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Quantifying soil nitrogen mineralization to improve fertilizer nitrogen management of sugarcane

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Abstract Intensive use of synthetic nitrogen (N) fertilizers for sugarcane (*Saccharum* spp.) production presents environmental challenges for water and air quality as well as impacts profitability for producers. Central to these concerns is a widespread reliance on yield-based recommendations that invoke generic models of crop N response but lack any means to account for variations in soil N-supplying power, a critical determinant of fertilizer N need for cereal, fiber, and tuber crops. The work reported herein was designed to ascertain the impact of soil N mineralization on sugarcane response to N fertilization and was carried out in conjunction with eight N-response trials conducted between 2006 and 2010 at field sites in the largest sugarcane-cultivated area in Brazil. Soil samples were utilized in categorizing the sites as highly responsive, moderately responsive, or nonresponsive to fertilizer N, based on two chemical indices of soil N availability, the Illinois Soil Nitrogen Test (ISNT) and direct steam distillation (DSD), and assessments of (1) net mineralization during aerobic incubation for 12 weeks and (2) incubation-induced changes in soil N fractions obtained by acid (total hydrolyzable N, hydrolyzable NH_4^+-N, amino sugar N, and amino acid N) or alkaline (ISNT-N) hydrolysis. Sugarcane varied widely in response to N fertilization, indicating that yield-based recommendations would often under- or overestimate N requirement and thus adversely impact sustainability of sugarcane-based ethanol production. In studies to evaluate feasibility of soil N testing to improve fertilizer N recommendations, mineral N production upon aerobic incubation was accompanied by significant decreases in hydrolyzable NH_4^+-N and ISNT-N, indicating that both fractions were liberating mineralizable forms of soil N. Test values by the ISNT and DSD were highly correlated, and both showed promise for differentiating soil responsiveness to fertilizer N.

Keywords Response curves · ISNT · DSD · Net mineralization · Soil N test

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Introduction

With current international emphasis on renewable energy sources, increasing attention is being given to cultivation of sugarcane for bioethanol synthesis, and the area under production is expanding (Nass et al. 2007; Hartemink 2008). Sugarcane-based ethanol has become a global energy commodity that is economically competitive with petroleum-based gasoline (Goldemberg 2007), and usage is more environmentally sustainable due, in part, to the mitigating effect of biomass production on greenhouse gas emissions (Macedo et al. 2008; Vries et al. 2010). Brazil leads the world in sugarcane production, utilizing approximately 9.5 million ha and producing 715 million megagrams in 2011.

Synthetic nitrogen (N) fertilizers are utilized extensively to increase sugarcane productivity (Franco et al. 2011; Thorburn et al. 2011). The usual practice in Brazil is to apply 40 to 60 kg N ha^{-1} at planting and to reapply N after cane cutting, a practice known as ratoon fertilization that supplies 60 to 150 kg N ha^{-1} $year^{-1}$. Application rates often follow yield-based recommendations developed to minimize risk of

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N deficiency (e.g., Legendre 2001; Schroeder et al. 2006), but this approach unfortunately lacks any means to account for variations in soil N-supplying power and thus tends to promote excessive N usage. Underfertilization can also occur, particularly when fertilizer N is subject to immobilization following ratoon fertilization of unburned sites (Rossetto et al. 2010).

The fundamental importance of soil N for crop uptake is apparent from the common finding that unfertilized (check plot) yields in N response studies often exceed the yield increase from fertilization (e.g., Lory and Scharf 2003; Mulvaney et al. 2006; Franco et al. 2010; Rossetto et al. 2010), except when soil N availability is depressed by accumulation of carbonaceous residues in a static plot design. Further evidence of this importance is readily available from numerous field studies using ^{15}N-labeled fertilizer because in most cases, crop N uptake is principally derived from soil rather than fertilizer (e.g., Takahashi 1964; Olson et al. 1979; Wilson et al. 1989; Norman et al. 1992; Omay et al. 1998; Schindler and Knighton 1999; Stevens et al. 2005; López-Bellido et al. 2006; Dourado-Neto et al. 2010; Franco et al. 2011). The implication is that the soil N supply must be taken into account if the efficiency of N fertilization is to be optimized (Cassman et al. 1998; Liu et al. 2005; Mengel et al. 2006; Mulvaney et al. 2006; Cui et al. 2008). Profile NO_3^- testing serves this purpose in arid or semiarid regions, whereas a far greater challenge exists for more humid climates, owing to the fundamental impact of microbial N transformations on plant N availability. Mineralization is of foremost importance, as the process by which mineral N is generated from soil organic N reserves (Nannipieri and Paul 2009); however, the product (NH_4^+) is subject to assimilation (immobilization) by heterotrophic microflora unless organic C is limiting, in which case, autotrophic oxidation (nitrification) supplies plant-available NO_3^- while increasing the risk of N loss through leaching or denitrification.

A common approach for quantifying mineralization is to measure the net rate of mineral N production during aerobic or anaerobic incubations at a constant temperature (typically 30–40 °C). This approach is necessarily time-consuming because of the need for a 1- or 2-week incubation, and the results cannot be assumed to represent field conditions, since (1) mineralization–immobilization turnover occurs in the absence of mineral N uptake by a growing plant that also affects soil moisture and aeration while acting as a rhizospheric source of C, (2) the static incubation conditions employed lack the natural temperature fluctuations and wetting and drying cycles that affect microbial N dynamics in the field (Zak et al. 1999; Carpenter-Boggs et al. 2000; Wienhold 2007), (3) only surface soil is typically sampled, and (4) measurements are affected by sieving and any other processing the samples receive before use. Interpretations can be further compromised because mineralization, and microbial processes in general, are inherently affected by a wide array of physical and chemical soil properties and also by many aspects of management, including the cropping system, tillage and fertilizer practices, and residue inputs (Wu et al. 2008).

Ideally, mineralizable N would be estimated by a chemical soil test, but for years, this approach was impeded by fundamental flaws in steam distillation methods commonly used to fractionate soil N by acid hydrolysis, which led to serious underestimation of two key fractions, amino acid N and amino sugar N. These flaws were eliminated by Mulvaney and Khan (2001) in developing simple diffusion methods that were subsequently utilized to compare N distribution analyses for soils that differed in the yield response by corn (*Zea mays* L.) to N fertilization. The results showed an invariably higher concentration of amino sugar N for nonresponsive than for responsive soils, whereas no consistent difference was detected in analyses for total hydrolyzable N, hydrolyzable NH_4^+-N, or amino acid N (Mulvaney et al. 2001). Based on this finding, Khan et al. (2001) developed a simple soil test (the so-called Illinois Soil N Test or ISNT) for estimating the NH_4^+-N liberated by alkaline hydrolysis using 2 M NaOH, which completely resolved 12 nonresponsive from 13 responsive soils assuming a critical test range of 225–235 mg kg^{-1}. In a much more extensive evaluation involving 102 on-farm N-response studies, Mulvaney et al. (2006) found that the ISNT was highly significant for prediction of check plot yield, the yield increment with optimal fertilization (delta yield), fertilizer N uptake efficiency (FNUE), and economically optimum N rate (EONR). The critical range established by Khan et al. (2001) proved highly effective in detecting 31 of 33 nonresponsive site years but was exceeded for 15 of 69 responsive sites with no apparent limitation from moisture stress. Some of these errors were attributed to soil acidity that would have impeded mineralization, whereas others occurred where high plant populations would have increased crop N requirement and generated a larger input of carbonaceous residues, thereby promoting immobilization that would have reduced plant N availability.

The ISNT has been evaluated independently in several studies for prediction of crop N response, with mixed results. On the positive side, Ruffo et al. (2006) recognized considerable potential for site-specific N management after finding that test values were highly predictive of spatial variability in corn yield, and Williams et al. (2007a,b) reported strong linear regressions relating the ISNT to delta yield and EONR. Successful results were also reported by Klapwyk and Ketterings (2006) and Lawrence et al. (2009), but their work showed the ISNT to be an adequate predictor of fertilizer N responsiveness only if interpreted in conjunction with organic matter estimates using loss on ignition. This type of interpretation addresses heterotrophic interaction of C and N that is so central to soil N cycling and

availability and should improve the predictive value of the ISNT unless mineralization is limited by soil moisture (Steckler et al. 2008).

Confounding of ISNT interpretations no doubt contributed to unfavorable evaluations by Barker et al. (2006), Marriott and Wander (2006), Laboski et al. (2008), and Osterhaus et al. (2008), as data were combined without regard to differences in climate, crop rotation, residue C input, tillage, manure management, and/or planting rate. Although two of these studies showed the ISNT to be significantly related to EONR (Laboski et al. 2008; Osterhaus et al. 2008), the relationship was rather weak, while a strong correlation was observed with total soil N, leading the authors to conclude that the ISNT is not selective for labile soil N. This concern is difficult to reconcile with the fact that alkali-hydrolyzable N can vary considerably in relation to total soil N (Roberts et al. 2009a) and is inconsistent with organic N fractionation and ^{15}N uptake studies by Kwon et al. (2009), which demonstrate that the ISNT is primarily a measure of bacterial amino sugar and amide N.

If alkaline hydrolysis liberates a readily mineralizable form of soil N, the ISNT should have broad application to many other crops besides corn. Encouraging evidence in this direction has recently been provided by Roberts et al. (2011) for direct-seeded, delayed-flood rice (*Oryza sativa* L.), with successful calibration of fertilizer N response (FR) based on the ISNT or direct steam distillation (DSD), a related technique that is highly correlated (Bushong et al. 2008; Roberts et al. 2009b). Distillation reduces the analytical period from hours to minutes but is much more labor-intensive when large numbers of samples must be processed in routine soil testing.

The present study explores the potential of soil-based N management for sugarcane production in Brazil, using soil samples from eight N-response trials at five on-farm sites. Several measures of soil N-supplying power were evaluated relative to FR, including the ISNT, DSD, and net N mineralization. Soil N fractionations were utilized to clarify the chemical nature of labile organic N and further evaluate the ISNT.

Materials and methods

Experimental sites

Eight N-response trials for ratoon fertilization, representing different soil textures, cultivars, climatic conditions, and management histories, were conducted from 2006 to 2010 at five field sites in São Paulo state (Fig. 1). A randomized complete block design was utilized in each case, with four to five N rates ranging from 0 to 200 kg ha^{-1}, and four (sites 1 to 4) or five (site 5) replications per N rate. The N doses were chosen to extend well beyond yield-based recommendations of 60 to 120 kg ha^{-1} that are typical for ratoon N fertilization in São Paulo. Between 1 and 2 months after harvest, NH_4NO_3 was manually applied to the surface of the straw, beside the cane row and without incorporation. Plots consisted of seven (sites 1, 4, and 5) to 12 (sites 2 to 3) rows of sugarcane, each 15 m long and spaced at 1.5-m intervals. In order to obtain sugarcane stalk yield (megagrams per hectare of fresh phytomass cut from soil level to the top of the stalk, excluding the tops), the four middle rows were harvested mechanically using a transport truck equipped with a loading cell to measure stalk weights, and the residual straw was left unburned after being evenly distributed over the area harvested. Table 1 provides further details about each study site, including the soil subgroup, sugarcane variety, harvest and soil sampling dates, and any return of sugarcane by-product material.

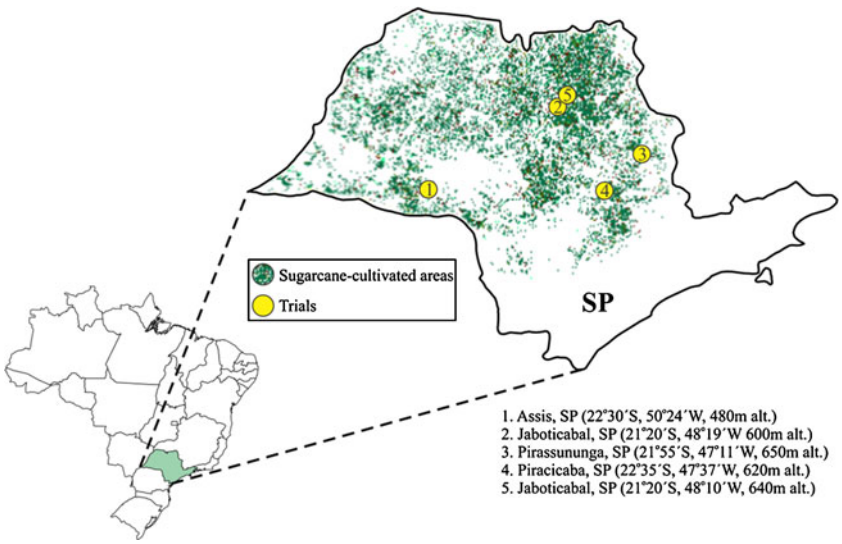

Fig. 1 Locations of the experimental sites in relation to sugarcane-cultivated areas in the state of São Paulo (SP), Brazil

Table 1 Soil classification and management summary for study sites

Site no.	Soil subgroup[a]	Sugarcane variety	Harvest date[b]			Soil sampling	Organic amendment[c]
			Trial 1	Trial 2	Trial 3		
1	Typic Hapludox	SP83 2847	8/2009 (1)	–	–	8/2008	N
2	Typic Kandiudox	SP81 3250	7/2007 (1)	7/2008 (2)	–	7/2006	V
3	Typic Hapludox	SP81 3250	6/2007 (1)	–	–	6/2006	N
4	Typic Hapludox	SP89 1115	6/2010 (1)	–	–	10/2009	V, FC
5	Rhodic Eutrudox	SP80 3280	9/2008 (1)	11/2009 (2)	11/2010 (3)	10/2009	V

N none, *V* vinasse, *FC* filter cake

[a] According to Soil Survey Staff (2010)

[b] Reported in month/year format, with a parenthetical value representing the number of previous harvests since sugarcane establishment

[c] No application of these residues during the experiment or within the preceding 2 years

Soil samples

To evaluate different methods of characterizing soil N-supplying power for prediction of sugarcane N response, soil samples were collected before N fertilizer application at the experimental sites. Sampling was done on the dates specified in Table 1 and followed any previous N fertilization by at least 12 months.

At each site, surface and subsurface samples were collected with a 7.5-cm (diameter) auger from individual plots, based on sampling depths of either 0–20 and 20–40 cm (sites 1, 4, and 5) or 0–30 and 30–60 cm (sites 2–3). Five subsamples per plot, each collected approximately 25 cm from the row, were composited in plastic bags that were left open during transport to the laboratory, and drying was subsequently carried out at 40 °C in a forced-air oven, so as to arrest microbial activity. The dried samples were crushed to pass through a 2-mm screen, followed by storage at room temperature in sealed polyethylene bags.

For physicochemical characterization (Table 2), composite surface and subsurface samples were prepared for each site by mixing equal weights of soil collected from all plots in different blocks. Only the surface-composited samples were utilized in estimating net mineralization or in fractionating hydrolyzable soil N. So as to better account for spatial variability in evaluating the ISNT and DSD, individual blocks as well as sampling depths were kept separate in combining equal weights of soil from different plots.

Table 2 Physicochemical properties of soils

Site no.	Sampling depth (cm)	pH[a]	Organic C[b] ($g kg^{-1}$)	Total N[c] ($g kg^{-1}$)	CEC[d] ($cmol_c kg^{-1}$)	Sand[e] ($g kg^{-1}$)	Silt[e] ($g kg^{-1}$)	Clay[e] ($g kg^{-1}$)	WHC[f] ($ml kg^{-1}$)
1	0–20	5.6	5.5	0.40	2.5	864	11	126	350
	20–40	5.0	4.8	0.34	2.6	858	16	126	360
2	0–30	5.4	7.8	0.53	4.4	661	54	285	460
	30–60	4.6	5.5	0.40	4.6	616	50	334	460
3	0–30	6.1	7.7	0.49	3.9	727	56	218	440
	30–60	5.6	5.5	0.37	3.1	693	61	247	440
4	0–20	4.9	21.0	1.33	11.5	470	20	510	490
	20–40	5.0	18.8	1.02	13.3	450	20	530	520
5	0–20	5.2	16.8	1.41	13.3	239	46	716	520
	20–40	5.3	14.1	1.18	12.7	242	42	716	520

Data reported as a mean of triplicate determinations, except for unreplicated textural determinations

[a] Soil:water ratio, 1:1

[b] Determined by the method of Mebius (1960)

[c] Determined by the regular Kjeldahl method using a block digester (Bremner 1996), followed by diffusion with NaOH (Stevens et al. 2000)

[d] Cation-exchange capacity (CEC) determined by NH_4^+ saturation–diffusion (Mulvaney et al. 2004)

[e] Textural analyses performed using the hydrometer method (Gee and Bauder 1986)

[f] Water-holding capacity (WHC) determined as described by Bremner and Shaw (1958)

Net mineralization

To estimate soil N availability from mineral N production during aerobic incubations, 18 samples of each soil (6 g sample^{-1}) were individually weighed onto a Whatman QM-A quartz filter disk in a 5.5-cm (diameter) polypropylene Büchner funnel, and mineral N was removed by leaching under vacuum with two 30-ml aliquots of 0.01 M $CaCl_2$, followed by 10 ml of "minus-N" nutrient solution (Stanford and Smith 1972). The soil moisture content was adjusted to 60 % water-holding capacity (WHC) by addition of deionized water, and funnel cups containing 15 moistened samples of each soil were then transferred to incubate for 1, 2, 4, 8, or 12 weeks at 25 °C and 80–90 % relative humidity, with periodic weighing for maintenance of soil moisture content. The remaining samples were processed as subsequently described to serve as a zero-time control.

Following incubation, or at 0 week, triplicate samples were transferred with the filter material to 125-ml polyethylene bottles, and mineral N was extracted by shaking the soil sample with 60 ml of 2 M KCl for 1 h and filtering the resulting suspension through Whatman no. 42 filter paper in a polypropylene Büchner funnel under vacuum. To reduce immobilization of mineralized N, samples incubated for 4, 8, or 12 weeks were leached under vacuum at biweekly intervals with two 30-ml aliquots of 0.01 M $CaCl_2$, the leachate was composited with any other collected for the same sample, and the sample was then leached with 10 ml of "minus-N" nutrient solution (Stanford and Smith 1972), readjusted to 60 % WHC with deionized water, and returned to the incubator. Extracts and leachates were analyzed for NH_4^+-N and (NO_3^-+NO_2^-)-N by accelerated diffusion methods (Khan et al. 1997).

Soil nitrogen fractionation

In conjunction with the study described for estimating net mineralization, samples incubated for 0 or 12 weeks were utilized to evaluate how incubation had affected soil N composition. Following vacuum filtration to collect the soil extract, residual soil on the filter paper was allowed to dry for several days at room temperature and was then scraped with a spatula from the paper into a polyethylene bag, crushed to a powder by rolling a glass bottle over the bag, and thoroughly homogenized. Soil hydrolysates were prepared by heating 4 g of soil under reflux at 110 °C for 12 h in 50-ml round-bottom flasks fitted with a 24/40 ground glass joint for attachment to a 40-cm Liebig condenser after treatment with 16 ml of 6 M HCl and two drops of octanol (to prevent foaming). Heating was carefully regulated by using a six-zone stirring hot plate (PMC Model 526C, Barnstead International, Dubuque, IA, USA) modified for individual electronic temperature control of stirred oil baths that each accommodated two flasks. As soon as possible, the hydrolysis mixture was transferred to allow vacuum filtration through Whatman no. 50 filter paper in a Büchner funnel, and the transfer was completed by rinsing the flask twice with 10 ml of deionized water from a wash bottle. The hydrolysate was collected in a 125-ml glass bottle, stored at 5 °C in a refrigerator, and subsequently neutralized in a 250-ml beaker by sequential additions of 10, 5, and 1 M NaOH to obtain a pH between 6.5 and 6.8 (Stevenson 1996). The neutralized hydrolysate was diluted to 100 ml with deionized water, returned to the storage bottle and then refrigerated.

Fractionations of hydrolyzable N were performed in triplicate using the diffusion methods described by Mulvaney and Khan (2001) for determination of total hydrolyzable N, NH_4^+-N, (NH_4^++amino sugar)-N, and amino acid N. Amino sugar N was determined as the difference between NH_4^+-N and (NH_4^++amino sugar)-N.

ISNT and DSD

The Illinois Soil Nitrogen Test (ISNT) originated by Khan et al. (2001) was performed in triplicate, with modifications to improve the uniformity of heating (^{15}N Analysis Service 2012), on block-composite samples of surface and subsurface soils and also on both sets of soil samples selected for N fractionation. To ensure data quality, every set of analyses included a chemical standard containing 250 μgN as glucosamine·HCl. Analyses by DSD were also performed in triplicate and followed the technique described by Roberts et al. (2009b).

Statistical analysis

Sugarcane yield data from the N-response studies were subjected to analysis of variance (ANOVA). When the F test showed significance at $P<0.10$, regression analyses were performed (Webster 2007) using a linear or quadratic regression model optimized for each data set. In cases where the F test was significant ($P<0.10$), FR was calculated from mean yield data as 100×(maximum yield−control yield)/control yield. With a nonsignificant F test at $P>0.10$, FR was considered to be zero. The study sites were thereby classified as nonresponsive (FR=0), moderately responsive (FR≤25 %), or highly responsive (FR>25 %).

Means and standard deviations were computed for replicate determinations, while incubation data were subjected to ANOVA assuming a factorial design involving soil as one factor. In cases where the F test was significant, mean values were compared on the basis of a least significant difference (LSD) at $P<0.05$. Pearson's correlation analyses were performed to quantify the relationship of different soil parameters to check plot yield or FR.

Results and discussion

Since the 1990s, there has been a growing trend in Brazil toward mechanical harvesting of sugarcane in areas where preharvest cane burning was prevalent. With the new system, aboveground crop residues, consisting of leaves and tops that typically range from 13 to 20 $Mg\,ha^{-1}$ of dry matter, remain on the soil surface to form a highly carbonaceous vegetative cover referred to as straw. This material contains an appreciable amount of N (70 to 100 $kg\,ha^{-1}$), but availability is very limited to the following ratoon crop, owing to immobilization of mineral N during microbial decomposition. The transition toward mechanical harvesting would, thus, be expected to increase the need for ratoon N fertilization although an offsetting effect may arise over the agricultural cycle (>4 years) as mineralization gradually liberates straw N (Fortes et al. 2011).

Although all five of the sites studied in our work were harvested mechanically without cane burning, Table 3 shows that only three (sites 1 to 3) were responsive to N fertilization at $P<0.10$. No response was observed in a single trial at site 4 or in three trials at site 5. Such variation is typical of sugarcane harvested without burning, according to previous studies documenting N response that occurs in many such cases (Rossetto et al. 2010; Vieira et al. 2010) but not in others. Rosetto et al. (2010), for example, reported considerable N response for 10 of 15 sites studied, whereas one site was nonresponsive and four others showed very limited but significant response.

Crop responsiveness to N fertilization can be limited by weather conditions that either promote fertilizer N loss through volatilization, leaching, or denitrification or that enhance the uptake of soil-derived N. No such limitation occurred for the eight trials reported herein, as conditions were adequate for optimum sugarcane growth with mean monthly temperatures of 15 to 26.5 °C and rainfall between 1,366 and 1,865 $mm\,year^{-1}$. Volatilization losses would have been negligible because NH_4NO_3 was applied to acidic soils (Table 2), while leaching losses were very limited when measured for two of the sites by Ghiberto et al. (2009, 2011).

A reduction in sugarcane response to synthetic N was expected for sites 2, 4, and 5, owing to previous inputs of vinasse and/or filter cake (Table 1), two widely used by-products of agroindustrial biofuel production that will be increasingly important due to regulations mandating environmental protection. These materials are much lower than straw in their C/N ratios and thus provide a more readily available source of N through short-term mineralization that reduces fertilizer N requirement by 50 to 150 $kg\,N\,ha^{-1}$ at typical application rates (Prasad 1976; Yaduvanshi et al. 1990) and can even eliminate FR, as occurred for sites 4 and 5. A similar effect has been reported for sites where sugarcane reestablishment follows a legume such as *Crotalaria juncea*, *Crotalaria spectabilis*, soybean (*Glycine max* L. Merr.), or peanut (*Arachis hypogaea* L.) (Resende et al. 2003; Ambrosano et al. 2005; Park et al. 2010).

Comparison of Tables 1 and 3 leaves no doubt about the importance of organic inputs to the range of FR observed for the five study sites. The greatest responses, ranging from 9 to 30 % FR, occurred at sites 1 and 3, where there was no application of vinasse or filter cake that would have moderated microbial N tie-up during decomposition of crop residues. Of the three sites that received one or both of these by-products, site 2 was moderately responsive at 5 to 7 % FR, while sites 4 and 5 were nonresponsive.

The importance of organic inputs is no less apparent for multiple trials conducted at sites 2 and 5, which necessarily followed a static plot design because individual plots received the same treatment in multiple years. This type of response study often leads to temporal inflation of FR (e.g., Swanson et al. 1973; Vanotti et al. 1997; Varvel and Wilhelm 2003), as check plot yield is depressed by a build-up of residue C in the absence of N fertilization. No such trend is apparent from Table 3. On the contrary, FR was consistent for both trials conducted at site 2 and for all three at site 5. The implication is that previous vinasse application supplied sufficient N to support heterotrophic oxidation of residue C and thereby prevented check plot yield depression by microbial immobilization.

Besides organic inputs, differences in soil N-supplying power probably contributed to the range of FR documented by Table 3. Such differences have long been recognized to affect crop response to N fertilization (e.g., Black et al. 1946; Pritchett et al. 1947; Fitts et al. 1953; Olson et al. 1960), which is consistent with considerable evidence from ^{15}N-tracer studies that mineralization often supplies the bulk of crop N uptake from fertilized soils (e.g., Takahashi 1964; Olson et al. 1979; Wilson et al. 1989; Norman et al. 1992; Omay et al. 1998; Schindler and Knighton 1999; Stevens et al. 2005; López-Bellido et al. 2006), as was the case in recent Brazilian studies by Dourado-Neto et al. (2010) and Franco et al. (2011). An increase in soil N mineralization capacity would, of course, be expected to decrease crop response to N fertilization although the consequences may not be apparent if climatic conditions are conducive to N loss or if N availability does not limit yield.

Biological methods have long been employed to estimate soil N-supplying power based on the net rate of mineral N production when soil samples are incubated under static conditions. A 12-week aerobic incubation of surface samples was adopted for this purpose in our work, following the technique employed by Mulvaney et al. (2001). The results (Table 4) show that for all five sites studied, mineral N production was most rapid in the first week after rewetting the dried samples and decreased considerably in the second

Table 3 Sugarcane productivity in eight N response trials conducted between 2006 and 2010

Site no.	Fertilizer N applied (kg ha^{-1})	Productivity[a] (Mg ha^{-1})		
		Trial 1	Trial 2	Trial 3
Highly responsive site				
1	0	69.1 (3.5)	–	–
	50	75.6 (6.5)	–	–
	100	77.3 (4.5)	–	–
	150	86.9 (4.6)	–	–
	200	90.0 (5.3)	–	–
	P	0.0004		
	Equation	$y=69.18+0.1060x$		
	R^2	0.96		
	FR[b] (%)	30.2		
Moderately responsive sites				
2	0	114.1 (7.9)	104.1 (5.4)	–
	50	120.8 (7.7)	104.1 (6.8)	–
	100	120.8 (10.9)	106.0 (8.1)	–
	150	121.9 (6.2)	109.7 (6.1)	–
	P	0.0399	0.0821	
	Equation	$y=114.52+0.1294x-0.00055x^2$	$y=103.15+0.0376x$	
	R^2	0.92	0.82	
	FR[b] (%)	6.8	5.4	
3	0	78.8 (5.9)	–	–
	50	81.1 (7.4)	–	–
	100	85.9 (7.4)	–	–
	150	86.4 (9.0)	–	–
	P	0.0823		
	Equation	$y=78.90+0.0055x$		
	R^2	0.93		
	FR[b] (%)	9.7		
Nonresponsive sites				
4	0	91.4 (12.3)	–	–
	50	95.6 (12.6)	–	–
	100	85.0 (16.5)	–	–
	150	78.6 (5.2)	–	–
	200	77.8 (10.9)	–	–
	P	0.1136		
	FR[b] (%)	0		
5	0	80.2 (6.7)	142.4 (19.5)	81.2 (7.1)
	50	80.3 (6.1)	138.3 (10.9)	75.8 (9.7)
	100	86.8 (7.2)	131.5 (12.4)	87.2 (2.1)
	150	80.5 (3.4)	132.3 (14.7)	82.6 (9.7)
	200	83.1 (7.0)	135.6 (14.0)	79.9 (7.0)
	P	0.3456	0.7738	0.2005
	FR[b] (%)	0	0	0

Trials conducted in 2006–2007 (sites 2 and 3), 2007–2008 (sites 2 and 5), 2008–2009 (sites 1 and 5), or 2009–2010 (sites 4 and 5)

[a]Data represent four (sites 1–4) or five (site 5) experimental blocks. Standard deviations are reported in parentheses

[b]FR, fertilizer N response calculated when $P<0.10$ as 100×(maximum yield−control yield)/control yield

week, during which net immobilization occurred for soils 1 to 3. Continued incubation usually led to net mineralization, but temporal fluctuations were common in the measured rates, no doubt reflecting the turnover of microbial biomass.

Table 4 shows that soils from the study sites differed significantly in mineral N production during the first 4 weeks of incubation and that only for the two nonresponsive soils (4 and 5) did net mineralization continue throughout this

Table 4 Capacities of surface soil samples for net N mineralization

	$(NH_4^+ + NO_3^- + NO_2^-)$-N produced[b] (mg kg^{-1} d^{-1})											
	0–1 week		1–2 weeks		2–4 weeks		4–8 weeks[b]		8–12 weeks[b]		0–12 weeks[b]	
Site no.	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Highly responsive site												
1	0.57c	0.03	−0.05ab	0.56	−0.02c	0.24	0.20	0.34	0.17	0.09	0.17	0.16
Moderately responsive sites												
2	1.25b	0.32	−0.27b	0.04	0.56a	0.18	0.31	0.08	−0.08	0.11	0.25	0.03
3	0.52c	0.13	−0.27b	0.36	0.12c	0.30	0.20	0.14	−0.03	0.02	0.10	0.04
Nonresponsive sites												
4	2.12a	0.41	0.07a	0.09	0.23bc	0.21	0.31	0.11	0.29	0.15	0.42	0.10
5	1.22b	0.10	0.08a	0.24	0.49ab	0.17	0	0.18	0.11	0.05	0.23	0.06
P<	0.001		0.1		0.01		NS		NS		NS	

6-g soil samples were moistened to 60 % WHC and incubated (25 °C, 85–90 % relative humidity) in triplicate for the period specified

SD standard deviation, *NS* not significant

[a] Mean values followed by the same letter within a column do not differ significantly according to the LSD ($P<0.05$)

[b] Values include $(NH_4^+ + NO_3^- + NO_2^-)$-N recovered by leaching soil samples with 0.01 M $CaCl_2$ and by extraction with 2 M KCl at the end of incubation. Leaching with $CaCl_2$ was performed every 2 weeks, once for samples incubated 4 weeks, three times for samples incubated 8 weeks, and five times for samples incubated 12 weeks

period and was never followed by net immobilization. Data from the first week of incubation gave better correlations with check plot yield ($r=0.89$***) and FR ($r=-0.67$**) than were obtained with longer incubation periods, but even so, the process would be much more time-consuming than is desirable for routine assessment of soil N availability.

Table 5 Concentrations of hydrolyzable N in surface soil samples before and after aerobic incubation for 12 weeks

Site no.	Incubation period (weeks)	Hydrolyzable N[a] (mg kg^{-1})											
		Total		AA		NH_4^++AS		NH_4^+		AS[b]		ISNT	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Highly responsive site													
1	0	306	15	100a	16	112a	3	61a	12	51a	15	50a	15
	12	321	5	82b	20	84b	2	49a	3	35b	4	38a	11
Moderately responsive sites													
2	0	465	17	137a	3	151a	9	97a	9	54b	1	94a	8
	12	490	30	136a	3	141a	1	62b	3	78a	3	80b	5
3	0	444	12	130a	13	146a	7	85a	2	61b	5	94a	2
	12	473	35	134a	5	142a	2	65b	4	76a	4	83a	8
Nonresponsive sites													
4	0	931	5	300a	1	334a	5	172a	10	162b	10	188a	14
	12	893	7	270b	17	302b	6	120b	8	182a	12	164b	11
5	0	1,040	32	351a	12	378a	17	210a	15	168b	11	225a	6
	12	1,012	74	340a	13	368a	12	113b	16	255a	4	207b	9
Mean for all sites													
	0	721	7	204a	3	224a	2	125a	3	99b	2	130a	2
	12	716	7	192b	3	207b	2	82b	3	125a	2	114b	2

AA amino acid, *AS* amino sugar, *ISNT* Illinois Soil Nitrogen Test, *SD* standard deviation

[a] Triplicate determinations. Mean values followed by the same letter within a column for a single site do not differ according to the LSD ($P<0.05$)

[b] Determined as $(NH_4^+ + AS)$-N$-NH_4^+$-N

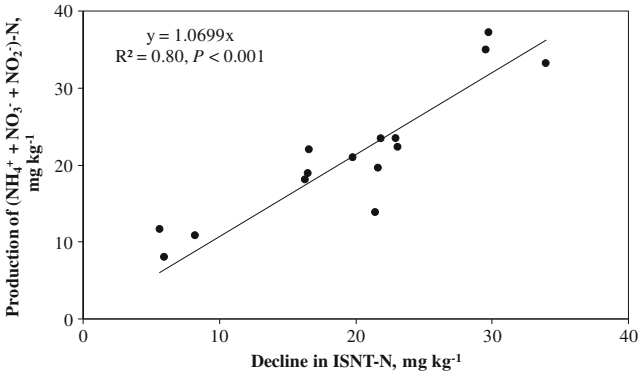

Fig. 2 Relationship between the magnitude of decline in the fraction of organic N determined by the Illinois Soil Nitrogen Test (ISNT) and inorganic N production following a 12-week aerobic incubation of the soils studied

A more practical option is to estimate a chemical quantity of plant-available or labile soil N in lieu of mineralization assays. The usual approach has been NO_3^- testing (e.g., Carson 1975; Magdoff et al. 1984; Blackmer et al. 1989; Bundy and Meisinger 1994), but the past decade has brought new hope that a chemical approach may also be useful for predicting a soil's mineralization capacity, based on a comparative study by Mulvaney et al. (2001) of soil N composition for sites that differed in whether corn had been responsive to N fertilization. Upon incubation, mineral N production was found to be much more extensive by nonresponsive than by responsive soils and to be accompanied by a net decrease in amino sugar N but not in amino acid N (Mulvaney et al. 2001).

So as to ascertain whether these findings are relevant for Brazilian soils under sugarcane production, acid hydrolysis was performed before and after the 12-week aerobic incubation utilized in estimating net mineralization. Table 5 summarizes the data subsequently obtained by N distribution analysis, documenting the expected trend toward lower concentrations of hydrolyzable N as total soil N (Table 2) decreased in the order: site 5>site 4>site 2>site 3>site 1. More importantly, Table 5 shows that incubation had different effects on the soil N fractions obtained by acid hydrolysis. No significant changes were observed in total hydrolyzable N and few changes in amino acid N, whereas a significant decrease in hydrolyzable NH_4^+-N was often accompanied by a significant increase in amino sugar N. As would be expected from their opposing directions, these changes were seldom reflected in (NH_4^++amino sugar)-N.

The results in Table 5 are consistent with the view that amino sugar N is more labile than amino acid N. This view rests on incubation evidence paralleling what was reported by Mulvaney et al. (2001), namely, a much greater net change in amino sugar N than amino acid N during aerobic incubations that involved periodic leaching to control immobilization. In contrast to the decreases observed in their work for amino sugar N, the net change was usually positive in the present study, reflecting biomass synthesis during microbial N assimilation. No definite explanation can be given for this disparity, but attention should be drawn to the soil, climatic, and cropping differences that existed for the Oxisols incubated in our work, as opposed to the Mollisols and Alfisols studied by Mulvaney et al. (2001). Such differences would be expected to affect the soil microfloral population and might also have affected the pattern of microbial cycling and the array of hydrolyzable amino compounds produced during incubation of the rewetted soil samples. Interestingly, incubation led to a decrease in hydrolyzable NH_4^+ with every soil studied, which implicates an easily degradable fraction in serving as a source of mineralizable N and suggests that mild hydrolysis should be feasible for estimating soil N-supplying power.

Table 6 Characterization of study sites by the Illinois Soil Nitrogen Test (ISNT) and Direct Steam Distillation (DSD)

Site no.	Sampling depth (cm)	ISNT[a] (mg N kg^{-1})		DSD[a] (mg N kg^{-1})	
		Mean	SD	Mean	SD
Highly responsive site					
1	0–20	57	6.0	51	4.5
	20–40	49	3.2	46	3.7
Moderately responsive sites					
2	0–30	85	6.3	78	3.4
	30–60	67	5.6	63	4.7
3	0–30	77	7.1	70	5.9
	30–60	62	4.2	55	6.7
Nonresponsive sites					
4	0–20	175	9.2	160	7.7
	20–40	146	10.3	132	11.6
5	0–20	209	5.5	193	12.9
	20–40	174	10.1	163	17.6

SD standard deviation

[a]Data represent 12 (sites 1–4) or 15 (site 5) values obtained when triplicate determinations were performed on soil samples collected from four (sites 1–4) or five (site 5) replicated field plots

The latter strategy becomes more promising when addressed with the ISNT, an alkaline diffusion technique performed directly on the soil itself without the use of acid hydrolysis. As shown by Table 5, incubation led to a significant ISNT decrease that occurred for every site studied. The decreases were strongly correlated with the corresponding changes in hydrolyzable NH_4^+-N (r=0.92***), indicating that both fractions were liberating similar forms of soil N. More importantly, Fig. 2 shows that net production of mineral N was closely related to the decline in ISNT values.

Given the evidence from Table 5 and Fig. 2 that a mineralizable form of soil N was being estimated by the ISNT, there was good reason to expect that soil testing for alkaline-hydrolyzable N would be useful for differentiating sites where sugarcane varied in yield response to N fertilization. This is verified by Table 6, which summarizes data obtained by the ISNT and also by DSD for surface and subsurface soil samples collected from the five study sites. With either technique, test values correctly ranked all five of these sites, decreasing in the order: nonresponsive sites 4 and 5>moderately responsive sites 2 and 3>highly responsive site 1. Both methods were highly correlated with FR (r=−0.78***), an encouraging sign of potential for adjusting sugarcane fertilizer N recommendations in Brazil and the first indication that these techniques may have broader application than has been explored in previous evaluations for corn or rice production (Khan et al. 2001; Barker et al. 2006; Klapwyk and Ketterings 2006; Mulvaney et al. 2006; Williams et al. 2007a,b; Laboski et al. 2008; Osterhaus et al. 2008; Spargo et al. 2009; Roberts et al. 2011).

As reported previously by Bushong et al. (2008), results obtained by the ISNT and DSD were significantly correlated (r=0.996***). In contrast to their findings, however, analytical precision was slightly lower by DSD than with the ISNT, according to a somewhat higher coefficient of variation that averaged 7.8 % as opposed to 6.6 %. Distillation reduces the analytical period to a few minutes, but daily sample throughput was actually higher with the ISNT, which is much less labor intensive. Automation will be necessary to realize the practical potential of DSD.

If the ISNT or DSD is to be utilized effectively for sugarcane N management, reliable calibrations must be developed to predict FR for a range of test levels. A replicated small-plot design similar to that employed in the present project is satisfactory for this purpose, provided that (1) study sites are located in production fields, such that all plots share the same management history; (2) care is taken to ensure that the same area is sampled for soil testing and yield measurement; and (3) productivity is not limited by adverse weather conditions. The importance of calibration is readily apparent, considering that sugarcane was nonresponsive in the present study with ISNT levels of 175–209 mgNkg^{-1} (Table 6), whereas Khan et al. (2001) found that temperate soils under corn production became nonresponsive above 235 mgNkg^{-1}. A lower critical threshold would be expected with the tropical climate of Brazil, owing to more intensive microbial activity and a much longer growing season that would prolong the cumulative uptake of mineralized soil N by sugarcane.

Conclusions

With the growing importance of sugarcane cultivation for energy production and with a shift from cane burning toward the return of crop residues and the use of by-products such as vinasse and filter cake, there is a growing need to improve fertilizer N management by accounting for the soil's capacity for mineralization. This strategy appears feasible according to the present study that utilized soil samples from five sites where eight ratoon N response trials were conducted in the state of São Paulo, Brazil.

Sugarcane yield ranged widely among the study sites in FR, indicating that yield-based recommendations would often under- or overestimate N requirement and thereby reduce the sustainability of sugarcane-based ethanol production. Our findings suggest that fertilizer N recommendations can be improved by accounting for differences in soil N availability, using the ISNT or DSD as a chemical index of alkaline-hydrolyzable N. This fraction was found to decrease during incubations that led to net production of mineral N and proved effective for differentiating soil responsiveness for ratoon N fertilization of sugarcane. Further evaluations are warranted, with a larger number of trials to ensure reliable calibration for soil-based N recommendations.

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