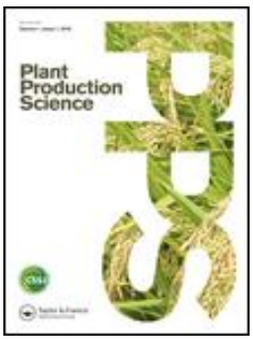

Plant Production Science

ISSN: 1343-943X (Print) 1349-1008 (Online) Journal homepage: http://www.tandfonline.com/loi/tpps20

Yield of sugarcane varieties and their sugar quality grown in different soil types and inoculated with a diazotrophic bacteria consortium

Nivaldo Schultz, Willian Pereira, Paulo de Albuquerque Silva, José Ivo Baldani, Robert Michael Boddey, Bruno José Rodrigues Alves, Segundo Urquiaga & Veronica Massena Reis

To cite this article: Nivaldo Schultz, Willian Pereira, Paulo de Albuquerque Silva, José Ivo Baldani, Robert Michael Boddey, Bruno José Rodrigues Alves, Segundo Urquiaga & Veronica Massena Reis (2017): Yield of sugarcane varieties and their sugar quality grown in different soil types and inoculated with a diazotrophic bacteria consortium, Plant Production Science, DOI: 10.1080/1343943X.2017.1374869

To link to this article: http://dx.doi.org/10.1080/1343943X.2017.1374869

Published online: 05 Oct 2017.

PLANT PRODUCTION SCIENCE, 2017
https://doi.org/10.1080/1343943X.2017.1374869

REGULAR PAPER

Yield of sugarcane varieties and their sugar quality grown in different soil types and inoculated with a diazotrophic bacteria consortium

Nivaldo Schultz[a], Willian Pereira[a], Paulo de Albuquerque Silva[b], José Ivo Baldani[c], Robert Michael Boddey[c], Bruno José Rodrigues Alves[c], Segundo Urquiaga[c] and Veronica Massena Reis[c]

[a]Soil Department, Agronomy Institute, Rio de Janeiro Rural Federal University, Rio de Janeiro, Brazil; [b]Research Performance Unit – UEP, Brazilian Agricultural Research Company – EMBRAPA, Rio Largo, Brazil; [c]Embrapa Agrobiologia, Seropédica, Rio de Janeiro, Brazil

ABSTRACT

Sugarcane is a crop of great economic, social, and environmental relevance in Brazil. The country is the largest sugar producer and the second largest bioethanol producer in the world. The goal of this study was to evaluate the efficiency of a sugarcane inoculant composed of five diazotrophic bacterial strains, as well as nitrogen fertilization of two sugarcane varieties. Two experiments were carried out on two varieties using an experimental design composed of complete randomized blocks in a factorial of two varieties and three treatments with four replicates. The treatments can be described as: inoculation with the consortium of five diazotrophic strains, or N fertilization with 120 kg ha^{-1}, and one control treatment. The following parameters were then evaluated: stem yield, accumulation of total dry matter, nitrogen content, quality of the sugarcane juice, and ^{15}N natural abundance on flag-leaves. Inoculation and N fertilization on the Sapucaia plantation promoted increases of stem yield equivalent to 22.3 and 26.5 Mg ha^{-1} in the RB867515 variety, in comparison to the control, respectively. Inoculation and N fertilizer used for the Coruripe plantation increased stem yield of 38.0 and 42.4 Mg ha^{-1}, respectively, with the RB867515 variety, while RB72454 showed increases of 16.7 and 37.5 Mg ha^{-1}, both compared to the control. Biological nitrogen fixation was not affected by the treatments, however, both treatments increased the total recoverable sugar yield. Benefits from inoculation appeared to promote plant growth due to the plant–bacteria interaction.

ARTICLE HISTORY
Received 20 January 2017
Revised 31 July 2017
Accepted 17 August 2017

KEYWORDS
Saccharum spp.; nitrogen; plant growth promotion; biological nitrogen fixation; ^{15}N natural abundance

CLASSIFICATION
Agronomy & Crop Ecology

Sugarcane (*Saccharum* spp.) is the most important crop for Brazilian production of renewable energy. In recent years it has expanded to new agricultural areas, especially on degraded pastures, decisively influencing the economic, social, and cultural development of most regions. According to the Brazilian Geography and Statistics Institute (IBGE-SIDRA, 2015), 10.3 million hectares were harvested during the 2014/2015 crop, with an average yield of 74.3 Mg ha^{-1} and total production of 755 million tons. Despite all the benefits from using renewable energy, the sugarcane industry still causes several negative environmental impacts, especially greenhouse gas emissions, such as CO_2, CH_4, and N_2O which result from the use of nitrogen fertilizers applied to the soil surface after harvesting (Boddey et al., 2008; Denmead et al., 2010). Thus, it is crucial to develop research in order to search for alternatives, ensuring a competitive and sustainable sugarcane industry.

Interactions between atmospheric N_2-fixing bacteria and sugarcane have been investigated by different researchers working with such crop since 1950s (Döbereiner, 1992). Various studies to quantify the contribution of biological N_2 fixation (BNF) to the crop have shown that approximately 50% of the total *N* accumulated in plant tissues comes from the air (Boddey et al., 2001; Lima et al., 1987; Urquiaga et al. 2012; Yoneyama et al., 1997). An inoculant composed of five diazotrophic strains was developed, in which the BNF contribution was of approximately 30%, using the SP701143 sugarcane variety grown in pots with inoculated soil containing a consortium of N_2-fixing bacteria (Oliveira et al., 2002). Field inoculation experiments with this consortium also showed yield increases, however, they were dependent on soil type and sugarcane variety (Oliveira et al., 2006). More recently, field inoculation studies showed that yield response varied with sugarcane varieties, and was due to both increases in the BNF input and the bacterial plant growth promotion effects (Schultz et al., 2012, 2014).

Several studies have shown that effects that promote growth due to diazotrophic bacterial inoculation are partly explained by phytohormone synthesis (especially

CONTACT Veronica Massena Reis veronica.massena@embrapa.br

Table 1. Geographical location, soil and climate data, cycles, treatments, and fertilization.

Mill and location	Soil and climate data	Cycle	Treatments	Fertilization
Sapucaia mill, Campos dos Goytacazes, RJ; (21°45′14″S and 41°19′26″O, altitude 14 m)	Ultisol. Aw climate, dry winter and hot and rainy summer; average annual temperature of 22.7 °C; precipitation during the cycle 1565 mm	Plant cane; planting in March 2006 and harvested in August 2007; 18 months of growing	Inoculated with diazotrophs; fertilization with 120 kg ha^{-1} N (Urea) and absolute control. Plots with 4 rows of 4 m, spaced at 1.4 m	120 kg ha^{-1} of P_2O_5; 100 kg ha^{-1} of K_2O; 40 kg ha^{-1} of FTE BR12 (micronutrients); 0.4 kg ha^{-1} of ammonium molybdate; 15 Mg ha^{-1} of filter cake applied in the groove bottom
Coruripe mill, Coruripe, AL; (10º 07′33″S and 36º10′33″W, altitude 16 m)	Ultisol. Tropical climate, hot, humid, and rainy between April and September; average annual temperature of 24.4 °C; precipitation during the cycle 1543 mm	Plant cane; planting in November 2009 and harvested in December 2010; 13 months of growing	Inoculated with diazotrophs; fertilization with 120 kg ha^{-1} N (Urea) and absolute control. Plots with 6 rows of 5 m, spaced at 1.0 m	100 kg ha^{-1} of P_2O_5; 144 kg ha^{-1} of K_2O; 30 kg ha^{-1} of micronutrients (B = 3%, Cu = 5%, Fe = 0.4%, Mn = 15%, and Zn = 5%); 0.4 kg ha^{-1} of ammonium molybdate

auxins) (Bashan et al., 2004; Videira et al., 2012), inorganic phosphate solubilization (Shukla et al., 2008; Singh et al., 2007), zinc compounds dissolution (Saravanan et al., 2007), increased rhizosphere retention of essential nutrients (Yadav et al., 2009), as well as biological control of plant pathogens (Spaepen et al., 2007).

The goal of this study was to evaluate the inoculation effect of the consortium composed of five diazotrophic strains over agronomic and industrial parameters of two varieties of planted sugarcane in two different soils and climate conditions, in the main sugarcane Brazilian producing regions.

Table 2. Chemical soil properties of both experimental areas.

Depth	pH	N	C	Ca	Mg	Al	H + Al	V	P	K
cm	H_2O	g dm^{-3}		$cmol_c$ dm^{-3}				%	mg dm^{-3}	
Sapucaia mill, RJ – ultisol										
0–20	6.2	.8	12.5	3.6	1.6	.0	2.3	70	66	49
20–40	5.2	.6	7.5	2.0	.7	.3	3.6	43	29	27
Coruripe mill, Al – ultisol										
0–20	6.7	.8	9.5	2.6	.9	.0	1.1	77	46	52
20–40	6.2	.5	5.9	1.7	.6	.0	1.7	58	34	19

Notes: (EMBRAPA, 2006). *V*% = 100 *S*/*T*. It represents the participation of the exchangeable bases in relation to the total cations in the complex. This value is used for the characterization of eutrophic and dystrophic soils. *V*% = 100 (Ca + Mg + K)/CEC = Cation exchange capacity.

Material and methods

General information

Tables 1 and 2 present the geographical location, soil and climate data, as well as soil.

Experimental design and varieties

Experiments were laid out in a complete randomized block factorial 2 × 3 (two varieties and three treatments) design with four replicates. RB867515 and RB72454 varieties were chosen based on their agronomic importance and contribution to the total sugarcane area cultivated in Brazil. Treatments imposed were: inoculation with the consortium of diazotrophic bacteria, N fertilization using 120 kg ha^{-1}, and the absolute control. The planting procedure employed stem pieces (setts) since it is a standard practice, at a density of approximately 15 buds per linear meter.

Inoculant preparation and inoculation procedure

The sugarcane inoculant was composed of five strains, isolated from different sugarcane varieties in Brazil and deposited at Embrapa Agrobiology Culture Collection (Seropédica, RJ, Brazil). The five diazotrophic bacterial strains were previously selected by Oliveira et al. (2002, 2003) and the species names are: *Gluconacetobacter diazotrophicus* (strain PAL5^T-BR11281) first described by Cavalcante and Döbereiner (1988), *Herbaspirillum seropedicae* (HRC54-BR11335 – Baldani et al., 1986), *Herbaspirillum rubrisubalbicans* (HCC103-BR11504 – Baldani et al., 1996), *Nitrospirillum amazonense* (Cbamc-BR11145) formerly *Azospirillum* described by Magalhães et al. (1983) and renamed by Lin et al. (2014); *Paraburkholderia tropica* (PPe8^T-BR11366) described by Reis et al. (2004) in the genus *Burkholderia* and renamed as new genus by Oren and Garrity (2015).

All strains were individually grown in DYGS liquid medium (Baldani et al., 2014) at 30 °C for 48 h in a rotary shaker at 150 rpm. Subsequently, the five bacterial cultures containing 10^9 cells mL^{-1} were mixed with the peat used as carrier in a proportion of 75 mL of DYGS culture medium mixed in 175 g of neutralized and sterilized milled peat packed in polyethylene bags. The total inoculum was composed of five packages of 250 g fresh weight containing each single strain. Three inoculant doses were diluted in 600 L of tap water and used to immerse sugarcane setts. The prepared inoculant suspension corresponded to a dose for one hectare. Pre-selected and standard size setts (with three buds) were packed for inoculation into bags according to the number of setts required per plant row (15 buds per meter), and immersed during 30 min in the inoculum suspension (600 L). Seedlings were then dried

in the shade for 30 min and immediately planted. Control plants were immersed in water for 30 min as well.

Assessments, preparation, and analyses of plant samples

Agronomic evaluations were performed to determine stem yield, dry matter yield, and total *N* in plant shoots (stems, straw, and flag-leaves). After weighing stems, straw, and flag-leaves, subsamples were taken from each fraction and dried until constant weight in an oven adjusted at 65 °C. Sub-samples were first ground in a Wiley mill (2 mm) and then similarly finely ground as described by Arnold and Schepers (2004). Nitrogen was determined according to the semi-micro Kjeldahl method (Nogueira & Souza, 2005) and ^{15}N natural abundance of sub-samples of flag-leaves was determined, which, according to Boddey et al. (2001), represent the whole-plant isotopic label. For analyses of samples containing between 30 and 50 µg of N, tin capsules for weighing were employed in the analyses by continuous flow isotope ratio mass spectrometry (Finnigan MAT, Bremen, Germany) at the Embrapa Agrobiology 'John Day Stable Isotope Laboratory' (Ramos et al., 2001).

Sugarcane juice quality (total recoverable sugar – TRS, soluble solids percentage – Brix, and apparent sucrose percentage – Pol), as well as TRS yield (kg per hectare) were determined according to the methodology proposed by Fernandes (2000) and the Instruction Manual for the São Paulo State Producers Council for Sugarcane, Sugar, and Alcohol (CONSECANA, 2006).

^{15}N natural abundance for available N in soil

Uniformity for ^{15}N isotopic labeling within the total N available in the soil for plants, considering the time and depth, is a pre-requirement for the ^{15}N isotopic dilution technique to assess BNF, provided that control plants have similar N uptake and roots as the target plant (Unkovich et al., 2008). However, Ledgard et al. (1984) and Urquiaga et al. (2012) found that ^{15}N isotope distribution of the N available in the soil is not uniform according to the depth, making it difficult to compare the plant ^{15}N natural abundance due to different root systems and consequent withdrawal of the N available in the soil at different depths.

Based on assumptions made by Unkovich et al. (2008), uniformity of the ^{15}N isotope of N available in the soil was assessed by following the methodology recommended by Ledgard et al. (1984) and Urquiaga et al. (2012). Soil samples were collected at three points from each experimental area in layers described as 0–15, 15–30, 30–45, and 45–60 cm (depth that contains most of the sugarcane root system). These samples were dried and sieved through a 2 mm mesh sieve. The soil prepared was conditioned into pots containing 400 g, as well as three reference plant species, which were non-N_2-fixing or had an insignificant fixation. Reference plants adopted for this study were *Sorghum bicolor, Panicum mileaceum*, and *Pennisetum glaucum*. The experimental design can be described by a randomized block in a factorial 3 × 4, three species of plants, four soil depths, and four replicates. Before planting, the soil was fertilized, except for N, by applying 100 mg kg^{-1} of P_2O_5 (superphosphate), 100 mg kg^{-1} K_2O (potassium chloride), 20 mg kg^{-1} of magnesium sulphate, and 50 mg kg^{-1} of FTE BR12 (micronutrients). Eight to ten seeds were planted per pot, with each pot containing only one plant species. Plants were grown for approximately 30 days until they presented yellowing of leaves, indicating depletion of the available N in the soil. Whole plants were harvested (root + shoot), washed, dried, weighed, ground, and analyzed as described for sugarcane samples.

The weighted average of ^{15}N natural abundance in the profiles was calculated through this methodology, since the N content available in the soil decreases according to profile depth, and almost all sugarcane roots are found only to a depth of 60 cm (Urquiaga et al., 2012). Thus, estimated ^{15}N natural abundance of soil N available to the sugarcane plant was calculated using the following Equation:

$$\text{Weighted average } \delta^{15}\text{N} = \sum (Rp\,\delta^{15}\text{N} * \text{TNrp}) / \sum (\text{Nfdrp})$$

$Rp\delta^{15}N$ = Reference plant $\delta^{15}N$, grown in soil samples of each layer, ‰.

TNrp = Total N available in each layer (15 cm), extracted by reference plant, mg.

Nfdrp = N available in full depth (0–60 cm), extracted by reference plant, mg.

Statistical analysis

At first, data obtained were statistically analyzed to verify normality and homogeneity of variance errors, by applying Lilliefors and Cochran & Bartley tests, respectively, using SAEG 9.1 software (Viçosa Federal University, MG, Brazil). Then, a variance analysis was performed, followed by application of the F test using the Sisvar 4.3 software program (Lavras Federal University, MG, Brazil). Means were compared by the Scott-Knott test at 5% probability. TRS yield in kg ha^{-1} was compared using the same test at 10% probability. Soil δ^{15} N values which were extracted by reference plants were compared through mean standard error bars.

Results

The response of the different sugarcane varieties to inoculation with the consortium composed of diazotrophic strains, as well as the application of 120 kg ha^{-1} of N, was

Table 3. Stem yield and total dry matter accumulation (stems, straw, and green leaves) of two grown sugarcane varieties inoculated with diazotrophs, fertilization with 120 kg ha^{-1} of N, and control in two Brazilian regions.

	RB867515		RB72454	
	Stems	Total dry matter	Stems	Total dry matter
Treatment	Mg ha^{-1}			
Sapucaia mill, RJ – crop 2006/2007				
Control	103.8b	48.4	141.2	59.2
Inoculated	126.1a	57.5	137.7	60.8
120 kg N	130.3a	61.5	145.1	59.7
CV (%)	9.2	16.5	9.2	16.5
Coruripe mill, AL – crop 2009/2010				
Control	115.9b	46.9b	132.0c	56.4
Inoculated	153.9a	68.4a	148.7b	63.2
120 kg N	158.3a	65.3a	169.5a	70.2
CV (%)	13.3	15.9	13.3	15.9

Notes: Means of four replicates. Means followed by different letters in the columns differ by Scott-Knott test at % probability. C.V: coefficient of variation.

Table 4. Total nitrogen content in stem dry matter and total dry matter (stems, straw, and green leaves) of two grown sugarcane varieties inoculated with diazotrophs, fertilization with 120 kg ha^{-1} of N, and control in two Brazilian regions.

	RB867515		RB72454	
	Stems	Total	Stems	Total
Treatment	kg ha^{-1}			
Sapucaia mill, RJ – crop 2006/2007				
Control	100.2	195.0	97.4	208.3
Inoculated	110.8	182.7	126.6	218.8
120 kg N	114.6	178.3	119.9	211.1
CV (%)	29.0	18.9	29.0	18.9
Coruripe mill, AL – crop 2009/2010				
Control	58.8	135.8	52.4b	148.3b
Inoculated	69.0	168.2	43.3b	138.1b
120 kg N	76.6	168.9	88.4a	195.9a
CV (%)	26.0	18.2	26.0	18.2

Notes: Means of four replicates. Means followed by different letters in the columns differ by Scott-Knott test at 5% probability C.V: Coefficient of variation.

Table 5. Total recoverable sugar (TRS), soluble solids (Brix), and apparent sucrose (Pol) of two grown sugarcane varieties inoculated with diazotrophs, fertilization with 120 kg ha^{-1} of N, and control in two Brazilian regions.

	RB867515			RB72454		
Treatment	TRS kg Mg^{-1}	Brix %	Pol %	TRS kg Mg^{-1}	Brix %	Pol %
Sapucaia mill, RJ – crop 2006/2007						
Control	138.4	20.9	14.0	136.9	20.5	13.8
Inoculated	130.6	19.9	13.2	134.9	19.8	13.5
120 kg N	131.6	20.2	13.2	136.1	19.6	13.8
CV (%)	7.2	6.4	7.9	7.2	6.4	7.9
Coruripe mill, AL – crop 2009/2010						
Control	159.8	23.1	16.4	164.3	23.4	16.9
Inoculated	157.2	22.6	16.1	167.0	23.7	17.2
120 kg N	150.1	21.8	15.3	165.2	23.7	17.0
CV (%)	5.8	5.3	6.2	5.8	5.3	6.2

Notes: Means of four replicates. The absence of letters means that there was no difference between treatments by Scott-Knott test at 5% probability. TRS: total recoverable sugar. Brix: soluble solids. Pol: apparent sucrose. C.V: Coefficient of variation.

variable when such varieties were grown in two diverse climatic Brazilian regions (Table 3). An expressive increase of fresh stems productivity was observed for the RB867515 variety grown in the Sapucaia mill (Southeast region), inoculated with the consortium or N fertilized. This increase was equivalent to 22.3 and 26.5 Mg ha^{-1}, respectively, in comparison to the control. However, there was no difference observed between the treatments for the RB72454 variety. In addition, there were significant effects on stem yields from inoculation and N fertilizer in Coruripe mill (Northeast region), with increases of 38.0 and 42.4 Mg ha^{-1}, respectively, in comparison to the control for RB867515. To the RB72454, the N fertilization effect was significantly higher to stem yields, in comparison to the control and inoculation (Table 3). Total dry matter accumulated presented a significant difference between the treatments, but only for the RB867515 variety in Coruripe mill, with increases of 21.5 and 18.4 Mg ha^{-1} by inoculation and N fertilization, respectively, in comparison to the control (Table 3).

Total N accumulated in the RB867515 variety at both mills was not significantly influenced either by inoculation or N fertilizer. A significant effect of N fertilizer over the total N accumulated in the whole plants (stems and total dry matter) was observed for RB72454 variety grown in Coruripe mill (Table 4).

Analysis of sugarcane juice quality showed that neither inoculation with the bacteria consortium nor fertilization with 120 kg ha^{-1} of N influenced the TRS, Brix, and Pol for the two sugarcane varieties grown in two diverse regions (Table 5). The results indicated that inoculation and N fertilizer did not influence sugar synthesis, despite increases of stem productivity and dry matter accumulation.

When TRS yield was expressed as kg ha^{-1} (Table 6), a significant response to inoculation and N fertilizer was observed for both varieties cultivated in the Coruripe mill. No difference was observed at the Sapucaia mill regarding total sugar yield (TRS) recovered from both sugarcane varieties which were inoculated or N fertilized. Despite the variability in the response of the varieties to inoculation and N fertilization, increases of 10,096 and 14,853 kg ha^{-1}, respectively, related to the total TRS accumulated in the plants cultivated at the two mills, were observed in comparison to the control. Real TRS gains with inoculation were 68% of that observed for nitrogen fertilization.

Table 7 shows delta ^{15}N values (δ^{15}N) for inoculated and non-inoculated sugarcane varieties with the consortium of diazotrophic bacteria. No significant differences in δ^{15}N

Table 6. Total recoverable sugar yield (TRS) of two grown sugarcane varieties inoculated with diazotrophs, fertilization with 120 kg ha^{-1} of N, and control in two Brazilian regions.

Treatment	RB867515 TRS kg ha^{-1}	RB72454 TRS kg ha^{-1}	Sum of the two varieties kg ha^{-1}	Increase compared to the control kg ha^{-1}
Sapucaia mill, RJ – crop 2006/2007				
Control	14,340	19,289	33,629	–
Inoculated	16,424	18,423	34,847	1218
120 kg N	17,024	19,706	36,730	3101
CV (%)	10.9		7.5	
Coruripe mill, AL – crop 2009/2010				
Control	18,376b	21,801b	40,177b	–
Inoculated	24,228a	24,827a	49,055a	8878
120 kg N	23,857a	28,072a	51,929a	11,752
CV (%)	15.7		14.0	
Sum of increase of the inoculation treatment compared to control				10,096
Sum of increase of nitrogen fertilization treatment compared to control				14,853

Notes: Means of four replications. Values followed by different letters in the columns differ by Scott-Knott test at 10% probability. Increases and the sums of the increases for treatments with inoculation and N fertilization compared to the control were not analyzed statistically. C.V: Coefficient of variation.

Table 7. ^{15}N delta (‰) of two grown sugarcane varieties with inoculation and non-inoculation of diazotrophs, as well as average delta ^{15}N of three reference plants grown in soil samples from the experimental areas.

Treatment	RB867515	RB72454
Sapucaia mill, RJ – crop 2006/2007		
Non-inoculated sugarcane	6.5 aB	8.7 aB
Inoculated sugarcane	6.6 aB	9.0 aB
Reference plants	11.2 A	
CV (%)	7.2	
Coruripe mill, AL – crop 2009/2010		
Non-inoculated sugarcane	2.3 aB	2.2 aB
Inoculated sugarcane	2.5 aB	2.4 aB
Reference plants	7.9 A	
CV (%)	7.5	

Notes: Means of three replicates. Small letters compare inoculated with non-inoculated sugarcane. Capital letters compare sugarcane (inoculated or non-inoculated) with reference plants. Scott-Knott test at 5% probability. C.V: Coefficient of variation. Reference plants: *P. mileaceum, P. glaucum,* and *S. bicolor.*

values were observed between inoculated and non-inoculated sugarcane varieties; although δ^{15}N values for inoculated and non-inoculated sugarcane varieties were lower than those observed in reference plants (*Panicum*, *Pennisetum*, and *Sorghum*). The isotope ^{15}N was not evaluated for the treatment with nitrogen fertilization, since the ^{14}N/^{15}N ratio is altered by the introduction of the synthetic nitrogen fertilizer, being thus cannot be used for the calculation of BNF.

Soil profiles for both regions showed an increase in ^{15}N natural abundance down to the layer between 15 and 30 cm depth, whereas available N increased within the layer between 30 and 45 cm depth for the Sapucaia plantation soil (Figure 1). On the other hand, available N in the Coruripe plantation soil was almost constant with depth, while ^{15}N natural abundance increased along the soil profile. Generally, ^{15}N natural abundance from available N found in the topsoil (0–15 cm depth) is lower than in subsoil (Unkovich et al., 2008). Results show that the soil does not provide ^{15}N isotope distribution uniformity according to the depth.

Discussion

Sugarcane variability in response to nitrogen fertilization, regardless of the N source, is a crop characteristic, for which there is still no conclusive explanation (Boddey et al., 2003; Franco et al., 2011; Vitti et al., 2008).

This study verified a response from both sugarcane varieties to nitrogen fertilization. Such effect was clear for RB867515 grown at Sapucaia mill, as well as both varieties grown at the Coruripe mill (Table 3). These results are distinguished from most literature reports, which usually do not show increases in productivity in the first year (planted cane) fertilized with nitrogen (Franco et al., 2010, 2011; Vitti et al., 2008). Several factors have been listed as being responsible for the lack of planted cane to respond

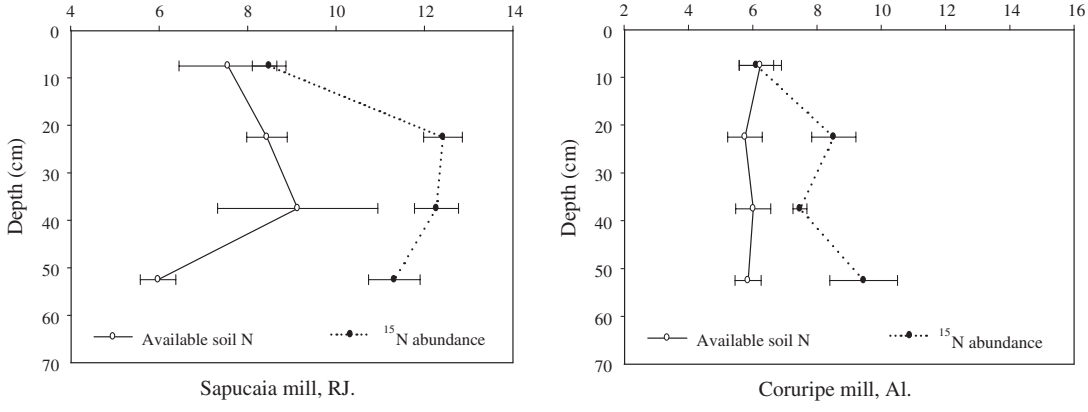

Figure 1. Available soil N (mg pot^{-1}) and δ ^{15}N natural abundance (‰), extracted by reference plants growing in pots with soil samples from the experimental areas.
Reference plants: *P. mileaceum, P. glaucum* and *S. bicolor*. Bars represent the standard error of the mean for three replicates.

to nitrogen fertilization, among them: organic soil matter mineralization during renewal of sugarcane plantations, N contained in planting stalks (Trivelin et al., 2002; Vitti et al., 2008), and BNF naturally associated to the crop (Boddey et al., 2001; Urquiaga et al., 2012; Yoneyama et al., 1997).

A proportional $\delta^{15}N$ value reduction in inoculated plants in comparison to non-inoculated plants should have been detected in case of a positive BNF contribution process (Unkovich et al., 2008). The hypothesis that the yield increase in the inoculated RB867515 variety did not result from the BNF is reinforced by the fact that N accumulated in stems and aerial part total dry matter did not differ between inoculated and non-inoculated plants, even when inoculation promoted significant increases in sugarcane yield. Very large differences between the ^{15}N abundance of the plant available N in the soil and that value in the sugar cane plants reinforces the belief that even without N_2-fixing bacteria inoculation, the sugar cane plants obtained large N contributions from BNF.

These results allow us to infer that inoculation promotes other benefits for sugarcane plants, which may be associated with actions of phytohormones that are synthesized by diazotrophs (Suman et al., 2001), solubilization of phosphate and zinc compounds (Shukla et al., 2008; Singh et al., 2007), or rhizosphere retention of essential nutrients (Yadav et al., 2009). Some other studies have stated that diazotrophs can act on plants, mainly by changing root system morphology, and thus influencing positively the crop development and productivity (Bashan et al., 2004). Muñoz-Rojas and Caballero Mellado (2003) evaluated micro-propagated sugarcane (MEX 57-473 variety) inoculated with *G. diazotrophicus* – PAL5^T strain and grown in sterile vermiculite. The authors verified increases in weight of dry matter roots, shoots, and total N accumulation, although total N levels were lower in comparison to the control. In addition, they concluded that benefits by *G. diazotrophicus* inoculation may have been derived from bacterial plant growth promoting effect.

Results from the current study corroborate other studies reported in the literature using the same multiple inoculum. Silva et al. (2009) evaluated the same sugarcane varieties grown in a Ultisol in the field in Seropédica, RJ, Brazil. They found that inoculation promoted the productivity of fresh stems for both varieties but did not affect the total N accumulated in plant tissues. No response to inoculation or to nitrogen fertilization was detected for either sugarcane varieties (planted cane) grown in an Inceptisol in the field in Campos dos Goytacazes, RJ, Brazil (Schultz et al., 2012). However, in the second ratoon, re-inoculated through aspersion on cut stems, the RB867515 variety responded to inoculation as well as with fertilization with 120 kg ha^{-1} of N. Nevertheless, ^{15}N delta values did not differ between inoculated and non-inoculated plants, allowing us to infer that the benefit provided by inoculation was not due to the BNF process associated with the addition of the biofertilizer.

A similar experiment carried out on an Alfisol at Embrapa Agrobiology, RJ, showed that the RB72454 variety responded to inoculation and nitrogen fertilization according to sugarcane yield parameters in contrast to the RB867515 that was not responsive to either treatment in the plant cane or first ratoon, but only to nitrogen fertilization in the second ratoon (Schultz et al., 2014).

This study revealed RB867515 and RB72454 variability in response to inoculation and soil types. Such results are similar to those of Oliveira et al. (2006). Sugarcane variability to respond to nitrogen fertilization may be due to environmental conditions, since precipitation and temperature data described in the Materials and Methods section are related to the regional level, which may not have been the experimental area actual condition at the time of fertilizer application and during the days after application. Environmental factors, especially drought, may influence the nitrogen fertilization efficiency negatively (Gava et al., 2001). Other authors observed that good water conditions along with the correct N supply may promote sugarcane root growth (Otto et al., 2009).

TRS, Brix, and Pol similarities among inoculated plants, N fertilization (120 kg ha^{-1}), and control treatments indicated that there was no effect on sugar synthesis in sugarcane plants. Among those researchers working in the area, there is no consensus concerning effects of the inoculation of diazotrophs on sugar synthesis in sugarcane plants. There are reports showing positive, negative, and non-applicable responses from nitrogen fertilization regarding sugarcane juice quality. These results have been attributed to differences between varieties, soil and climate conditions, crop management, N sources, and application procedures (Franco et al., 2010; Schultz et al., 2010).

Despite the variability from both sugarcane types to respond to inoculation and nitrogen fertilization, TRS total sum for both of them at the two regions showed a 10,096 kg ha^{-1} gain due to inoculation and 14,853 kg ha^{-1} due to nitrogen fertilization. Considering the U$ .138 (U$ 1.0 = R$ 3.323) reference value per kg of TRS paid to sugarcane producers (UNICA, 2015), inoculation provided a U$ 1393.25 gross profit, while nitrogen fertilization corresponded to U$ 2049.71. A higher economic gain (U$ 656.46) from nitrogen fertilization in comparison to inoculation represents approximately the cost of the commercial nitrogen fertilizer. Therefore, results suggest that the net return due to inoculation for these two experimental areas is the positive environmental balance which comes from inoculation, that can replace industrial nitrogen fertilizer.

In summary, this study suggests that bacterial inoculants benefit sugarcane plants by increasing the stems and sugar yield (TRS). However, inoculation did not alter the BNF naturally associated with the crop, suggesting that benefits derived from the inoculant may come from plant growth promoting substances which are synthesized by diazotrophs or other growth promotion effects described for the five strains used as a mixed inoculant.

Acknowledgments

The authors would like to thanks the Rio de Janeiro Rural Federal University, Agronomy Institute – Soil Department for the fellowships of the students. In addition, great consideration is given to the Brazilian Council for Scientific and Technological Development (CNPq) and Carlos Chagas Foundation (FAPERJ) for the research scholarship granted to the researchers from Embrapa Agrobiology. Sapucaia and Coruripe Mills are acknowledged for their support to conduct this research. The study publication was authorized by Embrapa Agrobiologia Editorial Publication Committee.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Embrapa [project number 03.13.00.016], [project number 02.13.08.004.00.03]; [grant number MCT/CNPq CT Agro 558329/2009-8], [grant number 470824/2013-1].

References

Arnold, S. L., & Schepers, J. S. (2004). A simple roller-mill grinding procedure for plant and soil samples. *Communication in Soil Science and Plant Analysis, 35*, 537–545. doi:10.1081/CSS-120029730

Baldani, J. I., Baldani, V. L. D., Seldin, L., & Döbereiner, J. (1986). Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. *International Journal of Systematic and Evolutionary Bacteriology, 36*, 86–93. doi:10.1099/00207713-36-1-86

Baldani, J. I., Pot, B., Kirchhof, G., Falsen, E., Baldani, V. L. D., Olivares, F. L., … Döbereiner, J. (1996). Emended description of *Herbaspirillum*; inclusion of [*Pseudomonas*] *rubrisubalbicans*, a mild plant pathogen, as *Herbaspirillum rubrisubalbicans* comb. nov.; and classification of a group of clinical isolates EF group 1 as *Herbaspirillum* species 3. *International Journal of Systematic and Evolutionary Bacteriology, 46*, 802–810. doi:10.1099/00207713-46-3-802

Baldani, I., Reis, V. M., Videira, S. S., Boddey, L. H., & Baldani, V. L. D. (2014). The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: A practical guide for microbiologists. *Plant and Soil, 384*, 413–431. doi:10.1007/s11104-014-2186-6

Bashan, Y., Holguin, G., & Bashan, L. E. (2004). *Azospirillum*-plant relationships: Physiological, molecular, agricultural and environmental advances (1997–2003). *Canadian Journal of Microbiology, 50*, 521–577. doi:10.1139/w04-035

Boddey, R. M., Polidoro, J. C., Resende, A. S., Alves, B. J. R., & Urquiaga, S. (2001). Use of the ^{15}N natural abundance technique for the quantification of the contribution of N_2 fixation to sugarcane and other grasses. *Australian Journal of Plant Physiology, 28*, 889–895. doi:10.1071/PP01058

Boddey, R. M., Soares, L. H. B., Alves, B. J. R., & Urquiaga, S. (2008). Bio-ethanol production in Brazil. Chapter 13. In D. Pimentel (Ed.), *Biofuels, solar and wind as renewable energy systems: Benefits and risks* (pp. 321–356). New York, NY: Springer.

Boddey, R. M., Urquiaga, S., Alves, B. J. R., & Reis, V. M. (2003). Endophytic nitrogen fixation in sugar cane: Present knowledge and future applications. *Plant and Soil, 252*, 139–149. doi:10.1023/A:1024152126541

Cavalcante, V., & Döbereiner, J. (1988). A new acid-tolerant nitrogen-fixing bacterium isolated from sugarcane. *Plant Soil, 108*, 23–31. doi:10.1007/BF02370096

CONSECANA. (2006). Conselho dos Produtores de Cana de Açúcar, Açúcar e Álcool do Estado de São Paulo. In *Manual de Instruções* (p. 112). Piracicaba: CONSECANA-SP.

Denmead, O. T., Macdonald, B. C. T., Bryant, G., Naylor, T., Wilson, S., Griffith, D. W. T., … Moody, P. W. (2010). Emissions of methane and nitrous oxide from Australian sugarcane soils. *Agricultural and Forest Meteorology, 150*, 748–756. doi:10.1016/j.agrformet.2009.06.018

Döbereiner, J. (1992). History and new perspectives of diazotrophs in association with non-leguminous plants. *Symbiosis, 13*, 1–13. https://agris.fao.org/agris-search/search.do?recordID=US201301767941

Empresa Brasileira De Pesquisa Agropecuária. (2006). *Sistema brasileiro de classificação de solos* (p. 306, 2nd. ed). Rio de Janeiro: Embrapa Solos.

Fernandes, A. C. (2000). *Cálculos na Agroindústria da Cana de açúcar* (p. 193). Piracicaba: STAB, Açúcar, Álcool e Sub produtos.

Franco, H. C. J., Otto, R., Faroni, C. E., Vitti, A. C., Oliveira, E. C. A., & Trivelin, P. C. O. (2011). Nitrogen in sugarcane derived from fertilizer under Brazilian field conditions. *Field Crops Research, 121*, 29–41. doi:10.1016/j.fcr.2010.11.011

Franco, H. C. J., Trivelin, P. C. O., Faroni, C. E., Vitti, A. C., & Otto, R. (2010). Stalk yield and technological attributes of planted cane as related to nitrogen fertilization. *Scientia Agricola, 67*, 579–590. doi:10.1590/S0103-90162010000500012

Gava, G. J. C., Trivelin, P. C. O., Oliveira, M. W., & Penatti, C. P. (2001). Crescimento e acúmulo de nitrogênio em cana-de-açúcar cultivada em solo coberto com palhada. *Pesquisa Agropecuária Brasileira, 36*, 1347–1354. doi:10.1590/S0100-204X2001001100004

Instituto Brasileiro de Geografia e Estatística, sistema IBGE de Recuperação Automática. (2015). Retrieved January 19, 2015, from https://www.sidra.ibge.gov.br/bda/prevsaf/default.Asp

Ledgard, S. F., Freney, J. R., & Simpson, J. R. (1984). Variations in natural enrichment of ^{15}N in the profiles of some Australian pasture soils. *Australian Journal of Soil Research, 22*, 155–164. doi:10.1071/SR9840155

Lima, E., Boddey, R. M., & Döbereiner, J. (1987). Quantification of biological nitrogen fixation associated with sugar cane using a ^{15}N aided nitrogen balance. *Soil Biology and Biochemistry, 19*, 165–170. doi:10.1016/0038-0717(87)90077-0

Lin, S. Y., Hameed, A., Shen, F. T., Liu, Y. C., Hsu, Y. H., Shahina, M., … Young, C. C. (2014). Description of *Niveispirillum fermenti* gen. nov., sp. nov., isolated from a fermentor in Taiwan, transfer of *Azospirillum irakense* 1989 as *Niveispirillum irakense* comb. nov., and reclassification of *Azospirillum amazonense* 1983 as *Nitrospirillum amazonense* gen. nov. *Antonie van Leeuwenhoek, 105*, 1149–1162. doi:10.1007/s10482-014-0176-6

Magalhães, F. M., Baldani, J. I., Souto, S. M., Kuykendall, J. R., & Döbereiner, J. (1983). A new acid-tolerant *Azospirillum* species. *Anais da Academia Brasileira de Ciências, 55*, 417–430. Retrieved from https://agris.fao.org/agris-search/search.do?recordID=US201302560110

Muñoz-Rojas, J., & Caballero Mellado, J. (2003). Population dynamics of *Gluconacetobacter diazotrophicus* in sugarcane cultivars and its effect on plant growth. *Microbial Ecology, 46*, 454–464. doi:10.1007/s00248-003-0110-3

Nogueira, A. R. A., & Souza, G. B. (2005). *Manual de laboratório: Solo, água, nutrição vegetal, nutrição animal e alimentos* (313pp). São Carlos: Embrapa Pecuária Sudeste.

Oliveira, A. L. M., Canuto, E. D. L., Reis, V. M., & Baldani, J. I. (2003). Response of micropropagated sugarcane varieties to inoculation with endophytic diazotrophic bacteria. *Brazilian Journal of Microbiology, 34*, 59–61. doi:10.1590/S1517-83822003000500020

Oliveira, A. L. M., Canuto, E. D. L., Urquiaga, S., Reis, V. M., & Baldani, J. I. (2006). Yield of micropropagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria. *Plant and Soil, 284*, 23–32. doi:10.1007/s11104-006-0025-0

Oliveira, A. L. M., Urquiaga, S., Döbereiner, J., & Baldani, J. I. (2002). The effect of inoculating endophytic N_2-fixing bacteria on micropropagated sugarcane plants. *Plant and Soil, 242*, 205–215. doi:10.1023/A:1016249704336

Oren, A., & Garrity, G. M. (2015). Notification that new names of prokaryotes, new combinations, and new taxonomic opinions have appeared in volume 65, part 2, of the IJSEM. *International Journal of Systematic and Evolutionary Microbiology, 65*, 1397–1399. doi:10.1099/ijs.0.000142

Otto, R., Franco, H. C. J., Faroni, C. E., Vitti, A. C., & Trivelin, P. C. O. (2009). Fitomassa de raízes e da parte aérea da cana-de-açúcar relacionada à adubação nitrogenada de plantio. *Pesquisa Agropecuária Brasileira, 44*, 398–405. doi:10.1590/S0100-204X2009000400010

Ramos, M. G., Villatoro, M. A. A., Urquiaga, S., Alves, B. J. R., & Boddey, R. M. (2001). Quantification of the contribution of biological nitrogen fixation to tropical green manure crops and the residual benefit to a subsequent maize crop using ^{15}N isotope techniques. *Journal of Biotechnology, 91*, 105–115. doi:10.1016/S0168-1656(01)00335-2

Reis, V. M., Estrada-De Los Santos, P., Tenorio-Salgado, S., Vogel, J., Stoffels, M., Guyon, S., … Caballero-Mellado, J. (2004). *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant-associated bacterium. *International Journal of Systematic and Evolutionary Bacteriology, 54*, 2155–2162. doi:10.1099/ijs.0.02879-0

Saravanan, V. S., Madhaiyan, M., & Thangaraju, M. (2007). Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. *Chemosphere, 66*, 1794–1798. doi:10.1016/j.chemosphere.2006.07.067

Schultz, N., Morais, R. F., Silva, J. A., Baptista, R. B., Oliveira, R. P., Leite, J. M., … Reis, V. M. (2012). Avaliação agronômica de duas variedades de cana-de-açúcar inoculadas com bactérias diazotróficas e adubadas com nitrogênio. *Pesquisa Agropecuária Brasileira, 47*, 261–268. doi:10.1590/S0100-204X2012000200015

Schultz, N., Pereira, G., Zonta, E., & Lima, E. (2010). Efeito residual da adubação na cana-planta e da adubação nitrogenada e potássica na cana-soca colhidas com e sem a queima da palhada. *Revista Brasileira de Ciência do Solo, 34*,811–820. doi:10.1590/S0100-06832010000300023

Schultz, N., Silva, J. A., Sousa, J. S., Monteiro, R. C., Oliveira, R. P., Chaves, V. A., … Urquiaga, S. (2014). Inoculation of sugarcane with diazotrophic bacteria. *Revista Brasileira de Ciência do Solo, 38*, 359–371. doi:10.1590/S0100-06832014000200005

Shukla, S. K., Yadav, R. L., Suman, A., & Singh, P. N. (2008). Improving rhizospheric environment and sugarcane ratoon yield through bio-agent amended farm yard manure in Udic Ustochrept soil. *Soil Tillage Research, 99*, 158–168. doi:10.1016/j.still.2008.02.007

Silva, M. F., Oliveira, P. J., Xavier, G. R., Rumjanek, N. G., & Reis, V. M. (2009). Inoculantes formulados com polímeros e bactérias endofíticas para a cultura da cana-de-açúcar. *Pesquisa Agropecuária Brasileira, 44*, 1437–1443. doi:10.1590/S0100-204X2009001100010

Singh, K. P., Suman, A., Singh, P. N., & Lal, M. (2007). Yield and soil nutrient balance of sugarcane plant ratoon system with conventional and organic nutrient management in subtropical India. *Nutrient Cycling Agroecosystems, 79*, 209–219. doi:10.1007/s10705-007-9108-4

Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews, 31*, 42–448. doi:10.1111/j.1574-6976.2007.00072.x

Suman, A., Shasany, A. K., Singh, M., Shahi, H. N., Gaur, A., & Khanuja, S. P. S. (2001). Molecular assessment of diversity among endophytic diazotrophs isolated from subtropical Indian sugarcane. *World Journal of Microbiology Biotechnology, 17*, 39–45. doi:10.1023/A:1016624701517

Trivelin, P. C. O., Oliveira, M. W., Gava, G. J. C., & Sarriés, G. A. (2002). Utilização de nitrogênio e produtividade da cana-de-açúcar (cana-planta) em solo arenoso com incorporação de resíduos da cultura. *Revista Brasileira de Ciência do Solo, 26*, 637–646. doi:10.1590/S0100-06832002000300008

UNIÃO DA INDÚSTRIA DE CANA-DE-AÇÚCAR. (2015). Retrieved January 19, 2015, from https://www.unica.com.brasp

Unkovich, M., Herridge, D., Peoples, M., Cadisch, G., Boddey, R., Giller, K., … Chalk, P. (2008). *Measuring plant-associated nitrogen fixation in agricultural systems* (p. 258). Canberra: ACIAR. (Monograph, 136).

Urquiaga, S., Xavier, R. P., Morais, R. F., Batista, R. B., Schultz, N., Leite, J. M., … Boddey, R. M. (2012). Evidence from field nitrogen balance and ^{15}N natural abundance data of the contribution of biological N_2 fixation to Brazilian sugarcane varieties. *Plant and Soil, 356*, 5–21. doi:10.1007/s11104-011-1016-3

Videira, S. S., Oliveira, D. M., Morais, R. F., Borges, W. L., Baldani, V. L. D., & Baldani, J. I. (2012). Genetic diversity and plant growth promoting traits of diazotrophic bacteria isolated from two *Pennisetum purpureum* Schum. genotypes grown in the field. *Plant and Soil, 356*, 51–66. doi:10.1007/s11104-011-1082-6

Vitti, A. C., Cantarella, H., Trivelin, P. C. O., & Rosseto, R. (2008). Nitrogênio. In L. L. Dinardo-Miranda, M. G. A. Landell, & A. C. M. Vasconcelos (Eds.), *Cana-de-açúcar* (pp. 239–269). Campinas: Instituto Agronômico.

Yadav, R. L., Suman, A., Prasad, S. R., & Prakash, O. (2009). Effect of *Gluconacetobacter diazotrophicus* and *Trichodermaviride* on soil health, yield and N-economy of sugarcane cultivation under subtropical climatic conditions of India. *European Journal of Agronomy, 30*, 296–330. doi:10.1016/j.eja.2009.01.002

Yoneyama, T., Muraoka, T., Kim, T. H., Dacanay, E. V., & Nakanishi, Y. (1997). The natural ^{15}N abundance of sugarcane and neighboring plants in Brazil, the Philippines and Miyako (Japan). *Plant and Soil, 189*, 239–244. doi:10.1023/A:1004288008199