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RESEARCH ARTICLE

Fertigated Sugarcane Yield and Carbon Isotope Discrimination ($\Delta^{13}C$) Related to Nitrogen Nutrition

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Abstract The aim of this study was to evaluate the response of sugarcane to nitrogen (N) application with drip irrigation and the relation with carbon isotope discrimination ($\Delta^{13}C$), aboveground dry matter cane yield and the N balance in consecutive ratoon crops of sugarcane. An experiment was set up in Jaú, SP, Brazil, in which the second and third ratoon crop cycles (2008/2009 and 2009/2010) were evaluated. The experiment included an unfertilized N control in both years (T1), and the following three nitrogen (N) fertilizer rates (in kg ha^{-1}) applied in 2008 and 2009, respectively: 70 and 50 (T2), 140 and 100 (T3), and 210 and 150 (T4). Fertilization with N caused a marked gain in stalk yields by 98 Mg ha^{-1} in 2 years. The N export with harvest was higher than N application in the control treatment T1 and at the lower rate (T2); this, in addition to the observed linear response to N, indicate the need to increase N fertilization in irrigated sugarcane. The values of $\Delta^{13}C$ decreased with the increase of N supply showing a significant negative correlation ($p < 0.05$) with stalk as well as whole plant aboveground dry matter yields. The values of $\Delta^{13}C$ in top leaves may be used as a tool to characterize the N status of sugarcane plants and its relation to aboveground dry matter and yield.

Keywords Drip-irrigation · C_4 plants · Water management · Sugar · N fertilization

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Introduction

The combination of nitrogen fertilizer and irrigation has been shown to have positive effect on sugarcane crop yield. Nitrogen is a structural constituent of many organic compounds, including amino acids and nucleic acids (Epstein and Bloom 2004). It is the nutrient that most limits the productivity of sugarcane, a plant of the *Poaceae* family which possesses C_4 carbon metabolism and is characterized by high net photosynthesis rates and dry matter production (Arruda 2012).

Brazil is currently the leading sugarcane producer in the world (FAO 2014). However, the yield of sugarcane in part of the country is low, around 70 Mg ha^{-1}, largely due to water deficit which occurs even in regions with adequate rainfall, but with irregular distribution. This limits the productive capacity of the crop, which is further reduced when N is limiting (Gava et al. 2011; Thorburn et al. 2003).

Traditionally, sugarcane in Brazil is grown under rainfed conditions. However, with the expansion of sugarcane towards the Brazilian Center-West regions, characterized by a long dry winter, the crop may require irrigation in order to guarantee high yields. In this case, salvage or supplementary irrigation is needed to provide water during periods of water stress and to ensure stability of production (UNEP 2011).

Globally, approximately 301 Mha are irrigated, which represents around 20 % of the cultivated land (Gunda and Youngs 2013). In Brazil, although land with irrigation potential was estimated at 29.3 Mha (AQUASTAT 2014), only 4.46 Mha are actually equipped with irrigation, of which sugarcane accounts for 24 % (IBGE 2006).

The carbon isotope discrimination ($\Delta^{13}C$) in atmospheric CO_2 fixation by higher plants can be evaluated by measuring the natural variation of the heavy carbon isotope ($\delta^{13}C$) assimilated. This technique can be used as an indicator to monitor and identify plants under environmental stress conditions such as plants under water deficit and N deficiency or excess (Meinzer and Zhu 1998; Clay et al. 2001; Monneveux et al. 2007).

The interpretation of the $\Delta^{13}C$ values in sugarcane is more complex when compared to plants of the C_3 cycle, as it does not depend only on the internal and external CO_2 pressure. $\Delta^{13}C$ of plants with C_4 cycle also depends on the leakiness of CO_2 between the PEPC and the RuBisCo compartments (Farquhar 1983; Henderson et al. 1992). Thus, the values of $\Delta^{13}C$ in C_4 plants are associated with the concentration of CO_2 in the bundle sheath cells, which is substantially greater than that of the leaf mesophyll cells and the atmosphere.

The conceptual model of the discrimination of stable carbon isotopes ($\Delta^{13}C$) in C_4 plants is represented by Eq. 1, proposed by Farquhar (1983), with alterations by Henderson et al. (1992):

$$\Delta^{13}C_4 = a + [b4 + \Phi(b3 - s) - a] \times pi/pa \quad (1)$$

where: *a* represents the discrimination of the stable C isotopes of atmospheric CO_2 diffusing through the stomata as in C_3 plants (+4.4 ‰); *b3* is the isotopic discrimination of C that occurs via carboxylation by RuBisCo (+29 ‰); *b4* is the isotopic discrimination of C during the dissolution and conversion to HCO_3^- and fixation by PEPC (−5.7 ‰); *s* is the isotopic discrimination of C of the CO_2 that is dispersed to the leaf mesophyll from the total CO_2 which is decarboxylated in the bundle sheath cells (+1.8 ‰); Φ is the leakiness of CO_2 due to decarboxylation in the bundle sheath cells; and *pi/pa* is the ratio of the partial pressure of CO_2, in the intracellular space (stomatal chamber) (*pi*) and in the environment (*pa*), respectively.

Values of $\Delta^{13}C$ in plants of the C_4 cycle and their relation to yield and the efficiency of water and nutrient use have been the subject of various studies (Hubick et al. 1990; Saliendra et al. 1996; Meinzer and Zhu 1998). However, for sugarcane, carbon isotope discrimination has not been well evaluated, considering different cultivars in contrasting conditions of water availability and nutrition. Nevertheless, $\Delta^{13}C$ if well understood will assist in the prediction of crop yields under different environmental conditions. $\Delta^{13}C$ is an important tool for use in genetic improvement, since it can predict plant responses in a non-destructive manner and throughout the crop cycle.

In light of the above, the aim of this study was to evaluate whether N availability in sugarcane irrigation management practices increases crop yield and has an effect on carbon isotope discrimination ($\Delta^{13}C$), as well as on aboveground dry matter yield and on the N balance in consecutive ratoon crops of sugarcane.

Materials and Methods

Characterization of the Experimental Area, Treatments and Experimental Design

The experiment was developed at the APTA West Center unit, located in the region of Jaú, in the state of São Paulo, Brazil (22°17′S, 48°34′W, at a mean altitude of 580 m a.s.l.). The second and third ratoons of a sugarcane crop (2008/2009 and 2009/2010 crop years) were evaluated. The cultivar used was SP80-3280, which is widely planted in the states of São Paulo and Mato Grosso do Sul (Chapola et al. 2011) in high fertility soils and is highly responsive to mineral fertilization.

The soil was classified as a Red Latosol in the Brazilian system (EMBRAPA 2013) or Typic Hapludox, according to the Soil Survey Staff (2010). Its chemical and physical characteristics determined in the 0–25 and 25–50 cm layers are shown in Table 1.

The experiment consisted of three doses of N under irrigation, as well as plots without N, namely, T1, unfertilized control; T2, 70 kg ha^{-1} of N in 2008 and 50 kg ha^{-1} of N in 2009; T3, 140 kg ha^{-1} of N in 2008 and 100 kg ha^{-1} of N in 2009; and T4, 210 kg ha^{-1} of N in 2008 and 150 kg ha^{-1} of N in 2009. A randomized block design with four replications was used. Nitrogen was split-applied as urea twice weekly by underground drip irrigation in small amounts throughout the crop cycle. Each month, a percentage of the total dose was supplied to the crop according to the phenological stage of the plants (Fig. 1); around 4 months before harvest (June/2009 and July/2010) the application of nitrogen was stopped. This phase corresponded to maturation of the sugarcane. All plots were fertilized with a common dose of 150 kg ha^{-1} of K_2O, as potassium chloride, throughout the crop cycle and application stopped 3 months before harvest. The doses of N in the third ratoon were smaller than in the second ratoon because nutrient requirements (and yield) decrease as the ratoons age.

Table 1 Chemical and physical characteristics of the soil in the experimental area

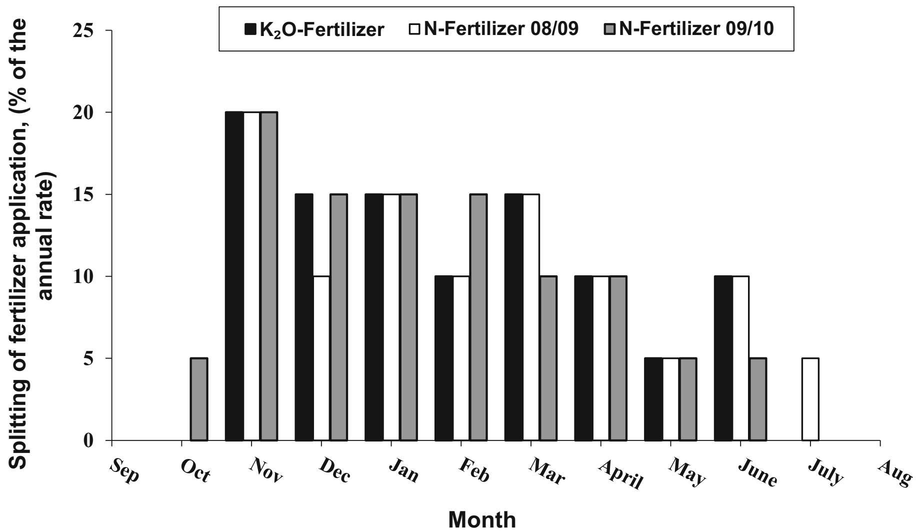
Depth (m)	pH	TOC (g dm^{-3})	N–IN (mg kg^{-1})	P (mg dm^{-3})	K (mmol$_c$ dm^{-3})	Ca (mmol$_c$ dm^{-3})	Mg (mmol$_c$ dm^{-3})	CEC (mmol$_c$ dm^{-3})	V (%)	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})
0–0.25	5.2*	8.7	6.0	17	1.7	15	7	70	56	660	70	270
0.25–0.50	4.8	7.5	6.6	20	1.2	9	4	32	44	570	100	330

Chemical and particle-size analysis according to Van Raij et al. (2001) and Embrapa (2006), respectively

TOC total organic carbon, *CEC* cation exchange capacity

*pH in 0.01 M $CaCl_2$

Fig. 1 The percentage distribution of N and K_2O annual rates applied through fertigation in 2008/2009 and 2009/2010



Spacing and Irrigation Management

The plots consisted of five 30-m rows of sugarcane. In all treatments the paired-row planting arrangement (planting in W) was used, with a spacing of 1.80 m between double rows and 0.4 m between paired sugarcane rows, with a dripline installed between them for irrigation. The drip-line adopted was the DRIPNET PC 22135 FL model (Adana, Turkey), with a 1.0 L h^{-1} flow rate and equipped with drip nozzles every 0.5 m, which were buried at a depth of 25 cm beneath the soil surface.

Water was applied to replace 100 % of the crop evapotranspiration (CET), according to the Penman–Monteith method (Howell and Evett 2004). The frequency of irrigation considered the available water capacity (AWC) of the soil of 70 mm, water supplied by rainfall (R), and the atmospheric demand due to evapotranspiration of sugarcane (CET). The 10-day water balance and the water deficit (DEF) estimated for both crop cycles are shown in Fig. 2A, B.

Total rainfall during the first cycle of the study (2008/2009 crop year) was 1740 mm, well above the historical mean for the region, which is 1460 mm. In this period, irrigation was performed with 293 mm of water to a total of 2033 mm. Crop evapotranspiration (CET) was 1250 mm, and the water deficit was 11 mm. The mean maximum and minimum temperatures observed were 29.3 and 15.2 °C, respectively. For the second crop cycle of this study (2009/2010), the measured rainfall was 1435 mm, which was close to the historical mean for the period. The quantity of water applied by irrigation was 390 mm, distributed throughout the crop cycle. The accumulated crop evapotranspiration (CET) was 1320 mm, with a water deficit of 28 mm. The maximum and minimum temperatures observed during the development cycle of 381 days were 29.2 and 16.4 °C, respectively.

Evaluations and Statistical Analysis

In the final harvest of each ratoon crop cycle, 2 m of the sugarcane row were collected per plot for the determination of above-ground plant biomass. Tops (green leaves), dry leaves and stalks were taken apart and weighed. The fresh material was subsequently ground in a forage chopper and subsamples was taken for the determination of plant dry matter after drying in a forced-air-circulation oven at 65 °C until constant mass. Dried samples were ground and the N content was determined by the Kjeldahl method (Malavolta et al. 1997). Subsequently, plants of 30 m of the sugar cane row were manually dehusked and cut at the soil surface

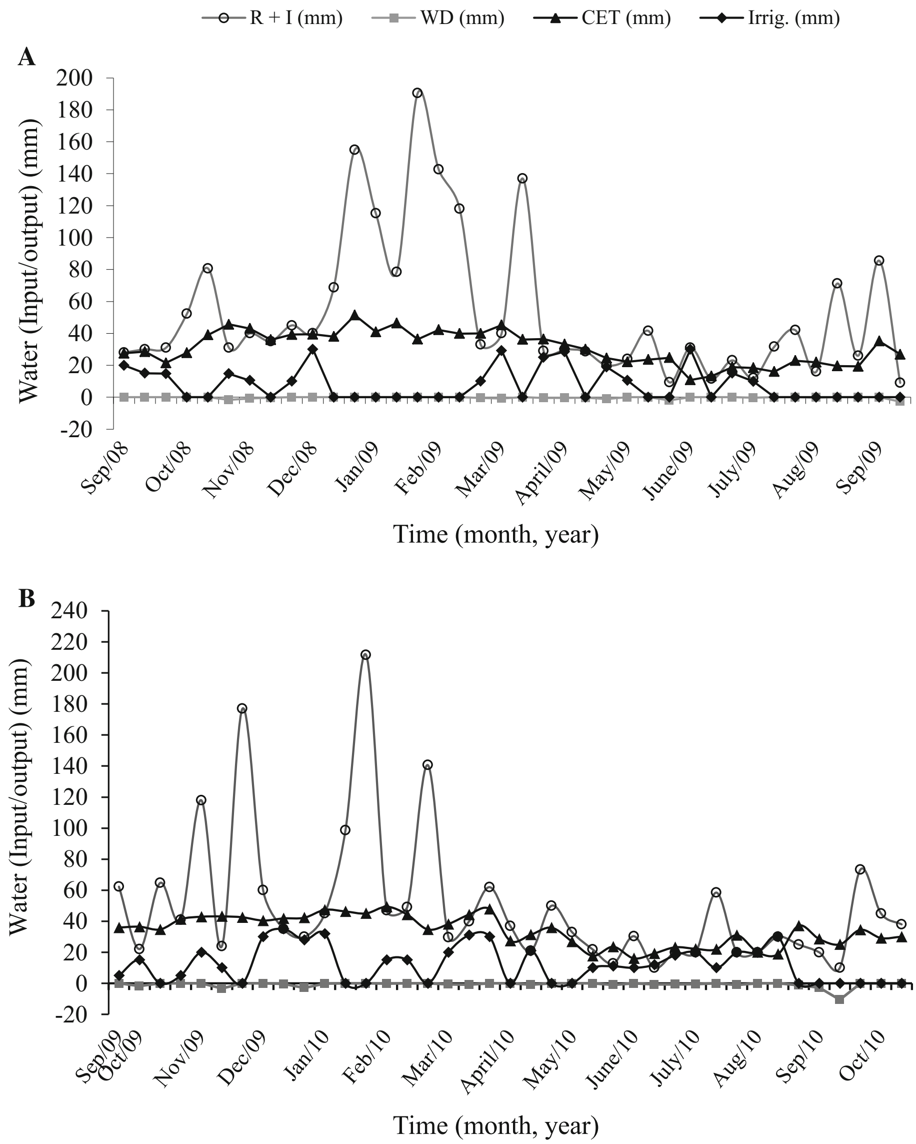


Fig. 2 Ten-day water balance for the 2008/2009 period (**A**), and 2009/2010 period (**B**). *R* + *I* rainfall + irrigation, *WD* water deficit, *CET* crop evapotranspiration, *irrig* irrigation

level (without burning), and were weighed for the determination of stalk yield Mega gram per hectare (Mg ha^{-1}). At the same time, ten stalks were collected per plot for determination of sugar and fiber content. Sugar yields were calculated taking into account the concentration of total sugars, measured as degrees Brix, sucrose concentration, determined by polarimetry (% pol), and fiber content, using the procedures described by Fernandes (2003).

Assuming that the N content in the soil at the beginning of the experiment was the same in all treatments, the N balance of the sugarcane crop was calculated based on the quantity of N fertilizer supplied via irrigation and that exported with the harvested stalks.

The results were subjected to analysis of variance ($p < 0.05$) and the treatments were evaluated by regression analysis. Regressions were determined using the SigmaPlot software (Version 11.0, Systat Software Inc., San Jose, USA).

For the determination of $\Delta^{13}C$, samples of top visible dewlap leaves (first leaf with visible ligule, as proposed by Kuijper in Van Dillewijn 1952) were collected from 360-day-old ratoon plants for the 2008/2009 cycle and at 208, 291 and 380 days for the 2009/2010 crop year (Meinzer and Zhu 1998). Leaves (15 per plot) were collected in the morning, preferentially at about 9:00 a.m. After collection, the mid-rib was removed and 20 cm blades of the middle region of leaves were used for analysis. After washing in deionized water, these samples were dried in a forced-air-circulation oven at 65 °C. The samples were then ground in a Wiley mill and stored in snap-cap plastic containers. The $\delta^{13}C$ isotope composition and the total N content of the samples were determined in a mass spectrometer (ANCA-GSL Hydra 20–20 model, SERCON Co., Crewe, GBR) coupled to a C and N automatic analyzer (Barrie and Prosser 1996).

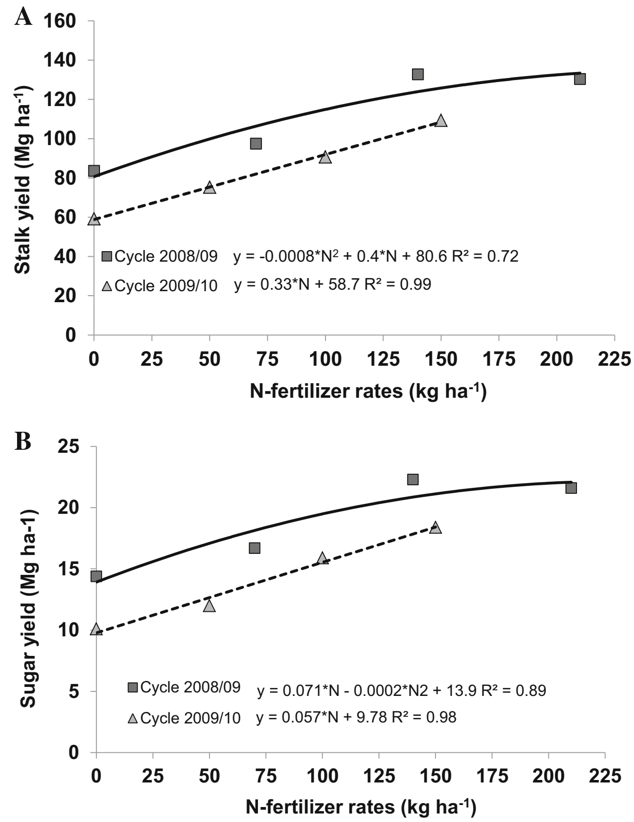


Fig. 3 Stalk (**A**) and sugar (**B**) yield as affected by N fertilization evaluated over two cycles of fertigated sugarcane

Values of $\Delta^{13}C$ (‰) were calculated with Eq. 2, described by Farquhar (1983), Henderson et al. (1992) and Cernusak et al. (2013).

$$\Delta^{13}C\permil = [(\delta^{13}C_a - \delta^{13}C_p)]/[1 + (\delta^{13}C_p/1000)] \quad (2)$$

where: $\delta^{13}C_a$ = reference atmospheric CO_2 isotopic composition ($\delta^{13}C$ of -8.0 ± 0.01 ‰) in relation to the Pee Dee Belemnite (PDB) international standard; $\delta^{13}Cp$ = isotopic composition of the sugarcane leaves.

Results

Stalk and Sugar Yield

Nitrogen fertilization promoted significant ($p < 0.05$) increments in stalk and sugar yield in the two crop cycles (Fig. 3A, B). Nevertheless, in each year, there were distinct responses in relation to the N doses. For the second ratoon (2008/2009 crop season) there was a quadratic response, whereas for the third ratoon (2009/2010 crop season) a linear response was observed.

In the 2008/2009 crop cycle, doses of up to 210 kg ha^{-1} of N were applied. However, in the second crop cycle, due to natural aging of ratoons and an increase in the number of dead plants, the N doses applied were reduced to a maximum of 150 kg ha^{-1} of N in the 2009/2010 crop season, but, it is apparent that yields would have responded to higher N rates as well (Fig. 3). An increase in the dose of N from 70 to 210 kg ha^{-1} (2008/2009) and from 50 to 150 kg ha^{-1} (2009/2010) increased stalk yield by a mean of 40 %.

Nitrogen fertilization did not affect fiber, total sugar (Brix) or the commercially recoverable sucrose (CRS) content of sugarcane (Table 2). In the 2008/2009 crop cycle the average values of fiber, CRS and total sugars were 13, 17 % and 22° Brix, respectively. The corresponding values for the 2009/2010 cycle were 16, 17 % and 24° Brix respectively.

Carbon Isotope Discrimination

There was a decrease in $\Delta^{13}C$ ‰ in sugarcane leaves with the increase of N application, with differences of 0.250 and 0.254 (Δ‰) between the control and the highest dose of N for the second and third ratoon, respectively ($p < 0.05$) (Fig. 4), with a mean reduction of 0.0014 ‰ in $\Delta^{13}C$ for each kg of N applied through fertigation.

Table 2 Fiber, commercially recoverable sucrose (CRS) and total sugars (° Brix) of stalks as affected by N rates applied under fertigation

Cycle 2008/2009				Cycle 2009/2010			
N rate (kg ha^{-1})	Fiber (%)	CRS	°Brix	N rate (kg ha^{-1})	Fiber (%)	CRS	°Brix
0	13.6	17.2	22.4	0	16.8	17.0	24.3
70	13.0	17.1	22.3	50	15.8	16.0	23.6
140	13.6	16.3	21.4	100	16.3	17.5	24.3
210	13.6	16.6	22.0	150	16.0	16.8	23.6
R^2 L.R.	ns	ns	ns		ns	ns	ns
R^2 Q.R.	ns	ns	ns		ns	ns	ns
CV (%)	5.4	2.9	1.9		4.8	5.8	5.5

ns not significant, *CV* coefficient of variation, *L.R.* linear regression, *Q.R.* quadratic regression

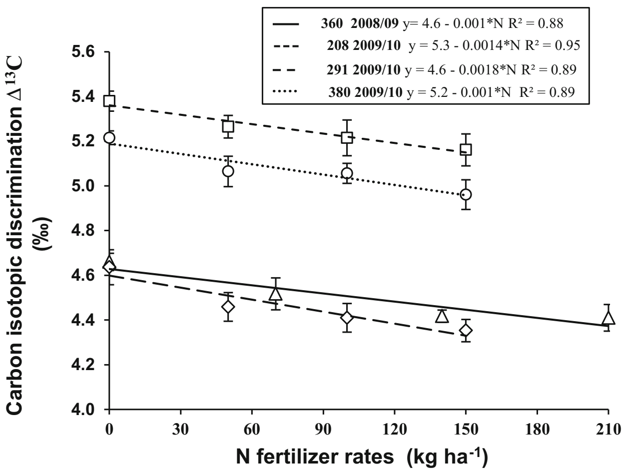


Fig. 4 Carbon isotope discrimination of ^{13}C ($\Delta^{13}C$ ‰) in sugarcane leaves as affected by N fertilization under drip irrigation in different cycles and sampling times. In the 2008/2009 cycle, leaves were collected 360 days after the previous harvest; the corresponding time intervals for the 2009/2010 cycle were 208, 291, and 380 days

Significant inverse correlations were obtained between stalk yield or total above-ground biomass yield and the values of $\Delta^{13}C$ in sugarcane leaves for the second ($R^2 = 0.57$ and $0.67, p < 0.05$) and third ($R^2 = 0.62$ and $0.66, p < 0.05$) ratoon, respectively (Fig. 5A, B). From the results of Fig. 4, it can be inferred that $\Delta^{13}C$ values relates to N concentration in sugarcane plants because a decrease in $\Delta^{13}C$ was observed for total residue biomass and for stalk yield.

Balance Between the N Applied as Fertilizer and Exported by Stalks

In the sum of two cycles, the balance between N fertilizer inputs and export with harvested stalks indicates that for the low and intermediate N rates (120 and 240 kg ha^{-1} N in 2 years) the deficits or surpluses of N were small. However, for the control treatment, about 50 kg ha^{-1} N was removed from the system with 142 Mg ha^{-1} stalks harvested in 2 years. This negative balance is relatively small because only 31 and 36 % of the N contained in the above-ground biomass was in the stalks in the unfertilized control whereas the corresponding figures for the fertilized treatments were 51–64 % (results not shown). For the treatment with the highest N rate (360 kg ha^{-1}) the average N surplus was 137 kg ha^{-1}, although stalk yields were much higher (240 Mg ha^{-1}) than that of the control (Fig. 6). All the trash was maintained over the soil. There was a marked response to fertigation with N: relative to the control, stalk yields increased by 69 % (98 Mg ha^{-1}) in the sum of 2 years when 360 kg ha^{-1} N was applied (linear response, $R^2 = 0.97$ and $p < 0.05$) (Fig. 6B).

In the unfertilized control treatment T1, the quantity of N in dry leaves and tops that are returned to the soil was high (around 70 kg ha^{-1} of N), representing 69 % of all N extracted by the crop. In contrast, in the following year, the values were lower, at about 30 kg N ha^{-1} (64 % of all N absorbed), y indicating further depletion of soil N supply in this sandy soil.

The lower proportion of N in dry leaves in the 2009/2010 cycle compared to that of the previous year may be due to time of harvest and weather conditions, which may have favored the loss of leaves and the translocation of nutrients in the 2008/2009 cycle (Fig. 7).

Discussion

The results herein obtained indicate that the demand for N in irrigated sugarcane ratoons might be greater than the doses currently recommended for dryland management,

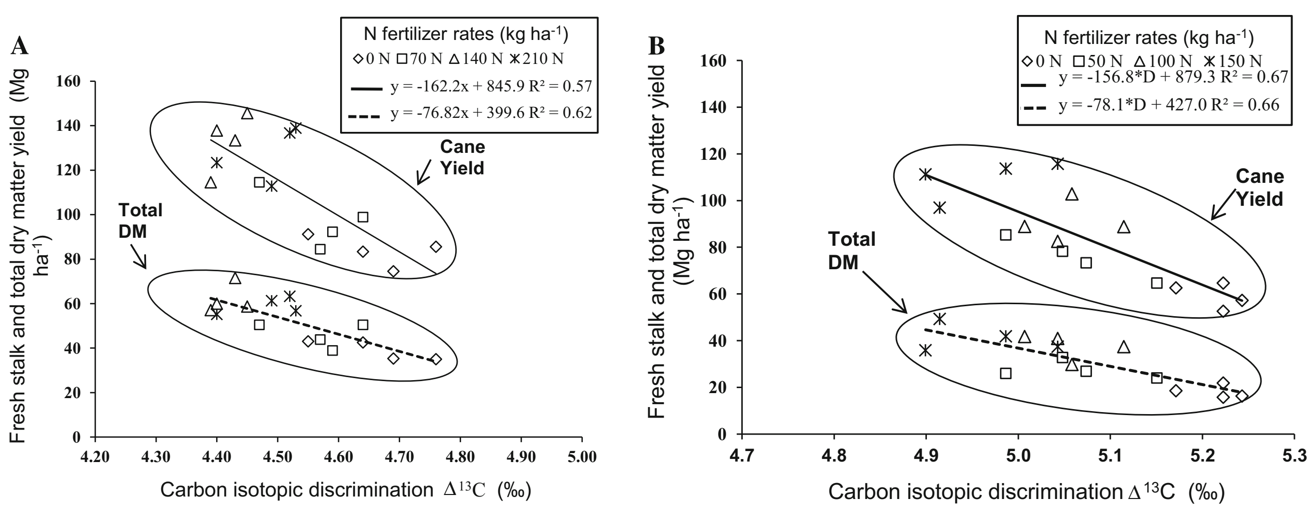


Fig. 5 Fresh stalk and whole plant above-ground dry matter (total DM) yields related to carbon isotope discrimination ($\Delta^{13}C$ ‰) over two sugarcane crop cycles (2008/2009 (**A**) and 2009/2010 (**B**)

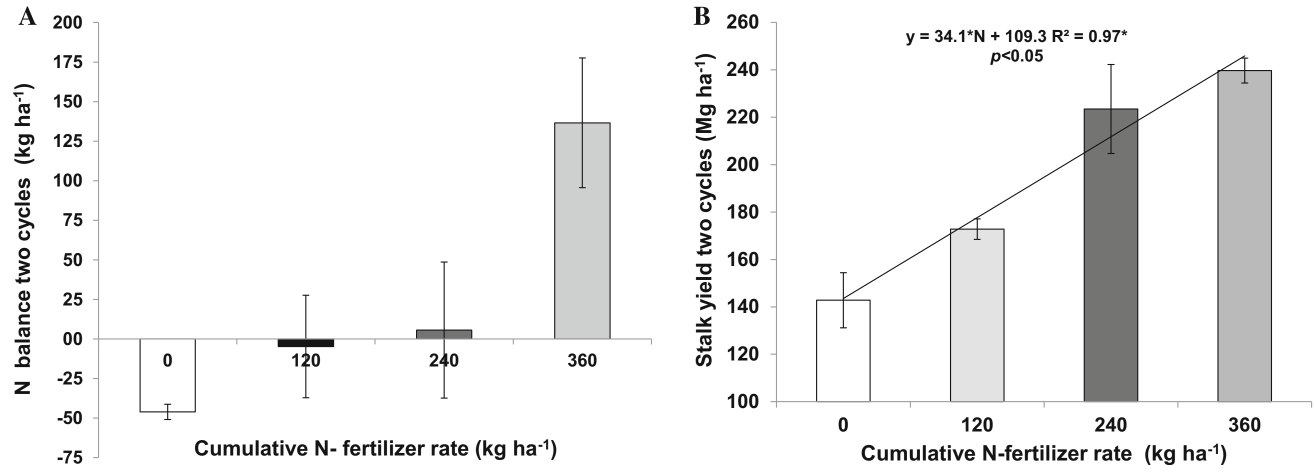


Fig. 6 Nitrogen balance between N inputs and exports (**A**) and cumulative stalk yields (**B**) as affected by N fertilization. Data are the sum of two cycles. Nitrogen balance is the difference between N applied as fertilizer and N removed with the stalks harvested. *Bars* indicate standard deviation in sum of two cycles

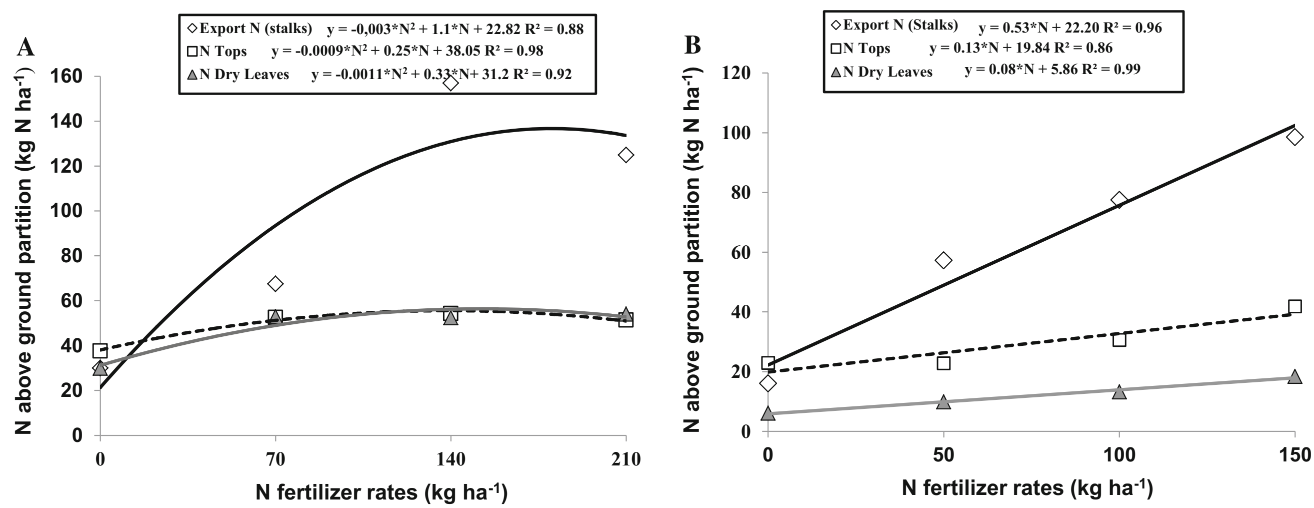


Fig. 7 Partition of N in the aboveground parts of sugarcane in two crop cycles: 2008/2009 (**A**), and 2009/2010 (**B**)

which range from 100 to 120 kg ha^{-1} N for expected yields greater than 100 Mg ha^{-1} of stalks (Van Raij and Cantarella 1996). A recent network of 15 field trials to evaluate the response of sugarcane ratoons to N rates up to 180 kg ha^{-1} under rainfed conditions in southwest Brazil showed that the average stalk yield gains were 9.6 Mg ha^{-1}, obtained with 148 kg ha^{-1} N, the rate for maximizing yields; the most economic N rate was 120 kg ha^{-1} (Rossetto et al. 2010). The stalk yield increases are much lower than the average 49 Mg ha^{-1} stalk gains obtained with the maximum N rates in the present study. Gava et al. (2011), working in the same region of our study, observed yield gains of 25.4 and 35.2 Mg ha^{-1} in plant cane and first ratoon, respectively, with fertigated sugarcane when compared to a rainfed crop.

Drip irrigation provides some benefits in relation to conventional rainfed management, such as reduction of nutrient losses, since the nutrient is applied in repeated small doses and meets crop demands for both nutrients and water. Other studies have indicated that supplying N by repeated small doses via irrigation increases the N use efficiency compared to a single application under rainfed farming (Wiedenfeld and Enciso 2008; Ng Kee Kwong et al. 1999; Gava et al. 2011). Yields of the second and thirty ratoons in the range of 100–120 Mg ha^{-1} are seldom observed under rainfed conditions in sandy soils in the state of São Paulo, such as those of the present study. Nutrients such as K could also be applied in repeated small doses (Dalri and Cruz 2008; Ng Kee Kwong et al. 1999). There is currently no recommendation for N fertilization in sugarcane in irrigated management practices in Brazil, and calibration of this fertilization is necessary.

Thorburn et al. (2003), using N rates up to 240 kg ha^{-1} N, obtained an increase in stalk yield using drip irrigation with the cultivar Q124 in four consecutive cycles in eastern Australia. Wiedenfeld and Enciso (2008) reported linear responses of stalk yields to increasing N rates with surface drip-irrigation in semi-arid subtropical climate conditions in Texas, USA. Crop response to N is intensified with drip irrigation (Singh and Mohan 1994; Wiedenfeld 1995; Wiedenfeld and Enciso 2008) due to a decrease in water restriction and an increase in the concentration of mineral forms of N in the soil solution, especially in the area near the emitter throughout the period of sugarcane growth (Stanley et al. 1990; Thorburn et al. 2003). One role of nitrogen in plants is to increase cell division and differentiation (Taiz and Zeiger 2004), which will increase sugarcane stalk yield.

Increasing rates of N did not affect fiber and sucrose contents and Brix measurements in sugarcane stalks (Table 2) as was also observed by Dalri and Cruz (2008), Korndörfer et al. (1997), Trivelin et al. (2002) and Franco et al. (2010). As sucrose concentrations in the juice were not affected by the N treatments, the sugar yield response to N followed the same trend as that of the stalk yield (Fig. 3B). In contrast, the studies of Singh and Mohan (1994) and Wiedenfeld (1995) indicated reduction in sugar content of the stalks with increasing rates of fertilizer N. This may happen for sugarcane cultivars that are not responsive to N or in situations with excess N supply, which was not the case of the present study since yield responses to N were linear (Fig. 6). According to Muchow et al. (1996), the supply of high quantities of N stimulates plant growth and dilutes the concentration of sugars in the stalk or delay maturation, and consequently, decreases its industrial quality. Wiedenfeld and Enciso (2008) observed a small sugar yield increase of about 27 % when N applied by underground dripline in Chernozems from Texas, USA increased from 60 to 180 kg ha^{-1}.

For each mega gram of stalk produced a mean of 1.46 kg of N accumulated in the plant and 50 % of this (0.73 kg N t^{-1}) was exported with the stalks. Bell and Garside (2014) showed that the sugarcane stalks contained 0.57 kg N t^{-1} cane under 100 % irrigation condition in Australia. The treatments had a direct effect on the N distribution percentage in the aboveground parts of the plant (Fig. 7). In the T2 treatment in the 2nd ratoon (Fig. 7A) and T1 in both cycles (Fig. 7A, B), the N contained in the stalks was less than 40 % of all the N absorbed by the sugarcane plants, whereas for the other treatments, the N exports were greater than 50 % of the total N of the aboveground parts. In the T1 treatment, the lack of N had a direct effect on stalk yield, resulting in lower percentage of N accumulated in the stalks.

Relevant amounts of N surplus in the balance of fertilizer inputs and exports were only observed for treatment 4 (Fig. 6). The difference between applied and exported N with the highest rate (360 kg ha^{-1} N in 2 years) was 137 kg ha^{-1} which could potentially be lost by leaching or in gaseous forms through denitrification. The trash left on the field in this treatment contained 128 kg ha^{-1} N, which can be recycled in the field (Fortes et al. 2012; Trivelin et al. 2013). Although usually more N taken up by sugarcane plants comes from soil than from fertilizer (Dourado Neto et al. 2010; Franco et al. 2011) and hence, losses of fertilizer N cannot be ruled out, in our study the overall N balance was quite tight, considering also the N that remained in the trash which returned to the field and not taking into account the N in the root system. Therefore, even for the highest N rate used it is likely that most N applied could be conserved in the soil and plant system with the split N application through fertigation. The linear nature of the yield response is an additional indication of that.

In maize, also a C4 plant, in the northern USA Clay et al. (2001) observed that the $\Delta^{13}C$ decreased when fertilization increased from 0 to 168, 201 and 234 kg ha^{-1} of N over 3 years of evaluation, respectively, with low, medium and high water availabilities. According to the authors, for each kg of N added, when water was not a limiting factor, there was a decrease of 0.01 ‰ in the $\Delta^{13}C$ of maize plants. The reduction in the $\Delta^{13}C$ per unit of applied N observed by these authors was ten times greater than that observed in this study. However, sugarcane has a longer growth cycle, exhibiting a much larger biomass yield compared to maize, thus showing a dilution effect of N that occurs as the plants grow (Oliveira et al. 2013); in addition, the N doses in the maize study were also greater than those used in sugarcane.

According to Meinzer and Zhu (1998), small variations in the leakiness of CO_2 from the sheath cells alter the $\Delta^{13}C$ of C_4 plants such as sugarcane. In our study, N fertilization altered the $\Delta^{13}C$ in plants, as shown by the significant ($p < 0.05$) inverse linear correlations ($R^2 = 0.88$) for the first and ($R^2 = 0.95$, 0.89 and 0.89) second crop cycles. Meinzer and Zhu (1998) observed a direct and linear relationship between the increase in Φ, the parameter for CO_2 leakiness, and $\Delta^{13}C$ in sugarcane leaves. In our study the correlation between these parameters was significant ($R^2 = 0.94$, $p < 0.01$) (Results not shown).

Meinzer and Zhu (1998), testing four commercial and four non-commercial clones of sugarcane, grown with different doses of N in Hawaii, USA, observed that the $\Delta^{13}C$ concentration in the leaves of sugarcane decreased linearly ($R^2 = 0.84$, $p < 0.05$) with an increase in quantum yield (photon absorption) of the plants. Saliendra et al. (1996) observed a reduction in the $\Delta^{13}C$ concentration from 4.87 to 3.55 with an increase in the rate of biomass growth from 45 to 110 mm day^{-1} in four varieties of irrigated sugarcane. Clay et al. (2001) also obtained an inverse linear correlation ($R^2 = 0.84$, $p < 0.01$) between the $\Delta^{13}C$ concentration and maize grain yield in a maize crop grown with three levels of irrigation and three levels of N fertilization.

Lower mean values of carbon isotopic discrimination were observed for all the treatments in the first crop cycle (Fig. 4), showing that the values of $\Delta^{13}C$ vary from year to year, and even according to the time period within the growth cycle. Consequently, an absolute value of $\Delta^{13}C$ cannot be used as indicator of stress to N and always requires a reference value. However, $\Delta^{13}C$ proved to be well correlated with sugarcane yield, especially in environments with reduced water deficit.

The results of treatment T1 (control) indicate the importance of N fertilization of sugarcane in soils with small N stocks in the organic matter. Under insufficient N nutrition the yield decline of subsequent ratoons will be accelerated and the need to replant the field will be anticipated. The difference in accumulated stalk yield between the control and the treatment with highest rate of N (98 Mg ha^{-1}) demonstrates the large response of sugarcane to nitrogen fertilization. This result draws attention to the effect of water availability and also to the potential increase in soil N availability with increasing moisture (Pilbeam and Warren 1995). Generally, sugarcane responses to N fertilization are not as large as those reported here (Carnaúba 1990; Franco et al. 2010). Soil N availability is strongly connected to soil moisture which favors N mineralization (Pilbeam and Warren 1995). However, in our study a possible increment in soil N mineralization due to favorable soil moisture throughout the growing season was not sufficient to supply all the N needed to support high sugarcane yields. Within the conditions of this study, in which N was split-applied associated with drip irrigation it is evident that there was greater efficiency of N fertilizer use than that usually measured under rainfed production where often there is a water deficit at least in some periods of the growing season.

Conclusions

The high response to N in ratoon sugarcane indicates that split N application with drip irrigation can substantially increase stalk and sugar yields even in a region with more than 1400 mm of annual precipitation. The values of $\Delta^{13}C$ in leaves were highly correlated with stalk and aboveground dry matter yield, and N status of sugarcane plants under fertigated conditions; thus, it can be used as a tool to characterize the relationship between N fertilization and yield of this crop. The supply of water via drip irrigation could not maintain high crop production without N fertilization; the practice of fertilization was fundamental for increasing crop yield and replacing the quantities of N exported by the stalks.

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References

AQUASTAT (FAO's Information System on Water and Agriculture). 2014. Brazil: Geography and population. Available from: http://www.fao.org/nr/water/aquastat/countries_regions/bra/index.stm. Accessed 05 May 2014.

Arruda, P. 2012. Genetically modified sugarcane for bioenergy generation. *Current Opinion Biotechnology* 23: 315–322. doi:10.1016/j.copbio.2011.10.012.

Barrie, A., and S.J. Prosser. 1996. Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In *Mass spectrometry of soils*, ed. T.W. Boutton, and S. Yamasaki, 1–46. New York: Marcel Dekker.

Bell, M.J., and A.L. Garside. 2014. Growth and yield responses to amending the sugarcane monoculture: interactions between break history and nitrogen fertilizer. *Crop & Pasture Science* 60: 287–299. doi:10.1071/CP13340.

Cernusak, L.A., N. Ubierna, K. Winter, J.A.M. Holtum, J.D. Marshall, and G.D. Farquhar. 2013. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist* 200: 950–965. doi:10.1111/nph.12423.

Carnaúba, B.A.A. 1990. O nitrogênio e a cana-de-açúcar. *STAB-Açúcar, Álcool e Subprodutos* 8: 4–41.

Chapola, R.G., H.P. Hoffmann, A.I. Bassinello, A.R. Fernandes Jr, and M.A.S. Vieira. 2011. Censo varietal 2010 de cana-de-açúcar nos estados de São Paulo e Mato Grosso do sul. *STAB-Açúcar, e Álcool e Subprodutos* 29: 42–45.

Clay, D.E., S.A. Clay, Z. Liu, and C. Reese. 2001. Spatial variability of C-13 isotopic discrimination in corn (*Zea mays*). *Communications Agronomy Journal* 32: 1813–1827. doi:10.2134/agronj2005.0066.

Dalri, A.B., and R.L. Cruz. 2008. Produtividade da cana-de-açúcar fertirrigada com N e K via gotejamento subsuperficial. *Engenharia Agrícola* 28: 516–524. doi:10.1590/S0100-69162008000300012.

Dourado Neto, D., D.S. Powlson, R.A. Bakar, O.O.S. Bacchi, M.V. Basanta, P.T. Cong, G. Keerthisinghe, M. Ismaili, S.M. Rahman, K. Reichardt, M.S.A. Safwat, R. Sangakkara, L.C. Timm, and J.Y. Wang. 2010. Multiseason recoveries of organic and inorganic nitrogen-15 in tropical cropping systems. *Soil Science Society of America Journal* 74: 139–152. doi:10.2136/sssaj2009.0192.

EMBRAPA. 2006. *CNPS—Centro Nacional de Pesquisa de Solos—Manual de métodos de análises de solo*, 2nd ed. Rio de Janeiro, RJ: Embrapa.

EMBRAPA. 2013. *CNPS—Centro Nacional de Pesquisa de Solos. Sistema brasileiro de classificação de solos*. Rio de Janeiro, RJ: Embrapa.

Epstein, E., and A.J. Bloom. 2004. *Mineral nutrition of plants: Principles and perspectives*, 2nd ed. Sunderland, MA: Sinauer Associates.

Farquhar, G.D. 1983. On the nature of carbon isotope discrimination in C4 species. *Australian Journal Plant Physiology* 10: 205–226.

Fernandes, A.C. 2003. Calculated on the ethanol plant cane. 2nd ed. *STAB. Açúcar, Álcool e Subprodutos.* Piracicaba, SP.

Fortes, C., P.C.O. Trivelin, and A.C. Vitti. 2012. Long-term decomposition of sugarcane harvest residues in Sao Paulo state, Brazil. *Biomass and Bioenergy* 42: 189–198. doi:10.1016/j.biombioe.2012.03.011.

Franco, H.C.J., R. Otto, C.E. Faroni, A.C. Vitti, E.C.A. Oliveira, and P.C.O. Trivelin. 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., P.C.O. Trivelin, C.E. Faroni, A.C. Vitti, and R. Otto. 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., M.A. Silva, R.C. Silva, E.M. Jerônimo, C.S. Cruz, and O.T. Kölln. 2011. Produtividade de três cultivares de cana-de-açúcar sob manejos de sequeiro e irrigado por gotejamento. *Revista Brasileira Engenharia Agrícola & Ambiental* 15: 250–255. doi:10.1590/S1415-43662011000300005.

Gunda, P., and H. Youngs. 2013. *Bioenergy and Water: Understanding Impacts. MIT Energy Initiative and Center for Strategic International Studies.* Energy Water Land Nexus Workshop. (May 6–7), 1–46. Washington, D.C.

Henderson, S.A., S.V. Caemmerer, and G.D. Farquhar. 1992. Short-term measurements of carbon isotope discrimination in several C_4 species. *Australian Journal Plant Physiology* 19: 263–285.

Howell, T.A., and S.R. Evett. 2004. *The Penman-Monteith method*, 14. Washington, DC: USDA-Agricultural Research Service, Conservation & Production Research Laboratory.

Hubick, K.T., G.L. Hammer, G.D. Farquhar, L.J. Wade, S. Von Caemmerer, and S.A. Henderson. 1990. Carbon isotope discrimination varies genetically in C_4 species. *Journal Plant Physiology* 91: 534–537. doi:10.1104/pp.92.2.534.

IBGE. 2006. Brazilian Institute of Geography and Statistics, 2006. Agricultural Census Survey. Available from http://www.ibge.gov.br/home/estatistica. Accessed 05 May 2014.

Korndörfer, G.H., M.R. Valle, M. Martins, and P.C.O. Trivelin. 1997. Utilization by planted cane of nitrogen from urea. *Revista Brasileira de Ciência Solo* 21: 23–26. doi:10.1590/S0100-06832008000700021.

Malavolta, E., G.C. Vitti, and S.A. Oliveira. 1997. *Avaliação do estado nutricional das plantas: princípios e aplicações*, 3rd ed. Potafos: Piracicaba, SP.

Meinzer, F.C., and J. Zhu. 1998. Nitrogen stress reduces the efficiency of the C_4 CO_2 concentrating system, and therefore quantum yield, in *Saccharum* (sugarcane) species. *Journal Experimental Botany* 49: 1227–1234. doi:10.1093/jxb/49.324.1227.

Monneveux, P., M.S. Sheshshayee, J. Akthter, and J.M. Ribaut. 2007. Using carbon isotope discrimination to select maize (*Zea mays* L.) inbred lines and hybrids for drought tolerance. *Plant Science* 173: 390–396. doi:10.1016/j.plantsci.2007.06.003.

Muchow, R.C., M.J. Robertson, and A.W. Wood. 1996. Growth of sugarcane under high input conditions in tropical Australia. II. Sucrose accumulation and commercial yield. *Field Crops Research* 48: 26–37. doi:10.1016/0378-4290(96)00042-1.

Ng Kee Kwong, K.F., J.P. Paul, and J. Deville. 1999. Drip-fertigation—a means for reducing fertilizer nitrogen to sugarcane. *Experimental Agriculture* 35: 31–37.

Oliveira, E.C.A., G.J. de Castro Gava, P.C.O. Trivelin, R. Otto, and H.C.J. Franco. 2013. Determining a critical nitrogen dilution curve for sugarcane. *Journal Plant Nutrition and Soil Science* 176: 712–723. doi:10.1002/jpln.201200133.

Pilbeam, C.J., and G.P. Warren. 1995. Use of 15 N for fertilizer N recovery an N mineralization studies in semiarid Kenya. *Fertilizer Research* 42: 123–128. doi:10.1007/BF00750506.

Van Raij, B., J.C. Andrade, H. Cantarella, and A.J. Quaggio (eds.). 2001. *Análise química para avaliação da fertilidade de solos tropicais*. Campinas, SP: IAC.

Rossetto, R., F.L.F. Dias, M.G.A. Landell, H. Cantarella, S. Tavares, A.C. Vitti, and D. Perecin. 2010. N and K fertilization of sugarcane ratoons harvested without burning. *Proceedings of the International Society of Sugar Cane Technologists* 27: 1–8.

Van Raij, B., and H. Cantarella. 1996. Outras culturas industriais. In *Recomendações de adubação e calagem para o Estado de São Paulo*, 2nd ed, ed. B. Van Raij, H. Cantarella, A.J. Quaggio, and A.M.C. Furlani, 8–13. Campinas, SP: IAC. **(Boletim Técnico, 100).**

Saliendra, N.Z., F.C. Meinzer, M. Perry, and M. Thom. 1996. Associations between partitioning of carboxylase activity and bundle sheath leakiness to CO_2, carbon isotope discrimination, photosynthesis, and growth in sugarcane. *Journal Experimental Botany* 47: 907–914. doi:10.1093/jxb/47.7.907.

Singh, P.N., and S.C. Mohan. 1994. Water use and yield response of sugarcane under different irrigation schedules and nitrogen levels in a subtropical region. *Agricultural Water Management* 26: 253–264. doi:10.1016/0378-3774(94)90012-4.

Soil Survey Staff. 2010. *Keys to soil taxonomy*, 11th ed. Washington, DC: USDA-Natural Resources Conservation Service.

Stanley, C.D., R.E. Green, M.A. Khan, and L.T. Santo. 1990. Nitrogen fertilization rate on soil nitrate distribution for micro-irrigated sugarcane. *Soil Science Society American Journal* 54: 217–222.

Trivelin, P.C.O., H.C.J. Franco, R. Otto, D.A. Ferreira, A.C. Vitti, C. Fortes, C.E. Faroni, E.C.A. Oliveira, and H. Cantarella. 2013. Impact of sugarcane trash on fertilizer requirements for São Paulo, Brazil. *Scientia Agricola* 70: 345–352. doi:10.1590/S0103-90162013000500009.

UNEP (United Nations Environment Programme), 2011. The Bioenergy and Water Nexus, Oeko-Institut and IEA Bioenergy Task 43. Available from: www.unep.org/greeneconomy/Portals/88/documents/ger/Water_Bioenergy_FINAL_WEB_VERSION.pdf. Accessed 01 June 2013.

Taiz, L., and E. Zeiger. 2004. *Fisiologia vegetal*, 3rd ed. Porto Alegre, RS: Artmed Editora.

Thorburn, P.J., I.K. Dart, I.M. Biggs, C.P. Baillie, M.A. Smith, and B.A. Keating. 2003. The fate of nitrogen applied to sugarcane by trickle irrigation. *Irrigation Science* 22: 201–209. doi:10.1007/s00271-003-0086-2.

Trivelin, P.C.O., M.W. Oliveira, A.C. Vitti, G.J.C. Gava, and J.A. Bendassolli. 2002. Perdas do nitrogênio da ureia no sistema solo-planta em dois ciclos de cana-de-açúcar. *Pesquisa Agropecuária Brasileira* 37: 193–201. doi:10.1590/S0100-204X2002000200011.

Wiedenfeld, B., and J. Enciso. 2008. Sugarcane responses to irrigation and nitrogen in semiarid South Texas. *Agronomy Journal* 100: 665–671. doi:10.2134/agronj2007.0286.

Wiedenfeld, R.P. 1995. Effects of irrigation and N fertilizer application on sugarcane yield and quality. *Field Crops Research* 43: 101–108. doi:10.1016/0378-4290(95)00043-P.

Van Dillewijn, C. 1952. *Botanys of sugarcane*. Waltham: Cronica Botanica Co.