

## Residual recovery and yield performance of nitrogen fertilizer applied at sugarcane planting

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**ABSTRACT:** The low effectiveness of nitrogen fertilizer (N) is a substantial concern that threatens global sugarcane production. The aim of the research reported in this paper was to assess the residual effect of N-fertilizer applied at sugarcane planting over four crop seasons in relation to sugarcane crop yield. Toward this end three field experiments were established in the state of São Paulo, Brazil, during February of 2005 and July of 2009, in a randomized block design with four treatments: 0, 40, 80 and 120 kg ha<sup>-1</sup> of N applied as urea during sugarcane planting. Within each plot, a microplot was established to which <sup>15</sup>N-labeled urea was applied. The application of N at planting increased plant cane yield in two of the three sites and sucrose content at the other, whereas the only residual effect was higher sucrose content in one of the following ratoons. The combined effect was an increase in sugar yield for three of the 11 crop seasons evaluated. Over the crop cycle of a plant cane and three ratoon crops, only 35 % of the applied N was recovered, split 75, 13, 7 and 5 % in the plant cane, first, second and third ratoons, respectively. These findings document the low efficiency of N recovery by sugarcane, which increases the risk that excessive N fertilization will reduce profitability and have an adverse effect on the environment.

**Keywords:** *Saccharum*, urea, nitrogen fertilization, isotopic technique

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### Introduction

In Brazil, sugarcane research has concentrated on assessing sugarcane as an annual crop, with few studies assessing the crop over a whole crop cycle (plant cane and ratoons). Many sugarcane technicians know that if N-fertilizer is not applied, productivity in the following ratoon will be adversely affected, and decrease the number of crops in a cycle. In a study over four crop seasons, Orlando Filho et al. (1999) verified this effect and affirmed that N fertilization of plant cane has a positive impact on the growth and vigor of the ratoon, and increases the yield of stalks in sugarcane ratoons. Vitti et al. (2007) also observed substantial residual effect in the fourth ratoon yield of N fertilization applied in the third ratoon.

Thus, it is appropriate to question N fertilizer strategies for sugarcane, in particular those applied to the first crop (plant cane), in relation to root growth, storage of nutrients in the belowground part of the crop, and whether this stored N is used by the following ratoon crop. Previous works have shown that N fertilization increases N storage in sugarcane root systems (Bologna-Campbell et al., 2013; Vitti et al., 2007); however, the importance of this increased storage of N in the root system depends on whether it is utilized by subsequent ratoons. Can the N-fertilizer applied at sugarcane planting be used by the ratoon? This issue has seldom been investigated in Brazil or elsewhere, because it requires the use of <sup>15</sup>N-labeled fertilizer as an isotopic tracer. Our aim was to assess the residual effect of <sup>15</sup>N-fertilizer applied at sugarcane planting through the evaluation of <sup>15</sup>N recovery by sugarcane ratoons in relation to sugarcane crop yield.

### Materials and Methods

#### Description of experiments

Three field trials were carried out in the southeast of Brazil, in the state of São Paulo, the largest sugarcane producing region in Brazil. Details regarding the experimental sites are shown in Table 1. All of the sites have a history of over 30 years under sugarcane cultivation.

The field experiments were established in February, 2005 at Pirassununga (São Luiz Sugar Mill - SL) (Typic Hapludox), in April, 2005 at Jaboticabal (Santa Adélia Sugar Mill - SA) (Typic Kandiodox) and in March, 2005 at Pradópolis (São Martinho Sugar Mill - SM) (Rhodic Eutrudox) (Table 1). Details of soil tillage practices, fertilizer applications, weed control and other management practices adopted in the experimental fields can be found in Franco et al. (2010, 2011) for the SL and SA sites, and in Fortes et al. (2012) for the SM site. For all three experimental sites, the predominant regional climate is classified as Koppen Aw Tropical Savanna.

For planting sugarcane, two stalk pieces (seeds) per meter were used, providing a distribution of 17-20 buds m<sup>-1</sup> of furrow. The stalks deposited in the furrow were cut into stalk pieces with 2-3 buds and covered with soil (mechanized operation). The sugarcane variety used was SP81 3250 since it is highly adaptable, very productive and one of the most common varieties planted in the centre-south region of Brazil. In all of the plots at the bottom of the furrow, 120 kg ha<sup>-1</sup> of K<sub>2</sub>O and 120 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> were applied in the form of potassium chloride and triple superphosphate, respectively.

Table 1 – Details of experimental sites.

	Experimental sites		
	SL	SA	SM
Location	Pirassununga, SP 21°55' S; 47 11' W	Jaboticabal, SP 21°20' S; 48°19'W	Pradópolis, SP 21°15' S; 48°18' W
Altitude (m)	650	600	580
Reference	Franco et al. (2010, 2011)	Franco et al. (2010, 2011)	Fortes et al. (2012)
Variety	SP81 3250	SP81 3250	SP81 3250
Replicates	16	16	16
Plot size (m <sup>2</sup> )	270	270	270
Space between rows (m)	1.50	1.50	1.50
Soil tillage	Herbicide application, chisel plowing (0.40 m), grading (0.25 m) and furrowing (0.35 m)	Deep plowing (0.40 m), grading (twice, 0.25 m) and furrowing (0.35 m)	Herbicide application, chisel plowing (0.40 m) and furrowing (0.35 m)
Soil Classification (Soil Survey Staff, 2010)	Typic Hapludox	Typic Kandiodox	Rhodic Eutrudox
Soil Texture	Sandy Clay Loam	Sandy Clay Loam	Clay
Month/Year			
Planted	Feb/2005	Apr/2005	Mar/2005
First harvest	Jun/2006	Jul/2006	Aug/2006
Second harvest	Jun/2007	Jul/2007	Aug/2007
Third harvest	Jul/2008	Jul/2008	Jul/2008
Fourth harvest	Jul/2009	Jul/2009	Jul/2009
Meteorological data	Average of four years (2006, 2007, 2008 and 2009)		
Rainfall	1476	1382	1580
ETc	1693	1573	1380
ETr	1359	1080	1002

SL – São Luiz Sugar Mill experiment located in Pirassununga; SA – Santa Adélia Sugar Mill experiment located in Jaboticabal; SM São Martinho Sugar Mill experiment located in Pradópolis; ETc = crop evapotranspiration; ETr = real evapotranspiration.

## Treatments

Fertilizer treatments at planting consisted of four N amounts (0, 40, 80 and 120 kg ha<sup>-1</sup> of N as urea) applied at the bottom of the planting furrow. These amounts were chosen based on Technical Bulletin 100 (Spironello et al., 1997), which recommends the application of up to 90 kg ha<sup>-1</sup> of N at planting. The treatments were arranged in a randomized block design with four replicates. The experimental plots were composed of 48 rows, 15 m in length with a space of 1.5 m between rows. Inside each treatment plot of 40, 80 and 120 kg ha<sup>-1</sup> of N, a microplot (2 m long and 1.5 m wide, totalling 3 m<sup>2</sup>) received urea labeled with 4.67 atom % excess <sup>15</sup>N. In the SM experiment, only the treatment with 80 kg ha<sup>-1</sup> N was evaluated in terms of <sup>15</sup>N-fertilizer recovery.

After plant cane harvest, no N-fertilizer was applied to three subsequent ratoons. Potassium chloride (at a rate of 150 kg ha<sup>-1</sup> K<sub>2</sub>O) was applied after every harvest, as well as triple superphosphate (at a rate of 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) in the SL experiment after the second harvest. Fertilizers were applied over the crop residue (trash) approximately 20 cm from the sugarcane row. The microplots that received <sup>15</sup>N-fertilizer at planting were kept to evaluate the residual effect of N applied at planting.

## Sampling and analyses

In the plant cane crop, the sugarcane harvest was performed 16, 15 and 17 months after planting in SL,

SA and SM sites, respectively. After the first harvest, the experimental sites were harvested successively after 12-months until the fourth harvest (Table 1). However, owing to an accidental fire, it was not possible to evaluate the 2008 crop at the SL site.

For yield measurements, four sugarcane rows of each plot were mechanically harvested and the stalks deposited in a truck coupled to a scale. The stalk mass registered was used to calculate stalk yield. Prior to harvesting, ten stalks per plot were collected and analyzed for sugar content. The sugar yield was calculated based on stalk yield and sucrose content, and was expressed in Mg ha<sup>-1</sup>.

The isotopic tracer technique allows for estimation of the percentage of N in the plant that is derived from fertilizer (NDFE) and the fertilizer use efficiency. The measurement of <sup>15</sup>N-fertilizer recovery by aboveground and belowground sugarcane was always taken at the end of each crop phase, one week before the mechanical harvest of sugarcane.

The evaluation was carried out within 1.0 m of the center of the microplot row and the two adjacent rows, keeping the samples separate according to the methods described by Trivelin et al. (1994). Whole plants from the 1.0 m sections were harvested to obtain separate samples of stalks, dry leaves and tops. The fresh mass of plant parts was evaluated directly in the field with a weighing scale, followed by mulching with a mechanical forage chopper. Homogenized sub-samples were prepared (Trivelin et al., 1994), dried at 65 °C to constant weight and finely ground with a Wiley mill to enable

determination of total N and  $^{15}\text{N}$  using a Hydra 20-20 mass spectrometer coupled to an automatic N analyzer ANCA-SGL (Barrie and Prosser, 1996).

For belowground measurements, root samples were collected in the same area that had been previously sampled for aboveground measurements, using a root sampling probe (5.5-cm internal diameter) that was inserted to a depth of 0.6 m at several points in relation to the row (Otto et al., 2009). The resulting cores were segmented in 0.2 m increments, as 50 % of sugarcane root biomass is typically found in the upper 0.2 m soil layer and 85 % in the upper 0.6 m layer (Blackburn, 1984). The decision to use the probe method was based on the need to minimize disturbance in the plot, so that measurements could be repeated over multiple years in the same plot. In previous work by Otto et al. (2009), the probe method enabled higher throughput than the monolith method, while giving similar results. Root samples were segregated by sieving (2 mm mesh), dried in an oven (65 °C) and then ground to < 0.595 mm using a Wiley mill. Analyses for total N and  $^{15}\text{N}$  were performed by continuous flow mass spectrometry.

Measurements of  $^{15}\text{N}$ -fertilizer recovery were repeated every year before the sugarcane harvest, using the previously presented methodology. Nitrogen recovery from fertilizer and crop residues was calculated as described in Trivelin et al. (1994).

### Meteorological data

Throughout the entire experimental period, meteorological data (rainfall, solar radiation, wind speed, relative humidity and temperature) were measured by means of automatic meteorological stations installed near the three experimental areas. Four-year average rainfall and evapotranspiration (ET) data are summarized in Table 1.

### Data analysis

For stalk yield, sucrose content and sugar yield, total N ( $\text{kg ha}^{-1}$ ) and NDFP (% and  $\text{kg ha}^{-1}$ ) data for individual site-years were analyzed by means of analysis of variance (ANOVA), considering differences to be significant when the probability was lower than 10 % ( $p < 0.10$ ). When the F test was significant, the Tukey test ( $p < 0.05$ ) was performed to compare the means.

## Results

There was a positive effect of N applied at planting on the stalk yield of plant cane in the SM and SL experiments, and also a positive effect on the sucrose content of plant cane at the SA site (Table 2). Reduced tillage may have contributed to the N response observed in the SM experiment, in comparison to conventional tillage that was in use at the remaining sites (Table 1).

None of the three sites showed any residual effect of N applied at planting on the yield of the following sugarcane crop cycles, nor there was an effect

Table 2 – Sugarcane stalk yield, sucrose content ( $\text{g kg}^{-1}$ ) and sugar yield ( $\text{Mg ha}^{-1}$ ) in four crop seasons as related to N rates applied at planting, for three experimental sites.

N rate $\text{kg ha}^{-1}$	Crop				
	Plant cane	1 <sup>st</sup> ratoon	2 <sup>nd</sup> ratoon	3 <sup>rd</sup> ratoon	Total
Stalk yield ( $\text{Mg ha}^{-1}$ )					
SA					
0	144.83	117.74	107.85	109.10	479.52
40	143.75	118.23	105.31	108.02	475.31
80	146.84	121.91	106.60	109.90	485.25
120	146.11	119.72	104.10	109.48	479.41
$p < n$	0.8582	0.4977	0.4756	0.9081	0.8106
SL					
0	134.16	80.10	#	99.72	313.98
40	141.63	85.90	-	101.63	329.17
80	138.61	84.24	-	102.60	325.45
120	141.32	81.98	-	100.76	324.06
$p < n$	0.0623	0.2612	-	0.7457	0.4431
SM					
0	141.26	81.56	63.81	88.61	375.25
40	152.64	76.52	60.45	88.40	378.02
80	155.69	78.10	58.40	89.79	381.98
120	159.12	81.66	63.99	90.45	395.22
$p < n$	0.0005	0.2547	0.1204	0.8512	0.2276
Sucrose content ( $\text{g kg}^{-1}$ )					
SA					
0	156.2	147.0	150.8	136.3	147.6
40	169.5	148.7	153.9	137.9	152.5
80	168.3	151.4	155.5	145.5	155.2
120	157.6	147.9	153.0	143.4	150.5
$p < n$	0.0251	0.1402	0.2002	0.0022	0.2474
SL					
0	145.4	138.8	#	140.3	141.5
40	147.3	135.9	-	141.9	141.7
80	146.5	134.8	-	141.1	140.8
120	138.4	135.0	-	140.9	138.1
$p < n$	0.3068	0.1633	-	0.8445	0.7073
SM					
0	166.6	161.2	169.2	169.7	166.7
40	175.4	161.2	166.1	167.7	167.6
80	166.6	158.7	168.2	165.7	164.8
120	172.2	161.9	168.0	168.1	167.6
$p < n$	0.2625	0.3297	0.5018	0.2571	0.8261
Sugar yield ( $\text{Mg ha}^{-1}$ )					
SA					
0	22.62	17.28	16.26	14.89	71.05
40	24.39	17.57	16.19	14.91	73.06
80	24.72	18.46	16.56	15.99	75.73
120	23.01	17.71	15.96	15.73	72.41
$p < n$	0.1024	0.0847	0.6912	0.0998	0.3535
SL					
0	19.48	11.11	#	13.99	44.59
40	20.86	11.68	-	14.41	46.95
80	20.34	11.37	-	14.47	46.17
120	19.50	11.09	-	14.19	44.78
$p < n$	0.4208	0.4674	-	0.7448	0.6924
SM					
0	23.54	13.14	10.80	15.04	62.51
40	26.78	12.33	10.04	14.78	63.93
80	25.91	12.38	9.82	14.86	62.96
120	27.42	13.22	10.76	15.22	66.61
$p < n$	0.0023	0.1053	0.1777	0.8039	0.3482

SL – São Luiz Sugar Mill experiment located in Pirassununga; SA – Santa Adélia Sugar Mill experiment located in Jaboticabal; SM – São Martinho Sugar Mill experiment located in Pradópolis;  $p < n$  indicates the probability by ANOVA analysis; #Data not obtained.

on the accumulated yield for the four harvests. The only residual effect was an increase in sucrose content for the third ratoon crop at the SA experiment (Table 2), an uncommon trend when compared to published results.

Due to an increase in stalk yield or in sucrose content (Table 2), the combined effect on sugar yield was an increase in 3 of the 11 site-years evaluated ( $p < 0.10$ ). Most of the positive results were found in the SA experiment, followed by SM. There was no effect of N applied at planting for any of the years evaluated in the SL experiment, or for any ratoon harvest at the SM site.

With regard to crop N recovery, after four years of evaluation it was found that 75 % of all fertilizer  $^{15}\text{N}$  uptake in the SA experiment was by the plant cane, whilst residual recoveries of  $^{15}\text{N}$  applied at planting were 14 %, 5 % and 6 %, respectively, for the first, second and third ratoon (Table 3). The results were very similar for the SM experiment, with 73 % of fertilizer  $^{15}\text{N}$  uptake being by plant cane, 11 % by the first ratoon, 8 % by the second ratoon and 8 % by the third ratoon (Table 4). As already mentioned for the SL experiment (Table 5), it was only possible to assess  $^{15}\text{N}$  recovery in the plant cane and first ratoon, because of an accidental fire during the second ratoon that led to the loss of biomass material from the microplots. However,

the results of two crops (plant and first ratoon) in this experiment showed the same trend observed in the SA and SM experiments, where the majority of fertilizer  $^{15}\text{N}$  uptake was by plant cane (86 %). Taken together, the data in Tables 3-5 show that N fertilizer applied at planting is quite limited in availability to the subsequent ratoons, especially in relation to total N uptake for sugarcane growth that can vary between 100 and 200 kg ha<sup>-1</sup> (Franco et al., 2011).

After four growing seasons, crop recovery of fertilizer  $^{15}\text{N}$  in the SA experiment totaled about 40, 35 and 30 % for N amounts of 40, 80 and 120 kg ha<sup>-1</sup>, respectively (Table 3). By comparison, total  $^{15}\text{N}$  recovery in the SM experiment was 45 % with 80 kg N ha<sup>-1</sup> (Table 4), while for two crops at the SL site, recoveries totaled 35, 40 and 27 %, respectively, for 40, 80 and 120 kg N ha<sup>-1</sup> (Table 5). Based on these data it is evident that 60 to 70 % of the fertilizer N applied at planting had another fate.

## Discussion

The present findings provide little evidence of a residual benefit from N applied to sugarcane at planting, and are consistent with a previous study by Vieira et al. (2010) but not with another by Vitti et al. (2007). Such disparities no doubt reflect differences due to soil

Table 3 – Recovery of  $^{15}\text{N}$ -fertilizer (kg ha<sup>-1</sup> and %) applied at sugarcane planting, as evaluated in four crop seasons. Santa Adelia Experiment - SA.

N Rates kg ha <sup>-1</sup>	Stalks		Dry Leaves		Tops		Above ground		Roots		Whole Plant	
	kg ha <sup>-1</sup>	%	kg ha <sup>-1</sup>	%	kg ha <sup>-1</sup>	%	kg ha <sup>-1</sup>	%	kg ha <sup>-1</sup>	%	kg ha <sup>-1</sup>	%
Plant Cane												
40	6.1 b	15.2	3.2	8.1	2.2	5.4	11.5 b	28.7	0.8	1.9	12.2 b	31
80	9.7 a	12.1	5.9	7.3	3.1	3.9	18.7 ab	23.4	1.3	1.6	20.0 ab	25
120	13.5 a	11.2	6.7	5.6	3.2	2.7	23.4 a	19.5	1.5	1.3	24.9 a	21
LSD	6.9	NS	NS	NS	NS	NS	11.4	NS	NS	NS	11.6	NS
1 <sup>st</sup> Ratoon												
40	1.0	2.4	0.4	1.1	0.6	1.4	2.0	4.9	—	—	—	—
80	1.8	2.3	1.1	1.4	1.5	1.9	4.4	5.5	—	—	—	—
120	1.8	1.5	1.2	1.0	1.5	0.4	3.5	2.9	—	—	—	—
LSD	NS	NS	NS	NS	NS	NS	NS	NS	—	—	—	—
2 <sup>nd</sup> Ratoon												
40	0.5	1.2	0.2	0.4	0.2	0.4	0.8	2.1	—	—	—	—
80	0.7	0.9	0.3	0.4	0.3	0.4	1.3	1.7	—	—	—	—
120	0.7	0.6	0.4	0.4	0.5	0.4	1.6	1.3	—	—	—	—
LSD	NS	NS	NS	NS	NS	NS	NS	NS	—	—	—	—
3 <sup>rd</sup> Ratoon												
40	0.3 b	0.7 b	0.04 b	0.11 b	0.3 b	0.7 ab	0.6 b	1.6 b	—	—	—	—
80	1.0 a	1.3 a	0.13 a	0.17 a	0.8 a	1.2 a	2.1 a	2.6 a	—	—	—	—
120	0.9 a	0.8 b	0.14 a	0.12 ab	0.9 a	0.6 b	1.8 a	1.5 b	—	—	—	—
LSD	0.5	0.48	0.07	0.05	0.4	0.5	0.9	0.9	—	—	—	—
Sum of crop seasons												
40	7.8 b	19.6	3.9	9.8	3.2	8.0	14.9 b	37.3	0.8	1.9	15.7 b	39
80	13.2 ab	16.6	7.4	9.2	5.9	7.3	26.5 ab	33.1	1.3	1.6	27.8 ab	35
120	16.9 a	14.1	8.5	7.0	5.0	4.2	30.4 a	25.3	1.5	1.3	31.9 a	27
LSD	8.0	NS	NS	NS	NS	NS	14	NS	NS	NS	14	NS

LSD: least significant difference; NS: not significant. Same letters in a column indicate no differences according to the Tukey test ( $p < 0.05$ ).

type, as N fertilization would be far more likely to have a carryover effect on ratoons when sugarcane is grown on soils low in organic matter and with limited capacity for mineralization.

The aerating effect of conventional tillage operations at the SA and SL sites would have created conditions conducive to microorganisms mineralizing N from soil organic matter, including the breakdown of organic matter linked to soil solid surfaces. Increased mineralisation of organic matter would have enhanced soil supplies of plant-available N, and reduced sugarcane responses to N fertilization as previously reported by Otto et al. (2013).

Many studies have found sucrose content to be unaffected by N fertilization (Franco et al., 2010; Thorburn et al., 2011; Yang et al., 2013), while in some cases a nega-

tive effect was observed when a high N amount ( $> 200 \text{ kg ha}^{-1}$ ) was applied at planting (Wiedenfeld, 1995), owing to a decrease in sucrose content. When the accumulated yield in four harvests was averaged over the three sites, there was an increase of 1.93, 2.23 and  $1.88 \text{ Mg ha}^{-1}$  in sugar yield for 40, 80 and  $120 \text{ kg N ha}^{-1}$  as compared to the unfertilized treatment; these gains were statistically different. Considering economic issues and also the potentially negative impact to the environment from excessive N doses, there is good reason for concern about the evidence in Table 2 documenting low residual availability of N applied at planting to the following ratoons.

Our results agree with previous findings that fertilizer N applied at planting is much more available in the first growing season than to subsequent ratoon crops. For example, Takahashi (1969) found in Hawaii that plant cane accounted for 79 % of total fertilizer  $^{15}\text{N}$  uptake, with 15 %, 2 % and 4 % recoveries by the first, second and third ratoon, respectively. In two experiments with sugarcane grown in either the summer or winter, Takahashi (1970a) found that crop recovery of fertilizer  $^{15}\text{N}$  applied at planting was largely due to plant cane (around 80 % on average for both experiments), the rest being partitioned among three subsequent ratoons. An additional study by Takahashi (1970b) showed the same trend; approximately 92 % of  $^{15}\text{N}$  uptake following fertilization at planting was by the plant cane, with the remainder being divided among the three ratoons. More recently, work in Brazil by Basanta et al. (2003) showed that plant cane accounted for 81 % of crop  $^{15}\text{N}$  uptake from fertilizer applied at planting, as compared to 14 % by the first ratoon and 5 % by the second ratoon. This means that the contribution of fertilizer N from the previous crop is very limited, and will typically account for less than 5 % of total N uptake by ratoons. Moreover, our data clearly show that the first ratoon dominates residual uptake of fertilizer N applied at planting.

Table 4 – Recovery of  $^{15}\text{N}$ -fertilizer ( $\text{kg ha}^{-1}$  and %) applied at sugarcane planting, as evaluated in four crop seasons. São Martinho experiment - SM.

	Stalks	Dry Leaves	Tops	Above ground
	$\text{kg ha}^{-1}$			
Plant Cane	18.9 a	3.1 a	2.7	24.7 a
1 <sup>st</sup> Ratoon	1.3 b	0.6 b	2.0	3.9 b
2 <sup>nd</sup> Ratoon	0.9 b	0.6 b	1.4	2.9 b
3 <sup>rd</sup> Ratoon	1.3 b	0.1 b	1.3	2.8 b
Sum of crop seasons	22.4	4.5	7.4	34.3
LSD	1.9	0.5	NS	3.0
	%			
	Stalks	Dry Leaves	Tops	Above ground
Plant Cane	24 a	3.9 a	3.4	31 a
1 <sup>st</sup> Ratoon	1.6 b	0.8 b	2.5	4.9 b
2 <sup>nd</sup> Ratoon	1.1 b	0.7 bc	1.8	3.6 b
3 <sup>rd</sup> Ratoon	1.7 b	0.2 c	1.6	3.5 b
Sum of crop seasons	28	5.6	9.3	43
LSD	7	0.5	NS	3.8

LSD: least significant difference; NS: not significant. Same letters in a column indicate no differences by the Tukey test ( $p < 0.05$ ).

Table 5 – Recovery of  $^{15}\text{N}$ -fertilizer ( $\text{kg ha}^{-1}$  and %) applied at sugarcane planting, as evaluated in two crop seasons. São Luis Experiment - SL.

N Rates $\text{kg ha}^{-1}$	Stalks		Dry Leaves		Tops		Above ground		Roots		Whole Plant	
	$\text{kg ha}^{-1}$	%	$\text{kg ha}^{-1}$	%	$\text{kg ha}^{-1}$	%	$\text{kg ha}^{-1}$	%	$\text{kg ha}^{-1}$	%	$\text{kg ha}^{-1}$	%
Plant Cane												
40	6.1 b	15 ab	2.0 b	5	2.7 b	6.8 ab	10.8 b	27 ab	1.7	4	12.5 b	31 ab
80	14.5 a	18 a	3.8 ab	5	7.4 a	9.3 a	25.8 a	32 a	2.3	3	28.1 a	35 a
120	12.0 a	10 b	5.4 a	5	5.5 ab	4.6 b	22.9 a	19 b	4.1	3	26.9 a	22 b
LSD	5.2	7.0	2.2	NS	3.7	4.0	6.8	8	NS	NS	7.6	9
1 <sup>st</sup> Ratoon												
40	0.5	1.0	0.5	1.4	0.4	0.9	1.4	3.4	—	—	—	—
80	0.8	1.1	1.3	1.6	1.6	1.9	3.7	4.6	—	—	—	—
120	1.9	1.6	1.7	1.4	1.4	1.2	5.0	4.1	—	—	—	—
LSD	NS	NS	NS	NS	NS	NS	NS	NS	—	—	—	—
Sum of crop season												
40	6.5 b	16.3 ab	2.5 b	6.2	3.1 b	7.8 ab	12.1 b	30.3 ab	1.7	4	13.8 b	34.6 ab
80	15.4 a	19.2 a	5.1 ab	6.4	9.0 a	11.2 a	29.5 a	36.8 a	2.3	3	31.7 a	39.7 a
120	13.9 a	11.6 b	7.0 a	5.8	6.9 ab	5.8 b	27.8 a	23.2 b	4.1	3	31.9 a	26.6 b
LSD	5.2	6.8	3.7	NS	4.7	5.1	7.5	7.7	NS	NS	8.9	10

LSD: least significant difference; NS: not significant. Same letters in a column indicate no differences by the Tukey test ( $p < 0.05$ ).

As expected, the efficiency of  $^{15}\text{N}$  recovery was decreased by higher application rates. The main fate of N fertilizer (about 20 to 40 %) applied to sugarcane is to remain in the soil, where the N immobilized by microorganisms resides in the organic fraction and is temporarily unavailable to the crop (Gava et al., 2005; Oliveira et al., 2000). N Fertilizer is also subject to loss through leaching, denitrification or  $\text{NH}_3$  volatilization, which can occur from senescing plant leaves as well as from soils amended with alkalinizing fertilizers. During leaf senescence, a reduction in the activities of the enzymes glutamine synthetase and glutamate synthetase leads to  $\text{NH}_4^+$  accumulation in the plant (Mattsson et al., 1998). As high  $\text{NH}_4^+$  concentrations are phytotoxic (Holtan-Hartwing and Bockman, 1994; Mattsson et al., 1998), this accumulation may result in natural losses of  $\text{NH}_3$  via the transpiration stream. For sugarcane,  $\text{NH}_3$  losses were estimated indirectly in Mauritius (Ng Kee Kwong and Deville, 1994) to be about  $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ .

The 60 to 70 % deficit in  $^{15}\text{N}$  recovery observed in our work was probably not associated with N losses through leaching, which were found to be very minor in previous studies carried out with sugarcane in Brazil. For instance, leaching losses of fertilizer  $^{15}\text{N}$  were only  $0.44 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in a study by Bologna-Campbell et al. (2013), and were attributed to N mineralization. In the SA experiment, Ghiberto et al. (2009) evaluated water flow through the soil using tensiometers and soil solution extractors to measure the amount of N lost by leaching. The results showed losses of  $15 \text{ kg N ha}^{-1}$  of N during the plant cane cycle, but only  $21 \text{ g ha}^{-1}$  of fertilizer-derived N. Outside Brazil, a lysimeter study by Ng Kee Kwong and Deville (1984) showed only small losses of N by leaching, even in the case of high rainfall events (about 3200 mm) during the experimental period. Their findings were attributed to microbial immobilization, which seems the most plausible explanation for our results. Besides reducing plant N availability, microbial immobilization of fertilizer N stimulates heterotrophic C oxidation and thereby promotes long-term depletion of soil organic C and N (Khan et al., 2007; Mulvaney et al., 2009).

Denitrification is another process that can lead to fertilizer N loss, particularly when organic matter has recently been incorporated into fine-textured soils that become waterlogged after heavy rainfall. This type of loss would have been more likely for the SL than the SA or SM experiments, owing to greater rainfall during the first month after N fertilization (data not presented). In relation to  $\text{NH}_3$  volatilization, this form of N loss was probably negligible in our experiments despite the use of urea as the N source, because the urea was placed at the bottom of the planting furrow and incorporated as it was applied.

### Conclusion

In three  $^{15}\text{N}$ -tracer experiments involving four crop seasons, N applied at planting increased plant-

cane yield for two of the three sites with no residual effect on ratoon production, while the other site showed a significant increase in sucrose content in two of the four growing seasons studied. The combined effect was an increase in sugar yield for three of the 11 site-years evaluated. Crop uptake of fertilizer  $^{15}\text{N}$  totaled 30 to 40 % over the entire study, 75 % of which was due to plant cane with 13 % in the first ratoon, 7 % in the second ratoon and 5 % in the third ratoon. The low residual benefits limit the value of N fertilization at planting, and enhance the need to avoid excessive N rates that have negative consequences for economic profitability and the environment.

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