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Effect of dietary protein concentration on ammonia and greenhouse gas emitting potential of dairy manure

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ABSTRACT

Two experiments were conducted to investigate the effect of dietary crude protein concentration on ammonia (NH_3) and greenhouse gas (GHG; nitrous oxide, methane, and carbon dioxide) emissions from fresh dairy cow manure incubated in a controlled environment (experiment 1) and from manure-amended soil (experiment 2). Manure was prepared from feces and urine collected from lactating Holstein cows fed diets with 16.7% (DM basis; HCP) or 14.8% CP (LCP). High-CP manure had higher N content and proportion of NH_3- and urea-N in total manure N than LCP manure (DM basis: 4.4 vs. 2.8% and 51.4 vs. 30.5%, respectively). In experiment 1, NH_3 emitting potential (EP) was greater for HCP compared with LCP manure (9.20 vs. 4.88 mg/m^2 per min, respectively). The 122-h cumulative NH_3 emission tended to be decreased 47% ($P = 0.09$) using LCP compared with HCP manure. The EP and cumulative emissions of GHG were not different between HCP and LCP manure. In experiment 2, urine and feces from cows fed LCP or HCP diets were mixed and immediately applied to lysimeters (61 × 61 × 61cm; Hagerstown silt loam; fine, mixed, mesic Typic Hapludalf) at 277 kg of N/ha application rate. The average NH_3 EP (1.53 vs. 1.03 mg/m^2 per min, respectively) and the area under the EP curve were greater for lysimeters amended with HCP than with LCP manure. The largest difference in the NH_3 EP occurred approximately 24 h after manure application (approximately 3.5 times greater for HCP than LCP manure). The 100-h cumulative NH_3 emission was 98% greater for HCP compared with LCP manure (7,415 vs. 3,745 mg/m^2, respectively). The EP of methane was increased and that of carbon dioxide tended to be increased by LCP compared with HCP manure. The cumulative methane emission was not different between treatments, whereas the cumulative carbon dioxide emission was increased with manure from the LCP diet. Nitrous oxide emissions were low in this experiment and did not differ between treatments. In the conditions of these experiments, fresh manure from dairy cows fed a LCP diet had substantially lower NH_3 EP, compared with manure from cows fed a HCP diet. The LCP manure increased soil methane EP due to a larger mass of manure added to meet plant N requirements compared with HCP manure. These results represent effects of dietary protein on NH_3 and GHG EP of manure in controlled laboratory conditions and do not account for environmental factors affecting gaseous emissions from manure on the farm.

Key words: ammonia, greenhouse gas, dairy manure

INTRODUCTION

Ammonia (NH_3) emitted from cattle manure has environmental and human health effects, including eutrophication of surface waters, acidification of ecosystems, and fine particulate matter formation in the atmosphere (US EPA, 2004). Livestock operations are considered to be the largest contributor to anthropogenic NH_3 emissions in the United States (50%; US EPA, 2004). Therefore, research efforts have been directed toward mitigating NH_3 emission from animal operations (Ndegwa et al., 2008). Decreasing dietary CP is one of the most effective strategies to decrease NH_3 emission from animal manure (Ndegwa et al., 2008; Hristov et al., 2011a). Studies have demonstrated substantial reductions of NH_3 emitted from dairy manure during simulated storage (Misselbrook et al., 2005; Agle et al., 2010) and from the barn floor (Li et al., 2009) with decreasing dietary CP concentration. Research investigating NH_3 emission from soil amended with manure from cows fed varying CP diets is limited. Manure application rates are usually nutrient (N or P)-based, thus avoiding over-application above crop

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requirements and the resulting water pollution this can create (Beegle, 2000). It is possible, however, that, at equal N application rates, type of manure N (urinary vs. fecal) may affect NH_3 emissions from soil. Manure N composition depends, among other factors, on dietary CP supply (Külling et al., 2001). As excess dietary CP is primarily excreted as urinary urea (Hristov et al., 2011a) and urea is the main source of NH_3 emission from manure (Lee et al., 2011a), it is likely that dietary CP has a major effect on NH_3 emissions from manure-amended soil.

Methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) are important greenhouse gases (**GHG**), of which cattle manure is a significant source (US EPA, 2010). Although the effects of manure storage conditions and type of feed on GHG emissions have been investigated (Jungbluth et al., 2001; Adviento-Borbe et al., 2010), studies on the specific effect of dietary CP are limited (Külling et al., 2001). Methane and CO_2 gases are generated in manure through microbial decomposition of fecal OM (Smith and Conen, 2004), a process that may be stimulated by organic or inorganic N sources along with available carbon. Ammonia, for example, is a critical N source for methanogens in various environments (Bryant, 1974) and its availability in manure may affect archaeal growth. Nitrous oxide can be directly produced from manure-amended soil through microbial nitrification and denitrification processes, or indirectly when N is lost through volatilization as NH_3, nitric oxide, and nitrogen dioxide (**NOx**), or run-off and leaching (US EPA, 2010). It is assumed that the indirect contribution of volatilized NH_3 and NOx occurs through redeposition of these N compounds onto the soil in the form of particulate ammonium, nitric acid, and NOx, which may enter the nitrification and denitrification cycle. On the other hand, volatilization losses of N will decrease the availability of N for nitrification and denitrification processes and consequently, N_2O formation (US EPA, 2010). In both cases, dietary CP concentration determines to a large extent manure N concentration and can have a significant effect on N_2O emissions (Cardenas et al., 2007). Therefore, manure N (specifically readily available urinary urea N) might play an important role in promoting GHG emissions from manure-amended soil.

Manure NH_3 and GHG emissions from the barn floor or manure storage are influenced by several important factors, including manure composition, environmental factors, and type of barn or manure storage facility (Ndegwa et al., 2008; Hristov et al., 2011a). Thus, if dietary effects on gaseous manure emissions are investigated, a good chance exists that results will be confounded by environmental and manure management factors, which make comparisons between studies and even within a study difficult. A recent review reported NH_3 flux rate from dairy farms varying from 0.03 to 17 g/m^2 per h; NH_3 flux rates from beef feedlots ranged from 0.09 (winter) to 0.25 (summer) g/m^2 per hour (Hristov et al., 2011a). With this large variability, it is difficult, or practically impossible, to categorize the effect of diet on manure emissions. Therefore, we proposed that investigating NH_3 (or GHG) emissions of fresh manure in a controlled environment [i.e., NH_3 (or GHG) emitting potential (**EP**) of manure], would provide a better understanding of the specific effect of diet on manure composition (mainly the relative proportions of urinary urea and fecal N) and the potential of a dietary treatment to affect gaseous manure emissions, providing environmental and manure management factors are equal (Hristov et al., 2009; Weiss et al., 2009; Agle et al., 2010).

Therefore, the objective of these experiments was to investigate the effects of dietary CP concentration on NH_3 and GHG EP of fresh dairy manure in a controlled environment, or following soil application. We hypothesized that fresh manure from cows fed a high-CP diet would emit more NH_3 and N_2O, and that GHG emissions may also be increased due to increased availability of N to fecal and soil microorganisms.

MATERIALS AND METHODS

Animals involved in these studies were cared for according to the guidelines of the Pennsylvania State University Animal Care and Use Committee (University Park). The committee reviewed and approved all procedures involving animals.

Experiment 1

This experiment was part of a companion experiment (Lee et al., 2011c) and was designed to examine the effect of dietary CP on the NH_3 and GHG EP of fresh manure in a controlled environment. In the companion experiment, 36 Holstein cows (average parity, 2.1 ± 1.0 lactations; BW, 618 ± 84 kg; DIM, 132 ± 7 d; milk yield, 44 ± 9.6 kg/d at the beginning of the trial) were fed 2 diets (Table 1): high CP (**HCP**, 16.7% CP) and low CP (**LCP**, 14.8% CP). Diets were fed as TMR and the HCP diet was formulated to meet the nutrient requirements of a lactating Holstein cow consuming 25 kg of feed DM/d and yielding 43.1 kg of milk/d with 3.6% milk fat and 3.0% true milk protein (NRC, 2001). The LCP diet was MP deficient (−156 g/d; based on NRC, 2001).

Fresh feces and urine samples were collected for the current experiment from individual cows (i.e., 12 cows fed the HCP diet and 12 cows fed the LCP diet) at

Table 1. Ingredients and chemical composition of the diets[1] fed to dairy cows to produce manure for experiments 1 and 2

	Diet[1]	
Composition	HCP	LCP
Ingredient, % of DM		
Corn silage	26.0	25.1
Alfalfa haylage	18.8	18.8
Grass hay	5.0	5.0
Corn grain, ground	14.3	19.5
Bakery byproduct meal	7.3	7.3
Canola meal (solvent extracted)	11.9	11.2
Cotton seed, hulls	5.3	6.2
Soybean seeds, whole, heated	4.5	0
Megalac[2]	2.6	2.6
Corn dry distillers grain with solubles	1.5	1.5
Molasses	1.5	1.5
Mineral and vitamin premix[3]	1.5	1.5
Chemical composition,[4] % of DM		
CP	16.7	14.8
RDP[5]	10.6	9.8
RUP[5]	6.1	4.9
NDF	31.7	31.9
ADF	21.1	21.2
NE_L, Mcal/kg	1.64	1.64
NFC	38.8	41.1
Ether extract	5.8	5.7
Ca	1.06	1.04
P	0.45	0.42

[1]HCP = diet containing 16.7% CP; LCP = diet containing 14.8% CP.

[2]Megalac (Church and Dwight Co. Inc., Princeton, NJ) contained 85% fat.

[3]The premix contained (%, as-is basis): trace mineral mix, 0.88; MgO (54% Mg), 8.3; NaCl, 6.4; vitamin ADE premix, 1.73; limestone, 35.8; selenium premix, 1.09; and dry corn distillers grains with solubles, 45.8. Composition: Ca, 14.9%; P, 0.37%; Mg, 4.84%; K, 0.44%; S, 0.32%; Se, 7.04 mg/kg; Cu, 377 mg/kg; Zn, 1,146 mg/kg; Fe, 191 mg/kg; Se, 6.67 mg/kg; Co, 5.4 mg/kg; vitamin A, 125,875 IU/kg; vitamin D, 31,418 IU/kg; and vitamin E, 946 IU/kg.

[4]Calculated from analyzed composition of individual feed ingredients (Cumberland Valley Analytical Services, Maugansville, MD).

[5]Calculated based on NRC (2001).

wk 5 and 7 of the companion experiment (which was 10 wk in duration). During each sampling week, feces (approximately 200 g per sampling) were collected from the rectum of each cow twice on d 1 (at 0700 and 1500 h) and once on d 2 (at 1800 h). Individual urine samples (approximately 200 g per sampling) were collected at the same times as the fecal collections by massaging the vulva. Samples were stored frozen at −20°C until analyzed. Fecal samples from the individual samplings were composited by equal weight within treatment to prepare one HCP and one LCP fecal sample. Urine was processed in a similar way. Thus, 72 individual fecal or urine samples were combined to make 1 composite HCP fecal or urine sample and 1 LCP fecal or urine sample.

The gas EP of manure was defined as the rate of gas emission (mg or $\mu g/m^2$ per minute) from cattle feces and urine mixed in a 1.7:1 (wt/wt, as-is basis) ratio incubated for 100 to 122 h in simulated storage under a controlled environment (room temperature, 25°C, and continuous air influx of 2 L/min), or after application to soil in a greenhouse under controlled temperature (20 to 26°C). The composited fecal and urine samples were thawed and mixed 1.7:1 (252 g of feces and 148 g of urine; ratio based on Hristov et al., 2011b) to produce HCP and LCP manure. In this experiment, NH_3, CO_2, CH_4, and N_2O EP of manure were analyzed using a steady-state flux chamber system (Wheeler et al., 2007). Briefly, the chambers (glass jars; surface area, 161.14 cm^2) were equipped with a lid consisting of 2 inlets, which were connected with a circular diffusion Teflon tube inside the chamber through which continuous-sweep airflow (2 L/min) was provided. The chamber outlets were attached to a multi-value switching apparatus, which allowed for automated, sequential gas measurements from each jar by an INNOVA 1412 photoacoustic gas monitor (AirTech Instruments A/S, Ballerup, Denmark). Emission data were collected approximately every 30 min; measurements were converted on a per-minute basis and these data were used in the statistical analysis. Manure (400 g) was placed in the chambers immediately before the beginning of the incubation, thus representing manure processes occurring on the barn floor immediately following excretion and mixing of feces and urine. Treatments were replicated twice and incubations were carried out at 25°C for 122 h.

Experiment 2

This experiment was conducted to investigate the effect of dietary CP level on the gas EP of fresh manure following soil (lysimeter) application. Separate sets of fecal and urine samples were collected from cows in the companion experiment (Lee et al., 2011c). Approximately 5 kg of feces and urine was collected from 2 cows fed the HCP diet and from 2 cows fed the LCP diet, at wk 5 and 7 of the companion experiment. Feces and urine were collected during 3 sampling events in 2 d of each sampling week. Each sampling event lasted for 3 h: from 0700 to 1000 and from 1500 to 1800 h (d 1) and from 1100 to 1400 h (d 2). Collected fecal and urine samples were immediately frozen at −20°C. After thawing, aliquots of the fecal and urine samples were composited on an equal-weight basis by cow and treatment (i.e., HCP or LCP diet), and week (wk 5 and 7) for chemical analyses (4 urine and 4 fecal composited samples per diet). Aliquots of the composited samples were freeze dried (VirTis Ultra 35 XL-70 freeze dryer; SP Industries Inc., Warminster, PA) and analyzed for total N (Costech ECS 4010 C/N/S elemental analyzer; Costech Analytical Technologies Inc., Valencia, CA).

Feces and urine were further composited by week to form 2 composite samples per treatment (i.e., HCP or LCP diet) and divided into 18 subsamples for lysimeter application.

Feces and urine within each of the 18 subsamples were mixed in a 1.7:1 ratio (as in experiment 1) and blended using a blender (Waring Products Division, Dynamics Corp., New Hartford, CT) for 30 s to prepare homogenous manure for lysimeter application. Aliquots of the freshly prepared manure (18 samples) were immediately frozen at −20°C and later freeze dried and analyzed for total N, NH_3-N (Chaney and Marbach, 1962), and urea-N (Stanbio Urea Nitrogen Kit 580; Stanbio laboratory Inc., San Antonio, TX) concentrations.

Manure was immediately applied after mixing feces and urine at a uniform rate of thickness to soil contained in a lysimeter system (Feyereisen and Folmar, 2009). In this experiment, 21 lysimeters were used (9 HCP manure, 9 LCP manure, and 3 with no added manure). The lysimeters were collected from the Pennsylvania State University's Russell E. Larson Agricultural Research Center; the soil was a Hagerstown silt loam (fine, mixed, mesic Typic Hapludalf) with average surface (0 to 5-cm depth) moisture content of 31.4 ± 0.19%. A brief description of the lysimeters and collection process follows. Steel cube-shaped casings open at the top and bottom (61 × 61 × 61 cm) were driven into the soil with a 1.1-Mg drop hammer. The soil-filled assembly was excavated and secured in a rollover device that allowed them to be flipped 180°. The bottom of the lysimeter was filled with 1 to 2 cm of dry sand to level the soil and then covered with a perforated polyvinyl chloride (**PVC**) bottom. The lysimeters were flipped upright and transported to the US Department of Agriculture-Agricultural Research Service Pasture Systems and Watershed Management Research Unit's greenhouse facility on the Pennsylvania State University's University Park campus. Lysimeters were arranged in the greenhouse in a 3-row by 7-column pattern with 0.9 m between rows and 1.2 m between columns and blocked by location in 3 blocks of 7. Air temperatures were maintained from 20 to 26°C throughout the experiment with exhaust fans. This experiment was part of a companion experiment studying ^{15}N-labeled manure N movement in soil and plant uptake. The companion experiment had 7 treatments, including blank (no added manure; 3 lysimeters), unlabeled HCP and LCP manure (3 of each, for a total of 6 lysimeters), ^{15}N-labeled feces HCP and LCP manure (6 lysimeters), and ^{15}N-labeled urine HCP and LCP manure (6 lysimeters). Lysimeters within a block were randomly assigned to 1 of the 7 treatments. The companion experiment was a randomized complete block design, split plot in time. For the purpose of experiment 2, all 9 lysimeters within manure type (i.e., HCP or LCP manure) were treated as replicates. The 9 lysimeters within manure type were 3 lysimeters with unlabeled manure and 6 with manure containing ^{15}N-labeled feces or urine. As N isotope fractionation naturally occurs during NH_3 volatilization from manure (see Hristov et al., 2009), experiment 2 data were also analyzed with the effect of ^{15}N-labeling of manure included in the statistical model. No difference was observed in the rate of NH_3 emission due to ^{15}N-labeling (1.28, 1.32, and 1.23 mg/m^2 per minute for manure containing ^{15}N-labeled feces, manure containing ^{15}N-labeled urine, and unlabeled manure, respectively; $P = 0.75$; SEM = 0.079). No interaction of ^{15}N-labeling × treatment (i.e., manure type; $P = 0.69$) was observed.

The planned manure application rate was 11.25 g of N/lysimeter (corresponding to 335 kg of N/ha), assuming an N availability factor of 0.3 (Pennsylvania State University, 2011; spring application of manure and soil incorporation within 5 to 7 d). At this application rate, N supply to a subsequent corn crop would be 101 kg/ha. The amount of mixed feces and urine needed to achieve this application rate was estimated based on N analyses of freeze-dried fecal and urine samples. Manure analysis indicated that the actual application rate was 9.3 g of N/lysimeter, or 277 kg of N/ha. The discrepancy between estimated and actual N application rates was probably a result of ammonia volatilization losses during application and handling of manure samples for analysis and variability in the N assay procedure. Because of the higher N concentration of HCP manure, a greater amount of LCP manure was applied to lysimeters to achieve equal N application rates (Table 2).

Emissions of NH_3, CO_2, CH_4, and N_2O were measured from the manure-soil surface at 3, 8, 23, 28, 54, and 100 h after manure application. Manure remained on the soil surface for the duration of gas measurements. Measurements were made using an INNOVA 1412 photoacoustic gas monitor (AirTech Instruments A/S) connected to a vented chamber. The chamber diameter was 25 cm, the height was 10 cm, and it was constructed from PVC pipe. To minimize NH_3 interaction with surfaces, the interior of the chamber was lined with Teflon tape and Teflon tubing was used to connect the chamber to the gas monitor. Chamber bases (25-cm diameter × 8 cm high) were also constructed from PVC and were inserted into the soil in each lysimeter shortly after manure application. The upper edge of the bases and lower edge of chambers had interlocking lips fitted with rubber gaskets to provide an airtight seal between the base and chamber. Each time the chamber was deployed, the gas monitor withdrew air samples and analyzed gas concentrations every minute for a 6-min

Table 2. Composition of feces and urine from cows fed high- and low-CP diets (n[1] = 8) and manure (mixture of feces and urine) applied to lysimeters in experiment 2 (n = 18)

	Diet[2]			
Item	HCP	LCP	SEM	*P*-value
Feces				
DM, %	16.9	17.2	0.19	0.37
N, % of DM	2.56	2.35	0.049	0.09
Urine				
DM, %	7.2	5.9	0.48	0.21
N, % of DM	14.9	9.2	0.26	0.02
Manure applied to lysimeters				
DM, %	13.2	13.7	0.45	0.41
N, % of DM	4.36	2.84	0.057	<0.001
Applied, g/lysimeter				
Feces	1,041	1,483	16.3	<0.001
Urine	612	872	9.6	<0.001
Manure[3]	1,653	2,356	25.9	<0.001
Manure DM	217	323	10.2	0.002
Fecal N	4.6	5.9	0.07	<0.001
Urinary N	6.2	4.7	0.06	<0.001
Manure N[4]	9.5	9.1	0.24	0.35
Total urea- and NH_3-N	4.9	2.8	0.06	<0.001
As proportion of total N applied				
Fecal N, %	42.4	55.5		
Urinary N, %	57.6	44.5		

[1]Indicates number of observations used in the statistical analysis (n = 8 represents 4 cows and 2 sampling weeks; n = 18 represents 18 manured lysimeters).

[2]HCP = manure from cows fed a diet containing 16.7% CP; LCP = manure from cows fed a diet containing 14.8% CP.

[3]Representing a 1.7:1 ratio (wt/wt, feces:urine).

[4]The estimated amount of manure N applied to lysimeters was lower than the sum of fecal and urinary N likely due to ammonia N losses during mixing of feces and urine and manure handling before application.

period. The gas measurements were conducted in the same order across lysimeters for all time points. Gas-emission rates were determined by regression of the change in gas concentration versus time. Two different calibration models were used because the emission rate curves had different shapes for NH_3 and GHG. Emissions curves for all gases were initially fit to both quadratic and linear models. A quadratic model provided the best fit for NH_3 and a linear model provided the best fit for the GHG. No significant emissions of NH_3, CH_4, and N_2O occurred from the blank, unmanured lysimeters (i.e., no significant change in the concentration of these gases above ambient). Therefore, it was assumed that emissions of these gases measured from the manured lysimeters were a response to manure application. Emission of CO_2 from the blank lysimeters was low but detectable and is, therefore, presented here for comparison (see footnote, Table 3).

Statistical Analysis

Ammonia and GHG EP data (experiments 1 and 2) were analyzed with the MIXED procedure of SAS (SAS Institute, 2003) as repeated measures assuming an autoregressive(1) covariance structure with overall mean, treatment, time, treatment × time, and error term in the model for experiment 1 and overall mean, treatment, time, treatment × time, block, block × treatment, and error term in the model for experiment 2. In the experiment 2 model, blocks and block × treatment interaction were random effects, whereas all other factors were fixed.

Feces and urine composition data, before being composited for lysimeter application (experiment 2; fecal and urine samples were not analyzed for experiment 1), were analyzed using the MIXED procedure of SAS with overall mean, cow, sampling week, treatment, and error term included in the model. Cow was a random effect, whereas all other factors were fixed. Manure composition and application data in experiment 2 were analyzed using the EP model, except that time and treatment × time terms were omitted.

Ammonia and GHG cumulative emissions in experiment 1 were estimated based on actual EP measurements. Emitting potential data from experiment 2 were fitted to various regression models (SigmaPlot 10.0; Systat Software Inc., San Jose, CA; NH_3, $R^2 = 0.95 \pm 0.020$; CH_4, 0.88 ± 0.064; N_2O, 0.79 ± 0.076; and CO_2: 0.92 ± 0.037, respectively) to estimate EP and cumulative emissions over the time period of emission

Table 3. Effects of dietary CP concentration on NH_3 and greenhouse gas emitting potential ($n^1 = 488$ for all gases in experiment 1; n = 72 for CH_4 and n = 107 for NH_3, N_2O, and CO_2 in experiment 2) and cumulative emissions (n = 4, experiment 1; n = 18, experiment 2) from manure

	Diet[2]			
Item	HCP	LCP	SEM	*P*-value
Experiment 1				
Emitting potential, mg/m^2 per minute[3,4]				
NH_3	9.20	4.88	0.733	0.02
CH_4	0.86	0.78	0.165	0.74
CO_2	65.6	70.1	2.79	0.31
Cumulative, 122-h emission, g/m^2				
NH_3	67.4	35.8	7.29	0.09
CH_4	6.3	5.7	0.99	0.72
CO_2	480	513	37.3	0.60
Experiment 2				
Emitting potential, mg/m^2 per minute (or as indicated)[5]				
NH_3	1.53	1.03	0.062	<0.001
CH_4	0.06	0.11	0.011	0.002
N_2O, μg/m^2	11.8	9.8	1.22	0.25
CO_2	34.7	39.2	1.31	0.07
Cumulative, 100-h emission, mg/m^2 (or as indicated)				
NH_3	7,415	3,745	469.8	<0.001
CH_4	138	167	41.4	0.65
N_2O	82	68	11.7	0.39
CO_2, g/m^2	163.8[6]	201.8	12.8	0.03

[1]Indicates number of observations used in the statistical analysis.

[2]HCP = manure from cows fed a diet containing 16.7% CP; LCP = manure from cows fed a diet containing 14.8% CP.

[3]N_2O was not detected in manure gas from experiment 1.

[4]Effect of time and time × treatment interaction: NH_3, $P < 0.001$ and 0.24; CH_4, $P < 0.001$ and <0.001; CO_2, $P < 0.001$ and 0.001, respectively.

[5]Effect of time and time × treatment interaction: NH_3, $P < 0.001$ and <0001; N_2O, $P < 0.001$ and 0.98; CH_4, $P < 0.001$ and 0.04; CO_2, $P < 0.001$ and <0.001, respectively.

[6]The cumulative CO_2 emission from the unmanured lysimeters (n = 3) was 16.7 ± 1.91 g/m^2.

measurements. Cumulative emissions data were analyzed using the EP models, except that the time and treatment × time terms were omitted.

The NH_3 EP curves from experiment 2 were fitted to various nonlinear models (SigmaPlot 10.0; average $R^2 = 0.95 \pm 0.014$) to estimate the area under the EP curves (AUC; AREA.XFM transform, SigmaPlot 10.0). Areas under the curve data were analyzed as the cumulative emissions data above.

In all models, the error term was assumed to be normally distributed with mean = 0 and constant variance. Statistical differences were declared at $P < 0.05$. Differences between treatments at $0.05 < P < 0.10$ were considered a trend toward significance.

RESULTS AND DISCUSSION

Manure Composition

The composition of feces and urine from cows fed HCP and LCP diets used in experiment 2 is shown in Table 2. The composition of feces, urine, and manure used in experiment 1 was not analyzed. Cows used to obtain feces and urine for experiment 1 were on the same companion experiment and fed the same diets as in experiment 2 and it is likely that feces and urine composition (and consequently manure) were similar between the 2 experiments. Fecal and urine DM contents were not different between diets. The concentration of N in feces and urine was greater (trend at $P = 0.09$ and $P = 0.02$, respectively) for HCP compared with LCP diets. As a result, manure from the HCP diet had 53% greater ($P < 0.001$) N concentration compared with LCP manure. Kebreab et al. (2002) reported an exponential response in urinary N output to increasing dietary CP concentration. Colmenero and Broderick (2006) also reported that urinary, but not fecal N, excretion increased with increasing CP concentration of the ration. In experiment 2, similar amounts of N from HCP and LCP manure were applied to the lysimeters (Table 2). Due to lower N concentration, the amount of manure DM applied was greater ($P < 0.001$) for LCP manure compared with HCP manure. The different N concentration in urine and similar N concentration in feces altered the proportions of urinary and fecal N in HCP and LCP manure. The proportion of fecal N was greater and that of urinary N was lower for LCP compared with HCP manure. As a result, the amount of urea- and NH_3-N added to the lysimeters was substantially greater ($P < 0.001$) for HCP than for LCP manure. As urinary urea is the main source of NH_3 emission from manure (Burgos et al., 2007; Lee et al.,

2011a), the different proportion of urinary N in HCP versus LCP manure was expected to have a major effect on NH_3 emissions.

Experiment 1

The average NH_3 EP of fresh manure was about 89% greater ($P = 0.02$) for HCP than LCP (Table 3). Peak EP was recorded at 6 h for LCP manure and at around 18 h for HCP manure (Figure 1; effect of time, $P < 0.001$; treatment × time interaction, $P = 0.24$). As a result of this greater EP, the cumulative NH_3 emission tended to be greater ($P = 0.09$) for HCP compared with LCP manure. Based on these cumulative losses and manure composition data (Table 2), an estimated 39 (HCP) and 30% (LCP) of the manure N was lost as NH_3 over the 122-h incubation period in this experiment. Decreased manure N concentration and NH_3 losses have been consistently reported as a result of decreased dietary CP concentration in dairy cattle (Misselbrook et al., 2005; van der Stelt et al., 2008; Agle et al., 2010). Swensson (2003) also found a linear relationship ($R^2 = 0.92$) between NH_3 release rate from manure and dietary CP concentration. The diet used to produce the LCP manure for the current experiment was not meeting the requirements of cows for MP according to NRC (2001). However, as discussed elsewhere (Huhtanen and Hristov, 2009), it is likely that the current NRC (2001) protein model overestimates the MP requirements of dairy cows. For example, based on actual nutrient intake and milk composition, NRC (2001) predicted MP-allowable milk yields of 38.8 ± 5.9 and 29.6 ± 4.8 kg/d for HCP and LCP in the companion experiment (Lee et al., 2011c). This represented an underestimation of the actual milk yield of the LCP group of 7.1 kg/d. In a subsequent trial, Lee et al. (2011b) were able to maintain milk production similar to the control (16% CP diet) of 38 to 39 kg/d with a 14% CP diet supplemented with ruminally protected AA. Thus, significant potential exists for decreasing the NH_3 EP of manure and, consequently, ammonia emissions from animal operations by decreasing dietary protein concentration without negatively affecting cow performance. It is expected that the magnitude of reduction in NH_3 EP observed in the current experiment would be even greater if dietary CP were decreased from the current industry standard of around 17 to 18% (Hristov et al., 2006). Ammonia EP data from this experiment, however, have to be interpreted with caution, as environmental factors, housing, and manure management all greatly affect on-farm emissions and these factors are not accounted for in our experimental setting.

The CH_4 and CO_2 EP and cumulative emissions were not affected by treatment in experiment 1 (Table 3). Peak CH_4 EP was recorded at 21 and 33 h for LCP and HCP manure, respectively (Figure 1; effect of time, $P < 0.001$). A significant treatment × time interaction ($P < 0.001$) was observed for CH_4. In effect, CH_4 EP was greater for LCP than for HighCP manure from 10 to 24 h (very low emissions were observed before 10 h) and then EP was greater for HCP up to 60 h when CH_4 emissions effectively ceased. Carbon dioxide EP increased rapidly from 0 to 30 h (effect of time, $P < 0.001$) and then plateaued for the HCP manure, but continued to slightly increase for the LCP manure (Figure 1), which resulted in a significant treatment × time interaction ($P = 0.001$). Although feces are the main source of CO_2 emissions from manure, urea hydrolysis per se can also be a source of CO_2. We did not attempt to distinguish sources of CO_2, but it is unlikely that the difference in urea concentration between LCP and HCP manure would have significantly affected the CO_2 emission data in this experiment. It was expected that higher N concentration in manure could increase CH_4 and CO_2 emissions as a result of greater availability of N for microbial growth. Results showed that our hypothesis was likely incorrect and N is not limiting the growth of manure microorganisms, particularly in these short-term experimental conditions.

Nitrous oxide was not detected in manure gas in experiment 1. Nitrous oxide emission is negligible on the barn floor (Adviento-Borbe et al., 2010; Arriaga et al., 2010) due to the lack of nitrifying and denitrifying microorganisms in cattle feces (Dowd et al., 2008). In addition, longer-term measurements of N_2O emission are necessary. For example, Külling et al. (2001) observed a significant decrease in N_2O from slurry with lower dietary CP concentration during a 3-wk manure storage experiment. Others have also reported low N_2O emissions during storage of dairy (Amon et al., 2006), swine (Park et al., 2006), and poultry manure (Li and Xin, 2010).

As discussed earlier, gaseous emissions from manure on the farm are influenced by a multitude of factors (temperature, wind, type of building, and manure-handling system; Ndegwa et al., 2008; Hristov et al., 2011a), confounding the effect of diet and CP intake. Analyzing emissions in a controlled environment (i.e., the EP of manure) is a more appropriate procedure for quantifying the effect of dietary factors. For example, in a current on-farm project with 12 commercial Pennsylvania dairy farms, we monitored barn floor NH_3 emissions in the spring and fall of yr 1 and then again in yr 2 of the project, after dietary CP concentrations were decreased by about 1 percentage unit (A.

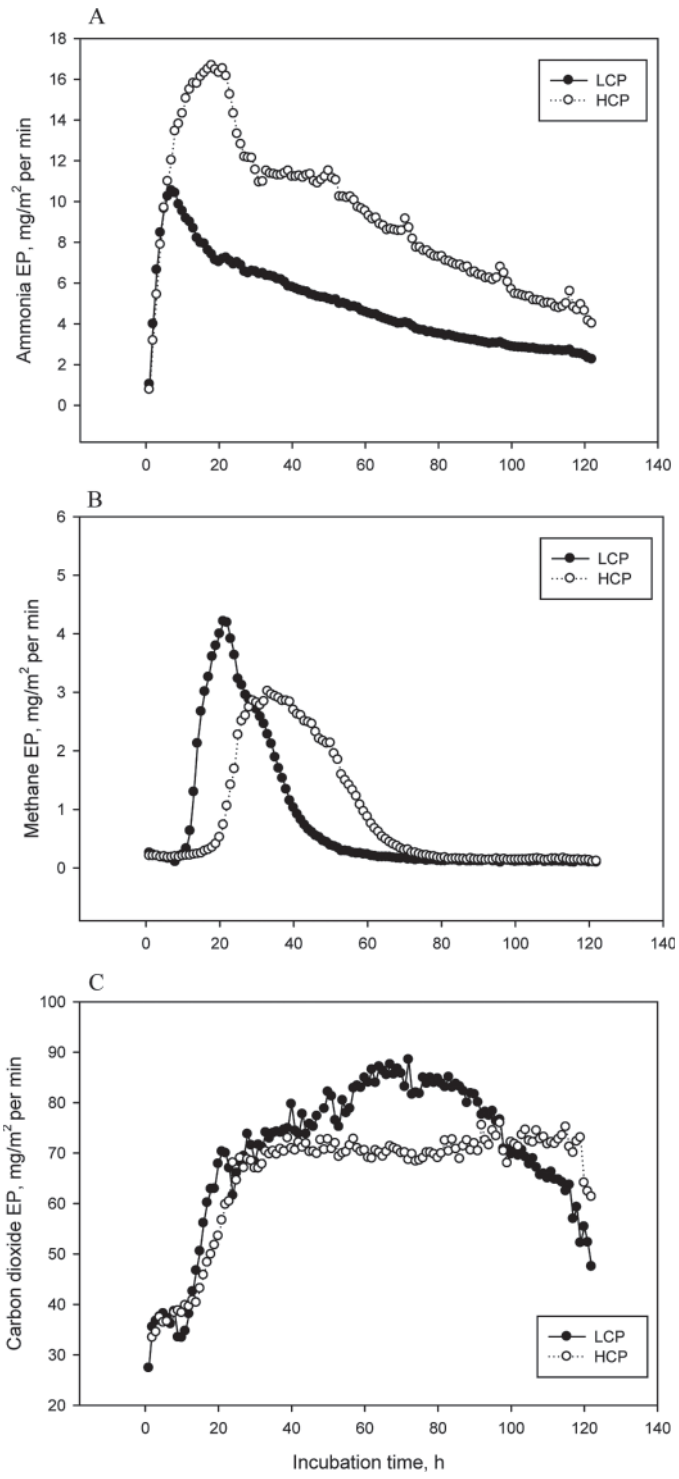

Figure 1. Effect of dietary CP concentration on NH_3 and greenhouse gas emitting potential (EP) of fresh manure incubated for 122 h (experiment 1; error bars are omitted for clarity, see Table 3 for variability and statistics). Panel A: ammonia; panel B: methane; panel C: carbon dioxide; nitrous oxide was not detected in manure gas from experiment 1. HCP = manure from cows fed a diet containing 16.7% CP; LCP = manure from cows fed a diet containing 14.8% CP.

N. Hristov, V. Ishler, K. Griswold, G. Schurman, S. Dinh, and E. F. Wheeler, Pennsylvania State University, University Park, unpublished data). On average, barn floor NH_3 emissions for the farms, in which the dietary CP reduction was documented by regular TMR sampling, decreased by about 65% (445 vs. 156, mg/m^2 per hour). However, average air temperatures during the emission measurements were 14 and 5°C (yr 1 and 2, 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 (feces and urine, collected separately, stored frozen, and later combined in the laboratory, as in the current experiments) were analyzed for NH_3 EP and showed unequivocally a decrease in emissions by about 36% for the low-CP period compared with the control, high-CP feeding period. This field study in progress demonstrates the potential of using EP as a tool for evaluating effect of diet and manure composition on NH_3 (and GHG) emissions from dairy farms.

Experiment 2

The decision to apply manure to the lysimeters immediately after mixing feces and urine was based on data from Lee et al. (2011a). In that trial, urea hydrolysis occurred very rapidly in manure with over 80% of the urea being hydrolyzed in 24 h (Lee et al., 2011a). As the difference in NH_3 emissions between the diets in the current experiment was expected to result from differences in urinary urea excretion, it was decided that manure should be applied to soil immediately following mixing of feces and urine. Thus, data from experiment 2 would be representative of situations where manure is applied to soil within 24 h after excretion (i.e., daily haul) and would not be representative of longer manure storage management systems.

The average NH_3 EP of manure following soil application was about 49% greater ($P < 0.001$) for HCP compared with LCP manure (Table 3). Ammonia EP remained relatively steady during the first 24 h after HCP manure application and decreased thereafter (Figure 2; effect of time, $P < 0.001$). A significant treatment × time interaction ($P < 0.001$) was observed. The EP for LCP manure sharply decreased during the first 24 h of manure application and at 23 h was, on average, 0.56 mg of NH_3/m^2 per minute compared with 1.92 mg of NH_3/m^2 per minute for the HCP manure. The average NH_3 EP during this period (23 and 28 h after manure application) was approximately 3.5 times greater for HCP than for LCP manure. The difference between the 2 manures remained large at 50 h (1.15 vs. 0.43 mg of NH_3/m^2 per minute, respectively) and disappeared by 100 h. As a result, the area under the

NH_3 EP curves was consistently larger ($P < 0.001$) for HCP than LCP throughout the 100-h monitoring period (see Figure 2 caption). This resulted in about 98% greater ($P < 0.001$) cumulative NH_3 emission from HCP compared with LCP manure-amended soil (Table 3). Based on these data and manure composition (Table 2), an estimated 23% of the HCP manure N and 12% of the LCP manure N applied to lysimeters were lost as NH_3 in 100 h. This greater NH_3 loss from the HCP manure can be explained by the greater concentration of urinary N in HCP than in LCP manure. Urea- and NH_3-N could represent from 50 to 90% of total urinary N in cattle (Bristow et al., 1992). Using ^{15}N-labeled urine or feces, Lee et al. (2011a) reported that urinary N contributed more than 90% of NH_3-N emitted from manure during the first 10 d after feces and urine were mixed (i.e., manure excretion). The decrease in NH_3 EP with LCP manure in experiment 2 was consistent with the decrease observed in experiment 1, but EP was considerably lower in experiment 2 than in experiment 1. Direct comparison of EP between the 2 experiments, however, is not possible because of different measurement protocols. The use of a static chamber to isolate emissions in experiment 2 was necessary to eliminate the effect of NH_3 drift among the closely placed lysimeters, but static chambers can alter conditions at the emitting surface and affect EP (Svensson, 1994). Moreover, manure in experiment 2 was uncovered (except for brief periods during gas-emission measurements) allowing for more rapid drying, and subsequent decrease in gas emission rates, than with the steady-state system used in experiment 1. As suggested by Jokela and Meisinger (2008), ammonium-N in manure is absorbed onto the soil exchange complex during application, which likely slowed the volatilization process in experiment 2. The main factor, however, causing the different emission rates between experiments 1 and 2 was likely the amount of manure-to-manure surface area ratio. For example, the ratio of amount of manure-to-manure surface area in experiment 1 was 2.48 g/cm^2, whereas in experiment 2, it was 0.44 and 0.63 g/cm^2 (HCP and LCP manure, respectively). Converted on a per-manure weight basis, NH_3 EP during the first 24 h were, in fact, similar between the 2 experiments for the HCP manure: 0.53 versus 0.44 μg of fresh manure/g per minute, but still about 50% lower in experiment 2 for the LCP manure (0.41 vs. 0.20 μg of fresh manure/g per minute, respectively).

The average CH_4 EP was greater ($P = 0.002$) for LCP compared with HCP manure-amended soil (Table 3). Overall, EP for both types of manure were very low in this experiment, but steadily increased from 6 to 23 h (effect of time, $P < 0.001$) and started decreasing thereafter in a similar manner for both manures (Figure 3; treatment × time interaction, $P = 0.03$). The cumulative CH_4 emission was not different between HCP and LCP manure (Table 3). The average CO_2 EP tended to be greater ($P = 0.07$) for LCP manure-amended soil and the cumulative CO_2 emission was greater ($P = 0.03$) compared with HCP manure. Carbon dioxide EP rapidly increased (effect of time, $P < 0.001$) for both manures up to 23 h, after which point CO_2 EP for LCP was markedly greater (55 vs. 34 mg/m^2 per minute at 50 h, respectively) compared with HCP (treatment × time interaction, $P < 0.001$). The increased CH_4 and CO_2 EP from LCP manure can be explained by the greater amount of manure OM applied to soil with this treatment. The proportion of fecal matter was greater in LCP than in HCP manure. Therefore, more undigested feed OM (and fecal microorganisms) was applied to the lysimeters with LCP manure. Synergistic activity between fecal and soil microorganisms that ferment fecal OM was likely responsible for the increased CH_4 and CO_2 EP from LCP manure. Clark et al. (2005) also reported greater emission (simulated storage conditions) of CH_4 and CO_2 from manure excreted from swine fed a low-CP diet. Furthermore, Külling et al. (2001) reported that lactating Brown Swiss cows fed a low-CP diet had decreased fiber digestibility, which resulted in more available carbon sources to form CO_2 and CH_4 in manure during storage. In the companion study, the LCP diet decreased total tract NDF and ADF digestibility, which resulted in greater ($P < 0.001$) concentrations of NDF and ADF in feces from cows

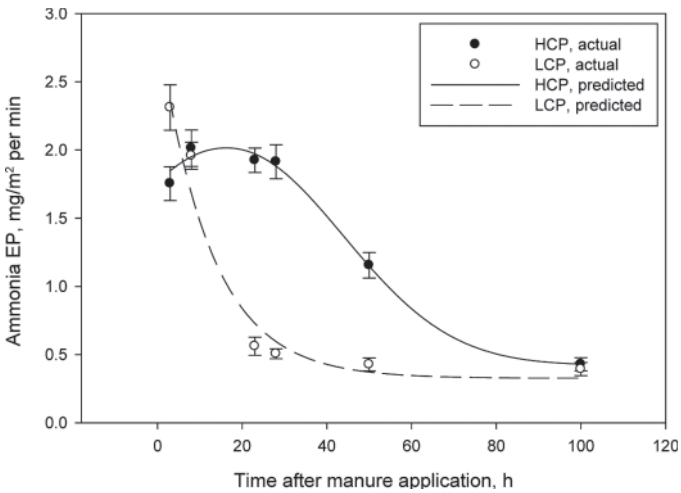

Figure 2. Ammonia emitting potential (EP) curves for manure applied to lysimeters in experiment 2 (means ± SE). Areas under the ammonia EP curves: HCP = 118.6; LCP = 55.4 mg of NH_3/m^2 per minute × h (n = 18, average $R^2 = 0.95 \pm 0.014$, SEM = 7.82, $P < 0.001$; effect of time, $P < 0.001$; treatment × time interaction, $P < 0.001$). HCP = manure from cows fed a diet containing 16.7% CP; LCP = manure from cows fed a diet containing 14.8% CP.

consuming LCP compared with HCP diets (51.4 vs. 48.2% and 39.3 vs. 25.9%, respectively; Lee et al., 2011c).

Methane EP measured in this experiment were much smaller in magnitude than on-farm emission rates. For example, CH_4 EP from experiment 2 were used to calculate annual manure CH_4 emissions per lactating cow. Using manure excretion data from a related experiment, in which total fecal and urine collections were performed (averaging 66 kg of fresh manure/cow per day; Lee et al., 2011d), annual CH_4 emission rates for HCP and LCP manure were estimated at 179 and 153 g/cow. These rates are not comparable to IPCC (2006) emission factors, which range (depending on the ambient temperature) from 63 to 98 kg of CH_4/dairy cow per year for North America. Our estimates, however, are comparable to rates reported by Külling et al. (2001; depending on diet, from 63 to 124 g/cow per year) for a manure storage system, similar to the one used in experiment 1. Annual CH_4 emission rates for experiment 1 were 1.2 and 1.1 kg/cow per year, respectively. It is apparent, that CH_4 emissions measured in the current study cannot be compared with IPCC (2006) estimates due to an array of factors, including primarily manure storage time and system (our on-farm measurements, for example, yielded CH_4 emission rates of 45 to 1,900 mg/m^2 per hour, depending on the manure storage system; flush and gravity flow, respectively), environmental effects, and measurement procedures, among others. Studies like ours, and that of Külling et al. (2001), are not designed to provide actual field gas-emission factors, but to compare emissions between dietary treatments.

The N_2O EP was low in this experiment and not different between treatments (Table 3). Nitrous oxide EP increased (effect of time, $P < 0.001$; treatment $\times$ time interaction, $P = 0.98$) up to 23 to 28 h and then again up to 50 h for both types of manure and decreased thereafter. Similar to our data, Cardenas et al. (2007) reported relatively low N_2O emissions from sheep slurry: from 0.05 (ryegrass diet) to 0.32 (kale diet) kg of N_2O-N/ha per day for the first 4 d (anaerobic fermentation). Assuming an average EP of 15 μg of N_2O/m^2 per minute (approximately between 23 and 50 h following manure application; Figure 3), one can calculate that N_2O emissions in experiment 2 were about 0.14 kg of N_2O-N/ha per day, which is within the range reported by Cardenas et al. (2007). A longer measurement period may be needed to document effects of dietary CP on N_2O emission. Formation and release of N_2O can occur immediately after manure is applied to soil due to denitrification of soil NO_3^- in the presence of easily degradable manure OM. However, much more additional N_2O can be produced by deni-

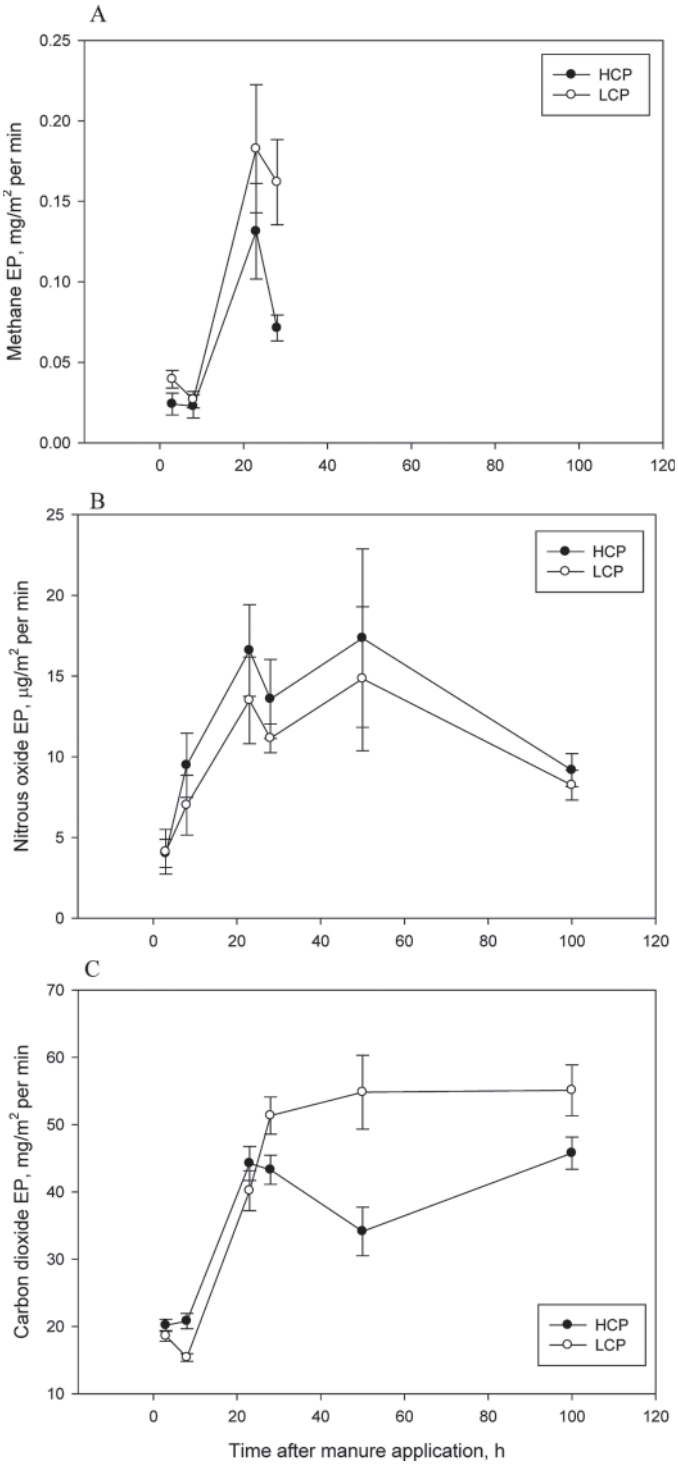

Figure 3. Effect of dietary CP concentration on greenhouse gas emitting potential (EP) of manure-amended soil in experiment 2 (mean ± SE, n = 9). Panel A: methane (methane emissions were not detected beyond 28 h); panel B: nitrous oxide; panel C: carbon dioxide. HCP = manure from cows fed a diet containing 16.7% CP; LCP = manure from cows fed a diet containing 14.8% CP.

trification of mineralized and nitrified manure N with time after manure application (Velthof et al., 2003). Nitrous oxide emissions ranged from 2.2 to 6.7 kg of N_2O-N/ha per day during the aerobic period (32 d) in the Cardenas et al. (2007) study. Thus, it is likely that following extended storage, N_2O emission from HCP manure-amended soil would be greater than that from LCP manure-amended soil. Cardenas et al. (2007), for example, reported the largest correlation between N_2O emissions and slurry protein concentration. In their study, slurry from sheep fed alfalfa had higher N content and about 45% greater N_2O emission than slurry from sheep fed ryegrass (although the largest emission was from sheep fed kale, which produced slurry with intermediate N concentration). Furthermore, NH_3 is an indirect source of N_2O. Ferm (1998) estimated that 5% of the global N_2O emissions originate from NH_3 oxidation in the atmosphere; in the present experiment, HCP manure emitted substantially greater NH_3 than did LCP manure in both experiments 1 and 2. Results from experiment 2 are applicable to NH_3 and GHG emissions from the barn floor, immediately after urine and feces are mixed, or to systems in which manure is rapidly removed and applied to soil (i.e., daily haul). Under most systems, however, manure is stored for various periods of time on farm before field application and our data are likely not accounting for important environmental factors affecting gaseous emissions from manure on the farm and changes in manure that occur during long-term storage.

CONCLUSIONS

The effect of dietary CP concentration on NH_3 EP of dairy cattle manure was significant in simulated storage as well as from the soil surface following manure application immediately after mixing feces and urine. More NH_3 was emitted from HCP manure-amended soil even though N application rates were similar for LCP and HCP manure. This can be explained by the proportionally greater concentration of urinary urea N in HCP than LCP manure. The largest difference in the NH_3 EP occurred approximately 24 h after manure application (approximately 3.5 times greater for HCP than LCP manure). Methane and CO_2 EP from manure-amended soil increased or tended to increase with LCP manure. This was likely a result of the greater addition of fecal matter with LCP compared with HCP manure. In this short-term experiment, emission of N_2O was not affected by dietary CP level. These results represent effects of dietary protein on NH_3 and GHG EP of manure in controlled laboratory conditions and do not account for the effect of storage or environmental factors affecting gaseous emissions from manure on the farm; therefore, applicability to most common farm manure management practices is limited.

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