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Nitrogen Isotope Fractionation and Origin of Ammonia Nitrogen Volatilized from Cattle Manure in Simulated Storage

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Abstract: A series of laboratory experiments were conducted to establish the relationship between nitrogen (N) isotope composition of cattle manure and ammonia emissions, potential contribution of nitrogenous gases other than ammonia to manure N volatilization losses, and to determine the relative contribution of urinary- vs. fecal-N to ammonia emissions during the initial stage of manure storage. Data confirmed that ammonia volatilization losses from manure are most intensive during the first 2 to 3 days of storage and this coincides with a very rapid loss (hydrolysis) of urinary urea. Long-term (30 days) monitoring of $\delta^{15}\text{N}$ of manure and emitted ammonia indicated that the dynamics of N isotope fractionation may be complicating the usefulness of the isotope approach as a tool for estimating ammonia emissions from manure in field conditions. The relationship between $\delta^{15}\text{N}$ of manure and ammonia emission appears to be linear during the initial stages of manure storage (when most of the ammonia losses occur) and should be further investigated. These experiments demonstrated that the main source of ammonia-N volatilized from cattle manure during the initial 10 days of storage is urinary-N, representing on average 90% of the emitted ammonia-N. The contribution of fecal-N was relatively low, but gradually increased to about 10% by day 10. There appears to be substantial emissions of nitrogenous gases other than ammonia, most likely dinitrogen gas, which may account for up to 25% of N losses during the first 20 days of manure storage. This finding, which has to be confirmed in laboratory and field conditions, may be indicative of overestimation of ammonia emissions from cattle operations by the current emissions factors.

Keywords: cattle manure; ammonia; urinary urea; isotope fractionation

1. Introduction

Ammonia (NH₃) emitted from animal feeding operations is a major air and water pollutant contributing to surface water eutrophication, soil acidity, and fine particulate matter (PM_{2.5}) formation [1,2]. Current estimates for livestock contribution to anthropogenic NH₃ in the U.S. are at 50% [1]. Some reports have indicated, however, that a significant portion of manure N lost during storage may be as non-NH₃ gases, such as dinitrogen gas (N₂) [3]. The latter authors suggested, for example, that N₂ emissions from swine lagoons are many times greater than emissions of NH₃. Emissions of N₂ from cattle manure may be also high, particularly during the initial stage of manure storage when the bulk of urinary N is volatilized. If this is the case, mass balance, or other indirect approaches (*i.e.*, not measuring NH₃ emissions directly; isotope, manure minerals:N ratios [4]) for estimating NH₃ emissions may not be accounting for gaseous non-NH₃-N losses and thus, NH₃ emissions from cattle operations may be overestimated. For example, 25 and almost 50% of the daily N flow in dairy and beef cattle operations, respectively, were unaccounted as milk, daily body weight gain, or manure [2]. How much of this loss is NH₃ and how much non-NH₃-N is unknown. It is important to point out that N₂ is an inert gas and, unlike NH₃, is not considered an air pollutant.

Of the two major N pools in cattle (or most farm animals) manure, feces and urine, the latter (specifically, urinary urea in cattle) is generally considered to be the major source of emitted NH₃ [5]. Although the biological and biochemical ground for such an assumption is solid, there is surprisingly little experimental data to support it. For example, the conclusions of Bussink and Oenema [5] are primarily based on a study with soil application of synthetic urinary N compounds [6]. To our knowledge, only one study directly investigated urinary *vs.* fecal N contribution to volatile N emissions from animal manure [7]. Nitrogenous gas emissions from manure are to a large extent dependent on manure composition [2], which in turn depends on the animals' diet. Thus, it is important to quantify the actual contribution of urinary N to these emissions, particularly in the initial stages of manure storage when emissions are most intensive, which would allow for successful mitigation of manure emissions through dietary means.

A substantial part of mitigating manure emissions, including NH₃, is the availability of accurate and practical methods for estimating emissions. Direct measurement techniques are "the gold standard", but are affected by a multitude of environmental factors (temperature, wind velocity; see later discussion) and are of limited value when, for example, the effect of diet on manure emissions is evaluated [2,8]. The U.S. Environmental Protection Agency (EPA) recently released data from the National Air Emissions Monitoring Study [9], in which gaseous emissions, including NH₃, from several commercial dairy operations were monitored. In this study, barn NH₃ emissions varied from 4.6 (a California dairy) to 78 g/cow/day (a Washington dairy). Similar large variability in directly monitored NH₃ emissions from dairy farms (0.82 to 250 g NH₃/cow/day) or beef feedlots (50 to 283 g NH₃/animal/day) was reported in a recent literature review [2]. With such large variability, determining the specific effect of diet is practically impossible. Therefore, we have

investigated indirect methods for estimating manure NH_3 emissions, utilizing minerals:N ratios and natural N isotope fractionation [4]. The isotope method appeared promising, however, the relationship between $\delta^{15}\text{N}$ of manure and NH_3 volatilization is a dynamic process and longer monitoring periods are necessary to determine the usefulness of this approach for practical applications.

In this study, a series of laboratory experiments were conducted with the following objectives: (1) establish the relationship between manure N isotope composition and NH_3 emissions beyond 10 days of storage; (2) investigate the potential contribution of nitrogenous gases other than NH_3 to manure N volatilization losses; and (3) determine the relative contribution of urinary- vs. fecal-N to NH_3 emissions during the initial stage of manure storage. We hypothesized that: (1) $\delta^{15}\text{N}$ of NH_3 and manure N will continue to increase beyond 10 days and will reach a plateau; (2) non- NH_3 gases, such as N_2 , may account for a significant portion of manure N losses, particularly during the initial storage phase; and (3) urinary urea-N is the primary source of NH_3 -N emitted from cattle manure during the initial, most intensive, phase of manure N volatilization losses.

2. Materials and Methods

2.1. Manure Preparation and Experimental Settings

Feces and urine for these experiments were collected from dairy cows fed a diet containing approximately 60% forage (corn silage, alfalfa haylage, and grass hay) and 40% concentrate (corn grain, whole-heated soybeans, canola meal, a bakery byproduct, cottonseed hulls, a sugar blend, a non-protein N source, and a mineral/vitamin premix) as a total mixed ration. The diet contained (as % of dry matter, DM): crude protein, 15.5; neutral-detergent fiber, 32.9; non-structural carbohydrates, 41.6, and total digestible nutrients, 72.3. Cows were on average 149 ± 40 days in milk, produced 44 ± 1.4 kg/day milk, and were housed at the Pennsylvania State University's dairy research center. All procedures involving animals were reviewed and approved by the Pennsylvania State University's Institutional Animal Care and Use Committee.

In each experiment, 2 cows were used as donors of feces and urine. Feces and urine were collected directly from the rectum and by massaging the vulva, respectively, and combined on an equal weight basis to produce one composite fecal and one urine sample for each experiment. The samples were stored frozen (-20°C) until needed. Feces and urine were thawed and mixed immediately before being used in a 1:1 ratio (w/w) to produce manure for each experiment. Combined feces and urine (800 g fresh weight) were incubated in a modified continuous culture fermenter system [10]. Briefly, the system consisted of 2 L capacity incubation vessels with ports allowing manure sampling and collection vessels containing 500 mL of 0.5 M H_2SO_4 to capture the released ammonia. Air, moisturized by passing through a sealed water jar, was continuously propelled through the system at a rate of 1 L/min to maintain positive pressure and carry manure gases through the acid solution. The acid solution was replaced daily and aliquots were analyzed for NH_3 -N and ^{15}N . All experiments were carried out at 25°C for 10, 20, or 30 days.

Two experiments (Exp. 1 and 2) were designed to quantify NH_3 -N volatilization losses, manure urea hydrolysis, and investigate N isotope fractionation during manure storage. In each experiment, 3 incubation vessels were used ($n = 3$). The incubations were carried out for 20 or 30 days

(Exp. 1 and 2, respectively). Manure samples (20 to 40 g each) were collected for total N, ^{15}N , and urea-N analyses on day 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 19 and 20 (Exp. 1), or day 0, 1, 3, 5, 8, 11, 16, 21, 26, and 31 (Exp. 2). Net manure N or ^{15}N loss was calculated with correction for the amount of N or ^{15}N removed with sampling (assuming an equivalent proportion of N or ^{15}N lost from the sample as from manure remaining in the incubation vessel). Similarly, $\text{NH}_3\text{-N}$ or $\text{NH}_3\text{-}^{15}\text{N}$ recovered in the acid trap was corrected for $\text{NH}_3\text{-N}$ or $\text{NH}_3\text{-}^{15}\text{N}$ that would have been emitted from the samples removed from the incubation vessels.

The N isotope composition of manure- and emitted $\text{NH}_3\text{-N}$ was expressed as delta ^{15}N ($\delta^{15}\text{N}$) and calculated as:

$$\delta^{15}\text{N} = \frac{\text{R sample} - \text{R standard}}{\text{R standard}}, \text{ where } \text{R} = \frac{^{15}\text{N}}{(^{14}\text{N} + ^{15}\text{N})}$$

Experiment 3 was designed to quantify the contribution of $\text{NH}_3\text{-N}$ to total N volatilization losses from manure. Incubation length and sampling were as for Exp. 1, except that the manure urea-N pool was labeled by incorporating 200 mg [$^{15}\text{N}_2$] urea (98 atom % ^{15}N ; Cambridge Isotope Laboratories Inc., Andover, MA) at day 0. Daily manure and acid- NH_3 solution samples were analyzed for ^{15}N -enrichment, expressed as atom % excess [APE; atom % ^{15}N - 0.3663 (the natural abundance of ^{15}N in air)].

Experiment 4 was designed to investigate the relative contribution of fecal and urinary N to $\text{NH}_3\text{-N}$ emitted from manure. Two-ruminally cannulated cows were used as donors of feces and urine. Feces and urine were collected in 2 separate sampling periods (Periods 1 and 2). In Period 1, unlabeled with ^{15}N feces and urine were collected. In Period 2, the cows received intraruminal doses of 99 atom % $^{15}\text{NH}_4\text{Cl}$ (Cambridge Isotope Laboratories Inc.) to produce ^{15}N -labeled feces and urine. A total of 4 g/day $^{15}\text{NH}_4\text{Cl}$ were dosed intraruminally to each cow for 5 consecutive days. The isotope was dissolved in 1 L distilled water and dosed twice daily (2 g at each dosing), immediately before the morning and afternoon feedings. Approximately 10 kg of ruminal contents were removed from the rumen of each cow, the isotope solution was mixed in, and the labeled contents were returned to the rumen. Feces and urine were collected on day 4 (at 0700 and 1500 h) and 5 (1100 h) of each sampling period (*i.e.*, allowing 3 day for labeling of animal excreta) and frozen. Samples of unlabeled or ^{15}N -labeled feces and urine were thawed and composited on an equal weight basis immediately before being used in Exp. 4. Manure containing ^{15}N -labeled feces (FLM) was prepared by mixing 400 g (fresh weight) of unlabeled urine and 400 g of ^{15}N -labeled feces (per incubation vessel). Manure containing ^{15}N -labeled urine (ULM) was prepared by mixing 400 g of ^{15}N -labeled urine and 400 g of unlabeled feces. Incubation conditions were as for Exp. 1, except incubation length was 10 days. Incubation vessels were replicated within incubation and incubation was repeated ($n = 4$ for the isotope data, or $n = 8$ for the manure composition and $\text{NH}_3\text{-N}$ emission data). Nitrogen-15 enrichment of manure and $\text{NH}_3\text{-N}$ recovered in the acid solution were used to calculate fecal and urinary N contribution to $\text{NH}_3\text{-N}$ emitted from manure as follows:

$\text{NH}_3\text{-N}$ originating from fecal N (FLM manure) = ^{15}N -enrichment (APE) of $\text{NH}_3\text{-N} \div$ ^{15}N -enrichment (APE) of ^{15}N -labeled feces

$\text{NH}_3\text{-N}$ originating from urinary N (ULM manure) = ^{15}N -enrichment (APE) of $\text{NH}_3\text{-N} \div$ ^{15}N -enrichment (APE) of ^{15}N -labeled urine

2.2. Sample Analyses

Daily manure samples were immediately acidified with 2 mL of 0.5 M H₂SO₄ and freeze-dried (VirTis Ultra 35XL freeze-drier; SP Scientific, Gardiner, NY) to determine DM content. An aliquot of the dried manure sample was pulverized using Mixer Mill MM 200 (Retsch, Newtown, PA) and analyzed for N and ¹⁵N on a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA) interfaced to a Delta V Advantage Isotope-Ratio Mass Spectrometer (ThermoFinnigan MAT GmbH, Bremen, Germany). Urine samples (60 µL) were weighed directly into tin capsules (Costech Analytical Technologies, Inc.), freeze-dried, and analyzed for N and ¹⁵N. Aliquots (20 mL) of the daily manure samples were centrifuged at 20,000 × g for 20 min, the supernatant was precipitated with 65% (w/v) trichloroacetic acid solution (5% w/v final concentration), recentrifuged at 20,000 × g for 20 min, and analyzed for NH₃-N [11] and urea-N (Stanbio Urea Nitrogen Kit 580, Stanbio laboratory, Inc., San Antonio, TX) concentrations. Samples for analysis of ¹⁵N enrichment of NH₃-N were prepared utilizing the diffusion method [12].

2.3. Statistical Analysis

Manure composition and ammonia losses data were analyzed by analysis of variance using the GLM procedure of SAS (2003; SAS Inst. Inc., Cary, NC) with experiment in the model. Data from Exp. 4 were analyzed by analysis of variance using the GLM procedure of SAS with treatment (*i.e.*, ¹⁵N-labeled feces or urine), incubation, and treatment × incubation interaction included in the model; the interaction was not significant for any variable. The ¹⁵N-enrichment data model included only treatment. Significant differences were declared at $P \leq 0.05$. Means are presented as least squares means. When the main effect was significant, means were separated by pairwise *t*-test (diff option of PROC GLM). Manure-, urea-, and NH₃-N concentrations and ¹⁵N data were fitted to various non-linear regression models (exponential decay, exponential rise to a maximum, or sigmoid; SigmaPlot 10.0, Systat Software Inc., San Jose, CA).

3. Results and Discussion

Dry matter and concentration of total and urea-N in manure used in this study varied significantly among experiments (Table 1). Manure N and specifically urea-N are important factors determining NH₃-N volatilization losses from cattle manure [2]. Manure in Exp. 2 had about 50 to 60% lower ($P < 0.001$) urea- and total-N concentrations compared with manure used in Exp. 1, 3, and 4. This led to significantly lower daily manure N losses in Exp. 2, compared with Exp. 1, 3, and 4. The highest ($P < 0.001$) daily N losses were in Exp. 4, which can be explained by the shorter duration of this experiment (10 days), compared with the other experiments (20 or 30 days). The most rapid loss of manure N and most intensive NH₃-N emissions occurred during the first 5 to 6 days (Figure 1A,C). This was matched by an equivalent rapid increase in NH₃-N concentration in manure, reaching a peak at day 2 to 5. Initial concentration of ammonium in manure was negligible, but rapidly increased (to about 3 to 5 mg/mL manure) through day 5 in both Exp. 1 and 2 due to hydrolysis of urinary urea (data not shown). The much more rapid decline in manure urea-N concentration (Figure 1B) suggests that although urea hydrolysis took place immediately following mixing of feces and urine, NH₃-N

volatilization was a slower process. As shown in Table 1 and Figure 1 (Panels A and C), the quantity and intensity of manure N losses and NH₃-N emissions were much lower in Exp. 2. As a proportion of manure N at day 0, N losses were similar ($P > 0.05$) between Exp. 1, 2, and 4, even though the duration of Exp. 2 was 30 days (compared with 20 or 10 days for Exp. 1 and 4, respectively). This again, emphasizes the importance of urinary urea-N concentration in the early stages of storage for the magnitude of NH₃-N losses from manure. The daily NH₃-N losses were the lowest ($P < 0.001$) in Exp. 2, but the highest as a proportion of manure N losses compared with the other experiments (Table 1). The lowest total recovery of manure N lost during the incubation was for Exp. 4, which had the shortest incubation length (10 days).

Table 1. Manure composition, nitrogen losses, and ammonia emissions in Experiments 1, 2, 3, and 4 (least squares means; $n = 3$ in Exp. 1, 2, and 3 and $n = 8$ in Exp. 4).

Item	Experiment				SEM	P-value ¹
	Exp. 1	Exp. 2	Exp. 3	Exp. 4		
Incubation length, day	20	30	20	10		
Manure composition						
Dry matter (DM), %	11.2 ^b	11.2 ^b	9.5 ^c	13.3 ^a	0.42	<0.001
Nitrogen, % of DM	7.16 ^a	4.95 ^d	6.41 ^b	5.71 ^c	0.230	<0.001
Urea-N, mg/mL manure	4.11 ^a	1.98 ^b	4.77 ^a	4.56 ^a	0.228	<0.001
Manure N lost, mg/day	154 ^b	76 ^c	137 ^b	292 ^a	14.4	<0.001
Manure N loss, % ²	47.9 ^b	48.6 ^b	56.3 ^a	47.3 ^b	1.53	<0.01
Emitted NH ₃ -N, mg/day ³	105 ^b	67 ^c	107 ^b	135 ^a	4.1	<0.001
Emitted NH ₃ -N, % ⁴	68.2 ^c	88.1 ^a	78.1 ^b	48.0 ^d	2.21	<0.001

¹ P-value for the main effect of experiment; ² Cumulative manure N lost as % of manure N at day 0;

³ Manure N recovered as NH₃-N in the acid solution; ⁴ Emitted NH₃-N, % of manure N lost (corrected for sampling); ^{a, b, c, d} Within a row, means without a common superscript letter differ ($P < 0.05$).

The higher manure N recovery as NH₃-N in Exp. 3 vs. Exp. 1 (both 20 days in length) can be related to the lower N concentration of manure in Exp. 3. The relationship between manure N concentration and manure N recovery as NH₃-N, which was linear and negative for Exp. 1, 2, and 3 [$147.7 - 11.0 \times \text{N concentration in manure (\%)}; R^2 = 0.86; P < 0.001$], presents an interesting phenomenon. Recovery of manure N as NH₃-N captured in the acid trap was generally low. Recovery was even lower in the initial stages of the incubation (on average, $19.2 \pm 0.72\%$ during the first 3 days of incubation) suggesting that: (1) the acid trap did not effectively capture NH₃-N emitted from manure, particularly when emissions were most intensive, or (2) N was being lost from manure in forms other than NH₃-N. We have investigated the factors affecting the NH₃-N trapping efficiency of acid solutions and reported that efficiency decreases with increasing the amount of NH₃-N being emitted [13]. Decreased trapping efficiency, however, could not explain the large discrepancy between manure N losses and NH₃-N captured in the acid trap in Exp. 1 through 3. To further eliminate the NH₃-N trapping capacity of the acid solution as a factor for the low recovery of manure N lost during the incubation process, we conducted a series of experiments comparing the acid trap system with direct measurement of NH₃-N emitted from the incubation vessels using a photoacoustic gas analyzer INNOVA 1412 (AirTech Instruments, Ballerup, Denmark), which allowed continuous monitoring of

NH₃-N concentration in the gas flowing out of the system. The conclusion from these experiments was that the 2 measurement methods gave similar NH₃-N recovery. For example, cumulative NH₃-N emissions were 108 vs. 121 mg in 24 h (SEM = 4.99; $P = 0.10$) and 128 vs. 136 mg in 72 h (SEM = 6.40; $P = 0.50$) for the acid trap and the INNOVA gas analyzer, respectively.

The possibility of a significant gaseous N loss, other than NH₃-N, was further investigated. Nitrous oxide emission is expected to be negligible in conditions as those utilized in the current study due to the lack of nitrifying and denitrifying microorganisms in cattle feces [14] and relatively short storage time. Adviento-Borbe *et al.* [15] and Arriaga *et al.* [16], for example, reported insignificant N₂O emissions off the barn floor in dairy farms. In an experiment related to this study, N₂O emissions were negligible from dairy manure stored in laboratory conditions or during the first 100 h following soil application [17]. If sufficient time (at least 3 weeks) is allowed, however, cattle manure will generate N₂O in simulated storage conditions [18]. Bussink and Oenema [5] and Harper *et al.* [19] indicated that some N may be lost from lagoons/retention ponds via reduction of nitrate to N₂O and dinitrogen gas (N₂). A number of possible chemical and biological mechanisms may exist for formation of N₂ during manure storage [20] and such processes, including chemical, non-biological conversion of ammonium to N₂ (termed “chemo-denitrification”) have been reported to be responsible for a significant amount of gaseous N losses from swine lagoons [3]. In a separate series of experiments we used pure argon gas (99.99%; GTS-Welco, Allentown, PA) instead of air to provide airflow in the manure storage system used in Exp. 1 through 4 and analyzed the composition of the gas flowing out of the system. Preliminary results from these experiments (data not shown) indicated very intensive N₂ emissions in the first 5 h of simulated manure storage, suggesting that N₂ gas may represent a significant N loss in the initial stages of the manure storage process and likely accounts for a significant part of the N losses observed in the current study.

As discussed earlier, farm NH₃ emissions are influenced by important environmental factors and such data are not suitable for evaluating the impact of dietary mitigating strategies. For example, in a current on-farm project with 12 commercial Pennsylvania dairy farms, we monitored barn floor NH₃ emissions in spring and fall of Year 1 and then again in Year 2 of the project, after dietary crude protein concentrations were reduced by about 1%-unit [21]. On average, barn floor NH₃ emissions for the farms, in which the dietary protein reduction was documented by regular sampling, were reduced by about 65% (445 vs. 156, mg/m²/h). However, average air temperatures during the emission measurements were 14 °C and 5 °C, respectively. Thus, in this particular project, it was impossible to distinguish the effect of diet from the effect of environment. Manure samples from the same farms (collected, stored, processed, and analyzed as in the current study) showed unequivocally a reduction in laboratory NH₃-N emissions by about 36% for the low-protein period compared with the control, high-protein feeding period. Laboratory methods, naturally, have the limitation of not accounting for the environmental factors affecting emissions, but are useful in quantifying the effect of dietary manipulations on the gas-emitting potential of manure [2].

One of the objectives of the current study was to further investigate the relationship between NH₃-N volatilization and N isotope composition of manure. We first reported a significant N isotope fractionation in cattle manure during storage due to NH₃-N volatilization [4,22]. The isotope fractionation factor associated with NH₃-N volatilization is one of the highest in the N cycle (~1.029, [23]), which would result, when conditions are favorable, in a rapid increase in $\delta^{15}\text{N}$ of manure during storage. The

process has been discussed in length [4]. Experiments 1 and 2 were used to determine N isotope ratios beyond the short incubation time utilized in our original studies [4,22].

Delta ^{15}N of manure rapidly increased from 0.09 ± 0.36 (day 0) to 10.1 ± 0.42 (day 5) in Exp. 1 and from -1.12 ± 0.61 (day 0) to 5.99 ± 0.40 (day 5) in Exp. 2 (Figure 2A). This rapid increase in $\delta^{15}\text{N}$ was due to the loss of depleted in ^{15}N $\text{NH}_3\text{-N}$. Delta ^{15}N of volatilized NH_3 was -22.5 ± 0.68 on day 1 in Exp. 1 and -15.1 ± 0.17 on day 2 (day 1 measurement was lost) in Exp. 2 and increased to -16.5 ± 0.09 (day 5) and $-1.3 \pm 2.55\text{‰}$ (day 20) in Exp. 1 and to -14.9 ± 0.90 (day 5) and $2.38 \pm 1.45\text{‰}$ (day 30) in Exp. 2 (Figure 2B). As hydrolysis of urea to ammonium (which as Figure 1B shows is a very rapid process), $\text{NH}_3\text{-N}$ volatilization, and N isotope fractionation take place, $\delta^{15}\text{N}$ of $\text{NH}_3\text{-N}$ and the dissolved in manure ammonium will continue to increase until the ammonium is exhausted and the $\text{NH}_3\text{-N}$ obtains the $\delta^{15}\text{N}$ value of the original ammonium. Indeed, as Figure 2B shows, $\delta^{15}\text{N}$ of volatilized $\text{NH}_3\text{-N}$ continued to increase in a sigmoid fashion through day 30 of Exp. 2. The lower asymptote levels for both Exp. 1 and 2 indicated highly depleted in ^{15}N $\text{NH}_3\text{-N}$ at the beginning of the manure storage process. The length of Exp. 1, however, was apparently too short to clearly observe the point of equilibrium visible in Exp. 2 ($\delta^{15}\text{N}$ upper asymptote: 19.8‰). The inflexion point (*i.e.*, the point of maximum rate of $\delta^{15}\text{N}$ increase) was around day 13 and day 16 for Exp. 1 and Exp. 2, respectively. Delta ^{15}N of manure reached a plateau beyond day 6 (Figure 2, Panel A), which coincided with the decline in $\text{NH}_3\text{-N}$ emission rates. Although changes in $\delta^{15}\text{N}$ of manure parallel $\text{NH}_3\text{-N}$ losses, the dynamics of $\delta^{15}\text{N}$ of $\text{NH}_3\text{-N}$ will likely make application of the N isotope approach for estimating manure $\text{NH}_3\text{-N}$ emissions difficult in practical farm conditions, which was our original goal [4]. Nevertheless, the relationship between manure $\delta^{15}\text{N}$ and $\text{NH}_3\text{-N}$ volatilization losses appears to be linear in the initial stages of manure storage, when $\text{NH}_3\text{-N}$ losses are most intensive, and deserves further investigation.

Figure 1. Manure N loss (A), manure urea-N concentration (B), and daily ammonia-N emission (C) in Experiments 1, 2, and 3 (means \pm SE; $n = 3$).

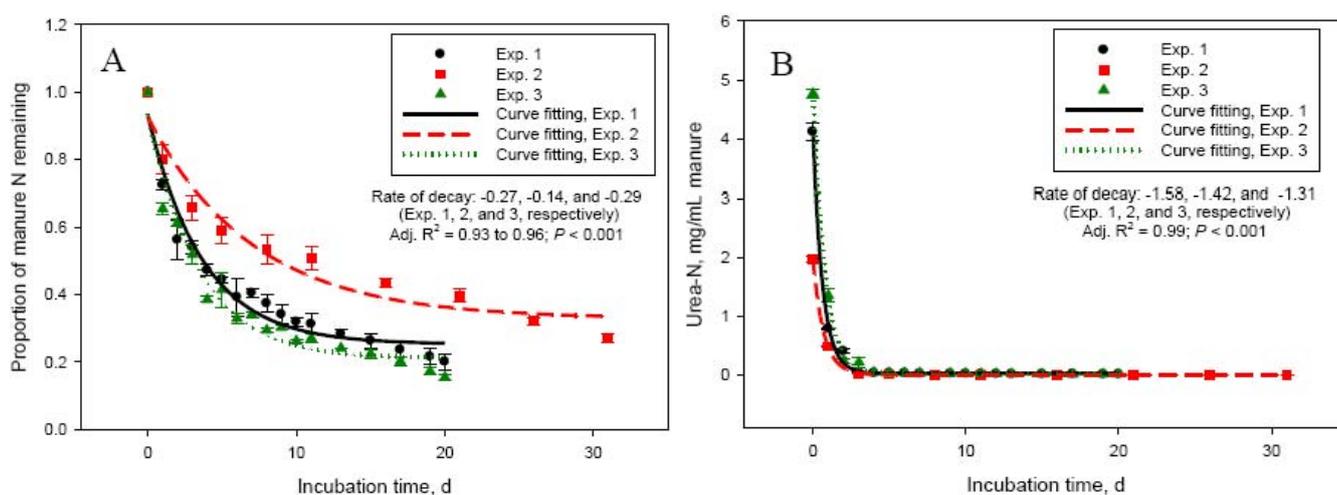


Figure 1. Cont.

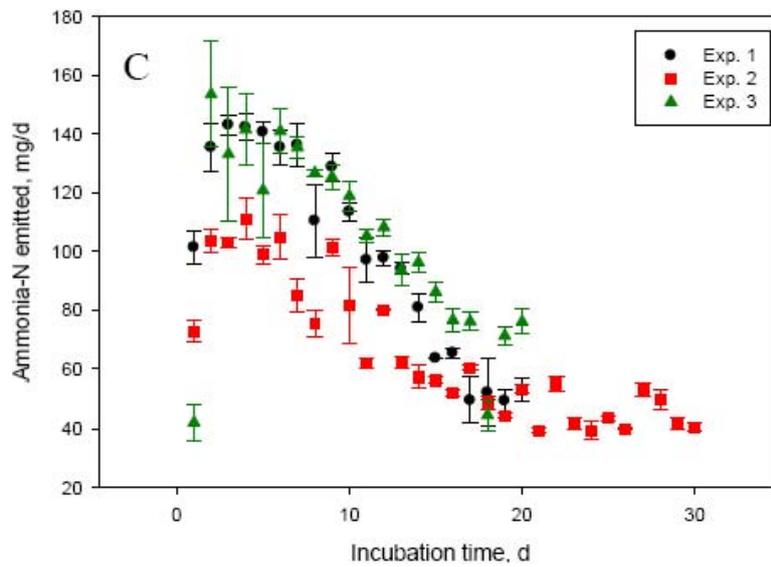
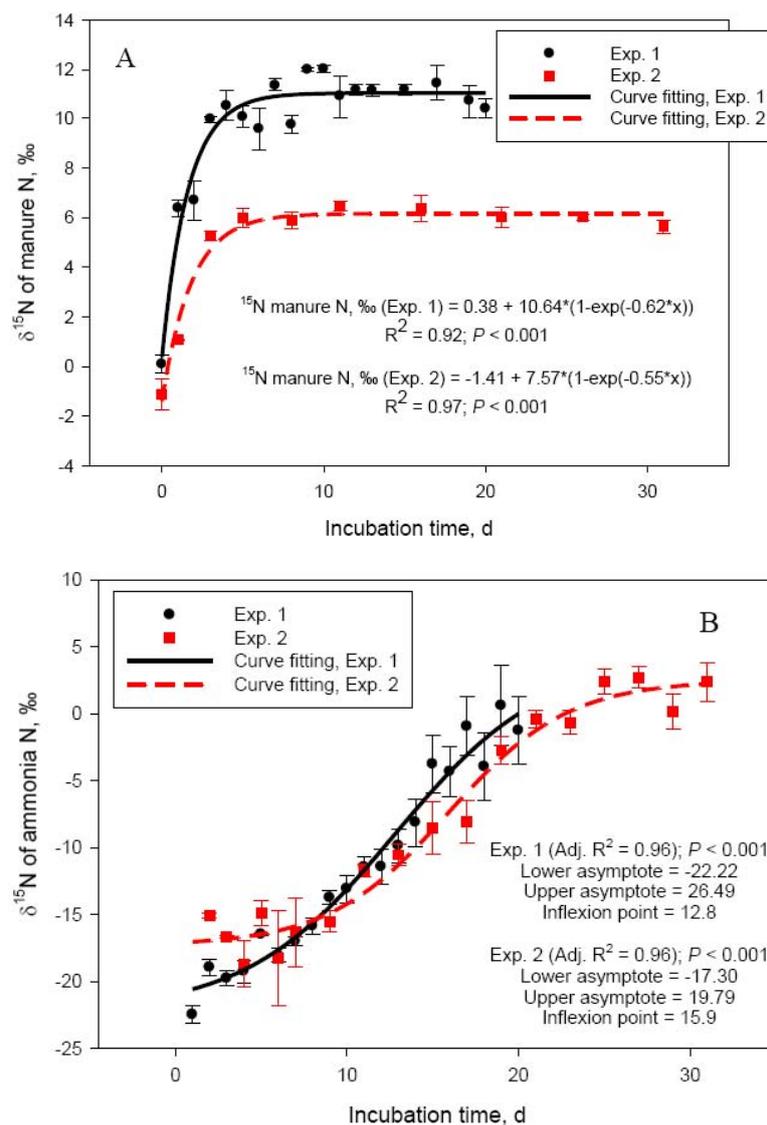
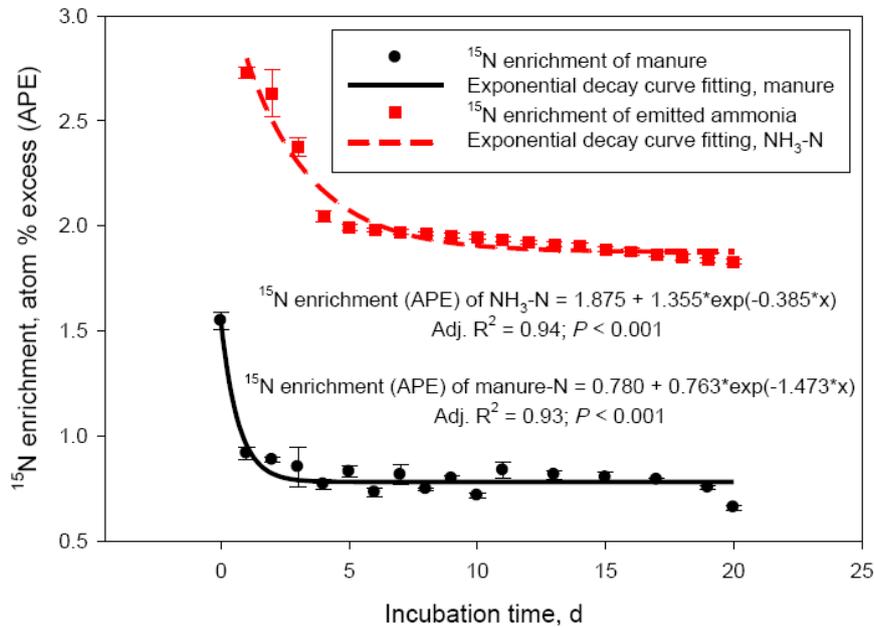


Figure 2. $\delta^{15}\text{N}$ (‰) of manure- (A) and ammonia-N (B) in Exp. 1 and 2 (means \pm SE; $n = 3$).



The discrepancy between net manure N losses and NH₃-N recovered in the acid trap was further investigated in Exp. 3. By labeling the urea N pool with ¹⁵N, the main source of emitted NH₃-N could be traced. Results of this experiment are shown in Figure 3. Nitrogen-15 enrichment of both manure- and NH₃-N pools rapidly declined within 5 days of the incubation (Figure 3), representing the most intensive phase of NH₃-N losses. For the manure-N pool, the ¹⁵N decay clearly reflected loss of highly enriched in ¹⁵N volatile N. The decline in ¹⁵N-enrichment of the NH₃-N pool followed the ¹⁵N decay of the source manure N pool and reflected the rapid decline in urea-N concentration observed in these experiments (Figure 1B). The decline in ¹⁵N-enrichment, however, was much more rapid for manure- compared with NH₃-N (rate constants of 1.473 and 0.385 APE/day, respectively). This would clearly represent ¹⁵N loss other than NH₃-N, which is in agreement with the suggested large N₂ loss in the initial hours of manure storage (see earlier discussion) and is supported by the studies of Harper *et al.* [3] with swine manure. Although isotope fractionation and discrimination against the heavier N isotope, as reported for Exp. 1 and 2, were undoubtedly taking place in Exp. 3, the δ¹⁵N values of the manure-N pool in this experiment (δ¹⁵N 4306 ± 77.7‰) was so much greater than the natural abundance of ¹⁵N in manure (δ¹⁵N -0.51 ± 0.42‰; Exp. 1 and 2) that these processes could not have had a measurable impact on the ¹⁵N-enrichment data from Exp. 3. The absolute losses of ¹⁵N during the 20 day simulated manure storage were on average 68.2 ± 2.45 mg. This represented approximately 70% of the 98 mg urea-¹⁵N introduced into each incubation vessel at day 0 (200 mg of 98 atom % ¹⁵N-urea). The amount of ¹⁵N recovered as NH₃-N was 51.2 ± 1.85 mg, or 75.2 ± 4.04% of the urea-¹⁵N lost in 20 days as NH₃-N. The difference of urea-¹⁵N lost and trapped as NH₃-N (about 25%) is supportive of the hypothesis that gaseous N losses other than NH₃ may be responsible for part of manure N losses during storage. This process is likely taking place exclusively in the initial days of manure storage. Averaged manure-¹⁵N and NH₃-¹⁵N losses data were fitted to a non-linear model (double exponential decay; data not shown) and the predicted value were used to calculate urea-¹⁵N recovery as NH₃-N during the initial 3 days of manure storage. As expected, recovery of daily urea-¹⁵N lost from manure as NH₃-N was the lowest at day 1 (30.4%) and day 2 (45.4%) of the incubation; recovery was complete (102.7%) by day 3. This trend supports the concept that volatile nitrogenous compounds other than NH₃ (likely N₂) could account for as much as 50 to 70% of the N losses during the initial 48 h of cattle manure storage. Harper *et al.* [3] reported 2 to 8 times greater N₂ than NH₃-N emissions from swine lagoons in Georgia and North Carolina. Dinitrogen gas emission would be still dependent on manure composition, specifically urinary urea excretion by the animal; lagoon ammonium concentration was the primary factor determining N₂ emissions in the Harper *et al.* [3] study. These authors concluded that swine lagoons emit much less NH₃-N than previously estimated. Based on results from the current study, similar conclusion may be drawn for cattle manure. Our laboratory data, indicating about 25% manure N losses unaccounted as NH₃-N (in 20 days), need to be confirmed in field experiments, where a multitude of environmental factors affect N volatilization losses from dairy and beef cattle operations [2].

Figure 3. ^{15}N -enrichment (atom % excess; APE) of ammonia- and manure-N in Exp. 3 (means \pm SE; $n = 3$).



One of the objectives of this study was to quantify the contribution of urinary- vs. fecal-N to $\text{NH}_3\text{-N}$ emitted from cattle manure. In Exp. 4, 2 types of manure were produced: FLM, ^{15}N -labeled feces and unlabeled urine and ULM, unlabeled feces and ^{15}N -labeled urine. Labeled and unlabeled feces or urine used to prepare FLM and ULM had similar N concentrations: on average 0.48 ± 0.01 and $1.01 \pm 0.02\%$, respectively. As a result, both types of manure had similar ($P = 0.14$ to 0.44) DM (data not shown), N, and urea-N concentrations (Table 2). Consequently, cumulative or daily $\text{NH}_3\text{-N}$ emissions were also not different ($P = 0.59$) between the 2 treatments. The goal of labeling feces or urine with ^{15}N was successfully achieved. Delta ^{15}N of feces in FLM manure was approximately 17-times higher ($P = 0.022$) than that of feces in ULM manure. Similarly, $\delta^{15}\text{N}$ of urine in ULM manure was drastically higher ($P < 0.001$) than that of urine in FLM, which resulted in $\delta^{15}\text{N}$ of ULM being higher ($P < 0.001$) than $\delta^{15}\text{N}$ of FLM. Delta ^{15}N of unlabeled feces and urine for both types of manure was within the range of natural $\delta^{15}\text{N}$ reported for dairy cows [4]. Nitrogen-15 enrichment of $\text{NH}_3\text{-N}$ followed a sigmoid trend for FLM and an exponential decay trend for ULM (adjusted $R^2 = 0.86$ and 0.92 , $P = 0.005$ and < 0.001 , respectively; Figure 4A). The estimated proportion of $\text{NH}_3\text{-N}$ originating from fecal N (FLM) was negligible in the first 48 h of manure storage, represented 0.04 ± 0.006 by day 5, and then gradually increased to 0.11 ± 0.019 of the emitted $\text{NH}_3\text{-N}$ by day 10 (logistic regression model; adjusted $R^2 = 0.91$, $P < 0.001$) (Figure 4B). The proportion of $\text{NH}_3\text{-N}$ originating from urinary N (ULM) represented 0.94 ± 0.027 at 24 h, 0.97 ± 0.002 at 48 h, 0.91 ± 0.004 at 72 h, and gradually decreased to 0.87 ± 0.005 by day 10 (exponential decay model; adjusted $R^2 = 0.92$, $P < 0.001$). This experiment clearly identified urinary N as the principal source of $\text{NH}_3\text{-N}$ volatilized from cattle manure during the initial 10 days of storage, accounting for an average of 90% of the emitted $\text{NH}_3\text{-N}$. The contribution of fecal N was relatively low, but gradually increased to about 10% by day 10. Using a similar approach, Thomsen [7] estimated that urinary N accounted for 79% of the total N losses from sheep manure after 7 days of composting, decreasing to 64% at the end of the 86-day storage period. In

manure stored anaerobically, urinary N accounted for 94% of the total N losses after 28 days and for 68% at 86 days [7].

Table 2. Manure characteristics in Exp. 4 (least squares means; $n = 8$).

Item	FLM ¹	ULM	SEM	P-value ²
Total N, % of dry matter	5.66	5.76	0.080	0.44
Urea-N, mg/mL manure	4.74	4.38	0.134	0.14
NH ₃ -N emission, g ³	1.4	1.3	0.05	0.59
Day 0 $\delta^{15}\text{N}$, ‰				
Feces	246	13.8	5.59	0.022
Urine	1.3	364	0.06	<0.001
Manure	94	256	1.94	<0.001

¹ FLM = manure with ¹⁵N-labeled feces; ULM = manure with ¹⁵N-labeled urine; ² P-value for the main effect of treatment (*i.e.*, FLM vs. ULM); ³ Cumulative, 10-d NH₃-N emissions. Daily emissions were 137 and 133 mg/day FLM and ULM, respectively; SEM = 5.1, $P = 0.59$.

Figure 4. ¹⁵N-enrichment (atom % excess; APE) of ammonia-N emitted from manure with ¹⁵N-labeled feces (FLM), or with ¹⁵N-labeled urine (ULM) (Panel A) and proportion of ammonia-N emitted from manure originating from fecal- or urinary-N (Panel B) in Exp. 4 (means \pm SE; $n = 8$).

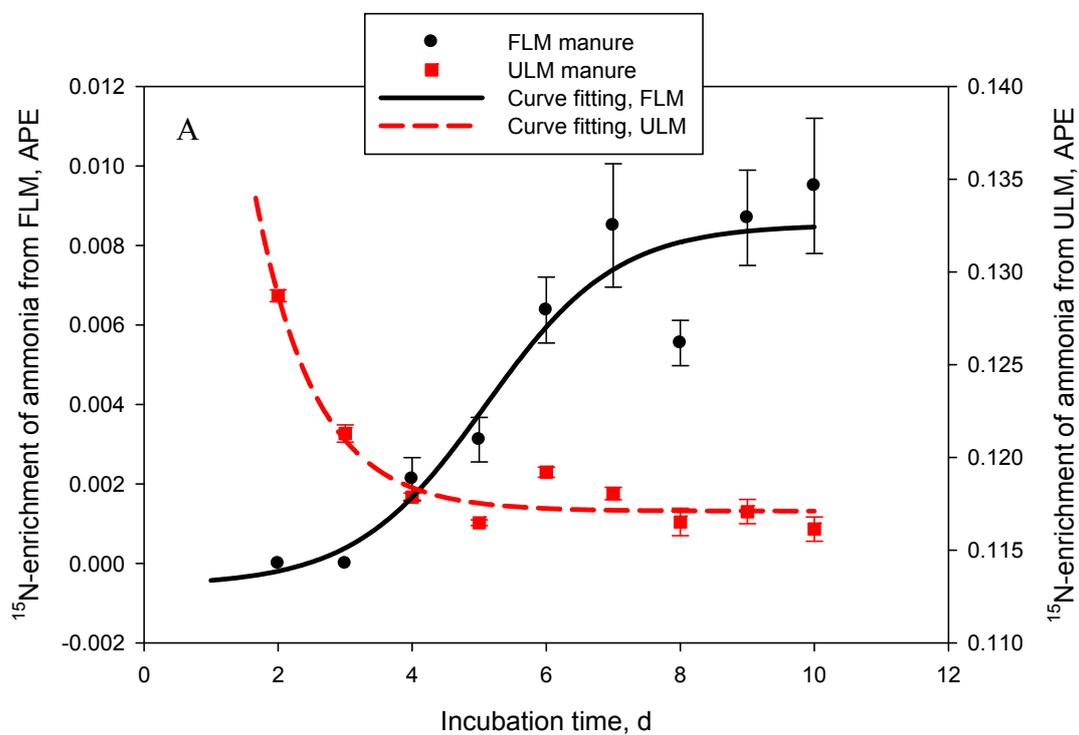
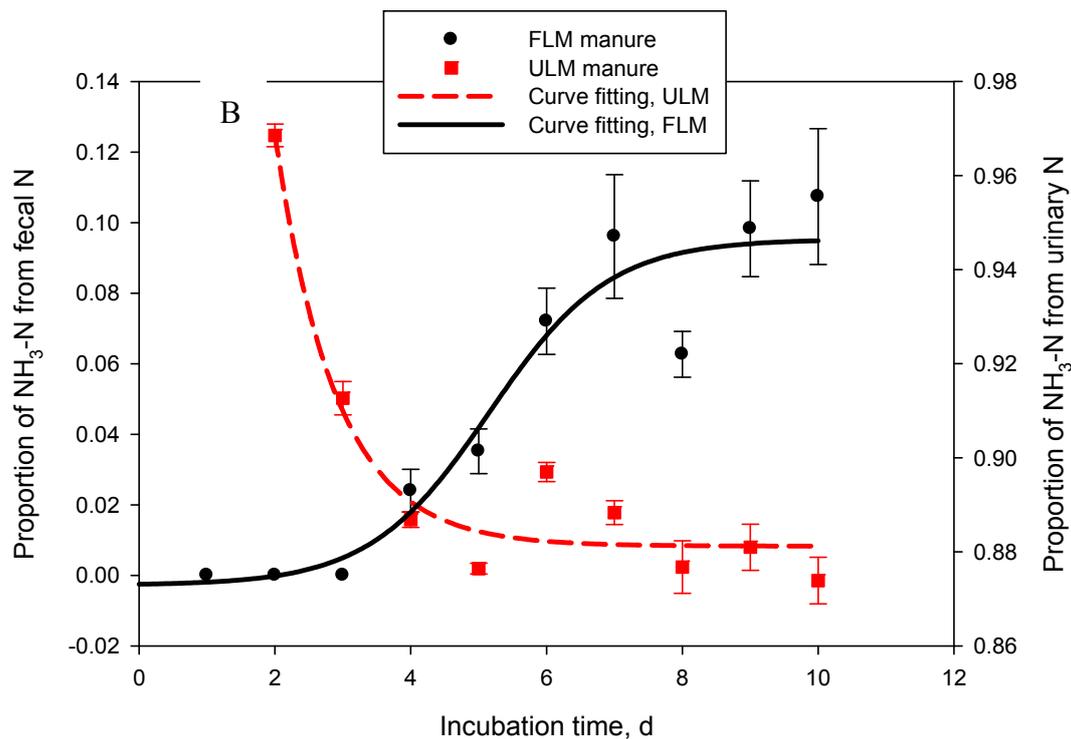


Figure 4. Cont.



4. Conclusions

These series of laboratory experiments confirmed that NH_3 volatilization losses from manure are most intensive during the first 2 to 3 days of manure storage and this coincides with a rapid loss (hydrolysis) of urinary urea. The relationship between $\delta^{15}\text{N}$ of manure and NH_3 emission appears to be linear during the initial stages of manure storage (when most of the NH_3 losses occur) and should be further investigated. The main source of $\text{NH}_3\text{-N}$ volatilized from cattle manure during the initial 10 days of storage is urinary-N, representing on average 90% of the emitted $\text{NH}_3\text{-N}$. The contribution of fecal N was relatively low, but increased to about 10% by day 10. There appears to be substantial emissions of nitrogenous gases other than NH_3 , most likely dinitrogen gas, which may account for up to 25% of N losses during the first 20 day of manure storage. This finding, which has to be confirmed in laboratory and field experiments, may be indicative of overestimation of NH_3 emissions from cattle operations by the current emissions factors.

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