DOI: 10.1590/S0103-90162011000300014

^{15}N-labeled nitrogen from green manure and ammonium sulfate utilization by the sugarcane ratoon

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ABSTRACT: Legumes as green manure are alternative sources of nitrogen (N) for crops and can supplement or even replace mineral nitrogen fertilization due to their potential for biological nitrogen fixation (BNF). The utilization of nitrogen by sugarcane (*Saccharum* spp.) fertilized with sunn hemp (*Crotalaria juncea* L.) and ammonium sulfate (AS) was evaluated using the ^{15}N tracer technique. N was added at the rate of 196 and 70 kg ha^{-1} as ^{15}N-labeled sunn hemp green manure (SH) and as ammonium sulfate (AS), respectively. Treatments were: (i) Control; (ii) AS^{15}N; (iii) SH^{15}N + AS; (iv) SH^{15}N; and (v) AS^{15}N + SH. Sugarcane was cultivated for five years and was harvested three times. ^{15}N recovery was evaluated in the two first harvests. In the sum of the three harvests, the highest stalk yields were obtained with a combination of green manure and inorganic N fertilizer; however, in the second cutting the yields were higher where SH was used than in plots with AS. The recovery of N by the first two consecutive harvests accounted for 19 to 21% of the N applied as leguminous green manure and 46 to 49% of the N applied as AS. The amounts of inorganic N, derived from both N sources, present in the 0-0.4 m layer of soil in the first season after N application and were below 1 kg ha^{-1}.
Key words: *Saccharum* spp., Brazil, sunn hemp, isotope technique

Aproveitamento do nitrogênio-15 da adubação verde e do sulfato de amônio pela soqueira da cana-de-açúcar

RESUMO: Leguminosas, como adubo verde, são fontes alternativas de nitrogênio para as culturas e podem complementar ou mesmo substituir a adubação mineral nitrogenada, devido ao seu potencial de fixação biológica de nitrogênio (FBN). A utilização do nitrogênio pela cana-de-açúcar (*Saccharum* spp.) fertilizada com crotalária (*Crotalaria juncea* L.) e sulfato de amônio (SA) foi avaliada utilizando a técnica de traçador ^{15}N. As quantidades equivalentes a 196 e 70 kg de N por hectare foram adicionados como adubo verde crotalária júncea (CJ) e como o sulfato de amônio (SA), respectivamente, nos seguintes tratamentos: (i) controle; (ii) SA^{15}N; (iii) CJ^{15}N + SA; (iv) CJ^{15}N; e (v) SA^{15}N + CJ. A cana-de-açúcar foi cultivada por cinco anos e colhida três vezes. A recuperação do ^{15}N foi avaliada nas duas primeiras colheitas. Na soma das três safras, os maiores rendimentos de colmos foram obtidos com uma combinação de adubos verdes e fertilizantes N inorgânicos, mas, no segundo corte rendimentos superiores foram observados nos tratamentos com CJ em comparação com os observados com SA. A recuperação de N nas primeiras duas safras consecutivas representou 19 a 21% do N aplicado como adubo verde e 46 a 49% do N aplicado como SA. As quantidades de N inorgânico derivado das fontes marcadas, presentes na camada 0-0,4 m do solo na primeira safra após a aplicação do N foram inferiores a 1 kg ha^{-1}.
Palavras-chave: *Saccharum* spp., Brasil, crotalária, técnica isotópica

Introduction

The area cropped with sugarcane (*Saccharum* spp.) in Brazil shows rapid expansion, with most of the increase for ethanol production. The area cultivated with sugarcane is now 9.6 Mha, with an increase of 5 Mha from 2000 and over 8.6 Mha of fresh sugarcane harvested per year (IBGE, 2010). Sugarcane crops in Brazil are replanted every five to ten years. In southeastern Brazil, the interval between the last sugarcane harvest and the new plantings occurs during the spring-summer season, under high temperature and heavy rainfall (almost 1,000 mm in six months - Ambrosano et al., 2010).

Green manure fertilization of the soil with legumes has been recommended before a sugarcane field is replanted. This practice does not imply on losing the cropping season, does not interfere with sugarcane germination, and provides increases in sugarcane and sugar yield, at least during two consecutive cuts (Ambrosano et al., 2005). Additionally, it protects the soil against erosion, prevents weed spreading and reduces nematode populations (Dinardo-Miranda and Fracasso, 2009).

Legumes usually accumulate large quantities of N and K, the nutrients which are taken up in the highest amounts by the sugarcane plants. The amounts of N fixed from the atmosphere by diazotrophic bacteria associated with legumes may be substantial and vary from 30 to more than 200 kg ha^{-1} N (Herridge et al., 2008, Silva et al., 2008). Ambrosano (2009) studied the fate of nitrogen from green manure legumes and found that the efficiency of using this nitrogen source by maize was 30%, for the above-ground part of sunn hemp incorporated to an Ultisol. Trivelin et al. (1995; 1996) showed that sugarcane recovery values of nitrogen applied in a mineral form ranged from 19 to 40%.

This study aimed at evaluating: (i) the utilization by sugarcane of the nitrogen from a leguminous green-manure crop and from mineral fertilization with ammonium sulfate, applied together or separately; (ii) its residual effect during three cane cuttings; and (iii) the impact of these N sources on agricultural productivity and recovery of the N by the sugarcane crop.

Material and Methods

The experiment was carried out in Piracicaba, state of São Paulo, Brazil (22°42' S, 47°38' W, and 560 m a.s.l). The soil, classified as an Arenic Hapludult, was chemically characterized at different depths after cutting the green manure crop, before the sugarcane first planting. The soil was acidic and had low amounts of nutrients (Table 1), typical of many sugarcane growing areas.

Sunn hemp (*Crotalaria juncea* L, cv IAC 1-2) was sown at the rate of 25 seeds per meter on the 4 Dec 2000 and emerged in nine days. Microplots, consisting of 6 rows, 2-m long and spaced by 0.5 m within the sunn hemp plots were used for ^{15}N enrichment as described by Ambrosano et al. (2003). After 79 days the sunn hemp was cut, and the fresh material was laid down on the soil surface. Total dry mass of sunn hemp was equivalent to 9.15 Mg ha^{-1}, containing 21.4 g kg^{-1} N, corresponding to 195.8 kg ha^{-1} N with an ^{15}N enrichment of 2.412 atoms % excess.

Table 1 – Soil chemical characteristics before the sugarcane planting, in plots without green manure, at depths of 0-0.2 and 0.2-0.4 m.

Soil characteristic	Soil depth, m	
	0-0.2	0.2-0.4
pH $CaCl_2$(0.01 mol L^{-1})	4.1	4.0
O.M. (g dm^{-3})	26	22
P (mg dm^{-3})	3	14
S (mg dm^{-3})	12	15
K ($mmol_c$ dm^{-3})	0.7	0.5
Ca ($mmol_c$ dm^{-3})	7	6
Mg ($mmol_c$ dm^{-3})	6	5
H + Al ($mmol_c$ dm^{-3})	50	68
Al ($mmol_c$ dm^{-3})	10	11
CEC ($mmol_c$ dm^{-3})	64	80
V %	22	14

O. M. = soil organic matter; CEC = cation exchange capacity; V% = percentage of base saturation.

Sugarcane cultivar IAC- 87-3396 was planted on the 1 Mar 2001 on plots with ten sugarcane rows, 10-m long and spaced at 1.4 m. The experiment consisted of four treatments with four replications in a randomized block design: a) control with no N fertilizer or green manure; b) ammonium sulfate (AS) at a rate of 70 kg ha^{-1} N; c) sunn hemp (SH) green manure; d) and sunn hemp plus ammonium sulfate (SH + AS). Microplots consisting of three rows of sugarcane 2-m long were set up in plots c and d with the ^{15}N-labeled sunn hemp. Microplots with AS-labeled fertilizer (3.01 ± 0.01 atoms % ^{15}N), with two contiguous rows 1-m long, were set up in plots b and also in plots d; therefore, these plots had microplots for both sunn hemp and AS-labeled materials.

Ammonium sulfate was sidedressed to sugarcane 90 days after planting in both main plots and microplots. N rate (70 kg ha^{-1}) is within the range (30 to 90 kg ha^{-1} N) recommended for the plant cane cycle in Brazil (Cantarella et al., 2007). A basal fertilization containing 100 kg ha^{-1} P_2O_5 as triple superphosphate and 100 kg ha^{-1} K_2O as potassium chloride was applied to all treatments to ensure a full sugarcane development.

Cane yield was determined outside the microplots by weighing the stalks of three rows of sugarcane, 2-m long. Stalks yields were measured after 18 months (plant-cane cycle, on 24 Aug 2002), 31 months (1st ratoon crop, on 8 Oct 2003), and 43 months after planting (2nd ratoon crop, on 20 Sep 2004). Samples consisting of ten stalks were used for the determination of apparent sucrose content (Pol) in the cane juice, according to Tanimoto (1964). The expressed cane juice was analyzed for Pol (apparent sucrose) by a saccharimeter. Just before harvesting of the plant cane (24 Aug 2002) and of the first ratoon (8 Oct 2003) whole plants were collected from 1-m row of plants in the center of the microplots. Leaves and stalks were analyzed separately for determination of ^{15}N abundance and N content in a mass spectrometer coupled to an N analyzer, following the methods described in Trivelin et al. (1994).

The fraction and amount of nitrogen in the plant derived from the labeled source (Ndff) and the fraction of N recovery of the labeled source (R%) were calculated based on the isotopic results (atoms %), according to Trivelin et al. (1994), Equations 1 to 3:

$$Ndff = (a/b)\ 100 \quad (1)$$

$$QNdff = [Ndff/100]\ TN \quad (2)$$

$$R\% = [Ndff/NF]\ 100 \quad (3)$$

where: Ndff (%) is the fraction of nitrogen in the plant derived from the labeled source, a and b are ^{15}N abundance values (atoms % excess) in the plant and in the labeled source (AS or SH), respectively; QNdff (kg ha^{-1}) is the amount of nitrogen in the plant derived from the labeled source, TN (kg ha^{-1}) is total cumulative nitrogen in the sugarcane plant (kg ha^{-1}); R% is the fraction of N recovery of the labeled source, NF is the rate of N (kg ha^{-1}) applied as AS or SH. For R (%) calculation purposes, rates of 70 kg ha^{-1} for N-ammonium sulfate and 196 kg ha^{-1} N for the organic source (SH) were taken into consideration.

Precipitation and temperature values were measured during the experimental period from a weather station located near the experimental field. Soil samples were taken from the 0-0.2 and 0.2-0.4 m depths in the microplots, 8, 12, 15, and 18 months after sugarcane planting. Soil mineral nitrogen (nitrate and ammonium) was extracted by shaking 50 g soil sample with 250 mL of a 2 mol L^{-1} KCl solution. An aliquot of 200 mL of the extract was distilled with MgO and Devarda's alloy, according to the method described by Buresh et al. (1982). The distilled solution was collected in a H_3BO_4 solution (20 g L^{-1}) and titrated with a standardized 0.005 M H_2SO_4 solution. The whole volume of the H_3BO_4 extract was treated with additional amounts of H_2SO_4 solution to lower the pH below 4.0, and was dried in an oven at ~90°C. The solid residue containing 120 to 400 μg N was used to determine isotopic-N concentration by mass spectrometry by the wet method with alkaline lithium hipobromite (Rittenberg, 1946). This procedure was carried out for soil samples collected in each sampling season. Soil samples were also collected for chemical characterization at the sugarcane planting and harvesting seasons.

Statistical analysis of cane yield considered four treatments in a randomized block design with four replications. For mineral N in the soil, a random blocks design was used, with four replicates organized as split plots, where plots represented treatments and subplots represented depths. For mineral N, nitrogen derived from fertilizer (Ndff) and quantity of nitrogen derived from fertilizer (Qndff), for nitrogen derived from fertilizer (Ndff) and quantity of nitrogen derived from fertilizer (Qndff), in the leaves and stalks the data obtained were transformed to log (x), since the assumptions of the mathematical model were violated, because the data were not in a normal distribution.

The statistical analysis of the data was performed using the concept of measurements repeated in time and the MIXED procedure in the SAS (Statistical Analysis System) version 8.2 for Windows software. (Littel et al., 1996) criterion was used to select the variance and covariance matrix, by choosing the matrix with the smallest value for that parameter (Akaike, 1974 and SAS, 2004). In the statistical model, the effects of treatment, sampling seasons, and their interactions were considered fixed, and the block effect was considered random. The adjusted means for the fixed effects were obtained with the "LSMEANS" option, and mean comparisons were made by the Tukey-Kramer test ($p < 0.1$).

Results and Discussion

Millable stalk yields of the first cycle (plant cane, harvested 18 months after planting) were higher than those of the second and the third cycle (Table 2). This yield decline over time is common, especially when only the first cycle crop was fertilized to evaluate the residual effect of N application in the mineral or green manure forms. In the first year, stalk yield was higher in plots fertilized with a combination of green manure and AS. However, in the second year the plots that received SH produced more cane than those fertilized only with AS or the control treatment, indicating that the green manure applied before planting still affected plant growth and yield after 34 months. In the third cycle, there were no differences among the treatments, i.e. the residual effect of both N sources had disappeared (Table 2). In the sum of three cuttings, the combination of AS and green manure resulted in highest yields.

Nitrogen derived from AS and SH in the leaves and top parts of the sugarcane plant, excluding stalks, varied from

Table 2 – Millable stalk yield and POL of sugarcane plants in three consecutive harvests as a function of N sources[1].

Treatment[2]	Harvests			Total of three cuttings	Mean ± SEM[3]
	24 Aug 2002	08 Oct 2003	20 Sep 2004		
	Stalk yield, Mg ha^{-1}				
Control	86.0 Ba	61.1 Bab	47.1 Ab	194.2^{b}	64.7 ± 4.6
$AS^{15}N$	106.2 ABa	64.7 Bb	42.3 Ab	213.2^{ab}	71.1 ± 4.6
$AS^{15}N$ + SH	128.7 Aa	84.5Ab	45.0 Ac	258.2^{a}	86.1 ± 4.6
$SH^{15}N$	92.4 ABa	83.8 Aa	41.2 Ab	217.3^{ab}	72.4 ± 4.6
Mean ± SEM	103.3 ± 3.8 a	73.5 ± 3.8 b	43.9 ± 3.8 c	215.4 ± 18.9	
	POL, Mg ha^{-1}				
Control	11.9	10.4	17.9	40.2^{b}	13.5 ± 0.7 B
$AS^{15}N$	14.9	11.1	17.5	43.5^{ab}	14.5 ± 0.6 AB
$AS^{15}N$ + SH	17.0	14.1	18.4	49.5^{a}	16.5 ± 0.6 A
$SH^{15}N$	12.9	14.2	18.1	45.2^{ab}	15.1 ± 0.6 AB
Mean ± SEM	14.2 ± 0.9 b	12.4 ± 0.9 b	18.0 ± 0.9 a	43.8 ± 2.4	

Means followed by a different lower-case letter, in the rows, and upper-case letter, in the columns, are different (Tukey-Kramer, $p \leq 0.1$). Means followed by superscript letters differ vertically (Tukey-Kramer, $p \leq 0.1$). [1]Cane was planted on 01 Mar 2001. [2]Treatments: control (no N fertilization applied); $AS^{15}N$ (^{15}N-labeled ammonium sulfate); $AS^{15}N$ + SH (^{15}N-labeled ammonium sulfate + Sunn hemp); $SH^{15}N$ (^{15}N-labeled Sunn hemp); [3]Standard error of the mean.

6.9 to 12.3% of the total N at the end of the first cycle (plant cane) and was not affected by the N source (Table 3). However, the amounts of N from both sources accumulated in the leaves and tops were in the range of only 4.5 to 6.0 kg ha^{-1}, which represent a recovery of 6.4 to 8.1% of the N applied as AS and 2.7 and 3.1% of the N from the green manure (Table 3). The recovery of ^{15}N in the second cycle decreased when the N source was the inorganic fertilizer. In the second year the fraction of N derived from sunn hemp was greater than that from the AS, indicating a slightly higher residual effect of the green manure (Table 3).

The fraction of N derived from the AS or SH accumulated in the stalks harvested in the first cycle were similar and ranged from 7.0 to 10.5% of the total N content. In the plant cane cycle, the N amounts in the stalks that had been applied as inorganic or organic fertilizers were higher than those measured in the leaves and tops and varied from 27.3 to 24.1 kg N ha^{-1} (Table 4). The recovery of N derived from inorganic fertilizer - 30.1 to 34.4% - was higher than that of the sunn hemp - 8.8 to 9.8%. On the other hand, the green manure supplied more N to the cane stalk than AS in the second harvest (Table 4). The difference in the amounts of N in the sugarcane plants derived from green manure and mineral fertilizer in the ratoon crop was around 1 to 2 kg ha^{-1} N in leaves and tops (Table 3) and 4 to 7 kg N ha^{-1} in the stalks (Table 4), which were relatively small compared to the amounts of N accumulated in the ratoon plants (179 kg N ha^{-1}in plants supplied with AS and 243 kg ha^{-1} N in the SH treatments, or a 64 kg N ha^{-1}difference - Table 4). These results suggest that the effect of green manure on the yield of the 2nd ratoon crop (Table 2) may not be only due to the extra N supply, but rather to other beneficial role of green manure on soil physical or biological properties.

Adding up the amounts of N taken up by the sugarcane plant and contained in the above-ground parts of the plant (leaves, tops, and stalks), AS supplied 32.4 to 34.2 kg N ha^{-1}or about 46 to 49% of N recovery; the N taken up by sugarcane from sunn hemp varied from 37.4 to 40.0 kg ha^{-1}, which represented 19.1 to 20.8% N recovery (Tables 3 and 4).

The average concentration of inorganic nitrogen in the 0-0.4 m layer of soil was relatively low in most samples taken after 8, 12, 15, and 18 months planting (Table 5). Samples taken in February, in the rainy and hot season, had somewhat higher values of (NH_4^+ + NO_3^-)-N probably reflecting higher mineralization of soil organic N (Figure 1). Later in the growing season (samples of May and Aug 2002) soil inorganic N content decreased again. This coincides with the

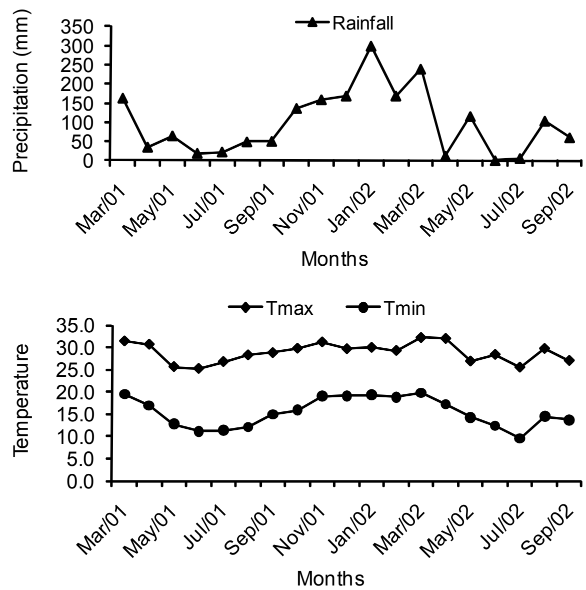

Figure 1 – Climatic data for maximum and minimum temperature and rainfall during the first sugarcane growing season (plant cane cycle-2001, 2002).

Table 3 – Fraction (Ndff) and quantity (QNdff) of nitrogen in the leaves derived from the labeled fertilizer source and nitrogen recovery (R) in samples taken in the first and second harvests[1].

Sampling dates	Treatment[2]				Mean ± SEM[3]
	AS^{15}N	SH^{15}N + AS	SH^{15}N	AS^{15}N + SH	
	---------- Ndff, % ----------				
24 Aug 2002	12.3 Aa	11.1 Aa	10.9 Aa	6.9 Aa	10.3 ± 1.1
08 Oct 2003	1.7 Bb	5.5 Aa	4.1 Aab	1.7 Bb	3.2 ± 1.1
Mean ± SEM	7.0 ± 1.6	8.3 ± 1.6	7.5 ± 1.6	4.3 ± 1.6	
	---------- QNdff, kg ha^{-1} ----------				
24 Aug 2002	5.7	6.0	5.2	4.5	5.4 ± 0.6 A
08 Oct 2003	1.8	6.8	4.6	2.9	4.0 ± 0.6 A
Mean ± SEM	3.7 ± 1.0 a	6.4 ± 1.0 a	4.9 ± 1.0 a	3.7 ± 1.0 a	
	---------- R, % ----------				
24 Aug 2002	8.1 Aa	3.1 Aa	2.7 Aa	6.4 Aa	5.1 ± 0.6
08 Oct 2003	2.6 Ba	3.5 Aa	2.3 Aa	4.1 Aa	3.1 ± 0.6
Mean ± SEM	5.3 ± 0.9	3.3 ± 0.9	2.5 ± 0.9	5.3 ± 0.9	

Means followed by a different lower-case letter, in the rows, and upper-case letter, in the columns, are different (Tukey-Kramer and F' tests, $p \leq 0.1$), respectively. [1]Cane was planted on 01 Mar 2001. [2]Treatments: AS^{15}N (^{15}N-labeled ammonium sulfate); SH^{15}N + AS (^{15}N-labeled Sunn hemp + ammonium sulfate); SH^{15}N (^{15}N-labeled Sunn hemp); AS^{15}N + SH (^{15}N-labeled ammonium sulfate + Sunn hemp). [3]Standard error of the mean.

Table 4 – Fraction (Ndff) and quantity (QNdff) of nitrogen derived from the labeled fertilizer source, nitrogen recovery (R) in the sugarcane stalks and nitrogen accumulated in the samplings carried out in the first and second harvestings[1].

Sampling dates	Treatment[2]				Mean ± SEM[3]
	$AS^{15}N$	$SH^{15}N$ + AS	$SH^{15}N$	$AS^{15}N$ + SH	
	Ndff, %				
24 Aug 2002	10.5 Aa	7.0 Aa	8.2 Aa	10.3 Aa	9.0 ± 1.2
08 Oct 2003	1.4 Bb	3.8 Aa	3.7 Aa	1.7 Bb	2.6 ± 0.1
Mean ± SEM	6.0 ± 1.2	5.4 ± 1.2	5.9 ± 1.2	6.0 ± 1.2	
	QNdff, kg ha^{-1}				
24 Aug 2002	24.1 Aa	19.3 Aa	17.3 Aa	21.1 Aa	20.4 ± 2.78
08 Oct 2003	2.7 Bb	8.6 Aa	10.3 Aa	3.9 Bb	6.4 ± 0.8
Mean ± SEM	13.4 ± 2.6	14.0 ± 2.6	13.8 ± 2.6	12.5 ± 2.6	
	R, %				
24 Aug 2002	34.4 Aa	9.9 Abc	8.8 Ac	30.1 Aab	20.8 ± 1.9
08 Oct 2003	3.9 Ba	4.4 Aa	5.3 Aa	5.6 Ba	4.8 ± 1.9
Mean ± SEM	19.1 ± 3.2	7.1 ± 3.2	7.0 ± 3.2	17.8 ± 3.2	
	Cumulative, N kg ha^{-1}				
24 Aug 2002	177.4	235.6	257.0	220.4	222.6 ± 9.2 A
08 Oct 2003	181.0	190.8	228.0	270.8	217.6 ± 9.2 A
Mean ± SEM	179.2 ± 12.0 a	213.2 ± 12.0 a	242.5 ± 12.0 a	245.6 ± 12.0 a	

Means followed by a different lower-case letter, in the rows, and upper-case letter, in the columns, are different (Tukey-Kramer and F tests, $p \leq 0.1$). [1]Cane was planted on 01 Mar 2001. [2]Treatment: $AS^{15}N$ (^{15}N-labeled ammonium sulfate); $SH^{15}N$ + AS (^{15}N-labeled Sunn hemp + ammonium sulfate); $SH^{15}N$ (^{15}N-labeled Sunn hemp); $AS^{15}N$ + SH (^{15}N-labeled ammonium sulfate + Sunn hemp). [3]Standard error of the mean.

Table 5 – Soil mineral N (NH_4^+ + NO_3^-) determined in four sampling dates during the plant cane cycle. Data are average of samplings of the 0-0.2 and 0.2-0.4 m soil layers.

Treatment[1]	Sampling dates				
	29 Oct 2001	20 Feb 2002	28 May 2002	24 Aug 2002	Mean ± SEM[2]
	mg kg^{-1}				
Control	2.7 Ab	7.3 ABa	2.3 Ab	2.8 Ab	3.8 ± 0.22
$AS^{15}N$	2.6 Ab	9.1 Aa	2.2 Ab	3.2 Ab	4.3 ± 0.22
$SH^{15}N$ + AS	2.9 Ab	7.0 ABa	2.7 Ab	3.1 Ab	3.9 ± 0.22
$SH^{15}N$	2.8 Ab	5.8 Ba	1.5 Bc	2.8 Ab	3.2 ± 0.22
$AS^{15}N$ + SH	2.7 Ab	7.2 ABa	3.1 Ab	2.6 Ab	3.9 ± 0.22
Mean ± SEM	2.7 ± 0.19	7.3 ± 0.19	2.4 ± 0.19	2.9 ± 0.19	

Means followed by a different lower-case letter, in the rows, and upper-case letter, in the columns, are different (Tukey-Kramer, $p \leq 0.1$). [1]Treatments: Control (no N fertilizer applied); $AS^{15}N$ (^{15}N-labeled ammonium sulfate); $SH^{15}N$ + AS (^{15}N-labeled Sunn hemp + ammonium sulfate); $SH^{15}N$ (^{15}N-labeled Sunn hemp); $AS^{15}N$ + SH (^{15}N-labeled ammonium sulfate + Sunn hemp). [2]Standard error of the mean.

beginning of the dry season with mild temperatures, when the sugarcane plant reached maturity and probably had already depleted the soil of most of the available N.

The fraction of the inorganic N derived from AS or SH present in the soil from the 8th to the 18th month after sugarcane planting represented only 1 to 9% of total inorganic N (Table 6). The proportion of N that was originated from AS decreased with time whereas that from the green manure increased, indicating that the mineralization of this organic source could supply more N at the end of the season (Table 6). Indeed, Ambrosano et al. (2005) showed that sugarcane stalks sampled in 15-month old plants had more N derived from AS than from SH; in the 18th month that difference had disappeared. Nonetheless, throughout the season, the amounts of inorganic N in the soil derived from either AS or SH were of very little significance for the nutrition of the sugarcane plant - less than 1 kg ha^{-1} of inorganic N in a 0.4 m soil layer (Table 6), indicating that little residual N is expected in soils grown with this crop. Although the rate of N applied as SH was almost 200 kg N ha^{-1}, little nitrate leaching losses are expected under the conditions of this experiment.

Mascarenhas et al. (1994) showed evidence of the positive effect of green manure fertilization with sunn hemp in sugarcane, with a greater sugarcane yield increase than with

Table 6 – Fraction (Ndff) and amount (QNdff) of soil mineral N ($NH_4^+ + NO_3^-$) derived from the labeled fertilizer source (Ndff). Data are average of samplings of the 0-0.2 and 0.2-0.4 m soil layers.

Treatment[1]	Sampling dates				
	29 Oct 2001	20 Feb 2002	28 May 2002	24 Aug 2002	Mean ± SEM[2]
	---------- Ndff, % ----------				
AS^{15}N	5.9 Aa	0.7 Aa	3.2 Aa	1.0 Ba	2.7 ± 0.57
SH^{15}N + AS	2.6 Ab	3.2 Aab	9.0 Aa	5.7 ABab	5.1 ± 0.62
SH^{15}N	2.9 Aa	4.3 Aa	7.0 Aa	5.9 Aa	5.0 ± 0.58
AS^{15}N + SH	2.9 Aa	0.3 Aa	4.0 Aa	1.3 ABa	2.1 ± 0.62
Mean ± SEM	3.6 ± 0.58	2.1 ± 0.56	5.8 ± 056	3.5 ± 0.55	
	---------- QNdff, kg ha^{-1} ----------				
AS^{15}N	0.3 Aa	0.3 Aa	0.4 Aa	0.5 Aa	0.40 ± 0.16
SH^{15}N + AS	0.1 Aa	0.2 Aa	0.2 Aa	0.2 Aa	0.18 ± 0.18
SH^{15}N	0.1 Aa	0.2 Aa	0.1 Aa	0.2 Aa	0.15 ± 0.16
AS^{15}N + SH	0.1 Aa	0.1 Aa	0.1 Aa	0.0 Aa	0.07 ± 0.16
Mean ± SEM	0.15 ± 0.09	0.21 ± 0.09	0.24 ± 0.09	0.22 ± 0.09	

For Ndff: means followed by a different lower-case letter, in the rows, and upper-case letter, in the columns, are different (Tukey-Kramer and F tests, $p \leq 0.1$), respectively. For Qndff: means followed by a different letter lower-case letter, in the rows, and upper-case letter, in the columns, are different by the Tukey-Kramer test ($p \leq 0.1$). [1]Treatments: AS^{15}N (^{15}N-labeled ammonium sulfate); SH^{15}N+AS (^{15}N-labeled Sunn hemp + ammonium sulfate); SH^{15}N (^{15}N-labeled Sunn hemp); AS^{15}N + SH (^{15}N-labeled ammonium sulfate + Sunn hemp). [2]Standard error of the mean.

the application of 40 kg ha^{-1} mineral N to the soil. Kanthack et al. (1991) evaluated lupine(*Lupinus* albus L.) in maize (*Zea Mays* L.) , and Muraoka et al. (2002) evaluated velvet bean (*Mucuna aterrima* (Piper & Tracy) Holland)and sunn hemp in rice (*Oriza sativa* L.), and these authors did not find a response to mineral N applied after green manure. No N fertilizer was needed when vetch (Vicia spp.) was grown after wheat (*Triticum* spp.), and when cotton (*Gossypium hirsutum* L.) followed faba beans(*Faba vulgaris* L.) (Rochester and Peoples, 2005).

The effect of fertilizer source on sugar concentration was less evident. In the average of three cuttings, pol value in canes from plots treated with both AS + SH was higher than in that observed in plots that received no N (Table 2). Pol in cane juice was higher in the third cutting than in the two previous ones. Variations in pol measurements among cropping seasons are usually affected by temperature and drought that determine cane maturation than by nutrition. However, high N tends to decrease sugar content and delay maturation (Silveira and Crocomo, 1990); therefore, after two years without N fertilization, sugar content in cane plants was more likely to be high.

The recovery of N from fertilizers by sugarcane is usually lower than that of grain crops: the latter varies from 50 to 70% (Freney et al., 1992) whereas for sugarcane the figures vary from 20 to 40% (Gava et al., 2003, Trivelin et al., 1995; 1996; Cantarella et al., 2007). The utilization of N from green manure by subsequent crops rarely exceeds 20% (Muraoka et al., 2002; Silva et al., 2006, Ambrosano et al., 2005, 2009) and most of the N remains in the soil, incorporated in the organic matter fraction. In this study, the application of AS along with SH increased N utilization by sugarcane plants. This result agrees with Muraoka et al. (2002), who used an organic source isolated or combined with an inorganic fertilizer in rice crops and concluded that the green manures improved the mineral N utilization, resulting in N use efficiency of up to 79%.

In a pot experiment, Ambrosano et al. (2009) observed that maize plants took up more N from sunn hemp incorporated to a sandy soil (Paleudalf) than to a clayey soil (Eutrudox) and that the N derived from the roots was more recalcitrant than that of the shoots. Between 50 and 68% of the ^{15}N of the sunn hemp shoots remained in the soil whereas the figures for roots varied from 65 to 80%. Unaccounted for ^{15}N (varied from 5 to 15% of the sunn hemp N) was assumed to be lost mainly by gaseous forms, (Ambrosano et al., 2009). In a detailed account of the first year of the present experiment, Ambrosano et al. (2005) showed that eight months after planting the recovery by sugarcane plants (above ground parts) of the N derived from AS or from sunn hemp was similar: 3 to 6% of the added N. However, 12 and 15-month-old sugarcane plants recovered between 20 and 35% of the AS but only 6 to 8% of the sunn hemp-derived N.

When sugarcane plants were harvested after 18 months of planting, the recovery of the inorganic fertilizer N contained in the stalk varied from 30 to 34%; the corresponding figures for the N derived from sunn hemp were lower: around 9 to 10% (Table 4). The residual effect of the N from both sources in the 2nd harvest of the sugarcane plant was similar: between 4 and 6% of the N supplied at planting as AS or SH was recovered in the stalks of the sugarcane plant 31 months after planting (Table 3).

About 69% of the N existent in the sunn hemp residues were from BNF. These data are in agreement with those obtained by Resende et al. (2003) for green manure produced in the field, in the inter-rows of the ratoon crop. Perin et al. (2006) found substantial amounts of N derived from BNF present in the above ground parts of sunn hemp (57.0%) grown isolated and 61.1% when intercropped with millet (*Pennisetum glaucum*, L. Brown) (50% seeded with each crop). The sunn hemp + millet treatment grown before a maize crop resulted in higher grain yield than when the sunn hemp alone was the preceding rotation. This effect was not observed when N-fertilizer (90 kg N ha^{-1}) was added. Intercropping legume and cereals is a promising biological strategy to increase and keep N into a production system under tropical conditions (Perin et al., 2006).

No difference was observed in the cumulative N (Table 4). The cumulative N results are similar to those found by Gava (2003), who obtained mean values of 252.3 kg ha^{-1} cumulative nitrogen during plant cane harvesting, with high N and plant material accumulation during the last three months, as also observed by Trivelin et al. (1996). N contents found in the above-ground part of sugarcane (Table 4) are in agreement with results of Gava (2003).

As the amounts of N applied as AS or SH to sugarcane in the first cycle were different (70 kg N ha^{-1}as AS and 196 kg N ha^{-1}as SH), the quantities of N derived from the green manure in the second harvest were larger than those from the inorganic fertilizer, although the N recovery was similar (Table 3). Because less N derived from the green manure was recovered by the sugarcane plant in the first cycle it would be expected that a higher proportion of that N would be taken up in the second cycle (1^{st} ratoon), but this did not happen. It seems that the residual N that is incorporated to the soil organic matter has a somewhat long turnover. Other authors have reported low recovery (about 3.5% of the N) by the second crop after sunn hemp cover crop (Silva et al., 2006) or hairy vetch (*Vicia villosa* Roth) plowed into the soil (Seo et al., 2006). Low recovery of residual N has also been observed for inorganic fertilizer sources: less than 3% of the N derived from fertilizers was taken up by soybeans (*Glicine max* (L) Merril) (Boaretto et al., 2004), maize (Silva et al., 2006), Seo et al. (2006) or sugarcane (*Saccharum* spp.) (Basanta et al., 2003). These results are similar to those obtained in this study (Table 3 and 4). Soil N is often the most limiting element for plant growth and quality. Therefore, green manure may be useful for increasing soil fertility and crop production. Regarding fertilization, organic matter such as a green manure can be potentially important sources of N for crop production (Asagi and Ueno, 2009).

Sugarcane is a fast growing plant that produces high amounts of dry matter. Therefore, it tends to rapidly deplete the soil of inorganic N, especially in soils fertilized with small rates of soluble N as in the case of this study. Cantarella et al. (2007) reviewed several Brazilian studies showing little nitrate losses by leaching in sugarcane. Recently, Ghiberto et al. (2009) showed that only 0.2 kg ha^{-1} NO_3^--N derived from 120 kg ha^{-1} of N as urea enriched to 5.04 ^{15}N At% applied to the planting furrow leached below 0.9 m in a sugarcane field, although the total N loss reached 18 kg ha^{-1} N, mostly derived from soil organic matter mineralization or residual N already present in the soil. As in this study, Ghiberto et al. (2009) refer to N applied at the end of the rainy season when excess water percolating through the soil profile is limited (Figure 1).

Conclusions

The combination of inorganic fertilizer and green manure resulted in higher sugarcane yields than either N source applied separately. The recovery of N from ammonium sulfate was higher in the first year, whereas in the green manure presented a longer residual effect and resulted in higher yields of cane in the second cycle. The recovery of ^{15}N- labeled fertilizers by two successive sugarcane crops summed up 19 to 21% of the N applied as sunn hemp and 46 to 49% of the N applied as ammonium sulfate. Very little inorganic N was present in the 0-0.4 m soil layer with both N sources.

Acknowledgements

To technicians Ângela Maria C. da Silva, Gilberto Farias, Benedito Mota, Isac Serafin and Maria Aparecida C. de Godoy. To interns Rogério Haruo Sakai, and Lais Ferraz de Camargo. To FAPESP and CNPq for the grants

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Received February 02, 2010
Accepted September 01, 2010