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Dairy Manure Type, Application Rate, and Frequency Impact Plants and Soils

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The impacts of dairy rations on manure chemistry, manure N mineralization in soils, and crop N uptake have been evaluated after single manure applications. No information is available on these impacts when manure is applied to soils at different rates and frequencies. Manure from lactating dairy cows (*Bos taurus*) fed three diets differing in crude protein (CP) content were applied to a Plano (fine-silty, mixed, superactive, mesic Typic Argiudolls) and a Rosholt (coarse-loamy, mixed, superactive, frigid Haplic Glossudalfs) soil at two application rates (225 or 450 kg total N ha^{-1}) and three application frequencies (once, twice, or thrice). Oat (*Avena sativa* L.), sorghum [*Sorghum bicolor* (L.) Moench] and sorghum ratoon were grown in succession during a 170-d period. Plant responses to manure from different CP diets were much less than responses due to soil type and manure application rate and frequency. Manure N uptake efficiency by plants was greatest for manure derived from a low-CP diet than manure derived from either the medium- or high-CP diets. Manure application rate and frequency increased NO_3 levels in oat and sorghum shoots, which if used as forage could have detrimental impacts on dairy cow health. No significant differences were measured in second-crop (sorghum) yield or N uptake due to a previous manure application at either N application rate. Third-crop (sorghum ratoon) yield and N uptake were significantly increased, however, due to previous manure applications, but only in pots that received two manure applications at the high rate. Longer term, repeated applications of dairy manure derived from different diets at lower rates could provide more pronounced impacts on plants and soils than those observed during this relatively short-term greenhouse study.

Abbreviations: ADIN, acid detergent insoluble nitrogen; CP, crude protein; DM, dry matter; F1, first manure application; F2, second manure application; F3, third manure application; HP, high protein diet; IN, inorganic N; LP, low protein diet; MNUE, manure nitrogen use efficiency; MP, medium protein diet; NDIN, neutral detergent insoluble nitrogen; OM, organic matter; R1, manure application at agronomic N rate (225 kg ha^{-1}); R2, manure application at twice agronomic N rate (450 kg ha^{-1}).

SOIL FERTILITY & PLANT NUTRITION

Under current dairy feeding practices, only 20 to 30% of the CP fed to dairy cows is secreted in milk. On average, a dairy cow annually produces 8200 kg of milk and excretes 21,000 kg of manure, which contains about 110 kg N (Van Horn et al., 1996). The type and amount of CP fed to dairy cows can have a large impact on total manure N excretion and the proportion of manure N that is excreted in feces and urine (Castillo et al., 2000; Broderick, 2003; Wattiaux and Karg, 2004), how much manure N is lost as NH_3 (Misselbrook et al., 2005), and manure N mineralization in soils and crop N uptake (Powell et al., 2006).

In the Northeast and Midwest regions of the USA, many dairy farmers are increasing the number of cows they milk and importing more feed. This increases the manure amount per unit cropland area. Manure is often applied year after year on land closest to barns (Powell et al., 2007) because transportation costs to spread manure on distant fields are higher than the perceived fertilizer value of the manure (Nowak et al., 1997; Saam et al., 2005). While the application of manure to agricultural land recycles plant-available nutrients and organic matter, which can improve crop production and soil quality, repeated manure application in excess of crop requirement may also have adverse effects on the feeding value of forages and soil quality, and increase the risks of environmental pollution (Chang and Janzen, 1996; Eghball and Power, 1999; Ferguson et al., 2005). To protect the environment, many states regulate when, where, and how much manure can be land applied. Unless targeted for P-based management, agronomic recommendations for manure application rates are based on the N requirement of the following crop.

Soil-manure incubations and greenhouse trials revealed that single applications of dairy manure derived from cows fed various diets had differential impacts on soil N mineralization, crop yield, and N uptake (Powell et al., 2006) and NH_3–N losses from land-applied manure (Misselbrook et al., 2005). Recent field studies in central Wisconsin (Cusick et al., 2006) revealed that single and multiple dairy manure applications (derived from uniform diets) have differential impacts on crop yield and N uptake the first and subsequent years after manure application. While these and other (e.g., Sørensen et al., 2003) experiments have evaluated dairy diet impacts on manure chemistry and its mineralization in soil after single applications, and how the manure application rate and frequency impact crop yield and N uptake, no information is available on how dairy manure derived from different diets impacts crop production and soil properties when these manures are applied to soil at different rates and frequencies. The objective of this study was to evaluate the

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interactive effects of dairy manure type (derived from different CP diets), application rate, and frequency on crop yield and N uptake, forage NO_3^- levels, and the chemical properties of two soils cultivated by Wisconsin dairy farmers.

MATERIALS AND METHODS

Soil Description

Representative samples were taken with a stainless steel shovel from the surface (0–15 cm) horizons of a Plano silt loam soil (45°35′ N, 90°10′ W) and a Rosholt sandy loam soil (44°15′ N, 89°5′ W). The two fields from which soil was taken had not received manure for at least the previous 5 yr. Soil samples were air dried, sieved to pass a 2-mm screen, and stored in plastic containers until used in the greenhouse trial. Soil pH (1:1 water) was measured using a calibrated portable pH meter (Accumet AP61, Fisher Scientific, Pittsburgh, PA), and total N and C were determined by combustion assay (Elementar VarioMax CN analyzer, Elementar, Hanan, Germany). Ammonium N and NO_3^- + NO_2^- were extracted with 2 *M* KCl (5 g of soil in 50 mL of 2 *M* KCl, shaken for 2 h and filtered), followed by analyses using QuickChem Methods 12–107–06–2-A (NH_4^+) and 12–107–04–1-B (NO_3^- + NO_2^-) on a Lachat automated N analyzer (Lachat Instruments, 1996). Soil pH, total C, and total and inorganic N were much higher in the Plano than in the Rosholt soil (Table 1).

Manure Types

Dairy urine and feces were collected separately from lactating Holstein dairy cows fed diets containing three CP levels: low (13.6% of dietary dry matter intake, LP), medium (17.1%, MP), or high (19.4%, HP). Details on diet composition (Broderick, 2003) and manure collection (Misselbrook et al., 2005) have been reported previously. In brief, feces were scraped by hand from metal catchment pans fitted into tie-stall manure collection gutters, and urine was collected using indwelling catheter tubes draining into plastic containers embedded in ice. Fresh fecal and urine samples were pooled among cows fed similar diets, and pooled fecal and urine subsamples were frozen separately until laboratory analyses and application to soils. The day before manure application to soils in greenhouse pots, the frozen subsamples of feces and urine were thawed and then recombined (hand mixed) into a slurry using the fecal/urine weight ratio determined at the time of excretion (Misselbrook et al., 2005). Triplicate slurry samples per diet type were analyzed for pH (1:2 manure/water mixture) and dry matter (DM; 100°C, 24 h), and subsamples were freeze-dried, ground to pass a 1-mm screen, and analyzed for total N and total C using the same combustion assay as outlined for soils. Total NH_4–N in slurries was extracted using KCl (5 g slurry in 50 mL 2 *M* KCl, shaken for 2 h and filtered through Whatman no. 42 filter paper) and analyzed on a Lachat automated N analyzer. Cell wall components of feces were determined using the detergent system (Goering and Van Soest, 1970) as neutral detergent fiber and acid detergent fiber. The N content of neutral detergent fiber (neutral detergent insolubel N; NDIN) and acid detergent fiber (acid detergent insoluble N; ADIN) was determined by combustion assay as outlined for soils. The NDIN is a measure of manure N associated with undigested dietary fiber and ADIN is a measure of manure N associated with the hemicellulose and cellulose fractions of undigested dietary fiber. Manure derived from the HP diet had higher concentrations of total N, NDIN, ADIN, NH_4, and NO_3, and a lower C/N ratio than manure from either the LP or MP diets (Table 2).

Experimental Design

Eight hundred grams of air-dried soil were placed in nondraining plastic 700 mL pots, P was applied at the rate of 40 kg P ha^{-1} as KH_2PO_4, and distilled water was added to achieve soil moisture contents of approximately 60% water-filled pore space (WFPS) using procedures outlined by Honeycutt et al. (2005). A complete factorial design was used that included treatment combinations of the two soil types (Plano or Rosholt), three manure types (LP, MP, or HP), two manure application rates (an agronomic rate equivalent to 225 kg total N ha^{-1} [R1], or twice the agronomic rate [R2]), and three manure application frequencies (a single application before the first crop [F1], a repeated application before the second crop [F1 + F2], or a third application before the third crop [F1 + F2 + F3]). Triplicate pots per treatment combination and triplicate unamended control pots (no manure) were used. Pots were watered every 2 to 3 d to maintain 60% WFPS and pot locations on the greenhouse bench were relocated randomly each week.

The first manure application (F1) was mixed with the upper approximately 2.5 cm of soil in each pot, followed by immediate watering. A 5-d initial fallow period followed F1 manure application, after which nine oat seeds were sown in each pot, and seedlings were thinned at 10 d to keep the five most robust plants per pot. After a 45-d growth period, oat shoots and roots were harvested. Oat roots were removed from pots to minimize their impact on N mineralization and crop growth during the next two cropping phases. Soil and organic debris were washed from roots and wash water returned to the pots. After oat harvest, one-third of the pots received no manure (F1 treatment) and F2 manures were applied in the same manner as F1 to the remaining two-thirds of the pots (one-third of which would eventually receive an F3 manure application just before the third cropping phase). The F2 manure application was followed by a 20-d fallow period, after which seven sorghum seeds were planted per pot and grown in the same manner as oat for 60 d. Sorghum shoots were harvested and F3 manure was applied within four slots, approximately 2.5 cm deep, to one-third of the pots (one-third of the remaining pots kept as F1 and the other one-third as F2) and sorghum plants were allowed to ratoon (regrow) for an additional 45 d, then sorghum ratoon shoots were harvested. Total N in oat and sorghum shoots and roots was determined using the same methods as outlined for the manure. Nitrates in oat and sorghum shoots were extracted with 2% glacial ascetic acid and analyzed using QuickChem Methods 13–107–04–1-A on a Lachat automated N analyzer. Ash residues were used to determine

Table 1. Total N (TN), total C (TC), C/N ratio, pH, NH_4–N, NO_3–N, and total inorganic N (IN) in Plano and Rosholt soils at the onset of the greenhouse trial.

Soil type	TN	TC	C/N	pH	NH_4–N	NO_3–N	IN
	—g kg^{-1}—				mg kg^{-1}		
Plano	2.70	23.19	8.59	5.79	8.21	32.04	40.25
Rosholt	1.22	9.42	7.71	5.08	14.89	5.33	20.22

Table 2. Dairy manure pH and concentrations of total C (TC), total N (TN), C/N ratio, neutral detergent insoluble N (NDIN), acid detergent insoluble N (ADIN), NH_4–N, and NO_3–N in manures derived from low (LP), medium (MP), and high (HP) protein diets used in the greenhouse trials.

Manure composition	Manure type		
	LP	MP	HP
pH	8.63	8.55	8.55
TC, g kg^{-1} dry matter (DM)	425	428	424
TN, g kg^{-1} DM	30.7	32.7	36.7
C/N, kg kg^{-1} DM	13.8	13.1	11.5
NDIN, g kg^{-1} DM	3.09	3.46	4.32
ADIN, g kg^{-1} DM	2.76	3.23	4.09
NH_4^+-N, g kg^{-1} DM	17.88	15.79	18.09
NO_3–N, g kg^{-1} DM	0.067	0.056	0.074

Table 3. Partial ANOVA for soil type, manure type, and manure rate and application frequency effects on crop dry matter (DM) and N uptake.

Source of variation	df	Crop DM						Crop N uptake					
		Oat		Sorghum		Sorghum ratoon		Oat		Sorghum		Sorghum ratoon	
		P	Trt.†	*P*	Trt.	*P*	Trt.	*P*	Trt.	*P*	Trt.	*P*	Trt.
			%		%		%		%		%		%
Soil type (S)	1	<0.0001	96.8	<0.0001	16.1	<0.0001	9.4	0.0348	4.9	<0.0001	28.9	NS‡	–
Manure type (T)	2	NS	–	NS	–	NS	–	NS	–	0.2807	0.1	NS	–
Manure rate (R)	1	0.0049	2.5	0.0728	1.5	<0.0001	13.4	<0.0001	91.8	<0.0001	4.2	<0.0001	21.0
Manure frequency (F)	2	NS	–	<0.0001	79.2	<0.0001	70.6	NS	–	<0.0001	53.9	<0.0001	69.1
S × T	2	NS	–	NS	–	NS	–	NS	–	NS	–	NS	–
S × R	1	NS	–	NS	–	0.0055	1.7	NS	–	0.0003	1.6	0.0011	1.1
S × F	1	NS	–	NS	–	NS	–	NS	–	<0.0001	7.8	0.0181	0.4
T × R	2	NS	–	NS	–	NS	–	NS	–	NS	–	NS	–
T × F	1	NS	–	NS	–	NS		NS	–	NS	–	0.0591	0.2
R × F	1	NS	–	NS	–	0.0322	0.8	NS	–	<0.0001	2.2	0.0001	1.0

† Percentage of variation attributable to source.

‡ Not significant.

possible soil contamination of oat and sorghum roots. Approximately 0.5-g root subsamples were combusted at 600°C for 24 h, and soil contamination was calculated (Potthoff and Loftfield, 1998) and subtracted from root DM to calculate root organic matter (OM) production per pot. After oat harvest and at trial's end, representative soil samples were taken from each pot and analyzed for pH and total soil inorganic N (IN) using the same procedures outlined for initial soil samples.

Calculations and Statistics

For oat (Harvest 1) manure N use efficiency (MNUE) was calculated as the percentage of shoot N uptake that could be associated with manure N applications. This calculation was made using

$$\text{MNUE} = \frac{\text{treatment oat N uptake} - \text{control oat N uptake}}{\text{total manure N applied}} 100 \quad [1]$$

For sorghum (Harvest 2), the numerator of Eq. [1] included N uptake by oat and sorghum shoots and the denominator included manure application from F1 and F2. For sorghum ratoon (Harvest 3), the numerator of Eq. [1] included N uptake by oat, sorghum, and sorghum ratoon shoots, and the denominator included manure application from F1, F2, and F3 (Mooleki et al., 2004).

Statistical analyses were performed using the SAS statistical package (SAS Institute, 1990). Differences in plant DM, N uptake, and NO_3 levels; root OM and N uptake; MNUE; and soil properties due to treatments were analyzed by generalized least squares analysis of variance, assuming replicates to be a random effect and soil type, manure type, application rate and frequency, and all interactions to be fixed effects. Where relevant, the protected LSD test was used to determine significant differences among treatments at $P < 0.05$.

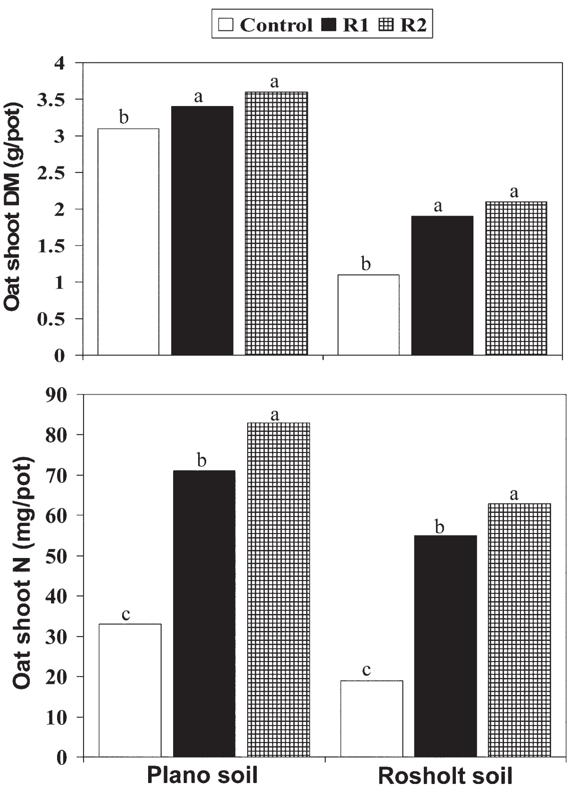

Fig. 1. Effects of manure application rate (recommended rate, R1, or twice the recommended rate, R2) on Harvest 1 (oat) dry matter (DM) and N uptake (within a soil type, application rate bar means having different letters differ at *P* < 0.05).

RESULTS

The partial analysis of variance (Table 3) revealed very few significant interactive effects of soil type, manure type, application rate, or frequency on oat, sorghum, or sorghum ratoon DM and N uptakes. Except for interactive effects of soil type and manure application frequency on sorghum (Harvest 2) N uptake, which accounted for approximately 8% of all treatment variability, other significant interactive treatment effects on plant DM and N uptake generally accounted for <2% of treatment variability. For this reason, the following results and discussion focus on the main effects of soil type, manure type, and manure application rate and frequency on observed plant DM and N uptake during the three plant growth cycles of this greenhouse study.

Treatment Effects on Plant Shoot Dry Matter and Nitrogen Uptake

For Harvest 1, soil type had the greatest impact (97% of treatment variability) on oat DM, and manure application rate had the greatest impact (92% of treatment variability) on oat N uptake (Table 3). Oat DM harvested from pots containing the Plano soil was, on average, two to three times greater than oat DM in pots containing the Rosholt soil (Fig. 1). In both soils, oat DM in pots that received R1 and R2 manure application rates were similar and significantly greater than oat DM in control pots. Also, in both soils, oat N uptake was increased significantly as manure N applications increased from the simulated agronomic rate (R1) to twice the agronomic rate (R2).

For Harvest 2, manure application frequency had the greatest impact on sorghum DM (79% of treatment variability) and N uptake (54% of treatment variability), followed by soil type, which accounted for 16 and 29% of treatment variability associated with sorghum DM and N uptake, respectively (Table 3). For both soils, the second manure application (F1 + F2) increased sorghum DM and N uptake significantly over pots that received only the first (F1) manure application (Fig. 2). Also for both soils, there were no significant differences between sorghum DM or N uptake in control pots (no manure) and pots that received the first manure application (F1), at either the low (R2) or high (R2) application rates. These results indicate no apparent residual effects of the first manure application on second-cycle plant DM and N uptake. As was observed with oat (Fig. 1), sorghum DM and N uptake (Fig. 2) in pots containing Plano soil were overall greater than in pots that contained the Rosholt soil.

The pattern of treatment effects on Harvest 3 (Fig. 3) were similar to observations made for Harvest 2 (Fig. 2). Manure application frequency had the greatest impact on sorghum ratoon yield (71% of treatment variability) and N uptake (69%), followed by soil type, which accounted for 13 and 21% of treatment variability associated with ratoon sorghum DM and N uptake, respectively (Table 3). For both soils, the third manure application (F1 + F2 + F3) applied at the high rate (R2) increased sorghum ratoon DM and N uptake significantly over pots that received either one (F1) or two (F1 + F2) manure applications. In the Rosholt soil, the third manure application at the low application rate (R1) had the same impacts on sorghum ratoon DM and N uptake as those noted for the high application rate. This was not the case, however, in the Plano soil. For the Plano soil, there were no significant differences between sorghum ratoon DM and N between pots that received F1 or F1 + F2 manure at the low application rate (R1). Results from the final harvest imply that although manure applications in excess of agronomic recommendations (R2) may not increase plant DM during the growing cycle immediately after application (e.g., F1 + F2 response depicted in Fig. 2), this high application rate may enhance plant DM and N uptake during a later plant growth cycle (e.g., F1 + F2 responses depicted in Fig. 3).

Treatment Effects on Plant Root Organic Matter and Nitrogen Content

Oat roots were harvested before planting sorghum, and sorghum roots were harvested at the trial's end. Somewhat similar to the observed treatment effects on shoot DM and N uptake, oat roots harvested from pots containing the Plano soil had, on average, significantly greater amounts of OM (grams per pot) and N (milligrams per pot) than roots harvested from pots that contained the Rosholt soil (Table 4). In the Plano soil, there were no significant differences in oat root OM between pots that received no manure (controls) and pots that received manure at either the low (R1) or high (R2) rates. Oat root N in the Plano soil was, however, significantly greater in manure-amended pots (R1 and R2) than in the control pots. Also in the Plano soil, N uptake by oat shoots was

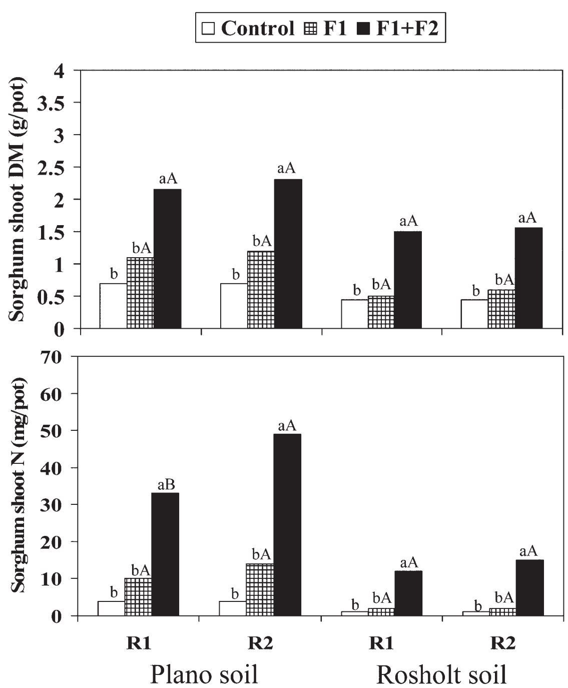

Fig. 2. Effects of manure application frequency (before first crop, F1, or before first and second crops, F1 + F2) and rate (recommended rate, R1, or twice the recommended rate, R2) on Harvest 2 (sorghum) dry matter (DM) and N uptake (within a soil type and application rate, application frequency bar means having different lowercase letters differ at $P < 0.05$; within a soil type and application frequency, application rate means followed by different uppercase letters differ at $P < 0.05$).

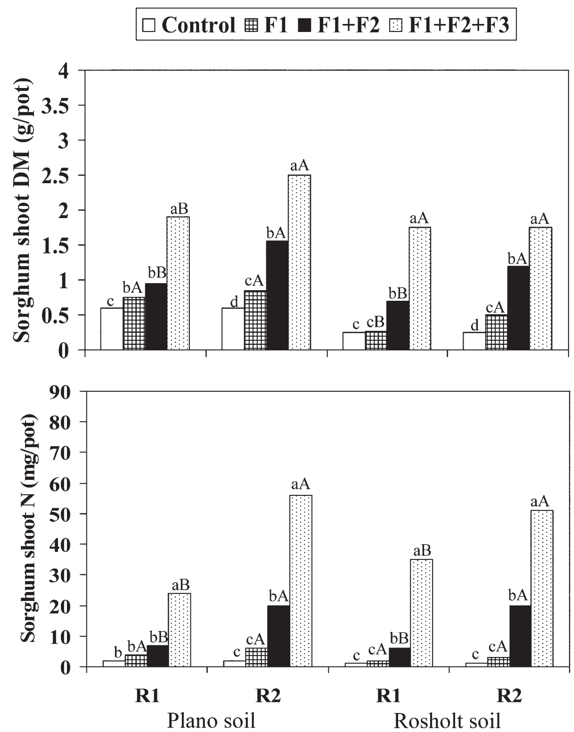

Fig. 3. Effects of manure application frequency (before first crop, F1, before first and second crops, F1 + F2, or before all three crops, F1 + F2 + F3) and rate (recommended rate, R1, or twice the recommended rate, R2) on Harvest 3 (sorghum ratoon) dry matter (DM) and N uptake (within a soil type and application rate, application frequency bar means having different lowercase letters differ at $P < 0.05$; within a soil type and application frequency, application rate bar means having different uppercase letters differ at $P < 0.05$).

Table 4. Oat and sorghum root organic matter weight (ROMW) and root organic matter N (ROMN) in Plano and Rosholt soils in pots receiving no manure (control) or manure applied once (F1), twice (F1 + F2), or thrice (F1 + F2 + F3) at the agronomic rate (R1) or twice the agronomic rate (R2).

Property	Harvest (crop)	Manure application frequency	Plano			Rosholt		
			Control	R1	R2	Control	R1	R2
ROMW, g pot^{-1}	H1 (oat)	F1	0.98	0.99	0.95	0.57 c†	0.74 b	0.87 a
	H3 (sorghum ratoon)	F1	1.06 c	1.80 bC‡	2.05 aB	0.71 b	0.70 bC	1.01 aB
		F1 + F2	1.06 c	3.79 Bb	5.30 aB	0.71 c	2.51 bB	3.80 aB
		F1 + F2 + F3	1.06 c	4.81 ba	5.67 aA	0.71 c	3.46 bA	4.04 aA
ROMN, mg pot^{-1}	H1 (oat)	F1	17.5 b	21.0 a	21.2 a	9.7 c	15.6 b	20.2 a
	H3 (sorghum ratoon)	F1	12.1 b	21.9 ac	25.8 aC	8.6 b	6.8 bC	10.7 aC
		F1 + F2	12.1 c	43.2 bb	67.2 aB	8.6 c	22.2 bB	46.9 aB
		F1 + F2 + F3	12.1 c	58.9 ba	97.8 aA	8.6 c	51.4 bA	79.1 aA

† Within a soil type, harvest (crop), and application frequency, row rate means followed by different lowercase letters differ at $P < 0.05$.

‡ Within a soil type, harvest (crop), and manure application rate, column frequency means followed by different uppercase letters differ at $P < 0.05$.

increased significantly as manure N applications increased from the simulated agronomic rate (R1) to twice (R2) the agronomic rate (Fig. 2). In the Rosholt soil, both oat root OM and N were highest in pots amended with R2, followed by R1 and the control pots.

In a pattern similar to that observed with oat, sorghum ratoon root OM and N were higher in the Plano than in the Rosholt soil (Table 4). In both soils, manure frequency and application rate had significant positive effects on sorghum root OM and N. In the Plano soil, manure application frequency generally increased sorghum OM and N at both manure application rates (R1 and R2). Pots that received all three manure applications (F1 + F2 + F3) had significantly more root OM and N than pots that received only two manure applications (F1 + F2), and pots that received two applications had higher root OM and N than pots that received only one manure application (F1). The one exception to this was root OM at the high manure application rate. Root OM was similar in pots that received manure at frequencies F1 and F1 + F2. The same patterns of treatment effects on oat root OM and N in the Plano soil were also observed in the Rosholt soil (including the one exception just mentioned).

Treatment Effects on Soil pH and Inorganic Nitrogen

Representative soil samples were taken from each pot the day following each harvest. As was observed with treatment impacts on shoot and root yields and N uptake, soil type had the greatest impact on soil pH and IN. Following oat harvest in the Plano soil, soil pH was greater in pots that received the high manure rate than in controls, which had, on average, a similar pH as pots that received manure at the low rate (Table 5). In the Rosholt soil, soil pH was lowest in the control, and higher in pots that received the low (R1) than in pots that received the high (R2) manure application rate. After sorghum harvest (Harvest 2), soil pH in pots containing the Plano soil was not impacted significantly by previous manure application rates or frequencies. In the Rosholt soil, however, soil pH in pots that received only the initial manure application (F1) was much higher than in control pots, indicating great carryover effects of manure application on soil pH. Rosholt soil pH was lower in pots that received two manure applications (F1 + F2) than in pots that received only one (F1) manure application. Also in pots that received both (F1 + F2) manure applications, soil pH was greater in pots amended at rate R1 than at rate R2. In pots that received only one (F1) manure application, however, soil pH was lower in pots amended with manure application at R1 than in pots amended at R2.

Observed treatment effects on soil pH following sorghum ratoon harvest (Harvest 3) were different than those observed after sorghum harvest (Harvest 2), especially in the Plano soil. In the Plano soil, there were no significant differences in soil pH in control pots and pots that received manure at the low rate (R1) and either F1 or F1 + F2 application frequencies. Plano soil pH was greater in pots amended with manure at the high (R2) than the low (R1) application rate, at all three manure application frequen-

Table 5. Soil pH and total inorganic nitrogen (IN) after Harvest 1 (H1), 2 (H2), and 3 (H3) in unmanured control pots and pots that received one (F1), two (F1 + F2), or three (F1 + F2 + F3) manure applications at either the agronomic rate (R1) or twice the agronomic rate (R2).

Soil property	Harvest (crop)	Manure application frequency	Plano			Rosholt		
			Control	R1	R2	Control	R1	R2
pH	H1 (oat)	F1	5.92 b†	6.00 ab	6.15 a	4.92 c	6.09 a	5.80 b
	H2 (sorghum)	F1	6.32	6.20	6.36	5.15 c	6.79 bA‡	7.05 aA
		F1 + F2	6.32	6.28	6.30	5.15 c	6.00 aB	5.66 bB
	H3 (sorghum ratoon)	F1	6.17 b	6.19 bB	6.26 aC	5.62 c	6.95 bA	7.17 aA
		F1 + F2	6.17 b	6.21 bB	6.37 aB	5.62 c	5.88 bB	6.77 aB
		F1 + F2 + F3	6.17 c	6.32 bA	6.62 aA	5.62 b	5.78 bB	5.94 aC
Total IN, mg pot^{-1}	H1 (oat)	F1	4.7 c	6.0 b	7.3 a	6.1c	6.9 b	10.6 a
	H2 (sorghum)	F1	8.4	6.6 B	7.8 B	4.6	3.8 B	3.7 B
		F1 + F2	8.4 b	10.1 bA	36.8 aA	4.6 c	13.7 Ab	51.8 aA
	H3 (sorghum ratoon)	F1	4.8	4.8	5.6 B	3.6	3.4	3.5 B
		F1 + F2	4.8	4.9	5.2 B	3.6	3.7	5.5 B
		F1 + F2 + F3	4.8 b	5.2 b	8.5 aA	3.6 b	4.8 b	17.7 aA

† Within a soil type, harvest (crop), and application frequency, row rate means followed by different lowercase letters differ at $P < 0.05$.

‡ Within a soil type, harvest (crop), and manure application rate, column frequency means followed by different uppercase letters differ at $P < 0.05$.

Table 6. Nitrate-N levels in oat and sorghum shoots harvested from pots containing Plano and Rosholt soils amended with no manure (control), and manure applied once (F1), twice (F1 + F2) or thrice (F1 + F2 + F3) at the agronomic rate (R1) or twice the agronomic rate (R2).

Harvest (crop)	Manure application frequency	Shoot NO_3–N					
		Plano			Rosholt		
		Control	R1	R2	Control	R1	R2
		mg kg^{-1}					
H1 (oat)	F1	0.40 c†	1.70 b	2.16 a	1.38 c	2.33 b	2.97 a
H2 (sorghum)	F1	0.36	0.22	0.20 B‡	0.34 b	0.44 aB	0.21 bB
	F1 + F2	0.36 b	0.35 b	1.35 aA	0.34 c	1.01 bA	1.33 aA
H3 (sorghum ratoon)	F1	0.08	0.114	0.109 B	0.19	0.215	0.143 aB
	F1 + F2	0.08	0.120	0.130 B	0.19	0.121	0.162 B
	F1 + F2 + F3	0.08 b	0.097 b	0.651 aA	0.19 b	0.156 b	1.313 aA

† Within a soil type, harvest (crop), and application frequency, row rate means followed by different lowercase letters differ at $P < 0.05$.

‡ Within a soil type, harvest (crop), and manure application rate, column frequency means followed by different uppercase letters differ at $P < 0.05$.

cies. In the Rosholt soil at the low application rate (R1), average soil pH was similar in pots that received two (F1 + F2) or three (F1 + F3 + F3) manure applications, which was lower than soil pH in pots that received only one (F1) manure application. Also in the Rosholt soil, pots that received the high manure application rate (R2) thrice (F1 + F2 + F3) had lower soil pH than pots that received R2 either once (F1) or twice (F1 + F2).

Manure application rate and frequency impacted soil IN levels in both soil types. After oat harvest (Harvest 1), soil IN levels in both soils were lowest in the control pots and increased significantly with each manure application rate. After sorghum harvest (Harvest 2), soil IN levels in both soils were similar in controls and pots that received only the first manure application at either application rate. Low soil IN after the first harvest was probably the reason why there was no difference in sorghum DM or N uptake in pots that received only the first manure application (Fig. 2). For both soils, soil IN levels after sorghum harvest were significantly greater in pots that received two manure applications (F1 + F2) at both the low (R1) and high (R2) rates. Plano and Rosholt soil IN levels were, on average, three to four times greater in pots that received the R2 than pots that received the R1 application rate. Observed effects of manure rate on soil IN levels after Harvest 2 were somewhat diminished after Harvest 3. At trial's end, soil IN levels for both soil types were similar in pots that received the first (F1) and second (F1 + F2) manure applications. Only in pots that received a third manure application (F1 + F2 + F3) at the high application rate (R2) were there elevated soil IN levels.

Treatment Effects on Plant Nitrate Levels

In patterns similar to those observed for soil IN levels (Table 5), manure application rate and frequency impacted shoot NO_3 levels (Table 6). In both soils, NO_3 levels in oat plants (Harvest 1) were greater at the high (R2) than the low (R1) manure application rate, and both manure application rates produced oat shoots that had higher NO_3 levels than pots that received no manure. Also in both soils, NO_3 levels in sorghum plants (Harvest 2) were generally higher in pots amended with the second (F1 + F2) than only the first (F1) manure application, and were also higher in pots that received the higher (R2) than the lower (R1) manure application rate. At trials end in both soils, NO_3 levels in sorghum ratoons (Harvest 3) were higher only in pots amended with all three manure applications (F1 + F2 + F3) at the high (R2) application rate.

Treatment Effects on Manure Nitrogen Use Efficiency

Manure type (diet CP level), soil type, and manure application rate and frequency all impacted MNUE in Harvests 1, 2, and 3 (Table 7). For all three harvests, manure derived from the low-CP diet (LP) provided MNUEs that were significantly greater than manure derived from the medium- (MP) or high-protein (HP) diets, which had statistically similar MNUEs. Also for all three harvests, MNUEs were significantly greater in pots that contained the Plano than pots that contained the Rosholt soil, and greater in pots amended with manure at the agronomic rate (R1) than in pots amended with manure at twice (R2) the agronomic rate.

The cumulative impacts of manure application frequency on calculated MNUE (Eq. [1]) depended on soil type (Fig. 4). In the Plano soil, MNUEs were significantly greater in pots amended with one (F1) than in pots amended with two (F1 + F2) or three (F1 + F2 + F3) manure applications. In the Rosholt soil, MNUEs were similar across all three manure application frequencies. In pots that received only the first manure application, MNUE was greater in Plano (65%) than Rosholt (50%) soils.

DISCUSSION

The two soils used in this study had a wide range of soil textures and chemical properties (Table 1), which had the greatest impact on

Table 7. Manure N use efficiency (MNUE) in pots containing Plano and Rosholt soils amended with manure derived from low (LP), medium (MP), and high (HP) protein diets, and in pots that received manure at the agronomic rate (R1) or twice the agronomic rate (R2).

Harvest (crop)	MNUE						
	Manure type			Soil type		Manure application rate	
	LP	MP	HP	Plano	Rosholt	R1	R2
	%						
H1 (oat)	51.1 a†	45.5 b	44.2 b	48.8 a	45.0 b	54.8 a	39.1 b
H2 (sorghum)	37.9 a	29.7 b	31.7 b	38.5 a	27.7 b	37.4 a	28.8 b
H3 (sorghum ratoon)	56.6 a	48.6 b	49.0 b	53.9 a	48.9 b	57.9 a	44.9 b

† Within a harvest (crop), manure type, soil type, or manure application rate, row MNUE means followed by different lowercase letters differ at $P < 0.05$.

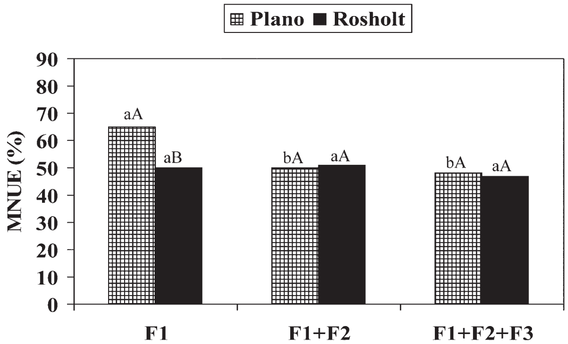

Fig. 4. Effects of manure application frequency on manure N use efficiency (MNUE) (across a soil type, application frequency bar means having different lowercase letters differ at *P* < 0.05; within an application frequency, soil MNUE bar means having different uppercase letters differ at *P* < 0.05).

crop yield and N uptake (Table 3). Under normal climatic and soil management conditions, agricultural soils in Wisconsin annually mineralize approximately 2.5% of total soil N (Vanotti et al., 1997). At this mineralization rate and using each soil's total N concentrations (Table 1), annual soil IN productions of approximately 54 and 24 mg kg^{-1}, or 43 and 19 mg pot^{-1}, would be expected for Plano and Rosholt soils, respectively. In the control pots (no manure), total plant N uptake during the 170-d growing period (Fig. 1, 2, and 3) plus net soil IN (soil IN at trial's end [Table 5] minus soil IN at beginning of trial [Table 1]) was 178 and 88 mg pot^{-1} for the Plano and Rosholt soils, respectively, or some four to five times greater than what would be expected under field conditions. The reasons for high plant N uptake and soil IN in the unamended controls were probably associated with multiple factors, including the drying, sieving, and rewetting of our soils, and the ideal greenhouse conditions (lighting, temperature, and soil moisture), which probably enhanced soil IN formation and plant N uptake.

A principal objective of this study was to determine if initial observations (Powell et al., 2006) of dietary CP impacts on manure N chemistry, N mineralization in soils, and plant DM and N uptake would become more accentuated when manures derived from the same diets (Table 2) were applied at higher application rates and frequencies. In the present greenhouse trial, manure application rate and frequency had much greater impacts on plant DM and N uptake than manure type (Table 3). The only notable impact of manure type was on MNUE (Table 2), whereby application of manure derived from the low-CP diet resulted in greater MNUE than manure derived from the other diets. A possible reason for this effect was that manure from the LP diet had higher levels of NH_4–N and lower levels of NDIN and ADIN (58, 0.31, and 0.28% of total manure N, respectively) than manures from the MP and HP diets (Table 2). These chemical differences perhaps made manure N from the LP diets more readily mineralizable and plant available than manure derived from the MP and HP diets (e.g., Powell et al., 1999; Sørensen et al., 2003; Sørensen and Fernández, 2003).

The observed oat and sorghum DM and N uptake increases associated with the manure application rates and frequencies corroborate previous field study findings (Motavalli et al., 2003). Kaffka and Kanneganti (1996) showed that orchardgrass (*Dactylis glomerata* L.) DM increased when liquid and solid dairy manure were applied to the soil surface annually in amounts equivalent to 150, 300, or 450 kg N ha^{-1} in one, two, or four equal applications. Application of cattle manure at 400 kg total N ha^{-1} increased crop DM and N uptake the application year and subsequent years (Mooleki et al., 2004).

Post-harvest soil pH and available N increased with manure application rate and frequency. The relatively smaller pH increases in the Plano than the Rosholt soil due to manure application rate and frequency (Table 5) were probably due to higher organic matter levels (Table 1) and therefore higher buffer capacity of the Plano soil. Post-harvest soil IN increased more sharply with the manure rate and frequency in the Rosholt than the Plano soil, indicating that risks of NO_3 leaching would be greater in the sandy loam Rosholt than in the silt loam Plano soil when manure is applied repeatedly in excess of agronomic recommendations. Previous research with dairy manure (Comfort et al., 1988) and sewage sludge (Kelling et al., 1977) on a Plano soil showed little leaching during the corn (*Zea mays* L.) growing season.

In both soils, manure application rate and frequency increased NO_3 levels in oat and sorghum shoots (Table 6), which if used as forage, could have detrimental impacts on dairy cow health. Nitrates can oxidize Fe(II) in blood hemoglobin to Fe(III), forming methemoglobin, which is unable to act as an O_2 carrier. If enough methemoglobin is created, livestock could die of tissue anoxia (Van Soest, 1987). Whereas the present study revealed NO_3 concentrations in plant shoots, NO_3 tends to accumulate in plant parts nearest to the ground, as well as in stalks and stems, which contain less of the enzyme that break it down (Robinson, 2006). Results from the present greenhouse trial indicate risks of NO_3 accumulation due to manure application rate and frequency, but these impacts warrant further investigation under operational, field-level conditions.

Approximately 3 to 5% of applied dairy manure N should be available for crop uptake the second and third cropping season after application (Kelling et al., 1998; Cusick et al., 2006). In both soils, however, no significant increases were detected in second crop (sorghum) DM or N uptake due to a previous manure application, at either the agronomic or twice the agronomic N application rate (Fig. 2). Variability (CV range of 32 to 40% for sorghum N uptake in both soils) was probably the reason for the inability to detect residual manure N availability in the second crop. Third crop (sorghum ratoon) DM and N were significantly increased, however, due to residual manure applications, but only in pots that received two manure applications at the high rate. In the Plano soil, the highest cumulative MNUEs were attained in pots that received only the initial (F1) manure application (Fig. 4). This result indicates that crops will continue to utilize manure N applied to previous crops. Residual manure N has been corroborated for the Plano soil under field conditions using ^{15}N-labeled dairy manure (Cusick et al., 2006).

Long-term manure applications have been found to increase the proportion of potentially mineralizable N in soils (Whalen et al., 2001). Perhaps this is why soil IN was higher after the second crop (sorghum) in pots that received F1 + F2 manure applications than in pots that received only the F1 manure application (Table 5), and why the third crop (sorghum ratoon) had higher shoot DM and N uptake in pots that received F1 + F2 manure applications than in pots that received only F1 application (Fig. 3). High initial manure applications may be appropriate, therefore, to build the soil organic N pool, followed by reduced manure N applications in subsequent years (Pang and Letey, 2000). For example, annual manure applications to supply 400 kg N ha^{-1} was deemed an appropriate agronomic practice for the first 3 or 4 yr on previously unmanured soil (Mooleki et al., 2004).

CONCLUSIONS

Soil type had the greatest impact on plant responses and soil chemical changes due to manure application rate and frequency. Plant responses and soil changes due to application of manure derived from different diets were not as pronounced, even after repeated manure applications at rates that simulated agronomic or twice agronomic N application levels. Lack of manure-type impacts may have been associated with greenhouse pots being closed systems, which retained applied manure N and maintained soil IN at fairly high levels, as also indicated by high shoot NO_3 levels. These conditions perhaps made it more difficult to detect any impacts of manure type. Responses to applied manure derived from different diets may be more pronounced under free-draining and more plant N-limiting conditions, and perhaps for a longer term. Longer term, repeated applications of dairy manure derived from different diets at lower rates could provide more pronounced impacts on plants and soils than what were observed during this relatively short-term greenhouse study.

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