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Research article

Effect of fertilizer rate and water irrigation quality on the recovery of ^{15}N-labeled fertilizer applied to Sudangrass

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Abstract – Wastewaters are increasingly used for irrigation of cropping systems in Tunisia. However, to develop environmentally sound practices the contribution of wastewater to crop N nutrition needs to be clarified, especially in cropping systems already receiving mineral fertilizers. For a better understanding of the interaction between fertilizer N and N originating from wastewater, experiments using ^{15}N were conducted. ^{15}N-labeled fertilizer was applied at different rates (0, 60, 100 and 140 kg N·ha^{-1}) and with different water irrigation qualities (tap water or treated wastewater) to sorghum grown in lysimeters during 1998 and 1999. Recovery of ^{15}N-labeled fertilizer in the above-ground crop at final harvest in treated wastewater irrigation was higher at the lowest rate of fertilizer application (54%), with the amount recovered in the crop increasing as the rate of ^{15}N-labeled fertilizer application increased up to the rate of 100 kg N·ha^{-1}. Nevertheless, in spite of this increase in ^{15}N-labeled fertilizer in the crop, total plant N uptake did not differ between rates. Treated wastewater irrigation had no negative effect on the recovery of ^{15}N-labeled fertilizer. About 62 and 55% of ^{15}N-labeled fertilizer was removed by Sudangrass in either tap water or treated wastewater. Neither fertilizer N rate nor water quality had an effect on the ^{15}N-labeled fertilizer remaining in the soil at final harvest. On average 20% in the wastewater treatment (19–24%) and 30% in the tap water treatment (26–31%) of the ^{15}N fertilizer applied were in the 0–60 cm layer of soil at final harvest in 1998 and 1999, respectively, and mostly present in the 0–20 cm layer. The proportion of applied ^{15}N-labeled fertilizer remaining in the soil at final harvest increased with increasing N rates. About 60, 69 and 72% of ^{15}N left in the soil at final harvest was in the surface 0–20 cm layer. Residual ^{15}N was greatly higher in soil following the first harvest than after the final harvest, with the greatest value (38%) measured at the lowest rate of ^{15}N-labeled fertilizer (30 kg N·ha^{-1}). Losses of ^{15}N-labeled fertilizer increased with application rate, but were unaffected by water quality irrigation. Approximately 13% of the applied ^{15}N fertilizer was lost following the application of 100 kg N·ha^{-1} with either treated wastewater or tap water irrigation.

1. INTRODUCTION

In Tunisia, wastewater reuse has been integrated into water resources management; it is considered as an integral part of the environmental pollution control and water management strategy. Municipal wastewater is mainly domestic (at about 82%) and generally goes through secondary biological treatment. Application of wastewater to agricultural land is continuing to gain public acceptance in Tunisia [3, 4]. However, only 24% of the available treated wastewater is used, which could be evolved. The reuse of treated wastewater is generally considered beneficial for crop production and, as a result of its nitrogen content, can help to reduce the requirements for commercial fertilizer [1, 13, 18, 22, 27, 40, 41]. However, both nitrogen deficiency and N excess, especially early and late in the growing season, can reduce crop yields. Thus, obtaining optimum crop yields and good nitrogen use efficiency requires careful management of N application. Olson et al. [25] suggested that since about 60% of the N used by the crop comes from soil, total N uptake is not reliant on wastewater N and fertilizer removal. It is the interest of a technique such as isotope labeling to distinguish the origin of N removed and to calculate the balance sheet of the different N sources. The direct measurement of the recovery of fertilizer in the soil, and the subsequent calculation of the N that is lost from the crop/soil system can only be made using ^{15}N-labeled fertilizer [21]. In a field study, Olson et al. [24] noted that after 4 years of applying 5.1 cm irrigation with lagoon water enriched with $(^{15}NH_4)_2SO_4$ to Bromegrass (*Bromus inermis*), 41% of the labeled N applied was used by the crop, 28% remained in the soil profile, 31% was lost as gaseous N, and 0.07% was leached below the 105-cm depth, but when twice as much lagoon water was applied (10.2 cm irrigation), 38% was removed by the crop, 20% was found in the soil profile, 40% was lost as gaseous N, and 1.3% was leached. A one-year ^{15}N balance after three years of irrigation indicated uptake, soil NO_3-N, and unaccounted-for N was about 10, 19 and 71% for ^{15}N-labeled wastewater and 15, 33, and 52% for ^{15}N-labeled fertilizer [6]. The greater

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Table I. Some physical and chemical properties and water contents of the experimental soil.

Sand %	Argil %	Loam %	EC mmhos·cm^{-1}	pH	$CaCO_3$ %	OM %	WHC (mm)	WWP (mm)	AW (mm)	Total N g·kg^{-1}
88	2	9	0.6	8.5	0.3	0.5	9.86	3.28	9.87	0.7

WHC: water-holding capacity. WWP: water at wilting point. AW: available water calculated for10 cm of soil (AW = da (WHC – WWP) with da: apparent density of soil).

Table II. Agricultural practices of the experimental design.

Year	Planting date	Emergence date	Thinned date	First fraction of N	First harvest	Second fraction of N	Last harvest
1998	23/06	27/06	09/07	10/07	18/08	04/09	30/09
1999	14/06	21/06	29/06	06/07	02/08	18/08	25/09

losses of wastewater N compared with fertilizer N were attributed to enhanced denitrification due to the presence of oxidizable C in the wastewater.

Very few data are published on the effects of treated wastewater on the balance sheet of the N fertilizer supplemented. In their study concerning the fate of N in a system irrigated with wastewater, Bole et al. [7] noted that Reed canarygrass recovered nearly 50% of applied fertilizer ^{15}N over 2 years with about 80% of total uptake in the first harvest after N application. Alfalfa only recovered 24% of applied ^{15}N at a low irrigation rate and 14% at a higher rate. About 25% of the fertilizer N was left in the soil after two irrigation seasons, with 60% of that N remaining in the surface 15 cm of soil independent of forage species or irrigation rate.

The aims of this study of lysimeters, using tap water and treated wastewater for irrigation were:

– to quantify the recovery of N fertilizer by Sudangrass, a forage crop grown on approximately 8000 ha in Tunisia (DGPA-Ministry of agriculture, 1999 cited by Dzifanu [12]);

– to quantify the N remaining in soil and lost from the soil-plant system;

– to discuss the fertilizer N balance by considering both isotope dilution and difference methods.

2. MATERIALS AND METHODS

This research was carried out on the experimental field of the Rural, Water and Forest Research Institute, "INRGREF", of Tunisia in 1998 and 1999. An experiment using lysimeters was conducted utilizing a completely randomized block design with four replicates in the first year of experimentation and three replicates in the second year. The square lysimeters were 1 m^3 volume made with brick walls tarred from inside. They were filled with 0.1 m of a coarse gavel and sand mixture in their bottoms. The remaining 0.9 m were filled with sandy soil samples taken from the first 20 cm depth from the experimental site of the Cap-Bon in Nabeul. Before filling up the lysimeter, the soil was sieved at 5 mm. Some physical and chemical properties of the experimental soil are presented in Table I.

Sudangrass (*sorghum sudanense,* Piper) was planted on 23 June 1998 and 14 June 1999. The lysimeters were arranged to include four rows of sorghum with ten plants per row. Irrigation water rates were applied to meet evapotranspiration demands, which were 6–7 mm/day in the period of the experiments. Approximately 40 mm/week were added to each lysimeter with a watering can, at a frequency of two irrigations per week. The different agricultural practices are summarized in Table II.

In the first year of the experiment in 1998, Sorghum was irrigated with treated wastewater, providing a total of 237 kg N·ha^{-1}. The ^{15}N-labeled fertilizer was added at three rates: 60, 100 and 140 kg N·ha^{-1}, with a corresponding ^{15}N excess of 9.778, 9.66 and 9.577 atom%. A control treatment (0N) was included. In 1999, two fertilizer rates (0 and 100 kg N·ha^{-1}) were compared in the presence of either treated wastewater, providing an additional of 250 kg N·ha^{-1}, or tap water that did not contain nitrogen. For both experiments, wastewater irrigation provided about 7.5 kg N·ha^{-1} as nitrate N. Wastewater was produced by the Cherguia treatment plant; it was mostly domestic (about 82% domestic) and had received a secondary biological treatment. The N composition of the treated wastewater ranged from 58.3 to 25.2 mg.L^{-1} of total N, with an average of 37 mg N·L^{-1} in the main part as NH^+_4 (about 35 mg N-NH_4·L^{-1}), but contained less than 5 mg·L^{-1} of NO_3^-. For both experiments, fertilizer N was added as urea and applied in two equal fractions, at emergence and after the first harvest. In 1998, both N applications were labeled with ^{15}N. In 1999, to determine the effect of time of application, only one urea application was labeled at 9.66% ^{15}N excess. Under the circumstances, for each kind of irrigation water, six lysimeters were established to receive nitrogen fertilizer. Three lysimeters received labeled urea N at emergence and unlabeled urea N after the first harvest, whereas the remaining three lysimeters received unlabeled urea N at emergence and ^{15}N-labeled urea N after the first harvest. The N treatments were applied during irrigation. The soil surface was first moistened with 15 liters of irrigation water, and ^{15}N-labeled urea N was uniformly applied with a watering can in 2 liters of distilled water, then the watering can was rinsed immediately with one liter of distilled water and the rinsing water was also applied, followed by an additional 2 liters of distilled water to wash ^{15}N from the Sudangrass foliage.

Two harvests were carried out in each experiment. At each harvest, the plant material was harvested when the Sudangrass was at the beginning of the flowering stage for yield measurement. Twelve central sorghum plants were used to estimate fertilizer

Table III. Effect of different rates of fertilizer application and water irrigation on the above-ground dry matter production and uptake of labeled and unlabeled N by Sudangrass and retention of labeled N in soil.

Year (water quality)	^{15}N-labeled urea	Total dry matter (DM)	Plant N uptake (grain, leaves, stem and roots)			Labeled N remaining in soil after harvest			Total ^{15}N recovered%
			Labelled N	Unlabelled N	Total	0–20 cm	20–40 cm	40–60 cm	
	kg·ha^{-1}					%			
1998	0	22324 a	–	310† ab	310 b	–	–	–	–
TWW	60	22814 a	35 b	335† ab	370 a	12 a	4 a	3 a	77 a
	100	24394 a	54 a	341† a	395 a	12 a	3 a	2 a	71 ab
	140	22322 a	61 a	303† b	363 a	15 a	4 a	2 a	64 b
	LSD*	2073	7	35	36	7	2	1	12
1999									
TW ϕ	0	14485 c	–	172‡	172 c	–	–	–	–
	100	27487 a	55 a	265‡	320 b	21 a	8 a	2 a	86 a
TWW ϕ	0	21542 b	–	306†	306 b	–	–	–	–
	100	29092 a	62 b	301†	363 a	18 a	5 b	3 a	88 a
	LSD	2312	8	–	33	9	2	2	12

‡ N originating from soil. † N originating from both treated wastewater and soil (^{14}N).
ϕ Average of three replicates. * Least significant difference at 0.05 level of probability.

N recovery. In the first cut, only the above-ground plants were cut, while in the final harvest the whole plants were harvested. All plant samples were dried at 70 °C, and weights were recorded. The plant tissue was ground and analyzed for total N using the Kjeldahl digestion method [8]. At the second harvest, soil samples were collected with an auger at three depths (0–20, 20–40 and 40–60 cm) from six locations in each lysimeter and composited. Soil samples were analyzed for total N with the Kjeldahl method, modified to include nitrate (Olsen 1929 cited by Guiraud and Fardeau [15]). The isotopic composition of nitrogen in the soil-plant system was determined by mass spectrometry (VG SIRA12. UK). The % ^{15}N abundance obtained by mass spectrometry was transformed into atom% ^{15}N excess by subtracting the natural abundance (0.3663 atom% ^{15}N) from the % N abundance of plant and soil samples. To reduce the likelihood of cross-contamination, all samples were ground and analyzed from least ^{15}N concentration to greatest ^{15}N concentration. The data set was statistically analyzed using SAS [36] to test the effects of N application levels, and of irrigation water type on fertilizer N recovery. Mean comparisons were performed using protected LSD tests at the 0.05 level of probability.

3. RESULTS

3.1. Plant production and nitrogen uptake

The lowest dry matter production obtained was recorded with tap water irrigation with no fertilizer added (1999) and was significantly higher with treated wastewater. Increasing the rates of N application from 0 to 140 kg N·ha^{-1} did not significantly increase dry matter production in the 1998 experiment, while plant N uptake was enhanced by a moderate fertilizer rate (60 kg N·ha^{-1}). By contrast, in 1999, the effect of the N rate was greater on both dry matter production and N uptake in tap water as compared with treated wastewater.

The assessment of irrigation water quality in 1999 proves a significant effect of treated wastewater in increasing dry matter production and total plant N uptake (Tab. III). The amount of unlabeled N originating from soil in 1999 increased by 93 kg N·ha^{-1} with fertilizer N application in the tap water treatment. Although unlabeled N originating from both soil and treated wastewater did not vary greatly with N application in 1999, it decreased significantly with an increase in the N rate from 100 to 140 kg ha^{-1} in 1998 (Tab. III). The amounts of soil N absorbed by Sudangrass irrigated with tap water were five-fold higher than the fertilizer N uptake, suggesting extensive turnover of soil and fertilizer N through immobilization and mineralization. The apparent increase in the uptake of soil N with application of 100 kg N·ha^{-1} rates in 1999 could be due to an increased mineralization from soil organic matter, pool substitution between ^{14}N and ^{15}N in the soil, or increased root development in the fertilized treatments.

3.2. Recovery of fertilizer N in soil

Neither the fertilizer N rate nor water quality had an effect on the ^{15}N-labeled fertilizer remaining in the 0–60 cm layer at final harvest (Tab. III). About 20 and 30% of the applied fertilizer were found in the 0–60 cm layer of soil at final harvest for all rates in 1998 and for both water qualities in 1999, respectively. Most of the residual ^{15}N-labeled fertilizer remaining in the soil was recovered in the surface 0–20 cm layer in 1998 and in 1999. As the rate of N fertilizer increased in 1998, 60 to 72% of total ^{15}N remaining in the soil at final harvest was found in the surface 0–20 cm layer (Fig. 1).

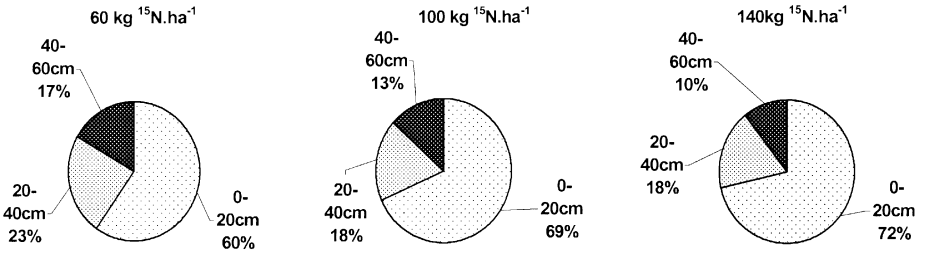

Figure 1. ^{15}N recovery in soil as a proportion of the total ^{15}N remaining in the 0–60 cm layer as related to fertilizer rates and depth.

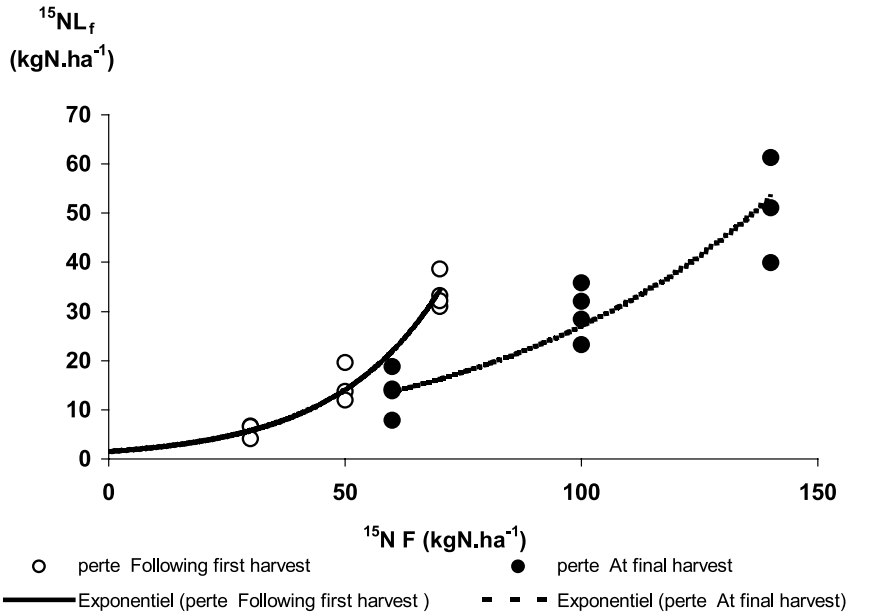

Figure 2. Loss of fertilizer nitrogen as a function of the rate and time of application of ^{15}N-labeled fertilizer (1998).

3.3. Fertilizer unaccounted for

The total recovery of ^{15}N-labeled fertilizer decreased significantly from 77% to 64% in 1998 as the rate of N application increased from 0 to 140 kg N·ha^{-1} (Tab. III). The type of water had no effect on the total ^{15}N recovered in 1999, so that about 86 to 88% of N fertilizer was recovered in the soil and the crop at final harvest (Tab. III). The losses of fertilizer nitrogen can be estimated from the differences between the amount of ^{15}N recovered in the soil and crop, and the known amount of ^{15}N applied (Tab. III). Losses estimated in this way increased at final harvest from 23 to 36% with increasing rates of fertilizer applied in 1998 (Tab. III). The proportion of ^{15}N-labeled fertilizer that was unaccounted for in 1999 was unaffected by the type of irrigation water, being 14% with tap water and 12% with treated wastewater (Tab. III).

Depending on whether losses of ^{15}N-fertilizer were measured at first harvest or at final harvest, they increased with increasing rates of fertilizer applied (Fig. 2). An exponential function of the amount of fertilizer N lost ($^{15}NL_f$) to the amount of fertilizer N applied (^{15}NF) resulted in: $^{15}NL_f = 1.5199\, e^{0.445\,(15NF)}$ after the first harvest, and $^{15}NL_f = 4.952\, e^{0.017\,(15NF)}$ at final harvest.

Nitrogen fertilizer losses tended to increase progressively with an increasing rate of fertilizer application, according to Figure 2. Losses of fertilizer nitrogen can affect nitrogen fertilizer efficiency, and it is important to know how most of the losses of fertilizer nitrogen occurred. All ^{15}N-labeled fertilizer in the experiment was applied as a solution of urea. The soil was moist when the fertilizer was applied, and the daily air temperatures ranged from 25 to 38 °C. Therefore, conditions were favorable for ammonia volatilization in the day following the application of fertilizer. Hence, it may be assumed that any nitrogen losses due to ammonia volatilization mainly occurred in a relatively short period following the fertilizer application.

3.4. Nitrogen fertilizer efficiency

The differences in fertilizer N use between the different fertilizer treatments and irrigation water qualities become clear when they are expressed on a percent recovery basis (Tab. IV). The most common method used to estimate fertilizer nitrogen taken up by a crop is the difference (or "indirect") method, in which the apparent recovery fraction (ARF) can be over 100% in some cases [14]; it is determined as nitrogen contents of the crops derived from the soil, measured in the control plot (NP_0),

Table IV. Fertilizer N recovery in the whole plant as estimated by the isotopic method.

Year (water quality)	^{15}N-labeled urea	Total N recovered	Fertilizer N recovery ^{15}NRF (Isotopic)
	(kg·ha^{-1})	(kg N)	(%)
1998 ‡	0	310 b	–
(TWW)	60	370 a	58 a
	100	395 a	54 a
	140	363 a	43 b
	LSD	37	8
1999 †			
(TW)	0	172 c	–
	100	320 b	55 a
(TWW)	0	306 b	–
	100	363 a	62 a
	LSD	32	8

‡ Recovery values are the average of four replicates. † Recovery values are the average of three replicates.

subtracted from the amounts of nitrogen taken up by the crops in the N-fertilized plots (NP) and divided by the amount of fertilizer nitrogen applied (NF): ARF = (NP – NP$_0$)/NF. Or, since wastewater irrigation provided a substantial amount of N and therefore there was no control treatment without treated wastewater added in 1998, the apparent recovery fraction was only determined when tap water was used. In the isotope-dilution (or "direct") method, the ^{15}N recovery fraction (^{15}NRF) for crops in fertilized plots is calculated from: $^{15}NRF = NP \times Y_p / NF \times Y_f$, where Y_p and Y_f are the atom percent excess ^{15}N in the crop and the applied fertilizer, respectively.

Fertilizer nitrogen recovery dropped from 58 to 43% when determined by the isotopic method as the fertilizer rate increased from 60 to 140 kg N·ha^{-1} in 1998 (Tab. IV). Water irrigation quality had no effect on the recovery of fertilizer N as determined by the isotopic method. Nearly 55% and 62% of the ^{15}N-labeled fertilizer was taken up by the Sudangrass when tap water and treated wastewater were used for irrigation in 1999. Nevertheless, when determined by the difference method, the apparent recovery fraction of 150% appears abnormal. It seems that the very low yield observed in tap water-0N, probably due to a bad development of roots (due to low N available) is the main cause of such a high and atypical apparent recovery observed in tap water-100. Especially in these experiments, true recovery is a more reliable parameter for measuring the absorption of fertilizer.

4. DISCUSSION

Sudangrass yields were not affected by an increasing fertilizer N rate when supplemented with treated wastewater. However, compared with tap water with no fertilizer added, dry matter yield showed a significant increase when irrigated with treated wastewater without fertilizer N addition. Many other studies have reported that higher crop yields were attained when irrigated with wastewater [9, 5, 27]. Hussain et al. [18] suggested that this could be attributed to the presence of appreciable amounts of N, P, K and some other micro-elements essential for plant growth compared with well water. Nitrogen fertilizer significantly increased dry matter production in tap water irrigation, but not when treated wastewater was used. Similar behavior was reported by Papadopolos and Stylianou [27], who concluded that Cotton yield could be higher with the effluent, particularly when supplemented with a lower N level. However, with the highest N level there was a reduction in yield obtained with the treated effluent in comparison with the yield obtained with borehole water supplemented with the same N level. According to Hussain et al. [18], a higher wheat grain yield and N use efficiency could be achieved with low N application rates if the crop is irrigated with treated effluent or wastewater containing nitrogen in the range of 20 mg·L^{-1} and above. Vaisman et al. [39] recommend that for good yields, Rhodes grass grown under field conditions required 250 kg N·ha^{-1} in addition to the 117.7 kg N·ha^{-1} added by the irrigation water.

Total nitrogen removed by the plant increased significantly when 60 and 100 kg N·ha^{-1} were supplemented with treated wastewater in 1998 and 1999, respectively. However, the difference in the total N removed by the plant was not significant between treatments supplemented with 60, 100 and 140 kg N·ha^{-1} in 1998. Otherwise, as reported by others [26, 32, 33, 1, 41, 20], treated wastewater irrigation with no fertilizer N added significantly increased the amount of N recovered by Sudangrass (TWW+0N – TW+0N = + 134 kg N·ha^{-1}) as compared with tap water irrigation. Nitrogen fertilizer efficiency as estimated by the isotopic method was similar to that reported in other investigations on Sudangrass [42, 16] and on maize [38, 31, 37]; it decreased with increasing rates of fertilizer N applied and was unaffected by water irrigation qualities. Most studies comparing nitrogen fertilizer use efficiency as determined by the isotopic and difference methods report higher recovery values using the difference method [42, 17, 16, 38, 31, 28], which is consistent with the finding of the present study when tap water was used in 1999. However, interpretation of the data is complicated by the fact that fertilizer N applied undergoes exchange with native soil N through mineralization-immobilization turnover [19], which accounts for the fact that estimates of N fertilizer efficiency based on ^{15}N uptake are usually lower than those calculated by difference [42].

Approximately 20 to 30% of the applied fertilizer were found in the 0–60 cm layer of soil at final harvest for all fertilizer N rates in 1998 and for both water qualities in 1999. In a previous study in Tunisia, 32% of the fertilizer N applied to wheat at two sites were recovered in the 0–80 cm soil layer [35]. Most of the ^{15}N-labeled fertilizer remaining in the soil was found in the surface 20 cm, with an average of 13% in 1998, and about 20% in 1999. Studies reported in the literature have also concluded that most of the fertilizer N that remained in the soil was located in the surface layer [23, 29]. The relatively high proportion of the fertilizer N remaining in the 0–20 cm layer suggests that fertilizer N retained in the soil was in organic forms. Allen et al. [2] found 97% of residual fertilizer N was recovered in organic combinations after a growing season. They found that 25 to 40% of the applied N remained in the surface 25 cm

of soil after one growing season and 15 to 19% remained after 5 years. Moreover, in the present study, the proportion of total ^{15}N remaining in the top 20 cm of soil increased from 60 to 72% as the ^{15}N-labeled fertilizer rate increased, which is in line with previous studies [34, 11]. According to Crozier et al. [10] and Pilbeam et al. [28], an enhancement in the apparent immobilization of ^{15}N-labeled fertilizer would be the most likely explanation for the reduced crop recovery as the urea N rate increased. Therefore, N recovery estimates based on the isotopic method are likely to be lower for all treatments than if the difference method had been used [30].

A nitrogen balance for this experiment shows that total recovery of ^{15}N-labeled fertilizer decreased significantly from 77% to 64% as the rate of N application increased in 1998 and about 86 to 88% of N fertilizer were recovered in the soil and the crop at final harvest for both types of water in 1999. From 23 to 36% of the ^{15}N-labeled fertilizer could not be accounted for at final harvest in 1998. Against total recovery of ^{15}N-fertilizer, labeled N unaccounted for increased as the ^{15}N-fertilizer rate increased in 1998. This is consistent with other studies on sorghum where losses increased with increasing rates of fertilizer N applied [16]. Water irrigation had no effect on the proportion of ^{15}N-labeled fertilizer unaccounted for. Approximately 13% of the applied ^{15}N-labeled fertilizer were lost following the application of 100 kg $N \cdot ha^{-1}$ to Sudangrass irrigated with either treated wastewater or tap water in 1999. Losses of ^{15}N-labeled fertilizer probably occurred to a great extent, shortly after application, materialized by gaseous losses through volatilization of N fertilizer, or through denitrification from wet soil after each irrigation period. However, because of the remineralization process, the immobilized N is not excluded from leaching. In addition, pertaining to the sandy nature of our soil, the most likely mechanisms responsible for the loss of 23 to 36% of urea N in 1998 and 12–14% in 1999 is leaching of nitrates below the sampling depth.

The results from this study have provided information on the likely behavior of fertilizer N in a soil/crop system under irrigation with tap water and wastewater. In two different lysimeter experiments, wastewater supplied a substantial amount of nitrogen so that the crop response to additional N fertilizer (urea) application was low. However, since limitations exist in relating experiments under more controlled environments to field conditions, the exact effect must be verified under field conditions. Such studies should be carried out over a number of years, so that long-term effects can be established. Moreover, consideration should be given to those wastewater constituents' behavior and especially to nitrogen, which if it is applied in amounts higher than crop needs may adversely affect crop growth or pollute groundwater.

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