^{15}N UPTAKE FOM MANURE AND FERTILIZER SOURCES BY THREE CONSECUTIVE CROPS UNDER CONTROLLED CONDITIONS[1]

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SUMMARY

There are several regions of the world where soil N analysis and/or N budgets are not used to determine how much N to apply, resulting in higher than needed N inputs, especially when manure is used. One such region is the North Central "La Comarca Lagunera", one of the most important dairy production areas of Mexico. We conducted a unique controlled greenhouse study using ^{15}N fertilizer and ^{15}N isotopic-labeled manure that was labeled under local conditions to monitor N cycling and recovery under higher N inputs. The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop at an equivalent rate of 30, 60 and 120 Mg ha^{-1} dry manure. The ^{15}N treatments were equivalent to 120 and 240 kg ha^{-1} (NH$_4$)$_2$SO$_4$-N for each crop. The total N fertilizer for each N fertilized treatment were 360, and 720 kg ha^{-1} N. We found very low N recoveries: about 9 % from the manure N inputs, lower than the 22 to 25 % from the fertilizer N inputs. The manure N recovered belowground in soil and roots ranged from 82 to 88 %. The low recoveries of N by the aboveground and low soil inorganic nitrate (NO$_3$-N) and ammonium (NH$_4$-N) content after the third harvested suggested that most of the ^{15}N recovered belowground was in the soil organic form. The losses from manure N inputs ranged from 3 to 11 %, lower than the 34 to 39 % lost from fertilizer N sources. Our study shows that excessive applications of

manure or fertilizer N that are traditionally used in this region will not increase the rate of N uptake by aboveground compartment but will increase the potential for N losses to the environment.

Index terms: denitrification, nitrogen use efficiency, annual ryegrass, salinity, sudangrass.

RESUMO: *ABSORÇÃO DE N¹⁵ DE FERTILIZANTES E ESTERCO POR TRÊS CULTIVOS SUCESSIVOS EM CONDIÇÕES CONTROLADAS*

No mundo existem várias regiões em que as fontes de N e as análises de solo não são levadas em consideração para determinar as necessidades desse elemento, o que resulta em aplicações excessivas, especialmente quando se utiliza esterco. Uma dessas regiões é a Centro-Norte do México, chamada de "La Comarca Lagunera", uma das maiores áreas produtoras de leite do país. Um experimento em casa de vegetação foi realizado com fertilizante e esterco marcados com o isótopo N¹⁵ para monitorar a ciclagem e a recuperação do N quando grandes quantidades são aplicadas. O tratamento N¹⁵-esterco foi aplicado somente uma vez e incorporado no solo previamente à plantação da primeira pastagem, com as seguintes doses: 30, 60 e 120 Mg ha⁻¹ de matéria seca. O tratamento N¹⁵-fertilizante consistiu na aplicação de 120 e 240 kg (NH₄)₂SO₄ ha⁻¹ em cada cultivo. O total de N-fertilizante para cada tratamento foi de 360 e 720 kg ha⁻¹ de N. A quantidade de N-esterco recuperado foi de 9 %, muito menor que a quantidade de N recuperado do fertilizante, que variou entre 22 e 25 %. A quantidade de N-esterco recuperado nas raízes e em profundidade no solo variou entre 82 e 88 %. A reduzida quantidade de N recuperado no solo nas formas de nitrato (NO₃-N) e amônio (NH₄-N) após a terceira colheita indica que a maior parte do ¹⁵N recuperado estava na forma orgânica. As perdas de N-esterco variaram entre 3 e 11 %, sendo bem menores que as do N-fertilizante (34–39 %). Este estudo mostra que as aplicações excessivas de esterco e fertilizantes, rotineiramente empregadas na região, não aumentam a taxa de absorção de N pela biomassa aérea das culturas, mas incrementam as perdas de N no ambiente.

Termos de indexação: denitrificação, eficiência de uso do nitrogênio, raygrass anual, salinidade, sudangrass.

INTRODUCTION

Losses of reactive N from agricultural systems that receive higher than needed N inputs negatively impact air, groundwater and surface water quality (Follett & Walker, 1989; Antweiler et al., 1996; Delgado et al, 2008). There are several regions of the world that tend to receive higher than needed N inputs, where soil testing and N budgets are not used as part of management practice planning. For example, several scientists have reported that N recoveries in China tend to be low due to excessive application of N fertilizer (Zhang et al., 1996; Zhu & Chen, 2002; Liu & Ju, 2003; Li et al., 2007). In some regions of China, applications of N can be as high as 750 to 1,900 kg ha⁻¹ yr⁻¹ N for greenhouse vegetables (Zhang et al., 1996; Zhu & Chen, 2002). Li et al. (2007) recommended best management practices that include soil testing and N budgets to increase N use efficiencies and decrease losses to the environment.

Another region that tends to receive higher than needed N inputs is located in North-Central Mexico.

In areas from the North-Central "La Comarca Lagunera" region, application of manure is traditionally excessive (Personal communications, Drs. Uriel Figueroa and Jose Cueto). This region is one of the most important dairy production areas of Mexico and generates an estimated 800,000 Mg yr⁻¹ of manure (dry basis). Manure fertilization operations are traditionally excessive, without any use of N budgets or soil and/or manure testing to determine N content.

In the USA, Sharpley et al. (1999, 2002, 2003) reported that confined animal operations helped change the roles of nutrient management operations of farms in the USA from those of sinks and users of nutrients to those of sources and exporters. This effect has also been reported for other regions of the globe such as La Comarca Lagunera that commonly have large numbers of animals and limited acreage, similar to some operations in the Southern United States. Based on the dry basis manure production in La Comarca Lagunera of 800,000 Mg yr⁻¹, we estimate that the region's yearly manure N resource is 16,000 metric tons of N with an estimated potential

cycling of 5,000 metric tons of available N during the first year after application. We also estimate that this manure N can supply the N needs for at least 17,000 ha of irrigated forage per year. Since there is limited data about N cycling of manure sources in La Comarca Lagunera, our objective was to assess the N recovery from dairy manure and fertilizer N sources using the isotopic ^{15}N technique.

State of the art isotopic ^{15}N techniques are available to quantify N losses and N cycling (Delgado 2002; Delgado et al., 2004). Comparatively few studies have been conducted with ^{15}N-labeled manure as opposed to ^{15}N-labeled fertilizer forms. Using ^{15}N-labeled manure, Sørensen et al. (1994) found that 20 % of the N in sheep manure was recovered during the first year, compared to 57 % recovered from the $^{15}NH_4^{15}NO_3$ applied to a barley (*Hordeum vulgare* L.)/grass catch crop. During the second year there were lower recoveries (5 and 6 %) of the ^{15}N-labeled manure and fertilizer sources, respectively (Sørensen et al., 1994). Thomsen et al., (1993) found a recovery of 23 % with barley from ^{15}N sheep slurry and 42 % with $^{15}NH_4^{15}NO_3$ during the first year. Both sources had a 3 % recovery during the second year.

Jokela (1992) studied the effect of N fertilizer and dairy manure on corn (*Zea mays* L.) yield. He found that the N recovery in corn from applied manure was 35, 49, and 43 % for three consecutive years. Recoveries were much larger than those measured by Muñoz et al., (2003) who used labeled ^{15}N manure and reported a recovery range from 13 to 22 % in three years of corn production.

The goal of this study was to trace the fate of N and determine how much N can be taken up under these high N input systems from Mexico. The only method for conducting such an assessment is with the use of isotopic ^{15}N techniques using labeled manure and labeled N fertilizer. Using these techniques, we can see how much ^{15}N is taken up by the aboveground shoots, how much stays as residual belowground and how much may be lost from the system.

MATERIALS AND METHODS

Field procedure to label the ^{15}N-manure

This study was established at "La Laguna" Research Station of INIFAP, Mexico, located in the semi-arid North Central region (25 ° 31 ' N, 103 ° 14 ' W, 1096 masl) with a mean annual temperature of 20.7 °C and precipitation of 210 mm per year. In order to conduct a ^{15}N-manure study, we first had to feed ^{15}N labeled forage to a cow, then collect the ^{15}N labeled manure.

In spring 2001, we established two 64 m^2 plots of sudangrass (*Sorghum sudanense* Piper Staff); one received non-labeled ammonium sulfate ($(NH_4)_2SO_4$), and the other received $(^{15}NH_4)_2SO_4$ labeled 5 atom % ^{15}N. The non-labeled sudangrass, with a background reading of 0.3726 ^{15}N atom %, was harvested first. The labeled sudangrass plot, with an enrichment of 0.7291 atom % ^{15}N, was harvested second to avoid cross-contamination.

A non-lactating cow was exclusively fed with the non-labeled sudangrass hay for the first 10 days. The manure obtained during the first three days was discarded, collecting only the manure of the following seven days. The non-labeled manure was piled separately from the cow in an enclosed area. Then, for the following ten days, the same cow was exclusively fed with the ^{15}N-enriched sudangrass hay. Similarly, the manure obtained during the first three days was discarded, and we collected and stored the ^{15}N labeled manure generated during the last seven days.

Manure from both sources was stored under similar conditions for three months to simulate commercial practices. Each pile was stored at a sufficient distance to avoid cross-contamination between labeled and non-labeled manures. The non-labeled manure pile was mixed with a spade to increase uniformity of manure after the three months. The ^{15}N labeled manure pile was also mixed with a different spade to avoid cross contamination. Because of the need for a highly intensive management system to produce ^{15}N manure and the large cost of ^{15}N material for handling and analysis, we only labeled enough ^{15}N manure material to conduct a controlled greenhouse study.

Greenhouse study

We used isotopic ^{15}N techniques to study the recovery and losses of manure-N and $(NH_4)_2SO_4$ sources applied to a forage rotation (annual ryegrass-sudangrass-annual ryegrass) grown in large pots. Each pot had a 15 cm internal diameter and a 50 cm height, and was filled with 14 kg of an Aridisol – a typical soil type in this agricultural region – collected from the top 30 cm arable layer. The soil clay, silt, and sand content were 52, 34, and 14 %, respectively and were determined with the hydrometer method (Bouyoucos) (Gee & Bauder, 1986).

The soil pH, soil organic matter, and electrical conductivity (EC) were 8.38, 1.05 % and 1.4 dS m^{-1}, respectively, with 15.9 % total calcium carbonates. Soil pH was determined electrometric in aqueous extract of saturated soil pastes. (ORION 3 STAR, Thermo Electronic Corporation[6]; Thomas, 1996). Soil organic matter was measured with the Walkley

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[6] Manufacture names are necessary to report factually on available data; however the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product and the use of a given name by the USDA does not imply approval of that product to the exclusion of others that may be suitable. Brand names are provided for the benefit of the reader and do not imply endorsement by the authors or USDA.

and Black method (Nelson & Sommers, 1996). The soil EC was determined using a conductance meter in aqueous extract of saturated soil pastes. (ORION 3 STAR, Thermo Electronic Corporation; Rhoades, 1996). The total carbonates was determined using the pressure calcimeter method (Loeppert & Suarez, 1996).

The NO_3-N content was 57.5 mg kg^{-1} while the NH_4-N was minimal. This high NO_3-N content is in agreement with other measured high residual soil NO_3-N across the region (personal communications from Dr. Figueroa, May, 2008). The soil NO_3-N content was measured with the colorimetric method from Robarge et al. (1983) and NH_4-N was determined with the steam distillation in soil extracts using 2 mol L^{-1} KCL (Mulvaney, 1996).

Pots were placed on greenhouse benches and received 100 to 150 mL of tap water with no detectable N every day during the summer and every other day in the winter. Irrigation kept soil moisture at an adequate level without drainage induction. For the manure treatments and in accordance with the application, soil and ground manure were mixed prior to pot filling.

Manure-N treatments were 0, 55, 110, and 220 g/pot dry basis manure (equivalent to 0, 30, 60, and 120 Mg ha^{-1} dry manure). The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. The N content of the manure was 1.97 %, equivalent to an application of 591, 1182 and 2364 kg ha^{-1} N for 30, 60 and 120 Mg ha^{-1} dry manure, respectively.

Fertilizer N treatments were 0, 220, and 440 mg $crop^{-1}$ $(NH_4)_2SO_4$-N, equivalent to 0, 120, and 240 kg ha^{-1} of N. For each of the three forage crops, $(NH_4)_2SO_4$ fertilizer was applied in four liquid applications: 25 % $(NH_4)_2SO_4$ at planting time and 25 % $(NH_4)_2SO_4$ after the first, second, and third harvest. The total N fertilizer applications for each pot were 0, 360, and 720 kg ha^{-1} $(NH_4)_2SO_4$-N. Treatments were arranged in a 4 x 3 factorial scheme, distributed in a randomized complete block design with three replicates.

We used ^{15}N isotopic techniques to assess the recovery and losses of N. For each of the three replicates of the manure-fertilizer combination, we applied the treatments in two combinations, one receiving a labeled manure (0.5335 ^{15}N atom %) and non-labeled fertilizer, and the other receiving non-labeled manure (0.3727 ^{15}N atom %) and labeled $(^{15}NH_4)_2SO_4$ (10 atom % ^{15}N). To avoid cross-contamination, we applied all the non-labeled $(NH_4)_2SO_4$ first, then we applied the $(^{15}NH_4)_2SO_4$ fertilizer using a different pipette. The N content and ^{15}N were determined on a continuous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL) coupled to an elemental analyzer (Costech) in the isotope laboratory of the University of Arizona (USA).

The first forage crop consisted of annual ryegrass (*Lolium multiflorum* Lam.) planted in the fall of 2001. The aboveground biomass was harvested four times, simulating a hay field production schedule. The second forage crop was sudangrass, which was planted in the spring of 2002, and was harvested four times for hay. The third forage crop was annual ryegrass planted in the fall of 2002 and harvested four times for hay until spring 2003.

At each of the four cuts, dry matter yield was measured by hand-harvesting the aboveground biomass, dried at 75 °C, weighed, and ground. Aboveground N and ^{15}N was measured as described before. At the end of the third forage crop a soil sample was taken from each pot for analysis.

We used the equations from Picchioni & Quiroga (1999) to determine the ^{15}N recoveries. The percentage of N derived from fertilizer (NDF_f) or from manure (NDF_m) was determined with equation 1. The amount of N derived from fertilizer (FDN_f) or from manure (FDN_m) was determined with equation 2. The percentage of N recovered (NR) was determined with equation 3. The N losses from manure (NL_{lm}) or fertilizer (NL_{lf}) inputs were calculated as the difference between 100 % minus NR.

$$\text{NDF (\%)} = \frac{\text{\% }^{15}N \text{ sample - \% }^{15}N \text{ control sample}}{\text{\%}^{15}N \text{ in fertilizer or manure - \%}^{15}N \text{ background}} \times 100 \quad (1)$$

$$\text{FDN (g)} = \frac{\text{\% NDF \%}}{100} \times \frac{\text{N sample}}{100} \times \text{DM (g)} \quad (2)$$

$$\text{NR (\%)} = \frac{\text{FDN (g)}}{\text{N (g) applied as fertilizer or manure}} \times 100 \quad (3)$$

The dry matter produced and recovered N by crop, were analyzed using the PROC ANOVA procedure and Least Significant Difference (LSD) for treatment analysis (SAS, 1990).

RESULTS AND DISCUSSION

Dry matter

There were no interactions between fertilizer and manure N inputs in dry matter production. For manure N inputs, the general trend for the first two crops was increased dry matter production as the manure N inputs increased; however there were no significant differences due to manure N input in the third crop (Table 1). Fertilizer N inputs of 120 and 240 kg ha^{-1} N increased dry matter production for the first and second forage crops by 11 and 25 %, respectively ($p \leq 0.05$). Although fertilizer N inputs of 240 kg ha^{-1} N increased dry matter production for the third forage crop by 94 % when compared to the

Table 1. Total dry aboveground matter production for the first (annual ryegrass), second (sudangrass) and third (annual ryegrass) forage crops grown under four manure and three fertilizer rates, labeled with [15]N

	First crop	Second crop	Third crop
Dry manure Mg ha^{-1} [(1)]	aboveground dry matter Mg ha^{-1}		
0	13.8 b [(2)]	14.2 b	5.5^{NS}
30	15.2 ab	15.0 ab	4.6
60	14.9 ab	14.9 ab	5.4
120	15.9 a	15.8 a	6.4
LSD$_{05}$ [(3)]	1.4	1.4	-
Fertilizer kg ha^{-1} N			
0	13.3 c	13.3 c	3.9 b
120	15.0 b	14.6 b	4.9 b
240	16.6 a	16.6 a	7.6 a
LSD$_{05}$	1.2	1.2	1.2

[(1)] The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. Fertilizer N treatments were equivalent to 0, 120, and 240 kg ha^{-1} $(NH_4)_2SO_4$-N, for each crop. The total N fertilizer applications for each pot were 0, 360, and 720 kg ha^{-1} $(NH_4)_2SO_4$-N. The manure rates were applied at an equivalent rate of 0, 30, 60 and 120 Mg ha^{-1} dry manure. The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. [(2)] Means with the same letter and N source in the same column are statistically equal. NS: no significant difference (p = 0.05). [(3)] LSD$_{05}$: Least Significant Difference (p = 0.05) Treatment main effects.

control or non-fertilizer plots, the harvested dry matter production for the third forage crop was still reduced by 63 % when compared to the first harvest crop (p ≤ 0.05).

Salinity

There were no interactions between manure and fertilizer N inputs and the residual salinity effect. At the end of the third forage crop, EC increased with manure and fertilizer N inputs, ranging from 1.8 dS m^{-1} for the absolute 0 treatment to 2.8 dS m^{-1} for the highest manure and fertilizer input combination. Chang et al. (1991), Ginting et al. (2003), and Eghball et al. (2004) reported soil EC increases as a result of manure and compost applications. Harivandi et al. (1992) reported that ryegrass is moderately sensitive to soil EC of 3 to 6 dS m^{-1}. We found a significant negative relationship between dry matter yields and EC ($r^2 = 0.69$; p ≤ 0.05). Because the all the pots were irrigated without drainage, we suggest that the reduction in dry matter yields for the third forage crop (Table 1) were due to an increase in soil EC, which is in concurrence with Chang et al. (1991), Harivandi et al. (1992), Ginting et al. (2003), and Eghball et al. (2004).

Percentage of NDF

The percentage of N derived from manure input (NDF$_m$) and fertilizer input (NDF$_f$) for each of the forage crops is shown in table 2. For the three forage crops, no significant interaction (p ≤ 0.05) was found between manure and fertilizer N sources for NDF$_m$. The NDF$_m$ increased significantly from 12 to 22 %

for the 30 to 120 Mg ha^{-1} manure. The fertilizer N inputs did not have any effects on NDF$_m$, with an average of 16 to 18 % for the three fertilizer N rates (Table 2). By the second forage crop, manure N significantly increased (p ≤ 0.05) NDF$_m$ with each rate: 15, 23, and 36 % for the 30, 60, and 120 Mg ha^{-1} rates, respectively. Fertilizer N decreased NDF$_m$, ranging from 29 % for 0 mg/pot N to 19.0 % for the 240 kg ha^{-1} N rate. During the last forage crop, the highest NDF$_m$ (27 %) was found at the highest manure N rate and it was significantly higher (p ≤ 0.05) than the NDF$_m$ resulting from each of the intermediate and lower manure N rates (20 and 18 %, respectively). Fertilizer N significantly decreases (p ≤ 0.05) NDF$_m$ with each rate increase (32, 21, and 12 % for the 0, 120, and 240 kg ha^{-1} N rates, respectively) (Table 2).

During the first forage crop without fertilizer N, 77 % of the total N uptake by the forage crop with the highest manure N input came from the soil (Table 2). Similarly, during the second forage crop, without fertilizer N, soil N contributed between 81 and 63 % of the total N uptake, for the 30 and 120 Mg ha^{-1} dry manure rates, respectively (Table 2). By the third forage crop without fertilizer N, about 70 % of the total N uptake came from the soil (Table 2).

FDN

There were no significant interactions between manure and fertilizer N inputs in total N uptake (Table 3). For manure N inputs, no significant differences (p ≤ 0.05) between manure N uptake levels were found. For the three forage crops, fertilizer N inputs of 240 kg ha^{-1} N significantly increased N

Table 2. Percentage of the N in aboveground dry matter that is derived from manure (NDF$_m$). The first (annual ryegrass), second (sudangrass) and third (annual ryegrass) forage crops grown under four manure and three fertilizer rates, labeled with ^{15}N

1st Crop (Ryegrass) Fertilizer [1]	Dry manure Mg ha^{-1} [1]			Means	LSD$_{05}$ [2]
	30	**60**	**120**		
kg ha^{-1} N		% ^{15}N recovered from manure			
0	10.6	21.4	23.0	18.3 [NS]	-
120	11.0	14.6	23.1	16.2	
240	14.8	13.8	18.5	15.7	
Means	12.1 b [3]	16.6 ab	21.5 a		
LSD$_{05}$	6.7				
2nd Crop (Sudan grass)					
0	18.8	31.5	36.9	29.1 a	6.9
120	16.1	21.6	39.7	25.8 ab	
240	9.0	16.4	31.6	19.0 b	
Means	14.6 c	23.2 b	36.1 a		
LSD$_{05}$	6.9				
3rd Crop (Ryegrass)					
0	30.6	31.7	33.1	31.8 a	6.7
120	17.3	15.2	31.0	21.2 b	
240	4.8	14.2	18.5	12.5 c	
Means	17.6 b	20.4 b	27.5 a		
LSD$_{05}$	6.7				

[1] The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. Fertilizer N treatments were equivalent to 0, 120, and 240 kg ha^{-1} (NH$_4$)$_2$SO$_4$-N, for each crop. The total N fertilizer applications for each pot were 0, 360, and 720 kg ha^{-1} (NH$_4$)$_2$SO$_4$-N. The manure rates were applied at an equivalent rate of 0, 30, 60 and 120 Mg ha^{-1} dry manure. The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. [2] Means with the same letter and N source in the same column are statistically equal. NS: no significant difference. (p = 0.05). [3] LSD$_{05}$: Least Significant Difference (p = 0.05). Treatment main effects.

Table 3. Shoot N uptake by aboveground dry matter production by each forage crop, fertilized with four manure and three N fertilizer inputs. The first (annual ryegrass), second (sudangrass) and third (annual ryegrass) forage crops grown under four manure and three fertilizer rates, labeled with ^{15}N

	First crop	Second crop	Third crop
	Aboveground forage crop N content		
Dry manure Mg ha^{-1} [1]		kg ha^{-1} N	
0	276 [NS(2)]	160 [NS]	56 [NS]
30	274	164	53
60	261	166	59
120	296	163	68
LSD$_{05}$	-	-	-
Fertilizer kg ha^{-1} N [1]			
0	261 b	147 c	46 b
120	274 ab	166 b	51 b
240	295 a	177 a	80 a
LSD$_{05}$ [3]	26	10	17

[1] The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. Fertilizer N treatments were equivalent to 0, 120, and 240 kg ha^{-1} (NH$_4$)$_2$SO$_4$-N, for each crop. The total N fertilizer applications for each pot were 0, 360, and 720 kg ha^{-1} (NH$_4$)$_2$SO$_4$-N. The manure rates were applied at an equivalent rate of 0, 30, 60 and 120 Mg ha^{-1} dry manure. The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. [2] Means with the same letter and N source in the same column are statistically equal. NS: no significant difference (p = 0.05). [3] LSD$_{05}$: Least Significant Difference (p = 0.05). Treatment main effects.

uptake by aboveground biomass (p ≤ 0.05). Nitrogen uptake decreased with each crop, probably as a result of the salt accumulation that was caused by conducting the study without drainage.

The average total N uptake by the first forage was 277 kg ha^{-1} N, ranging from 261-296 kg ha^{-1} N. Since there was initially an average of 443 kg ha^{-1} NO$_3$-N in the soil, enough to supply all the N needs for the first forage crop, we cannot explain why we got an N response to fertilizer N inputs of (NH$_4$)$_2$SO$_4$. We suggest that the ryegrass root system may have a greater affinity for NH$_4$-N uptake, showing a response pertaining to the presence of NH$_4$. Therefore, this hypothesis should be tested with nutrient solutions and combinations of different NH$_4$-N/NO$_3$-N concentrations.

N recovery

The total N recovered by the aboveground dry matter after three extraction cycles is shown in table 4. No interactions (p ≤ 0.05) between the applied manure and fertilizer N were found for NR. Only manure N inputs had significant (p ≤ 0.05) effects on N recovered from the manure source (NR$_m$) (Table 4), which was higher at the lower rates, decreasing as the manure N input increased. The average recoveries from the manure N inputs were about 9.3 % NR$_m$. Nitrogen recovery from the fertilizer source (NR$_f$) was significantly affected (p ≤ 0.05) only by the fertilizer rates. As an average over the three crops, NR$_f$ ranged from 22 to 25 % for the lower and higher fertilizer rates, respectively.

Manure N amounts remaining in the soil (NR$_{sm}$) after three growing cycles ranged from 82 to 88 %, and the N from the fertilizer source (NR$_{sf}$) ranged from 37 to 43 %. Losses ranging from 4 to 11 % from

manure inputs (NL$_{lm}$) in our study were lower than the 34 and 39 % of N losses were from the fertilizer sources (NL$_{lf}$). We suggest that these losses were mainly lost through atmospheric pathways, since no leaching was induced.

The total N uptake for the three crops ranged from 486 to 552 kg ha^{-1} N (Table 5). The initial inorganic soil NO$_3$-N available was 443 kg ha^{-1} N. Traditional practices in this region do not account for inorganic soil NO$_3$-N; however this study shows that the initial soil NO$_3$-N was high and almost enough to supply the total N uptake for these three crops.

These data and recoveries of N from the soil show that the initial soil NO$_3$-N are important N sources for this region. Although we did not conduct a soil mineralization rate study, we suggest that the mineralized N from the soil organic matter is also an important N source for this region. This data shows that if nutrient managers do not account for these sources, the recovery efficiencies due to excessive manure applications can be lower than 10 % of the applied N (Table 4 and 5). Excessive applications of manure will not increase the rate of N uptake and will increase the potential for N losses to the environment.

The unaccounted for N loss from the 30 and 120 Mg ha^{-1} dry manure were 21 and 248 kg ha^{-1} N, respectively (Table 5). The unaccounted-for N loss from the pots receiving 360 and 720 kg ha^{-1} N were 139 and 245 kg ha^{-1} N, respectively (Table 5). The average N losses were higher for the readily available N fertilizer. These results clearly show that the

Table 4. Total N recovered from manure and fertilizer in the aboveground dry matter (NR$_m$ & NR$_f$), N recovered belowground (soil and roots) (NR$_{sm}$ & NR$_{sf}$), and unrecovered N or estimated losses (NL$_{lm}$ & NL$_{lf}$) after three extraction cycles with annual crops (annual ryegrass-sudan grass-annual ryegrass)

	Aboveground Dry Matter		Belowground Soil-Roots		Non-recovered (lost)	
	NR$_m$	NR$_f$	NR$_{sm}$	NR$_{sf}$	NL$_{lm}$	NL$_{lf}$
Dry manure Mg ha^{-1} [1]			% ^{15}N recovered or lost			
0	-	25.9	-	37.1	-	36.9
30	9.3 a[2]	23.0	87.2	38.4	3.5	38.5
60	6.5 b	23.1	82.6	41.1	10.8	35.8
120	5.2 b	22.2	84.3	43.6	10.5	34.2
LSD$_{05}$[3]	1.8	-	-	-	-	-
Fertilizer kg ha^{-1} N [1]						
0	7.3	-	84.0	-	8.7	-
360	7.0	21.9 b	87.9	39.5	5.2	38.6
720	6.7	25.2 a	82.2	40.6	11.0	34.2
LSD$_{05}$	-	2.3	-	-	-	-

[1] The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. Fertilizer N treatments were equivalent to 0, 120, and 240 kg ha^{-1} (NH$_4$)$_2$SO$_4$-N, for each crop. The total N fertilizer applications for each pot were 0, 360, and 720 kg ha^{-1} (NH$_4$)$_2$SO$_4$-N. The manure rates were applied at an equivalent rate of 0, 30, 60 and 120 Mg ha^{-1} dry manure. The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. [2] Means with the same letter and N source in the same column are statistically equal. NS: no significant difference (P = 0.05). [3] LSD$_{05}$: Least Significant Difference (P = 0.05). Treatment main effects.

excessive manure rate applications increased N losses to the environment and are in agreement with the Delgado et al. (2008) assessment of N losses to the environment from higher manure rates.

Interaction between salinity and nitrogen inputs

The initial conductivity of the unamended soil used for the greenhouse cylinder study was slightly saline at 1.4 dS m^{-1}, above the threshold that is considered the lower limit for optimal microbial activity (Smith & Doran, 1996). Table 6 presents the data at the end of the study showing that the application of N inputs increased to about 2.8 dS m^{-1} for the higher N rates, levels that likely changed the balance of aerobic and anaerobic microbial activity (Smith & Doran, 1996). Smith & Doran (1996) reported that these conductivity levels can severely inhibit nitrification, and decrease the N loss by denitrification and may enhance the loss of N$_2$O. The choice of ryegrass as a bioassay crop probably masked the effects of high salinity, because this crop is much less susceptible to salt damage than many other agronomic and horticultural crops (Harivandi et al., 1992; Smith & Doran, 1996).

The initial level of NO$_3$-N was 443 kg ha^{-1} NO$_3$-N (Table 5), while at the end of the study the total inorganic soil N was lower than 60 kg ha^{-1} N (Table 6). The aboveground N uptake was also lowered from more than 260 kg ha^{-1} N with the first harvest, to about 60 kg ha^{-1} N with the last harvest (Table 3). However, we would have expected about 45 % mineralization from the applied manure according to Eghball et al. (2002). If we apply these guidelines to the equivalent manure rates applied in this study, we should have expected that about 236, 473, and

947 kg ha^{-1} N from the dry manure applied at 30, 60 and 120 Mg ha^{-1} N, respectively, would be available for uptake as inorganic N. Despite the high manure and N fertilizer application, the ^{15}N labeled data showed that there still was not high residual soil NO$_3$-N in this study or high N uptake and/or high N losses to the environment from manure. These results suggest that the mineralization of organic N was significantly reduced at these levels of electrical conductivity, in agreement with Smith & Doran (1996).

Our ^{15}N study clearly shows the following: (a) There was no significant ^{15}N uptake by the aboveground biomass as was expected because of the large mineralization pulse (Table 4), (b) there was not a significantly high residual NO$_3$-N or NH$_4$-N at the end of the study as was expected from the high mineralization rate assumed; (c) there was high rate of manure N recovery (over 82 % still in the soil, probably as organic N because the inorganic N content at the end of the study was minimal) (Tables 4, 6); (d) because the pots had no drainage, making leaching impossible, we suggest that the atmospheric N losses were most likely driven by denitrification since the N inputs were incorporated in the soil that was expected to reduce NH$_3$-N volatilizations losses (Meisinger & Randall, 1991).

Our mass balance approach with this ^{15}N recovery study demonstrates that these excessive manure applications impact electrical conductivity, resulting in an unbalanced biological situation that affects N transformations. This unique ^{15}N study conducted under controlled environment with large high labeled ^{15}N manure applications shows that the N losses from the manure applications were 127 and 248 kg ha^{-1} N for the equivalent dry manure rates of 60, and 120 Mg ha^{-1}, respectively. The ^{15}N recovery rates

Table 5. Assessment of N budget. Total N uptake shoots, initial soil NO$_3$-N, input of ^{15}N manure or fertilizer, fate of recovered ^{15}N in aboveground forage, belowground (soil and roots) and lost. after three extraction cycles with annual crops (annual ryegrass-sudan grass-annual ryegrass). Studies were conducted under irrigated greenhouse pots using a representative typical Aridisol from North Central "La Commarca Lagunera" region of Mexico

	Total N uptake shoot	Initial NO$_3$ N	Input ^{15}N	Fate of ^{15}N		
				Aboveground shoot	Belowground soil + roots	Lost
Dry manure Mg ha^{-1} [1]			kg ha^{-1} N			
30	491	443	591	55	515	21
60	486	443	1,182	77	987	127
120	527	443	2,367	123	1,996	248
Fertilizer kg ha^{-1} N [1]						
360	491	443	360	79	142	139
720	552	443	720	182	293	245

[1] The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. Fertilizer N treatments were equivalent to 120, and 240 kg ha^{-1} (NH$_4$)$_2$SO$_4$-N, kg ha^{-1} N for each crop. The total N fertilizer applications for each pot were 360, and 720 kg ha^{-1} (NH$_4$)$_2$SO$_4$-N. The manure rates were applied at an equivalent rate of 30, 60 and 120 Mg ha^{-1} dry manure. The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop.

Table 6. Final soil nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), pH, and electrical conductivity (EC) measurements conducted at the end of the study after three extraction cycles with annual crops (annual ryegrass-sudan grass-annual ryegrass)

	NO_3-N	NH_4N	pH	EC
Dry manure Mg ha^{-1} [1]	— kg ha^{-1} N —			dS m^{-1}
0	15	28	8.5	1.8 d
30	20	37	8.5	2.2 c
60	21	35	8.5	2.5 b
120	22	26	8.5	2.8 a
Fertilizer kg ha^{-1} N [1]				
0	19	31	8.5	2.0 c
360	18	33	8.5	2.3 b
720	23	28	8.5	2.6 a
LSD$_{05}$ [2]	-	-	-	0.2

[1] The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. Fertilizer N treatments were equivalent to 120, and 240 kg ha^{-1} (NH_4)$_2$$SO_4$-N, for each crop. The total N fertilizer applications for each pot were 360, and 720 kg ha^{-1} (NH_4)$_2$$SO_4$-N. The manure rates were applied at an equivalent rate of 30, 60 and 120 Mg ha^{-1} dry manure. The manure-N treatment was applied only once and was incorporated in the soil before planting the first forage crop. [2] LSD$_{05}$: Least Significant Difference (p = 0.05). Treatment main effects.

(aboveground biomass) were 77 and 123 kg ha^{-1} N, for the equivalent dry manure rates of 60, and 120, respectively. The amount of ^{15}N lost was nearly twice the amount of ^{15}N recovered by the aboveground biomass with the equivalent dry manure rates of 60, and 120 Mg ha^{-1}. Since there was no drainage from the pots, the N losses were via atmospheric pathways.

The lower application of manure, combined with a smaller increase in salinity, reduced atmospheric N losses to 21 kg ha^{-1} N, nearly two and half times lower than the 51 kg ha^{-1} N recovered by the aboveground biomass. The data set suggests that management is the key to reducing the development of conditions that may increase the potential for atmospheric nitrogen losses. Since there was no accumulation of nitrate and/or ammonium at the end of the study, we suggest that these N losses were due to denitrification, created by the unbalanced biological situation resulting from high salinity at these high manure rates (Smith & Doran, 1996). Other recent studies have shown that these changes in electrical conductivity affect the rates of N_2O emissions by way of a shift in microbial populations that drive nitrification and denitrification (Amos et al., 2005; Adviento-Borbe et al., 2006). Additional studies need to be conducted to see if these changes in microbial activity affect potential losses of N_2, N_2O, and/or NO_x, which was beyond the scope of this study.

CONCLUSIONS

1. Excessive manure and/or fertilizer N applications result in minimal N use efficiencies (< 10 %) and increased potential for losses of reactive N to the environment.

2. More efficient N use by crops and fewer negative effects from salinity will be achieved through lower-rate, more frequent manure applications, rather than higher rate, infrequent applications.

3. When management practices increase electrical conductivity and soil salinity, there is potential for an unbalanced biological situation that may reduce nitrification.

4. Management and application of manure rates that are more aligned with N uptake demands may minimize atmospheric N losses (lower than 3.5 %).

ACKNOWLEDGMENTS

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