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ORIGINAL ARTICLE

Legume nitrogen credits for sugarcane production: implications for soil N availability and ratoon yield

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Abstract One of the steps needed to achieve sustainable bioenergy is to reduce our reliance on synthetic nitrogen (N). Despite the fact that legume cover crops have the potential to increase soil quality and sugarcane (*Saccharum* spp.) yield, much information is still needed to determine amount of N available from cover crops to sequential ratoon cycles. This study was designed to assess the impacts of sunn hemp (*Crotalaria spectabilis*) cover crop on soil N dynamics and sugarcane ratoon response to N fertilization during two harvest seasons across three contrasting soil and climatic conditions in southern Brazil. The treatments consisted of cover crop and fallow established prior to sugarcane replanting; in addition to three N-fertilizer rates 60, 120 and 180 kg N ha^{-1} and a 0-N control applied during the first and second ratoons. Although there was increased sugarcane yield (8–13 Mg ha^{-1} in first ratoon and 10–16 Mg ha^{-1} in second ratoon) in plots planted with cover crop, it was not possible to detect significant increases in soil inorganic N, microbial biomass C and Illinois Soil N Test content under cover crop compared with fallow. Cover crop with sunn hemp increased the accumulated two-year yields by

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14–25 Mg ha^{-1} at all sites and NUE (Mg stalks kg^{-1} N) across all N rates at two sites. Our findings support the conclusion that cover crop did not change the N requirement of succeeding ratoon crops but increases the yield, thereby improving NUE in sugarcane systems.

Keywords *Crotalaria spectabilis* · Biological N fixation · Illinois soil nitrogen test · Inorganic N · Cover crop · *Saccharum* spp.

Introduction

The ever-increasing need for food, fiber and energy to a continuously crescent population presents challenges for soil, water and air conservation. Increasing bioenergy production is an important strategy to boost energy security and to mitigate the negative implications of climate change (Goldemberg 2007). In this context, sugarcane is the cleanest and most viable alternative biomass source to mitigate greenhouse gas (GHG) emissions (Vries et al. 2010). Changing fossil fuels with sugarcane-based ethanol could potentially reduce GHG emissions by as much as 85% (Cavalett et al. 2013).

In Brazil, the sugarcane sector has been improved over recent years with the main goals of enhancing yield, maintaining sustainability and minimizing the agricultural footprint on the ecosystem. The gradual elimination of pre-harvest sugarcane burning has led to increases in crop residue retained on the soil surface annually (10–20 Mg ha^{-1}) (Franco et al. 2013). The higher amount of crop residue in the soil after harvest has generated fertilizer management challenges, particularly related to nitrogen (N) fertilizer application and incorporation (Borges et al. 2019). As a result, new fertilizer management strategies are needed to increase N use efficiency (NUE) by sugarcane and reduce the amount of fertilizer N needed for optimum yield. Of high interest are practices that minimize N losses by volatilization and the use of N-fixing crops (soybean—*Glycine max* L., peanuts—*Arachis hypogaea* L., sunn hemp species) (Otto et al. 2016). Brazil relies heavily on external raw materials for N fertilizers production, and currently as much as 70% of the raw materials need to be imported to supply the country's demand for N fertilizer (MME 2017). Sugarcane ranks third in fertilizer consumption (Conab 2013) and, therefore, improved NUE will reduce production costs by reducing fertilizer inputs, which is critical to maintain Brazil as the world's major sugarcane producer and increase the supply of ethanol and its derivatives.

Nitrogen is an important nutrient for stimulating sugarcane growth and production (Thorburn et al. 2011; Franco et al. 2011; Robinson et al. 2011). Historically, sugarcane has shown to have low NUE when compared with annual crops, such as corn (*Zea mays*). It has been estimated in Brazilian conditions that 32% of total N applied annually is immobilized in the soil microbial biomass, 26% is absorbed by the plant, 16% is lost by volatilization (NH_3), 5.6% is lost by leaching, 1.84% is lost by denitrification, and 19% by other loss pathways, such as NH_3 and NO_2 volatilization through the leaves (Otto et al. 2016). Due to the small proportion of N-fertilizer absorbed by crops and the strong evidences that N supply from soil mineralization is a significant N source for crops (Dourado-Neto et al. 2010; Franco et al. 2011; Vieira-Megda et al. 2015), a satisfactory management approach to increase soil fertility and N storage may help reduce the dependence on synthetic N for sugarcane production. Recently, farmers have used N-fixing crops before the sugarcane is replanted as an option to supply part of the crop's requirements for N (Ambrosano et al. 2011a, b, 2013a, b). There are evidences that the N provided by legumes can minimize the requirements for N fertilization by the successor crops (Cherr et al. 2006). For example, Park et al. (2010) found a synergetic effect of reducing sugarcane ratoon cycles response to N fertilizer beyond the supply of legume N through biological fixation. Currently in Brazil, several sugarcane producers are using a legume cover crop prior to sugarcane replanting but without reducing N fertilization at the ratoon crop cycle. Much information is still needed about the implications of this crop management on N fertilizer requirements and response along ratoon cycles.

The N uptake from mineral fertilizers by sugarcane usually vary between 20 and 40%, while from green manures it is around 20% (Trivelin et al. 2002; Vitti et al. 2007; Franco et al. 2008; Ambrosano et al. 2011b; Lima Filho et al. 2014). Although legumes residue decomposition seems to provide a low NUE, the slow residue breakdown and slow N release might provide a steady amount of N throughout the growing season leading to a greater N uptake by the subsequent crops (Dinnes et al. 2002). In addition, others have found that sugarcane present a limited or low response to N fertilization in soils previously cultivated with legumes or receiving organic byproducts for long period of time, such as vinasse and filter cake (Otto et al. 2013). Similar reduction response of crops to N fertilizer application were observed after legume cover crops in a rice study in India (Aulakh et al. 2000), grain sorghum in the United States (Mahama et al. 2016) and sugarcane (cane-plant cycle) in Australia (Garside & Bell 2001).

Nitrogen rates applied in ratoon crop cycle are moderate in Brazil (80–150 kg N ha^{-1}; Otto et al. 2016) but expressive in other large-scale producers, for example, in Australia (160–200 kg N ha^{-1}), India (150–400 kg N ha^{-1}) and China (100–755 kg N ha^{-1}) (Robinson et al. 2011). The possibility of using cover crops to reduce the need of N for ratoon cycles without yield loss is appealing worldwide. However, the available information regarding the amount of N that *Crotalaria* spp. species can supply to sugarcane ratoons is limited.

This study was set up to provide information regarding the potential of sunn hemp as an alternative N source for ratoon crops. We hypothesized that using sunn hemp as a cover crop before sugarcane replanting will increase soil N availability and reduce sugarcane ratoons response to N fertilization. The objective of this study was to assess detailed changes of soil N dynamics and ratoon response to N rates during first and second ratoon cycles in areas subjected to cover crop rotation or under fallow in the renovation period.

Materials and methods

Characterization of study areas

The field trials were installed between December/2012 and January/2013 in commercial sugarcane areas at Quatá/SP (site I—22°14′S; 50°42′W), Chapadão do Céu/GO (site II—18°25′S; 52°33′W) and Quirinópolis/GO (site III—18°32′S; 50°26′W). Local sugarcane mills managed all sites. These locations were selected because they provide diverse edaphoclimatic conditions in southern Brazil and are regions of high sugarcane production in the country. Site I (altitude above sea level of 560 m; mean temperature of 23.7 °C; historical rainfall of 1.391 mm per year and a humid subtropical climate characterized by hot summer without dry season accordingly Alvares et al. (2013)) represents a traditional area of sugarcane production in Brazil and has a Arenic Kandiudult soil with sand-loam texture (Soil Survey Staff 2014). Site II (831 m; 22.5 °C; 1.654 mm; tropical monsoon climate with a brief dry season and heavy rains for the rest of the period) has a Rhodic Hapludox soil with clay texture (Soil Survey Staff 2014) and site III (541 m; 24.4 °C; 1.378 mm; tropical climate with dry winter) are areas where sugarcane production is expanding in the Brazilian Cerrado region, with a Rhodic Eutrudox soil with clay texture (Soil Survey Staff 2014).

Experimental design and treatments

Before treatment establishment (October 2012), the field experiments were submitted to a renovation period. The renovation period started with glyphosate application (6 L ha^{-1}) in the entire area, following lime (2 Mg ha^{-1}) and gypsum (1 Mg ha^{-1}) application and incorporation (up to 0.3 m) by chiseling. At each site, the cover crop treatment was established by seeding with sunn hemp for the cover crop treatment or keeping the area under a fallow condition (in this case, weed infestation was controlled by herbicide application as described below) during sugarcane-replanting period. The sunn hemp was sowed between December and January/2013 using a cereal planter at a rate of 25 kg seed ha^{-1}. In March/April 2013, both areas were sprayed with herbicides (5 L ha^{-1} of glyphosate, 1.2 L ha^{-1} of 2,4-D and 0.5 L ha^{-1} of triomax) at the flowering stage, following recommended practices adopted by sugarcane growers. After herbicide application reduced tillage was performed by opening planting furrows at 0.3-m soil depth, and fertilizers were applied at the base of the planting furrow according to recommended management practices (fertilization rates in sites I and III were: 40 kg N ha^{-1}, 125 kg P_2O_5 ha^{-1}, 125 kg K_2O ha^{-1} and in site II: 40 kg N ha^{-1}, 140 kg P_2O_5 ha^{-1}; 100 kg K_2O ha^{-1}). Sugarcane was planted manually by placing 15–20 buds per meter of the variety RB96-6928 at all three sites, and control of pests and weeds followed management practices by mills. The yields of first crop cycle (cane-plant) were 58, 170 and

168 Mg ha^{-1} in the cover crop treatment and 48, 144 and 151 Mg ha^{-1} in the fallow treatment for sites I, II and III, respectively.

After harvesting the cane-plant cycle (between June–August 2014), three N rates (60, 120, 180) in addition to a control treatment (without N-fertilizer) were arranged in a split plot design with four replicates. The split plot was N rates and the whole plot was cover crop. The split plot treatments were randomly applied within each of the cover crop whole plot. The experimental units consisted of five sugarcane rows, 9-m long and spaced at 1.5-m. Sixty days after harvesting the plant-cane cycle, N fertilizer treatments were applied manually over the residue without incorporation next to one side of the sugarcane row using ammonium nitrate (32% N) to avoid losses of NH_3 by volatilization. Potassium chloride (KCl) was applied at a rate of 120 kg K_2O ha^{-1} to avoid nutrient deficiency. After harvesting of first ratoon (between June–July 2015), N treatments were reapplied following the same methodology. The climatic data for mean temperature (maximum and minimum) and rainfall during the two sugarcane growing seasons were collected monthly from a meteorological station near to the experimental plots (Suppl. Figure 1).

Soil sampling was performed before the N fertilizer rates were established (after plant-cane harvest) for baseline characterization and included determination of physical and chemical properties (See Suppl. Table 1) following the procedures of Gee and Bauder (1986) and Raij et al. (2001), respectively. For the analysis of soil total N (TN) and total C (TC) samples were analyzed by dry combustion according to the methodology described in Nelson and Sommers (1996), using a Carbon Analyzer—LECO TruSpec CN.

Soil measurements

Soil samples for inorganic N determination were collected only in the control treatment (without N) to avoid influence of the N-fertilizer applied. Four samples per plot were collected at random positions using an auger at a distance of 0.25-m from the planting row every 6 months over 2 years at depths of 0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6 and 0.6–1.0-m. The samples were immediately preserved in styrofoam box with ice, and as soon as possible, the samples were frozen (− 17 °C) while still moist (to maintain field conditions) until chemical analyses could be performed. For extraction, triplicate 5-g subsamples were added to 25-mL of an extracting solution containing 2 mol L^{-1} KCl (Buresh et al. 1982), shaked for 1 h and gravity filtered on slow filter papers previously leached with 2 mol L^{-1} KCl. Another sub-sample of 20-g was weighed before and after oven drying (at 105 °C for 24 h) to determine the moisture content, in order to convert the results to a dry-weight basis (mg kg^{-1}). Ammonium N ($N–NH_4^+$) and $N–NO_3^{--}$ + $N–NO_2^-$ content were determined in soil extracts by flow injection analysis system (FIA) according to Kamogawa and Teixeira (2009). In our study, inorganic N is presented as the sum of $N–NH_4^+$, $N–NO_3^-$, and $N–NO_2^-$.

The hydrolysable fraction of soil organic N was determined by the Illinois Soil Nitrogen Test (ISNT-N) according to Khan et al. (2001). For glucosamine N standards, > 95% recovery was obtained in each batch of analysis. Further details of the methodology can be found in 15N Analysis Service (2011). Microbial biomass carbon (MBC) was evaluated by the fumigation-extraction method (Vance et al. 1987) in samples collected at the 0–0.1 and 0.1–0.2-m soil depths.

Crop measurements

In order to evaluate the N fertilization influence on the sugarcane nutritional diagnosis, the determination of relative chlorophyll index (SPAD) was performed in all treatments. The quantification of non-destructive chlorophyll content was performed between 150 and 180 days after each harvest (between January and February) using a SPAD Chlorophyll Meter (SPAD-502, Minolta Co., Ramsey, Japan) in the middle third of 15 diagnostic leaves (Top Visible Dewlap Leaf). Undeveloped tillers (very thin and less than 0.5-m high) and attacked by pests or diseases were not used in the SPAD meter measurements.

Stalk yield (Mg ha^{-1}) was quantified by harvesting three central rows of each plot between June and July in 2015 (first ratoon) and 2016 (second ratoon). The harvest was performed with a mechanical harvester and stalk yield were computed using an instrumented truck equipped with a loading cell for accurate yield assessment. The N use efficiency index (NUE) was calculated according to Dobermann (2005) using the equation:

$$\mathrm{NUE}\left(\mathrm{Mg\ stalks\ kg^{-1} of\ N}\right) = \mathrm{Yield\ of\ stalk}\left(\mathrm{Mg\ ha^{-1}}\right) \div \mathrm{N\ rate}\left(\mathrm{kg\ ha^{-1}}\right)$$

Statistical and data analysis

The effect of crop management system (cover crop or fallow), N rate, days after planting and their interactions on the soil properties and plant parameters were assessed using repeated measures ANOVA. As indicated previously, the three-sugarcane mills used different fertilizer formulations and thus it was most appropriate to analyze each location by itself. All statistical analyses were performed using the PROC GLIMMIX procedure in SAS (SAS 9.3 2010) (SAS Institute 2010). The variable year and soil depth were considered the repeated variables, while crop management (the whole plot term) and N rates (split-plot term) were considered as fixed variables, and block was considered a random variable in the model. The Akaike index (AIC) was used as selection criterion to determine the most appropriate covariance model for the repeated variable. The cut-off probability level selected was $P < 0.05$. The Fisher's LSD test for mean comparison was used when $P < 0.05$.

Results

Sunn hemp cover crop yield

The biomass production and N content of the legume cover crop were found to be 6.5 Mg ha^{-1}, 169 kg N ha^{-1} at site I; 8.3 Mg ha^{-1}, 127 kg N ha^{-1} at site II; and 8.1 Mg ha^{-1}, 192 kg N ha^{-1} at site III.

Soil inorganic-N ($NH_4^+ + NO_3^- + NO_2^-$) content

The soil inorganic-N was highly affected by the interaction of year × management treatments in all locations ($P = 0.005$; < 0.0001; < 0.0001 for sites I, II and III, respectively). In summary, soil inorganic N content decreased over ratoon cycles (from Jul/2014 to Jul/2016) in sites II and III with a slight increase only in site I, regardless of the crop management systems. The fallow treatment showed high levels of soil inorganic N at soil surface (0–0.20 m) at the beginning of the experiment until Jul/2015. Further increases in soil inorganic N were observed in the cover crop treatment at depths below 0.20 m (from 0.2 to 1.0 m) in all sites compared to fallow in similar periods (Fig. 1, see Suppl. Figures 2, 3, 4).

The results indicate high variability in inorganic N availability among the soils. At site I (sandy-loam soil), for example, inorganic N content rarely exceeded 13 mg kg^{-1} at all sampling times. In contrast, sites II and III (clayey soil) showed intermediate inorganic N levels, varying from 10 to 24 mg kg^{-1} in most soil depths and sampling times. The inclusion of legume cover crop showed different effects as a function of contrasting soil types. For instance, at site I inorganic N was positively affected by cover crop in the soil profile of 0.2–1.0 m and was detected only during Jul/2014. However, at the site II inorganic N content was higher under cover crop in soil depths of 0.6–1.0 m in Jul/2014 and 0.2–1.0 m in Jan/2015. At site III, cover crop increased inorganic N content in a depth of 0.4–1.0 m in Jul/2014 and also in a depth of 0.6–1.0 m in Jan/2015 and Jul/2015 (Fig. 1).

Soil hydrolysable-N content (ISNT-N)

Cultivation of cover crops in the renovation period of sugarcane fields resulted in small changes in soil ISNT-N content in the succeeding ratoon crop cycles. The ISNT-N showed temporal changes for both management treatments and locations with a decrease as a function of soil depth (Fig. 2). The ISNT-N content was affected by the interaction of year × management treatments in all locations ($P < 0.0001$; 0.0005; 0.0004 for sites I, II, III, respectively).

The planting of cover crop promoted greater ISNT-N content in the sandy-loam soil (site I) in the depth of 0–0.2 m between Jan and Jul/2015 when the ISNT-N content under cover crop (52 mg kg^{-1}) exceeded fallow (44 mg kg^{-1}) by 18%. Also, the highest amount of soil ISNT-N associated to cover crop was found in some periods such as in Jan/2016 at 0.2–0.4 m depth and in Jul/2016 at 0.4–0.6 m.

In the soils with high clay content (sites II and III) ISNT-N contents were variable and showed different patterns between management systems in comparison to site I. For instance, ISNT-N contents were higher under fallow treatment at the depth of 0–0.2-m in sites

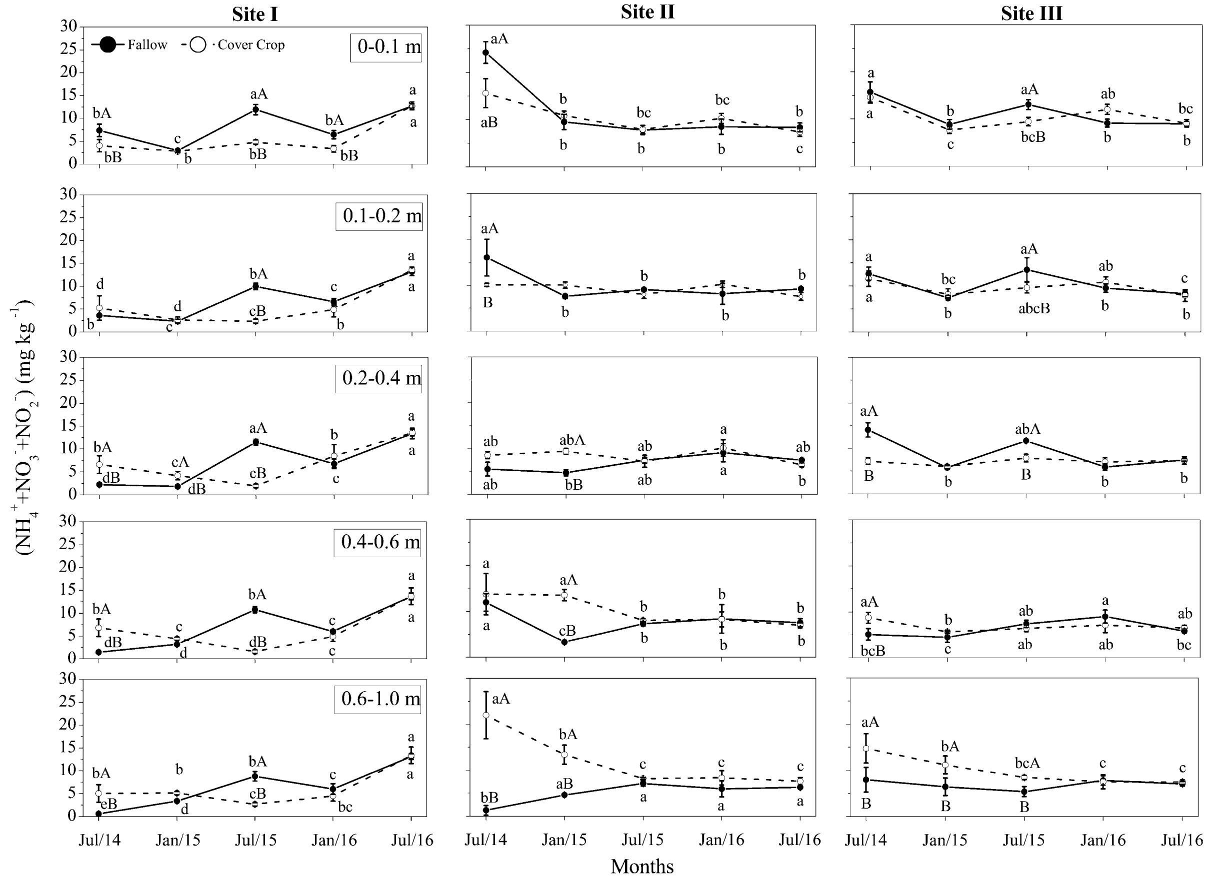

Fig. 1 Mean of inorganic-N concentration in the soil profile during ratoon crop cycles as a function of cover crop or fallow at site I (Quatá/SP), site II (Chapadão do Céu/GO) and site III (Quirinópolis/GO). Means followed by similar lowercase letters do not indicate differences between sampling times, while similar capital letters do not indicate differences between management system accordingly Fisher test ($P < 0.05$). Values represent average of four repetitions of soil samples collected in the control plots. Bars represent standard error (n = 4)

II and III. In site II, this pattern also occurred in Jan/15 at 0–0.1 m depth and at 0–0.2 m in Jun/14, Jan/16 and Jul/16. Site II exhibited cover crop positive effects on ISNT-N only in Jul/2014 at 0.4–0.6 m, in Jul/2015 at 0.2–0.4 m and in Jul/2016 at 0.6–1.0 m. At site III, there was also high variability in ISNT-N content between depth and sampling times. The fallow treatment showed higher ISNT-N content at the depth of 0–0.1 m in Jan/15 and Jul/15 (Fig. 2). In contrast, cover crop treatment presented higher ISNT-N content than fallow at the depths of 0–0.1-m in Jul/14; 0.2–0.4-m in Jul/15; 0.4–0.6-m in Jul/16; and 0.6–1.0-m in Jul/15 and Jul/16.

Microbial biomass C

The MBC was significantly affected by interaction of year × rotation management systems in all locations ($P = 0.0157$; 0.027; 0.0007 for sites I, II, III, respectively). The MBC content showed significant increase in Jan/16 at sites II and III (averages of 873 and 794 mg kg^{-1}, respectively) and in Jul/16 at site I (average of 551 mg kg^{-1}) (Fig. 3). Greater MBC was observed under cover crop in relation to fallow in topsoil of 0–0.2 m. During the first ratoon cycle (Jan–Jul/15), positive differences for cover crop over fallow treatment were observed at site I (148 and 113 mg kg^{-1}, respectively) and site II (480 mg kg^{-1} and 427 mg kg^{-1}, respectively). However, at site III significant differences were observed during the

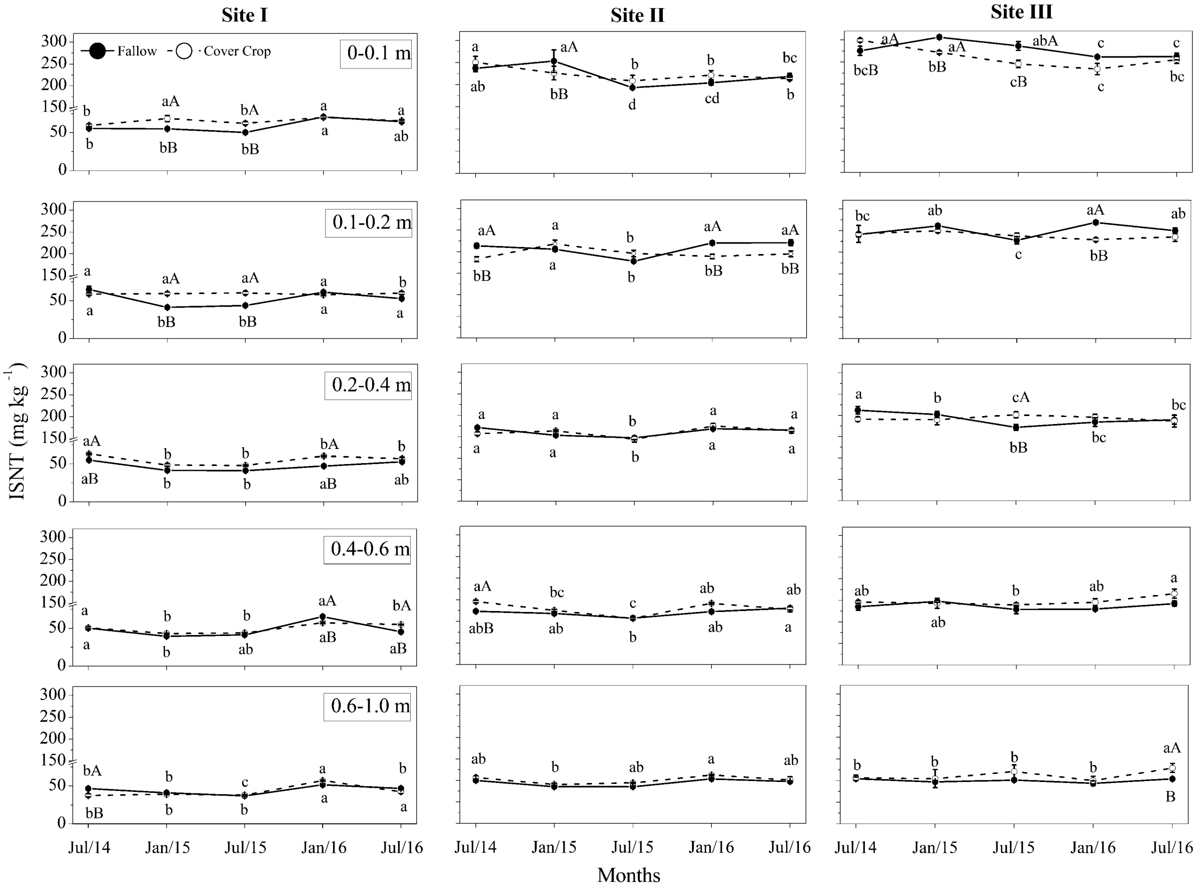

Fig. 2 Illinois Soil Nitrogen Test (ISNT) in the soil profile during ratoon crop cycles as a function of cover crop or fallow at site I (Quatá/SP), site II (Chapadão do Céu/GO) and site III (Quirinópolis/GO). Means followed by similar lowercase letters do not indicate differences between sampling times, while similar capital letters do not indicate differences between management system accordingly Fisher test ($P < 0.05$). Values represent average of four repetitions of soil samples collected in the control plots. Bars represent standard error (n = 4)

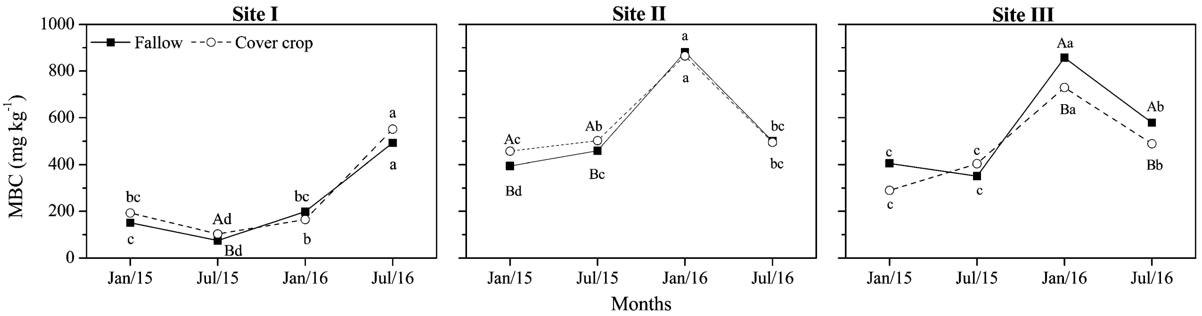

Fig. 3 Microbial biomass carbon (MBC) at depths of 0–0.1 and 0.1–0.2-m during first and second ratoon crop cycles as a function of cover crop or fallow at site I (Quatá/SP), site II (Chapadão do Céu/GO) and site III (Quirinópolis/GO). Means followed by similar lowercase letters do not indicate differences between sampling times, while similar capital letters do not indicate differences between management system accordingly Fisher test ($P < 0.05$). Values represent average of four replications of soil samples collected in the control plots

second ratoon (Jan–Jul/2016) and the mean result of sampling times revealed higher MBC content by 9% for fallow over cover crop (378 mg kg^{-1} and 347 mg kg^{-1}, respectively).

SPAD index

The SPAD index value was significantly ($P = 0.0450$) affected by the interaction of cover crop × N rates during the second ratoon cycle at site I, with an increase of 3% in SPAD index under cover crop in comparison to fallow. A reduction in SPAD values occurred with the increase in N rates from 60 to 180 kg N ha^{-1} under cover crop but not under fallow (Table 1).

The interaction of year × cover crop × N rates was observed in SPAD index at site II ($P = 0.0408$). Higher SPAD values under N fertilizer rates of 120 kg ha^{-1} and 180 kg ha^{-1} were observed during the first ratoon by 8% and 2%, respectively, compared to the second ratoon cycle. The SPAD index was significantly affected by the year main effect at site III ($P < 0.0001$). The mean SPAD value of the first ratoon was higher by 5% in comparison to the second ratoon.

Sugarcane yield

Considering the main effect of cover crop in the ANOVA, the sugarcane yield was positively affected by cover crop across both years in sites I and II ($P = 0.0066$; $P = 0.0003$, respectively) (Table 2). For the main effect N management, there was a significant increment of yield as a function of increasing N rates in sites I and II on the average of both years ($P = 0.0384$; $P = 0.0123$, respectively). Considering

Table 1 SPAD index during first and second ratoon crop cycles as a function of cover crop (CC) or fallow and N fertilization at site I (Quatá/SP,) site II (Chapadão do Céu/GO) and site III (Quirinópolis/GO). Values represent average of four replications

	Management system								
N rates	**Cover Crop**	**Fallow**	*Mean*	**Cover Crop**	**Fallow**	*Mean*	**Cover Crop**	**Fallow**	*Mean*
		Site I			Site II			Site III	
kg ha^{-1}					1st ratoon cycle				
0	38	34	*36*	42	42	*42*	42	42	*42*
60	38	36	*37*	42	42	*42*	41	40	*41*
120	35	37	*36*	42	43^{a}	*42*	42	41	*41*
180	36	36	*36*	41	42^{a}	*42*	41	42	*41*
Mean	*37*	*36*		*41*	*42*		*42*	*41*	
					2nd ratoon cycle				
0	37 aA	36 B	*36*	42	41	*42*	40	40	*40*
60	36 ab	36	*36*	40	42	*41*	39	39	*39*
120	35 b	36	*36*	41	40^{b}	*41*	39	39	*39*
180	36 ab	36	*36*	41	41^{b}	*41*	39	40	*40*
Mean	*36*	*36*		*41*	*41*		*39*	*40*	
					Mean of two years				
1st ratoon	*37*	*36*	*36*	*41*	*42*	*42^{a}*	*42*	*41*	*41^{a}*
2nd ratoon	*36*	*36*	*36*	*41*	*41*	*41^{b}*	*39*	*40*	*39^{b}*
Mean	*37*	*36*		*41*	*42*		*41*	*41*	
P_{CC}		0.3106			0.9579			0.5127	
P_{rate}		0.6717			0.8766			0.2192	
$P_{CC\ x\ rate}$		0.0450			0.5174			0.5477	
P_{year}		0.4937			0.0048			<0.0001	
$P_{year\ x\ CC}$		0.4707			0.0164			0.3657	
$P_{year\ x\ rate}$		0.8935			0.2022			0.7453	
$P_{year\ x\ CC\ x\ rate}$		0.4338			0.0408			0.5105	

Means followed by similar capital letters in the same line do not indicate differences between management system, similar lowercase letters in the same column do not indicate differences between N rates application accordingly Fisher test ($P < 0.05$); Means followed by different superscript letters differ vertically (Fisher, $P < 0.05$)

Table 2 Sugarcane stalk yield (Mg ha^{-1}) at first and second ratoon crop cycles as a function of cover crop (CC) or fallow and N fertilization at site I (Quatá/SP,) site II (Chapadão do Céu/GO) and site III (Quirinópolis/GO). Values represent average of four replications

	Management system								
N rates	**Cover Crop**	**Fallow**	*Mean*	**Cover Crop**	**Fallow**	*Mean*	**Cover Crop**	**Fallow**	*Mean*
		Site I			Site II			Site III	
kg ha^{-1}	Mg ha^{-1}								
					1st ratoon cycle				
0	84	82	*83*	138	130	*134 b*	150	140	*145 bc*
60	97	90	*93*	144	142	*143 b*	164	147	*155 ab*
120	91	90	*91*	157	153	*155 a*	168	151	*159 ab*
180	92	87	*90*	150	132	*141 b*	171	163	*167 a*
Mean	*91*	*87*		*147 A*	*139 B*		*163 A*	*150 B*	
					2nd ratoon cycle				
0	73	54	*63*	150	130	*140*	145	130	*138*
60	71	67	*69*	152	134	*143*	146	130	*138*
120	77	69	*73*	152	145	*148*	125	130	*127*
180	80	70	*75*	151	133	*142*	119	135	*127*
Mean	*75 A*	*65 B*		*151 A*	*135 B*		*134*	*131*	
P_{CC}		0.0066			0.0003			0.0624	
P_{rate}		0.0384			0.0123			0.7048	
$P_{CC\ x\ rate}$		0.8158			0.4792			0.3399	
P_{year}		<0.0001			0.9603			<0.0001	
$P_{year\ x\ CC}$		0.0981			0.0443			0.1707	
$P_{year\ x\ rate}$		0.2939			0.1555			0.0193	
$P_{year\ x\ CC\ x\ rate}$		0.3157			0.3780			0.4077	

Means followed by similar capital letters in the same line do not indicate differences between management system, while similar lowercase letters in the same column do not indicate differences between N rates application and years accordingly Fisher test ($P < 0.05$)

the main effect year, sites I and III showed highest yields at the first ratoon as compared to the second ratoon ($P < 0.0001$). The interaction of year × cover crop ($P = 0.0443$) in site II revealed that the yield under cover crop was superior to yield under fallow in both years. In addition, the year × N rate interaction ($P = 0.0193$) observed in site III showed that N rate increased yield on this site only at first ratoon. Considering the mean yield across all N rates (Table 2), cover crop treatment obtained a yield increment of 5.8% (8 Mg ha^{-1}) and 8.7% (13 Mg ha^{-1}) compared to fallow in first ratoon stages at sites II and III, respectively. In the second ratoon, average yield across all N rates under cover crop treatment at sites I and II was 15.4% (10 Mg ha^{-1}) and 11.9% (16 Mg ha^{-1}) superior when compared to fallow, respectively.

Regarding the year × N rates interaction, the results show that N fertilization had a significant effect on yield at sites II and III during the first ratoon, but not in the second ratoon (Table 2). In the first ratoon of site II, highest yield (155 Mg ha^{-1}) was obtained with 120 kg N ha^{-1}, a yield gain of 21 Mg ha^{-1} when compared to control yield (134 Mg ha^{-1}). In the first ratoon of site III, highest yield was obtained with 180 kg N ha^{-1} (167 Mg ha^{-1}), representing a yield gain of 22 Mg ha^{-1} when compared to the control yield (145 Mg ha^{-1}). Considering the N rate main effect across both years at site I, the yield under N rates of

120 and 180 kg N ha^{-1} (82 Mg ha^{-1} for both treatments, data not shown) resulted in yield gain of 9 Mg ha^{-1} when compared to control (73 Mg ha^{-1}, data not shown). In terms of percentage and considering the control treatment as baseline, the sugarcane response to fertilization reached 12% at site I across both years and totaled 15% at sites II and III during the first ratoon. However, in the second ratoon cycle there was no significant effect of N rates at sites II and III (Table 2).

Different from the expected, no significant interaction between cover crop × N rate was observed in any of the sites (Table 2), indicating that cultivation of cover crop did not affect the response of ratoon cycles to N fertilization. However, the yield gain promoted by cover crop cultivation in some situations (Table 2) resulted in improved NUE across all N rates for plots cultivated previously with cover crop at sites I and II (Fig. 4). At site III, NUE did not differ among management systems but decreased with the increasing N rates (Fig. 4).

Considering the accumulated yields over the two harvests, there was a yield response to N rates at sites I and II ($P = 0.0382$; $P = 0.0123$, respectively). At site I, the accumulated N rates of 120, 240 and 360 kg ha^{-1} provided comparable yields among them (162; 164; 164 Mg ha^{-1}, respectively) but higher than the control (147 Mg ha^{-1}) (Fig. 5). At site II, the accumulated rate of 240 kg ha^{-1} N provided the highest yield (303 Mg ha^{-1}) compared to other treatments (0 N = 274 Mg ha^{-1}; 120 N = 286 Mg ha^{-1}; 360 N = 283 Mg ha^{-1}). Regarding the management systems effect, the average yield across all N rates under cover crop showed increases of 14 and 25 Mg ha^{-1} in two years compared to fallow at sites I and II, respectively (Fig. 5).

Discussion

Effect of cover crop on soil N dynamics

This study was focused on the hypothesis that cultivation of cover crop during the sugarcane replanting period would reduce the consecutive ratoon cycle's response to N fertilization due to an improvement of soil N pool by cover crop cultivation. However, the fallow treatment showed higher inorganic N content at topsoil than cover crop in selected periods of the growing season for all evaluated sites (Fig. 1). It is possible that this unexpected pattern is associated to an increase in sugarcane yield with the cultivation of cover crop. The improvement in yield promoted by cover crop occurred not only in the first and second ratoon crop cycles (Table 2), but also in plant cane cycle. In plant cane cycle, the yield gain promoted by cover crop cultivation compared to fallow totaled 10, 26, and 17 Mg ha^{-1} at sites I, II and III, respectively (Tenelli 2016). Such difference in yield may have improved the uptake of N from soil in the cover crop plots, due to the well know effect of cover crops in increasing the N surplus by biological fixation (Park et al. 2010). In our study, the uptake of N by sugarcane was not measured in any of the years evaluated. Apart from that, the improvement in SPAD index in sugarcane leaves at site I in plots cultivated with cover crop is an indicator of the improvement in N nutrition by sugarcane in cover crop plots.

Plots that received cover crop were found to have greater inorganic N content at deeper soil layers (especially 0.6–1.0-m) in sites II and III compared to fallow before July 2015 (Fig. 1). The amount of N incorporated into the soil through biomass of sunn hemp totaled 169, 127, and 192 kg N ha^{-1} at sites I, II and III, respectively. It is possible that cover crop improved inorganic N content in the whole soil profile, but the improved growth of sugarcane in cover crop treatment caused a decline in inorganic N content in topsoil but not in deeper soil depths. Sugarcane has a well-developed root system, but many of the fine roots responsible for nutrient uptake are located near the soil surface (Otto et al. 2011; Barbosa et al. 2018).

The ISNT-N evaluates an easily mineralizable fraction of soil organic matter (SOM) associated with amino-sugars that is present in the bacteria cellular content and the soil N-amidic forms (Kwon et al. 2009). Thus, it was expected that areas under cover crop would show higher levels of ISNT-N, since cover crop promotes an increase in soil microbial biodiversity (Dalal 1998). The ISNT-N content was highly variable from site to site (Fig. 2), which corroborates with Roberts et al. (2009). The highly variable ISNT-N observed may be more associated with soil texture and soil TC and TN content, as previously pointed out by Laboski et al. (2008). Only the sandy-loam soil (site I) showed higher ISNT-N in the cover crop plots as compared to fallow in selected periods of evaluation (Fig. 2). This could be due to the fact that this soil is

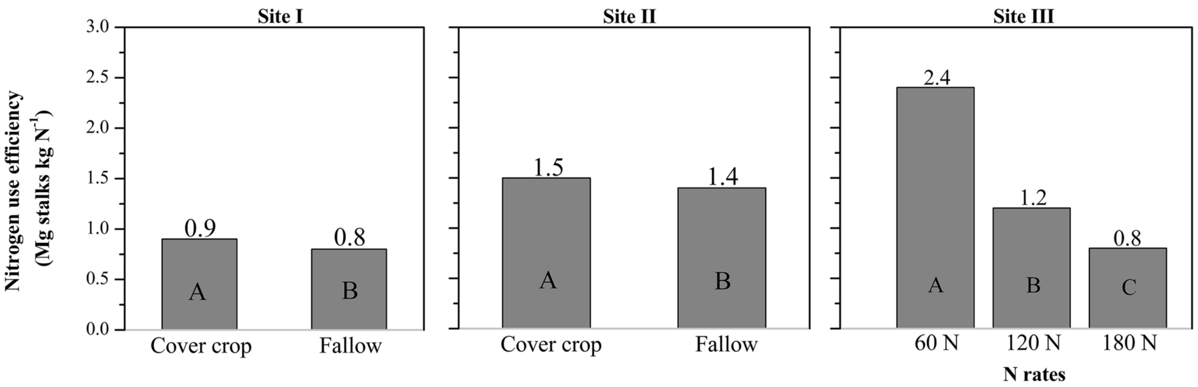

Fig. 4 N use efficiency index (Mg stalk kg^{-1} N) in first and second ratoon crop cycles at site I (Quatá/SP), site II (Chapadão do Céu/GO) and site III (Quirinópolis/GO). Different letters indicate differences among treatments within sites (Fisher´s test at $P < 0.05$)

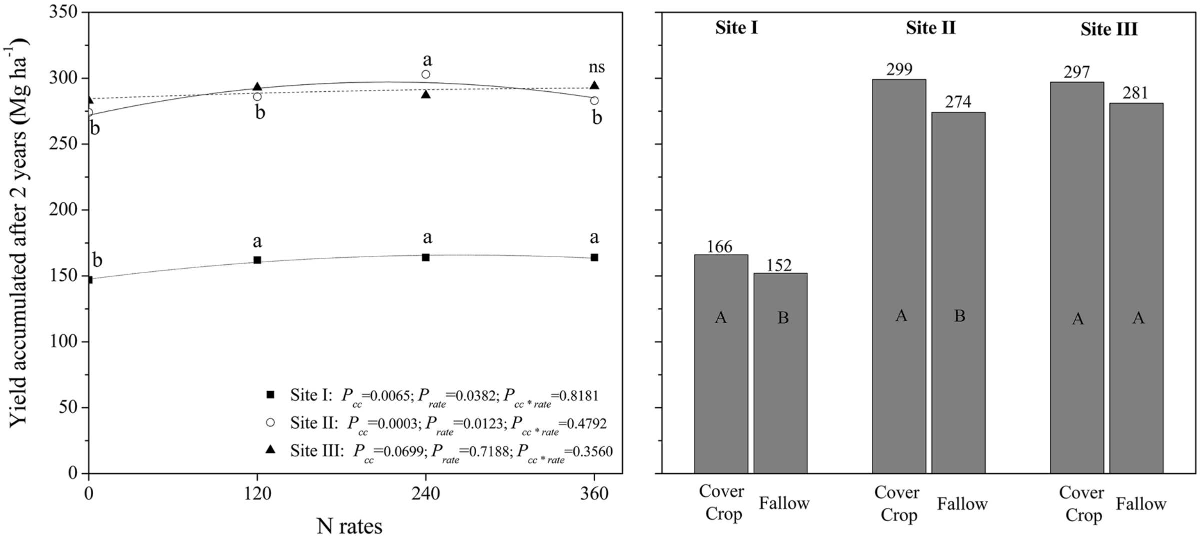

Fig. 5 Accumulated sugarcane yields (Mg ha^{-1}) as a function of N fertilization and cover crop or fallow after a 2-year period at site I (Quatá/SP), site II (Chapadão do Céu/GO) and site III (Quirinópolis/GO). Means followed by different capital letters (gray bars) indicate significant differences in sugarcane yields among management systems and those followed by different lowercase letters indicate differences among treatments within N rates (Fisher's test at $P < 0.05$)

characterized by poor soil fertility and lower water retention capacity, and cover crop management could have enhanced soil organic N content. In sites II and III, the same behavior observed for inorganic N content occurred for ISNT-N. The ISNT-N values were higher in fallow at topsoil. Apparently, the continuous uptake of N from sugarcane caused a decline in ISNT-N content in the plots with cover crop, in which yields were higher than those in the fallow treatment. The ISNT-N content of site I was lower than others sites and can be explained by the lower total C and N in the soil profile at that site (Suppl. Table 1). The ISNT-N values observed at site I were close to values observed in sandy soils cultivated with sugarcane in Brazil (Mariano et al. 2015; Otto et al. 2013), while the ISNT-N values observed at sites II and III were similar to those observed in temperate climate conditions (Khan et al. 2001; Barker et al. 2006; Laboski et al. 2008) or heavy clay content soils of Brazil (Otto et al. 2013).

Considering the averages over soil profile, it was observed that ISNT-N values decreased as a function of sampling times in all sites (Fig. 2). This behavior was expected since the soil samples were collected in the control plots, without N fertilization. The continuous uptake of inorganic N by the crop possibly decreased the ISNT-N content, which is related to a labile pool of the SOM (Kwon et al. 2009), promoting and accumulation of more recalcitrant SOM compounds over time. The high variability of ISNT-N content along the soil profile can be explained by the complex transformations of organic N evaluated by this methodology. For example, the variation of ISNT-N content at greater soil depths may be related to the conversion of organic compounds into more recalcitrant SOM forms in the first 0.15-m depth, formed by the high rate of microbial activity and humification (Roberts et al. 2009). Stratification of ISNT-N content in the soil profile was also demonstrated in other studies (Barker et al. 2006; Roberts et al. 2009; Wall et al. 2010) and can be related to movement of organic and inorganic N forms to the subsoil. The ISNT-N evaluations performed up to 1.0-m in this study differ from those performed by other authors, who focused the evaluations in topsoil. Evaluating ISNT-N variation in deeper soil layers is interesting since sugarcane has a well-developed root system that explores the subsoil (Battie-Laclau and Laclau 2009; Barbosa et al. 2018). Finally, these results indicate that ISNT-N content is quite variable in tropical conditions and is more related to soil characteristics such as texture and TC and TN content than short-term management practices.

Microbial biomass is responsible for SOM transformations, as well as its cycling, since it represents a potential source for N and other nutrients (Bünemann et al. 2006). In our study, MBC was slightly affected by cover crop during the periods evaluated (Fig. 3). This result contrasts with the initial expectations that MBC would be improved by cover crop since it improves soil quality via C return by crop residues to soil (Dalal 1998; Balota et al. 2003; Paul 2014). In our study, the large period from cover crop cultivation to soil sampling, which varied from two to 3.5 years, may have reduced the cover crop effects in MBC. The MBC values found in our study are similar to other studies in soils cultivated with sugarcane in Brazil (Galdos et al. 2009; Silva et al. 2012; Mariano et al. 2015). In general, the lower soil total C and N content at site I may be the reason for the lower MBC content in comparison to other sites. The large variation in MBC over time can possibly be related to fluctuation in soil temperature and moisture that ultimately affect microbial activity (Paul 2014). Interestingly, as soil ISNT-N levels increased under cover crop during the first ratoon (Jan-Jul/15) at site I, the MBC also increased, which expresses that there may be a relationship between MBC and ISNT indicators. At site III, the decrease in soil MBC and ISNT under cover crop during the second ratoon (Jan-Jul/16) suggests that the low microbial growth may have been caused by limitations in soil nutrients availability associated with higher yields observed in these conditions.

Effect of cover crop and N rates on sugarcane production

Small changes in SPAD index were observed in this study. At the first ratoon cycle, the results observed at site I suggest that cover crop may have increased N uptake by sugarcane when there was no fertilizer N applied. At the sites II and III, the results suggest that the N supplied by the soil was enough to maintain high levels of chlorophyll in the leaves. This effect can be related to the higher clay and SOM content at sites II and III and the favorable conditions of temperature and soil moisture to N mineralization process. Another possible reason for the lack of difference in SPAD index is the improved growth of sugarcane in cover crop treatment, which reduced the N and chlorophyll contents in the leaves through the dilution effect (Jarrel and Beverly 1981).

Maximum yields were always obtained in the cover crop compared to fallow in all sites (Table 2). Cover crop promoted an improvement in yield at site II from first to second ratoon cycle in addition to reducing the *common* yield decline from one season to another in the remaining sites. Such results reveal the potential of cover crop cultivation not only in improving yields but also in prolonging the lifetime of the sugarcane field by reducing the yield loss over time. It is interesting to note that cover crop cultivation is usually recommended for low yielding environments in order to improve soil physical and chemical properties and potential yield (Fernandes et al. 2012) rather than being adopted in more productive areas that already show better soil conditions. There is a misconception that high yielding lands will not respond favorably to cover crop addition. The results of this study show that areas with high productive potential (such as sites II and III) can respond favorably to cover crop implementation.

Although there was increased sugarcane yield in plots cultivated with cover crop, the rapid N uptake by the developing plants (in the cane-plant cycle, as well as first and second ratoon) hindered our ability to detect significant increases in inorganic N, MBC and ISNT-N values in these plots compared with plots under fallow. Since there was not a substantial increase in soil inorganic N content by cover crop, it is not possible to expect a reduction in sugarcane responsiveness to N under cover crop treatment; the greater yields obtained in this treatment resulted in higher N uptake by sugarcane growth detected by SPAD values. The absence of significant interaction between N rates and cover crop treatment in the three study sites demonstrate that cover crop does not reduce sugarcane responsiveness to N (see Table 2). In fact, cover crop increases maximum yield and consequently the demand of N for biomass production.

Park et al. (2010) estimated a potential reduction of N-fertilizer rates following cover crop with soybean (without grain removal) by 100%, 60%, 25% and 10% for the first, second, third and fourth ratoon cycles, respectively. However, those authors do not consider the increase in yield potential promoted by cover crop cultivation that certainly would deliver much lower estimates of reduction in N rates. Otto et al. (2013, 2016) also found that areas managed under cover crop, in addition to areas that receives continuous application of organic residues, present a limited to null response to N fertilization. In opposite, Ambrosano et al. (2011a, b) found a synergistic interaction between sunn hemp and N fertilization that produced greater sugarcane yield. Such disparities indicate that the contribution of legume N to the reduction of N fertilizer rates in sugarcane systems is still a research priority. Meanwhile, the cultivation of cover crops in replanting period of sugarcane fields is not used exclusively to reduce N fertilizer usage but also to protect soil surface against erosion, increase water storage, suppress weeds, pests and diseases, and improve soil health in terms of physical, biological and chemical attributes (Fernandes et al. 2012). The advantageous effect of better soil structure by cover crop may have been an important aspect to enhance nutrients uptake from the deeper soil layers to plants' growth (Rosolem et al. 2017).

The results reported herein indicated improvement in NUE promoted by crop rotation in two (sites I and II) of the three sites evaluated. According to Rosolem et al. (2017), cover crops can be an interesting option to increase NUE in cropping systems. At site III, there was no effect of crop rotation on NUE, but the increase in N rates promoted reduction in NUE. Our results corroborate with Dobermann (2005), which observed reduction of NUE with the increase in N fertilizer rates. This is also in line with Thorburn et al. (2017), who found that N fertilizer application rate in ratoon is the major driver of NUE. Our findings suggest that the cultivation of sunn hemp during the renovation period of sugarcane fields have the potential to increase NUE of the following sugarcane ratoon cycles. However, the improvement in sugarcane yield potential promoted by crop rotation did not reduce N-fertilizer demand by succeeding ratoons.

Conclusion

The results of this study showed that cultivation of cover crop during sugarcane replanting did not consistently increase soil N availability for ratoon cycles due to an increase in sugarcane yield that possibly increased N demand for biomass production. The use of a legume cover crop showed significant potential to increase sugarcane ratoon yield in soils with high fertility and high clay content. There were evidences that cover crop with sunn hemp can enhance NUE but did not reduce N-fertilizer demand by ratoon cycles. These results provide important insights into the impact of increasing NUE by sugarcane from sunn hemp cover crop on minimizing N-fertilizer inputs and environmental impacts associated to N losses for sugarcane bioenergy production.

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