N (NDSN) fraction in feed (cell-wall contents) and feces (endogenous N) was estimated as the difference between total N and NDIN. Differences in ^{15}N content of total N and NDIN in feces were determined using a t-test for paired observations (Little and Hills, 1978).

RESULTS AND DISCUSSION

For alfalfa, the lowest N and highest atom % ^{15}N contents were found with the first harvest, and the highest N and lowest atom % ^{15}N contents were associated with the third harvest (Table 1). The pattern of increasing N content in successive alfalfa harvests was due to

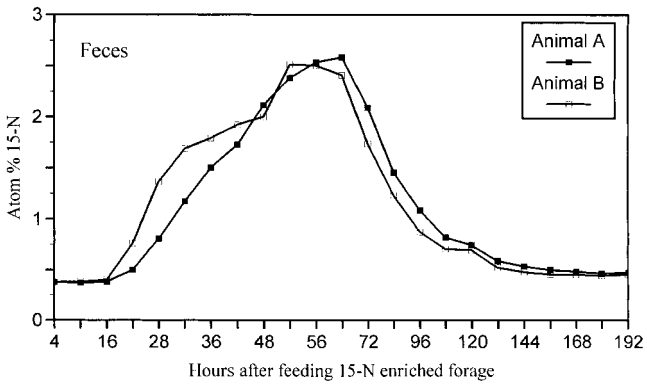

Fig. 2. Concentration of ^{15}N in feces after feeding ^{15}N-enriched forage.

Table 1. Composition of ^{15}N-enriched diet for nutrient cycling studies with dairy cattle.

Forage	Leaf:stem weight	Plant part	Total N	Atom % ^{15}N
			g kg^{-1}	%
Alfalfa				
Harvest 1	1:1.37	Leaves	42.44	3.980
		Stems	20.29	3.891
Harvest 2	1:0.69	Leaves	48.41	3.587
		Stems	24.13	3.664
Harvest 3	1:0.39	Leaves	49.42	2.386
		Stems	26.86	2.567
Corn silage	not recorded	Leaves	22.41	8.620
		Stems	3.50	7.609
Alfalfa–corn silage	not applicable	Total feed mix	19.42	4.026
		NDF in feed	2.74	3.745

† NDF, neutral-detergent fiber.

relative changes in production and N content of leaves and stems. The proportion of stems was two to four times greater in the first harvest than in the second or third harvests. Alfalfa stems contained only about half the N of alfalfa leaves. At the end of the growing season, approximately 36% of the applied fertilizer ^{15}N was accounted for in the three alfalfa harvests and 73% in corn silage. For corn, this recovery of fertilizer N was higher than 62% fertilizer N use efficiency (FNUE) by corn found by Bundy and Andraski (1998) at the study site using ^{15}N-depleted fertilizer. The apparent high FNUE of corn silage found in our nonreplicated study plot can probably be attributed to the careful, three-way split application of fertilizer N, and to the provision of irrigation to optimize water availability, forage growth, and N uptake.

The ^{15}N-enriched diet consisted of 55% alfalfa and 45% corn silage dry matter and had a total N content of 19.42 g kg^{-1}, of which 4.026 atom % was ^{15}N (Table 1). The pattern of ^{15}N excretion in urine (Fig. 1) and feces (Fig. 2) was similar for both cows. Nitrogen-15 began to appear in urine between 4 to 8 h and in feces between 16 to 24 h after the initial offer of ^{15}N-enriched feed. Peak ^{15}N concentrations were attained by 30 h in urine (1.642 % ^{15}N) and by 54 h in feces (2.341 % ^{15}N). A more rapid ^{15}N excretion in urine than feces reflected rapid absorption of labeled $^{15}NH_3$ from the rumen and its conversion into urea in the liver. Nitrogen-15 enrichment approached basal levels 132 h after feeding for both urine and feces. Peak ^{15}N concentrations attained 41% in urine and 58% in feces of the ^{15}N concentration in feed.

Of the total ^{15}N fed, 60% was recovered by 192 h: 31% in urine, and 29% in feces (Fig. 3). This 60% ^{15}N recovery corresponds to the 50 to 60% ^{15}N recovery in feces and urine from lactating Holstein cows fed an inorganic ^{15}N supplement during a 72-h sampling period (Sadik et al., 1990). The 40% ^{15}N retained by the nonlactating, mature cows, reflecting only the N consumed during the initial 36 h, began its depletion at 60 h (Fig. 2). Approximately 30% of the ^{15}N fed was recovered in feces within 96 h. Because all unconsumed ^{15}N-enriched feed was hand-entered into the rumen at 36 h, and most of this ^{15}N-enriched feed probably passed the rumen and was excreted within 60 h thereafter (Church, 1976, p. 99–114), one can assume that approximately 30% of feed ^{15}N was undigested. The ^{15}N excreted in feces and urine after 96 h mainly reflected recycling of ^{15}N that was absorbed from the digestive tract as NH_3 or amino acids.

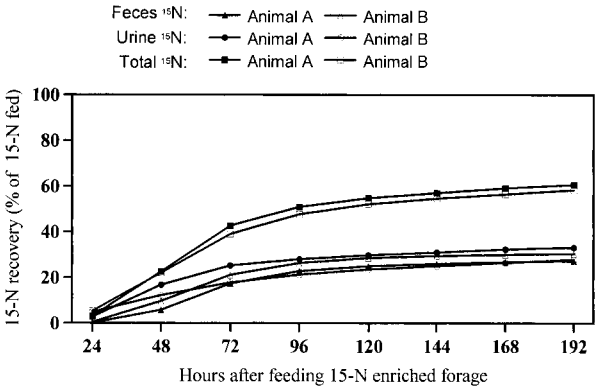

Fig. 3. Recovery of ^{15}N in dairy feces and urine.

The decomposition of feces (and other organic material) added to soil is best described by the two-pool exponential model

$$y = P_1 e^{-k1t} + P_2 e^{-k2t} \quad [2]$$

where y equals the proportion of original material remaining after time t, P_1 is a readily decomposable pool, P_2 is a less decomposable pool, and k_1 and k_2 are decomposition constants for P_1 and P_2, respectively (Molina et al., 1983; Van Veen et al., 1984; Deans et al., 1986). When sheep (*Ovis aries*) feces are applied to soil, NDSN mineralizes readily, followed by NDIN (Somda et al., 1995; Sørensen et al., 1994). These potential differences in N mineralization necessitate a uniform ^{15}N labeling of fecal N components for nutrient cycling studies (Sørensen et al., 1994). Uneven ^{15}N labeling of fecal N components could cause significant errors in estimating the rate and amount of fecal N mineralized in soil. Feces having a greater labeling of NDSN (endogenous N) than NDIN (undigested feed N) would falsely appear to mineralize more rapidly in soil than feces having equal NDSN and NDIN labeling; likewise, feces having a greater labeling of NDIN than NDSN would falsely appear to mineralize more slowly in soil than feces with equal labeling.

From 77 to 83% of the total N excreted in dairy feces under the dietary condition was NDSN and 17 to 23% as NDIN (Table 2). Across a wide range of diets, sheep feces contained from 52 to 75% NDSN (Powell et al., 1994). Feces from sheep fed annual ryegrass (*Lolium multiflorum* L. cv. Ninak) contained 57% NDSN (Sørensen et al., 1994).

The homogeneity of ^{15}N labeling of fecal N components was evaluated by comparing ^{15}N concentrations in total N and NDIN. The NDSN in feces can be calculated as the difference between total fecal N and NDIN (Mason and Frederiksen, 1979). Our present results (Fig. 4) indicate that ^{15}N labeling of NDIN and NDSN fecal components was similar 60 h after initiating the feeding of ^{15}N-enriched forage. The ratio of average NDI^{15}N to total ^{15}N (0.87:1.00) from 28 to 52 h was significantly ($P < 0.05$) less than the NDI^{15}N:total ^{15}N

Table 2. Neutral-detergent insoluble (NDIN) and soluble (NDSN) nitrogen in dairy feces (dry matter basis).†

Time after feeding ^{15}N forage	NDIN		NDSN‡	
	Animal A	Animal B	Animal A	Animal B
h	g kg^{-1}			
4	3.59	3.37	15.81	16.49
28	3.16	3.75	15.63	15.76
36	3.17	3.49	15.63	17.46
52	4.07	4.40	14.03	14.65
60	4.38	4.20	14.81	14.21
84	4.37	4.02	15.63	15.47
108	4.29	3.57	15.81	18.05
132	3.63	4.24	15.86	18.35
180	3.62	3.94	16.67	15.51
Means	3.84	3.95	15.51	16.19

† From two mature nonlactating cows.
‡ NDSN = Total N − NDIN.

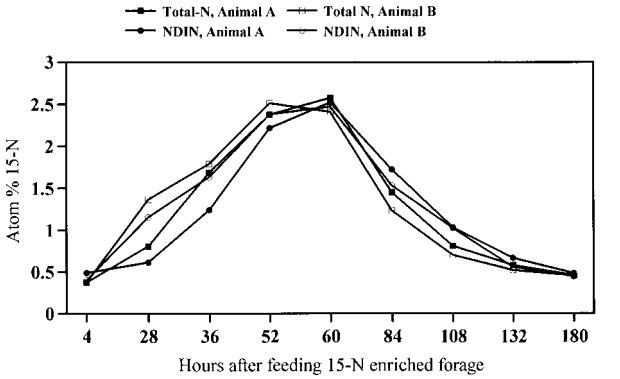

Fig. 4. Atom % ^{15}N in total nitrogen (TN) and neutral-detergent insoluble nitrogen (NDIN) of dairy feces.

ratio (1.28:1.00) obtained from 84 to 108 h. Mixing feces excreted between 16 and 122 h would provide feces having a ratio of NDI^{15}N to total ^{15}N near unity (1.02:1.00).

Most current methods of estimating the contribution of manure N to crop N requirements rely on indirect measurements. One example is the *fertilizer equivalent* method whereby yields and crop N uptake in manure-amended plots are compared to yields and crop N uptake in adjacent fertilizer-amended plots. The fertilizer equivalent of manure is the amount of fertilizer N required to achieve the same yield and N uptake achieved with manure. The fertilizer equivalent approach assumes that crop N uptake in the fertilizer-amended and manure-amended plots are accomplished with the same efficiencies. However, whereas most manure N is organically bound and must be mineralized by soil microbes before becoming available for crop uptake, fertilizer N is more water soluble and potentially more readily available for crop uptake. Also, fertilizer may create a greater priming effect than manure (Broadbent, 1984). In their review of research on the priming effect, whereby additions of energetic material along with (labeled) fertilizer N stimulate soil organic matter (SOM) transformations, Jansson and Persson (1982) concluded that such effects are normal features of mineralization–immobilization turnover (MIT). When fertilizer ^{15}N is added to soil, SOM turnover will cause this pool to lose labeling by immobilization and to gain unlabeled soil N by mineralization. It does not necessarily mean that a real stimulation of mineralization by the fertilizer ^{15}N occurred. Most investigations where a priming effect has been recorded have ignored immobilization of ^{15}N, a common feature of MIT. The potential differential effects of fertilizer N and manure N on MIT calls into question the use of fertilizer equivalents to estimate manure N availability to crops.

The various ^{15}N-enrichment levels of urine and feces offer possibilities for differential ^{15}N use in short- and long-term nutrient cycling studies involving animal excreta. For example, highly enriched material, such as urine captured between 24 and 72 h (Fig. 1) or feces captured between 36 and 84 h after feeding (Fig. 2 and 4), could be used for long-term field trials aimed at determining crop uptake of manure N during the first, second, and third year after initial manure application. Uniformly labeled feces and urine of lower ^{15}N enrichment could be used for shorter-term studies, such as manure and soil incubations and greenhouse trials.

The minimum atom % ^{15}N abundance of urine or feces required for a particular nutrient cycling study would depend on the expected ^{15}N content of soil extracts and crops after manure application and the detection limit of the mass spectrophotometer. For example, using ^{15}N-enriched manure, at least 0.376 atom % ^{15}N (i.e., 0.366 natural abundance + 0.01 detection limit of the mass spectrophotometer) would be needed in corn harvested 3 years after manure application. If we assume a manure N decay series of 25–10–5 (Klausner et al., 1994), approximately 5% of manure ^{15}N applied in Year 1 would be taken up by corn in Year 3. For corn to have 0.376 atom % ^{15}N abundance in Year 3, the manure applied in Year 1 would have to have at least 0.566 atom % ^{15}N abundance as calculated by the equation $0.376 = (0.366 \times 0.95) + (0.566 \times 0.05)$. Of the total N in corn harvested during Year 3, 95% would be derived from ^{15}N at natural abundance (0.366) and 5% would be derived from manure at 0.566 atom % ^{15}N abundance that was applied in Year 1. Expected ^{15}N content of corn in Years 1 and 2 would be 4.16 and 3.86 g kg^{-1}, respectively, if manure applied during Year 1 contained 0.566 atom % ^{15}N abundance.

SUMMARY AND CONCLUSIONS

The use of the stable isotope ^{15}N allows for direct measurement of nutrient flow in various components of the feed–animal–manure–soil and crop–environment continuum. This work evaluated the conversion of forage N into dairy urine N and fecal N, and the relative uniformity with which forage N is incorporated into the undigested feed N (NDIN) and endogenous N (NDSN) components of feces. Information on the ^{15}N-enrichment pattern of feces and urine is needed to select material of adequate enrichment for use in short- or long-term nutrient cycling studies. Similar labeling of the rapidly decomposable pool of N in feces (NDSN) and the less decomposable pool (NDIN) must be obtained to accurately determine the rate and extent of fecal N mineralization in soils. Uniform labeling of fecal components

can be achieved by the proportionate combination of feces having N components of different ^{15}N enrichments (i.e., feces excreted between 16 and 122 h under the experimental conditions of this study) or by feeding ^{15}N-enriched forage over a long period (Sørensen et al., 1994). The relative effectiveness of using ^{15}N-labeled urine and feces in nutrient cycling studies will depend on their ability to more accurately measure N mineralization in soils than the classical, indirect measurements (e.g., fertilizer equivalent) currently in use.

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