Revista Brasileira de Ciência do Solo
ISSN: 0100-0683
revista@sbcs.org.br
Sociedade Brasileira de Ciência do Solo
Brasil

Mariano, Eduardo; Ocheuze Trivelin, Paulo Cesar; Leite, José Marcos; Vieira Megda, Michele Xavier; Otto, Rafael; Coutinho Junqueira Franco, Henrique

INCUBATION METHODS FOR ASSESSING MINERALIZABLE NITROGEN IN SOILS UNDER SUGARCANE

Revista Brasileira de Ciência do Solo, vol. 37, núm. 2, 2013, pp. 450-461
Sociedade Brasileira de Ciência do Solo
Viçosa, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=180226346016

DIVISÃO 3 - USO E MANEJO DO SOLO

Comissão 3.1 - Fertilidade do solo e nutrição de plantas

INCUBATION METHODS FOR ASSESSING MINERALIZABLE NITROGEN IN SOILS UNDER SUGARCANE[1]

Eduardo Mariano[2], Paulo Cesar Ocheuze Trivelin[3], José Marcos Leite[2], Michele Xavier Vieira Megda[2], Rafael Otto[4] & Henrique Coutinho Junqueira Franco[5]

SUMMARY

Considering nitrogen mineralization (N) of soil organic matter is a key aspect for the efficient management of N fertilizers in agricultural systems. Long-term aerobic incubation is the standard technique for calibrating the chemical extraction methods used to estimate the potentially mineralizable N in soil. However, the technique is laborious, expensive and time-consuming. In this context, the aims of this study were to determine the amount of soil mineralizable N in the 0-60 cm layer and to evaluate the use of short-term anaerobic incubation instead of long-term aerobic incubation for the estimation of net N mineralization rates in soils under sugarcane. Five soils from areas without previous N fertilization were used in the layers 0-20, 20-40 and 40-60 cm. Soil samples were aerobically incubated at 35 °C for 32 weeks or anaerobically incubated (waterlogged) at 40 °C for seven days to determine the net soil N mineralization. The sand, silt and clay contents were highly correlated with the indexes used for predicting mineralizable N. The 0-40 cm layer was the best sampling depth for the estimation of soil mineralizable N, while in the 40-60 cm layer net N mineralization was low in both incubation procedures. Anaerobic incubation provided reliable estimates of mineralizable N in the soil that correlated well with the indexes obtained using aerobic incubation. The inclusion of the pre-existing NH_4^+-N content improved the reliability of the estimate of mineralizable N obtained using anaerobic incubation.

Index terms: *Saccharum* spp., net N mineralization, aerobic incubation, anaerobic incubation.

---

[1] Part of the Master Thesis of the first author. Received for publication in April 26, 2012 and approved on February 5, 2013.

[2] Doctoral student, Luiz de Queiroz College of Agriculture, University of São Paulo. Av. Pádua Dias, 11. Caixa Postal 9. CEP 13418-900 Piracicaba (SP), Brazil. E-mail: emariano@cena.usp.br; mxvieira@cena.usp.br; jmleite@cena.usp.br

[3] Associate Professor, Center for Nuclear Energy in Agriculture - CENA, University of São Paulo - USP. Av. Centenário, 303. Caixa Postal 96. CEP 13416-000 Piracicaba (SP), Brazil. E-mail: pcotrive@cena.usp.br

[4] Postdoctoral student, CENA, USP. E-mail: rotto@usp.br

[5] Researcher, Brazilian Bioethanol Science and Technology Laboratory, Brazilian Center of Research in Energy and Materials. Rua Giuseppe Máximo Scolfaro, 10.000. Caixa Postal 6192. CEP 13083-970 Campinas (SP), Brazil. E-mail: henrique.franco@bioetanol.org.br

RESUMO: *MÉTODOS DE INCUBAÇÃO PARA AVALIAR O NITROGÊNIO MINERALIZÁVEL EM SOLOS CULTIVADOS COM CANA-DE-AÇÚCAR*

Considerar a mineralização do nitrogênio (N) da matéria orgânica do solo é questão fundamental para o manejo eficiente dos fertilizantes nitrogenados nos sistemas agrícolas. A incubação aeróbia de longa duração é o método de referência para estudos que envolvem a calibração de métodos químicos de extração, a fim de estimar o N potencialmente mineralizável do solo. Contudo, a técnica é laboriosa e de alto consumo de tempo e custo. Os objetivos deste trabalho foram determinar o N mineralizável do solo na profundidade de 0-60 cm e avaliar a possibilidade de adotar a incubação anaeróbia de curta duração no lugar da aeróbia de longa duração, para estimar a mineralização líquida de N em solos cultivados com cana-de-açúcar. Utilizaram-se cinco solos, coletados nas camadas de 0-20, 20-40 e 40-60 cm, em locais que não receberam fertilização prévia com N. As amostras de solo foram incubadas aerobiamente a 35 °C, durante 32 semanas, e anaerobiamente (saturadas com água) a 40 °C, por sete dias, para determinar a mineralização líquida de N do solo. Os teores de areia, silte e argila apresentaram alta correlação com os índices usados para predizer o N mineralizável. O N mineralizável do solo foi mais bem estimado na profundidade de 0-40 cm, enquanto a camada de 40-60 cm apresentou baixa mineralização líquida de N em ambos os procedimentos de incubação. Na incubação anaeróbia, estimou-se adequadamente o N mineralizável do solo, correlacionando-se com o N mineralizado acumulado e o N potencialmente mineralizável do solo, ambos obtidos por meio da incubação aeróbia. A manutenção do N-NH_4^+ preexistente da amostra melhorou a estimativa do N mineralizável por meio da incubação anaeróbia.

Termos de indexação: Saccharum *spp., mineralização líquida de N, incubação aeróbia, incubação anaeróbia.*

INTRODUCTION

Several strategies are currently being developed to improve N fertilizer efficiency in agriculture worldwide, including in sugarcane (*Saccharum* spp.) systems (Thorburn et al., 2011). One of the strategies with particular relevance for temperate regions emphasizes the N supply from the soil - specifically, N mineralization from soil organic matter (SOM) (Khan et al., 2001; Roberts et al., 2011). The finding that soils, rather than fertilizers, are the main source of N to plants (Dourado-Neto et al., 2010; Franco et al., 2011) underscores the practicality of this approach.

Long-term aerobic incubation is the standard method used for the estimation of potentially mineralizable N (N_0) (Stanford & Smith, 1972). The estimate resulting from this particular method represents the amount of N that is likely to be released in mineral form from soil organic reserves to a solution in a 32-week period. Most studies provide results of aerobic incubation for the calibration of chemical methods used to estimate the N supply from SOM mineralization (Gianello & Bremner, 1986; Sharifi et al., 2007; Schomberg et al., 2009). Although long-term aerobic incubation is widely recognized as the standard reference methodology, it is expensive, time-consuming and impractical for routine use (Curtin & Campbell, 2007; Sharifi et al., 2007).

The short-term (waterlogged) anaerobic incubation method developed by Waring & Bremner (1964) and modified by Keeney & Bremner (1966) involves the determination of available NH_4^+ after soil samples were incubated under anaerobic conditions for seven or 14 days. Compared to aerobic incubation, the advantages of this short-term method include the following: only the available NH_4^+-N needs to be determined; appropriate moisture and water levels during incubation are easily stabilized; and a higher amount of N is mineralized (Keeney, 1982).

The long-term aerobic incubation and short-term anaerobic incubation are assessed for many decades and the results obtained using these two methods are highly correlated (Keeney & Bremner, 1966; Soon et al., 2007; Yagi et al., 2009). Nevertheless, most studies evaluate only the surface soil layer, neglecting the deeper layers. The evaluation of mineralizable N in subsurface soil layers is especially important for perennial and semi-perennial crops, such as sugarcane, which has an aggressive root system, with evidence of root activity below 2 m (Smith et al., 2005).

Thus, the objectives of this study were to determine soil mineralizable N levels in the 0-60 cm layer and to evaluate the use of short-term anaerobic incubation instead of long-term aerobic incubation as standard estimation method of net N mineralization in soils under sugarcane.

MATERIALS AND METHODS

The soil samples used in this study were collected from five trials testing N-response of sugarcane in São Paulo State, Brazil (Figure 1), carried out between

2007 to 2010. At all sites, sugarcane had been cultivated for 20-50 years and in the last eigth years, mechanical harvesting without burning had been used. Composite soil samples (four replications per trial) were collected from control plots (without N fertilizer application), between the ratoon cane rows (layers 0-20, 20-40 and 40-60 cm). The composite samples from each plot resulted from the blending of eight single samples. The samples were air-dried, ground, sieved (≤ 2 mm) and stored at room temperature (soil physical and chemical properties in Table 1). The soil pH was determined in 0.01 mol L^{-1} $CaCl_2$ (1: 2.5 soil: solution, m/v) (Raij et al., 2001), and soil texture determined by the densitometer method (Gee & Bauder, 1986). Soil organic carbon (SOC) and soil total nitrogen (STN) were determined by a mass spectrometer with an automated C and N analyzer (PDZ Europa, ANCA-GSL connected to a PDZ Europa 20-20, Crewe, UK) (Barrie & Prosser, 1996). The soil bulk density was determined using the volumetric ring method (Blake & Hartge, 1986).

Long-term aerobic incubation was performed according to the procedure described by Stanford & Smith (1972) with some modifications. In a 200 mL

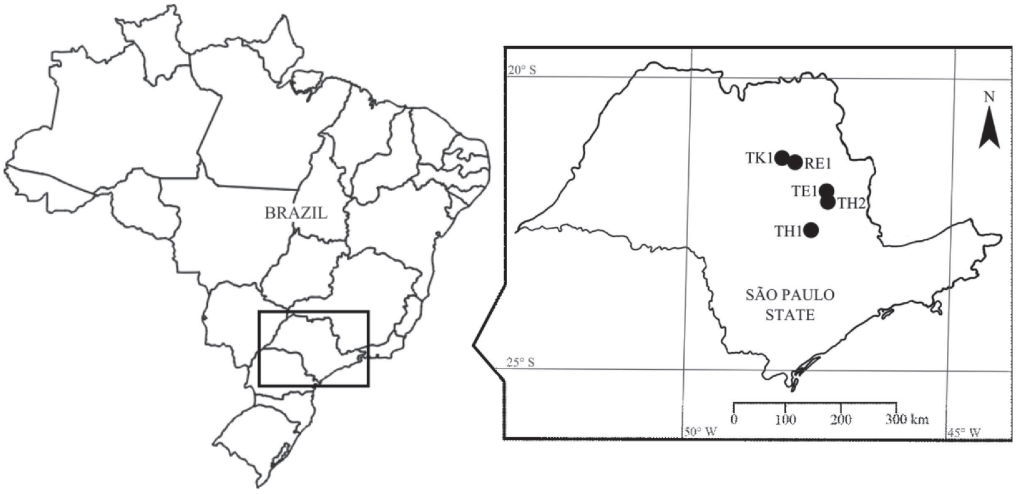

Figure 1. Locations of the soil sampling sites under sugarcane cultivation in São Paulo State, Brazil.

Table 1. Physical and chemical properties of soils under sugarcane in São Paulo State, Brazil, sampled in the layers 0-20, 20-40 and 40-60 cm

Soil[1]	Municipality	Location	Depth	pH ($CaCl_2$)	Sand	Silt	Clay	SOC[2]	STN[3]	C/N[4]	ρ[5]
			cm		g kg^{-1}						kg m^{-3}
Rhodic Eutrustox (RE1)	Pradópolis	21° 15' S 48° 18' W	0-20	5.1	142	181	677	20.4	1.5	13.3	1300
			20-40	5.2	137	185	678	15.2	1.2	12.5	1300
			40-60	5.6	131	143	726	9.2	0.7	13.3	1330
Typic Eutrustox (TE1)	Santa Cruz das Palmeiras	21° 47' S 47° 11' W	0-20	5.5	320	50	630	17.3	1.1	15.9	1450
			20-40	4.8	320	50	630	13.6	0.9	14.3	1470
			40-60	4.6	320	40	640	9.8	0.6	16.4	1320
Typic Hapludox (TH1)	Piracicaba	22° 35' S 47° 37' W	0-20	4.6	470	20	510	23.8	1.4	17.0	1350
			20-40	4.6	450	20	530	19.8	1.0	20.4	1340
			40-60	4.3	480	20	500	15.4	0.8	19.9	1160
Typic Kandiustults (TK1)	Jaboticabal	21° 20' S 48° 10' W	0-20	5.4	637	37	326	10.4	0.5	19.0	1300
			20-40	4.6	604	20	376	6.6	0.5	13.1	1460
			40-60	4.2	546	27	427	6.4	0.4	17.4	1390
Typic Haplustox (TH2)	Pirassununga	21° 55' S 47° 10' W	0-20	6.0	696	28	276	9.3	0.6	16.3	1640
			20-40	5.5	687	12	301	6.6	0.5	14.7	1630
			40-60	4.9	675	24	301	4.9	0.3	16.1	1630

[1] The classification refers to the Soil Taxonomy (Soil Survey Staff, 2010). [2] SOC: soil organic C. [3] STN: soil total N. [4] C/N: C/N ratio in the soil. [5] ρ: soil bulk density.

plastic funnel, a mixture of soil (30 g) and washed sand (30 g) was sandwiched between 20 and 5 mm of glass wool (above and below, respectively); four replicates were prepared for this analysis. The native mineral N from the samples was removed by leaching with 200 mL of 0.01 mol L^{-1} $CaCl_2.2H_2O$ and applied in 100 mL fractions, followed by an application of 50 mL N-free nutrient solution [0.002 mol L^{-1} $CaSO_4.2H_2O$, 0.002 mol L^{-1} $MgSO_4$, 0.005 mol L^{-1} $Ca(H_2PO_4)_2.H_2O$ and 0.0025 mol L^{-1} K_2SO_4]. Excess moisture was removed using a vacuum pump at a pressure of 10 cm Hg, according to Yagi et al. (2009). The funnels were sealed with plastic paraffin film (Parafilm® 'M', American National Can™, Chicago, USA) and incubated in a biological oxygen demand (BOD) chamber (MA-415 model, Marconi Equipamentos para Laboratório LTDA, Piracicaba, BRA) at 35 °C. After 2, 4, 6, 8, 10, 12, 16, 20, 24 and 32 weeks of incubation, mineral N forms were removed, a N-free nutrient solution was added and excess moisture was removed with a vacuum pump, as described above. To keep the incubated samples moist during incubation, water was replenished every three days or as needed. The leachates were analyzed for mineralized N (NH_4^+-N + NO_3^--N) by distillation with MgO and Devarda's alloy (Cantarella & Trivelin, 2001). Following the previously established methodology, the determined values of mineralized N were discarded after two weeks to avoid errors introduced by the initial NH_4^+-N flow after sample preparation and handling (Stanford & Smith, 1972; Matar et al., 1991; Sharifi et al., 2007). After 32 weeks of aerobic incubation, the accumulated mineralized N data (N_{mac}) were adjusted to the first-order exponential model (Equation 1) described by Stanford & Smith (1972):

$$N_{mac} = N_0(1 - e^{-kt}) \quad (1)$$

where N_{mac} = mineralized N accumulated at time t; N_0 = potentially mineralizable N; and k = mineralization constant.

The short-term anaerobic soil incubation method, developed by Waring & Bremner (1964) and modified by Keeney & Bremner (1966), involves the determination of NH_4^+-N in incubated and non-incubated soil and water suspensions. Five-gram soil samples were placed in test tubes (length 15 cm, inner diameter 1.6 cm) with 12.5 mL of deionized water. The tubes were sealed with plastic paraffin film and incubated in a BOD chamber at 40 ºC for seven days. Following the incubation, the test tubes were stirred manually for approximately 15 s and the contents were transferred to distillation tubes using 12.5 mL of a 4 mol L^{-1} KCl solution. After diluting the samples with water, the final KCl concentration was 2 mol L^{-1}. The samples were divided into three aliquots and distilled by the Kjeldahl method (Cantarella & Trivelin, 2001). Prior to short-term anaerobic incubation, the NH_4^+-N content of the soil samples was determined by extraction with 2 mol L^{-1} KCl. The anaerobically mineralized N was determined counting the NH_4^+-N content before incubation.

For the determination of N_0, the first-order exponential equation (Stanford & Smith, 1972) was adjusted using SigmaPlot software (Version 11.0, Systat Software Inc., San Jose, USA). The Levenberg-Marquardt algorithm and an iterative process that estimates the nonlinear parameters were used to minimize the sum of squared residuals from the regression. Pearson correlation coefficients were calculated for the physical and chemical soil properties and for the results from the aerobic and anaerobic incubation.

RESULTS AND DISCUSSION

An abrupt increase in mineralized N (N_m) was observed during the initial periods of long-term aerobic incubation, especially for soil samples in the 0-20 cm layer compared to those from subsurface depths; a subsequent reduction and relative stabilization of N_m was observed after the 6th week (Figure 2). In the 0-20, 20-40 and 40-60 cm sampling layers, 38, 45 and 48 % of N_m were obtained by the 6th week of incubation, respectively. These findings were similar to those of Rhoden et al. (2006), who observed an average of 52 % N_m by the 4th week of incubation for 15 flooded soils of the state of Rio Grande do Sul in southern Brazil. These kinetics of N mineralization are typical for deformed soil samples analyzed in long-term incubation studies (Yagi et al., 2009). That the values of N_m were greatest during the initial periods is attributable to the mineralizable organic fractions and, possibly, to the recycling of microbial biomass (Bonde et al., 1988; Mengel, 1996). Previous studies found that sample preparation and handling stimulate aeration and induce microbial activity (Stanford & Smith, 1972; Cabrera & Kissel, 1988; Yagi et al., 2009).

In the 0-20, 20-40 and 40-60 cm sampling layers, N_{mac} ranged from 29.9 to 84.0, 23.1 to 51.8 and 14.5 to 22.0 mg kg^{-1}, respectively (Figure 2 and Table 2). In the surface layer (0-20 cm), N_{mac} was on average approximately 1.5 and 2.9 times greater than the values in the 20-40 and 40-60 cm layers, respectively. As previously reported by Salcedo et al. (1985), the largest fluctuations in N_{mac} values were observed in the 0-20 cm layer, possibly due to variations in the organic matter content, chemical composition and microbial activity in this soil layer. There was less fluctuation in the subsurface N_{mac} values, particularly in the 40-60 cm layer, which may have been due to the presence of SOM in relatively stabilized fractions (humic substances) that are not easily attacked by soil microorganisms (Qualls, 2004). The decrease in SOC and STN contents in the profile (Table 1) may also have contributed to this circumstance.

The differences in N_{mac} behavior among different soils reflected their textural compositions. For example,

the highest N_{mac} values were observed in soils with high clay content (RE1 and TE1), and lower N_{mac} values were observed in soils with high sand content (TK1 and TH2). These results may be due to the formation of stable organo-mineral complexes between clay and SOM (Ladd & Jackson, 1982; Oades et al., 1987; Bayer et al., 2002), which promote the gradual release of mineral N to the soil solution (Camargo et al., 1997; Rhoden et al., 2006; Yagi et al., 2009). In soils with high sand content, the formation of these organo-mineral complexes is minimized by the absence or low quantities of clay. Differences in organic matter content may also alter the stability of organo-mineral complexes in variable charge soils (Stevenson, 1994), which are prevalent in Brazil and other tropical regions.

The N_{mac} values were adjusted to the first-order exponential model proposed by Stanford & Smith (1972) for all analyzed soils and sampling depths. The coefficient of determination (R^2) of the N_{mac} results adjusted to the exponential growth equation was higher for the samples from the 0-20 cm layer than those from the other layers (data not shown). This finding reflects the higher rates of SOM mineralization in this layer during the initial incubation period and the subsequent decrease in the amounts of mineralized N in the following weeks. N_0 ranged from 39.9 to 89.7 mg kg^{-1} (average 63.4 mg kg^{-1}) in the 0-20 cm layer and from 25.5 and 58.9 mg kg^{-1} (average 36.2 mg kg^{-1}) in the 20-40 cm layer (Table 2). Lower values of N_0, 14.7 to 24.4 mg kg^{-1} (average 19.5 mg kg^{-1}) were observed in the 40-60 cm layer. The N mineralization potential typically decreases with increasing soil depth (Campbell et al., 1981; Salcedo et al., 1985; Alves et al., 1999) because deeper layers of soils have a lower organic matter content and lability.

On the basis of the estimates of mineralizable N obtained from the five experimental sites, N_0 stock ranged from 232 to 451 kg ha^{-1} (average 336 kg ha^{-1})

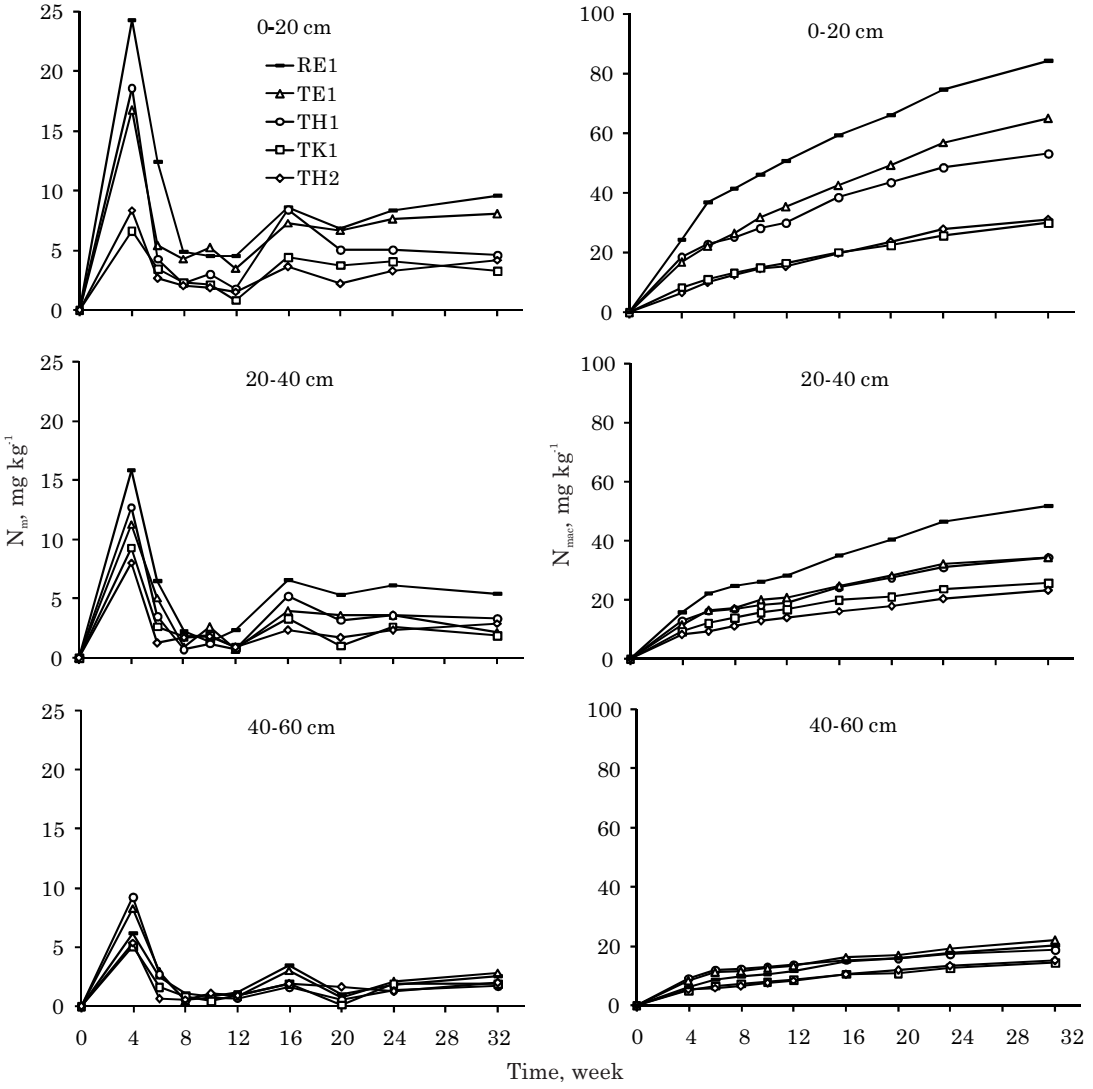

Figure 2. Mineralized nitrogen (N_m) and accumulated mineralized nitrogen (N_{mac}) in long-term aerobic incubation of soils (at three sampling depths) under sugarcane.

Table 2. Mineralized N in soil samples subjected to long-term aerobic incubation (N_{mac}), N mineralization potential (N_0), percentage ratio of mineralizable N to total soil N (N_0/N_{total}), mineralization constant (k) and N_0 half-life ($t_{1/2}$) at three soil depths (mean ± standard deviation)

Variable	N_{mac}	N_0	N_0/STN	k	$t_{1/2}$
	mg kg^{-1}		%	per week	week
			0-20 cm		
RE1	84.0 ± 12.2	89.7 ± 13.3	5.8 ± 0.8	0.074 ± 0.009	9.4 ± 1.2
TE1	64.9 ± 11.1	82.1 ± 7.9	7.5 ± 0.7	0.048 ± 0.010	14.9 ± 2.7
TH1	53.1 ± 8.2	60.5 ± 9.2	4.3 ± 0.6	0.070 ± 0.020	10.8 ± 4.1
TK1	30.9 ± 2.6	44.7 ± 12.5	8.1 ± 2.2	0.043 ± 0.017	17.8 ± 6.1
TH2	29.9 ± 5.6	39.9 ± 5.7	7.0 ± 1.0	0.063 ± 0.009	11.2 ± 1.6
Mean	52.6 ± 22.5	63.4 ± 22.1	6.5 ± 1.7	0.059 ± 0.017	12.8 ± 4.5
			20-40 cm		
RE1	51.8 ± 7.5	58.9 ± 3.9	4.8 ± 0.3	0.063 ± 0.018	11.5 ± 2.6
TE1	34.2 ± 8.5	35.6 ± 8.9	4.1 ± 0.3	0.080 ± 0.011	8.8 ± 1.1
TH1	34.4 ± 3.5	35.6 ± 2.3	3.6 ± 0.2	0.086 ± 0.009	8.1 ± 0.9
TK1	25.6 ± 4.7	25.6 ± 5.1	5.1 ± 1.0	0.108 ± 0.004	6.4 ± 0.2
TH2	23.1 ± 5.5	25.5 ± 4.5	5.6 ± 1.0	0.070 ± 0.025	11.0 ± 4.0
Mean	33.8 ± 11.6	36.2 ± 13.4	4.6 ± 0.9	0.081 ± 0.021	9.2 ± 2.8
			40-60 cm		
RE1	20.3 ± 8.0	24.4 ± 9.4	3.5 ± 1.3	0.058 ± 0.021	13.3 ± 4.1
TE1	22.0 ± 4.2	22.7 ± 5.8	3.8 ± 0.9	0.086 ± 0.013	8.2 ± 1.4
TH1	18.8 ± 2.5	17.3 ± 2.0	2.2 ± 0.2	0.162 ± 0.022	4.3 ± 0.6
TK1	14.5 ± 4.2	14.7 ± 4.2	3.9 ± 1.1	0.085 ± 0.012	8.3 ± 1.1
TH2	15.2 ± 2.2	18.7 ± 2.5	6.2 ± 0.8	0.056 ± 0.012	12.9 ± 2.5
Mean	18.2 ± 5.1	19.5 ± 6.1	3.9 ± 1.6	0.089 ± 0.042	9.4 ± 4.0

in the 0-60 cm layer during the 32-week aerobic incubation period. Although the root system of sugarcane is located primarily in the surface layer (0-20 cm), the occurrence of roots in deeper layers is considerable (Battie-Laclau & Laclau, 2009) and promotes the absorption of subsurface mineral N by the plant (Thorburn et al., 2003). The hypothesis that the N mineralization that occurs during the crop development cycle can meet the total N demand of sugarcane plants is supported by the amount of N extracted by sugarcane during the ratoon cycle (from 142 to 179 kg ha^{-1}) (Coale et al., 1993; Oliveira et al., 2010). However, it is worth pointing out that the incubation assays for predicting mineralizable N are performed under conditions of controlled humidity, temperature and aeration that are optimal for the soil microbiota. Soil N mineralization rates are usually lower in the field than in the laboratory (Johnson et al., 1980) due to the varying conditions that affect microorganisms.

The percentage ratios of N_0 to total soil N (N_0/N_{total}) at the sampling depths 0-20, 20-40 and 40-60 cm were 6, 5 and 4 %, respectively (Table 2). In the subsurface layers, the highest percentages were observed in soils with lower clay content (TK1 and TH2). The most likely explanation for this result is the reduced level of organic N in these soils, with higher N mineralization rates in relation to the total N content in deeper layers (Camargo et al., 1997; Rhoden et al., 2006). Moreover, the SOM is virtually unprotected against microbial attack in soils with low clay content. Thus, the main factors influencing N mineralization in the subsurface layers (20-60 cm) are the recalcitrance of SOM, microbial activity and soil management (Rhoden et al., 2006).

The average k (mineralization constant) values were 0.059, 0.081 and 0.089/week in the layers 0-20, 20-40 and 40-60 cm, respectively (Table 2). Lower k values in the surface layer can be explained by the deposition of leaves and stalk tips on the soil surface during the mechanized harvest of sugarcane. The high C/N ratio of this fresh organic matter promotes N mobilization by heterotrophic microorganisms and a subsequent reduction in SOM mineralization rates in the surface layer. The observation that k values were greater in the subsurface layers is consistent with the findings of Salcedo et al. (1985), Cassman & Munns (1980) and Campbell et al. (1981). The values reported by Salcedo et al. (1985) for soils under sugarcane were as follows: 0.090/week in both the 20-40 and 40-60 cm layers and an average of 0.075/week in the surface layer.

The half-life ($t_{1/2}$) is an estimate of the amount of time required for the mineralization of 50 % of N_0.

Table 2 shows the $t_{1/2}$ values for this study: in the 0-20 cm layer, the estimated half-life is approximately 13 weeks, while in the 20-40 and 40-60 cm layers, the estimated half-life is only nine weeks. Because $t_{1/2}$ is inversely proportional to k, the highest $t_{1/2}$ values were observed in the 0-20 cm layer. As mentioned above, this may have been due to the presence of plant material with a high C/N ratio in the soil surface layer. The results of this study for the 0-20 cm layer were similar to those reported by Stanford & Smith (1972), who evaluated the N mineralization potential of 39 soils in the USA and measured an average $t_{1/2}$ of 12.8 weeks.

As shown in table 3, the NH_4^+-N concentration in the soil prior to anaerobic incubation ranged from 5.1 to 10.8 mg kg^{-1} (average 7.4 mg kg^{-1}) in the 0-20 cm layer, from 4.8 to 7.4 mg kg^{-1} (average 6.2 mg kg^{-1}) at 20-40 cm and from 2.5 to 5.7 mg kg^{-1} (average 3.8 mg kg^{-1}) at 40-60 cm. One explanation for the decrease in NH_4^+-N content with increasing soil depth is the lower SOM lability and microbial activity in the subsurface layers. It should also be noted that the mineral N content at the time of sampling is not preserved by air-drying the samples, as done in this study, due to N immobilization and, or, mineralization that may occur during the drying period (Mattos Junior et al., 1995).

The amount of mineralized NH_4^+-N (taking the initial soil NH_4^+-N into account) in soil samples from the 0-20 cm layer after short-term anaerobic incubation (N_{an}) ranged from 21.8 to 42.6 mg kg^{-1} (Table 3). These results are similar to the values of 35.7 mg kg^{-1} and 29.1 mg kg^{-1} reported by Soon et al. (2007) and Yagi et al. (2009), respectively. However, they are lower than the value of 65.8 mg kg^{-1} reported by Narteh & Sahrawat (1997) and higher than the value of 8.1 mg kg^{-1} reported by Cantarella et al. (1994). N_{an} ranged from 9.8 to 21.3 mg kg^{-1} in the 20-40 cm layer and from 3.5 and 9.0 mg kg^{-1} in the 40-60 cm layer. As previously discussed for N_{mac}, the largest variations in N_{an} were observed in the 0-40 cm layer, which may have been due primarily to the particle-size composition of the soils.

The contribution of pre-existing NH_4^+-N in the soil to the total NH_4^+-N released after the seven-day anaerobic incubation increased with depth (Table 3). The pre-existing NH_4^+-N represented, on average, 25, 48 and 58 % of the NH_4^+-N released after anaerobic incubation in the 0-20, 20-40 and 40-60 cm layers, respectively. The pre-existing NH_4^+-N in the soil was presumably derived from the alkaline hydrolysis of organic N (relatively stabilized in the form of organo-mineral complexes) that occurred during the distillation of soil samples (Sahrawat & Ponnamperuma, 1978). These results further support the hypothesis that organic N is at an advanced humification stage in deeper soil layers and that the presence of high-molecular-mass compounds of different solubilities increase the complexity of organic N cycling. It has been proposed that the addition of this N is advantageous because it represents an integral part of the mineralizable N supply in the soil (Keeney, 1982).

Low pH values have been associated with reductions in microbial activity and SOM (Paul et al., 2001), and pH values greater than 6 have been found to considerably reduce the N mineralization (Adams & Martin, 1984). Despite these effects of pH, several studies investigating the use of aerobic and anaerobic soil incubation for the estimation of mineralizable N failed to identify any correlations between the indexes for mineralizable N and soil pH (Sahrawat, 1983; Dessureault-Rompré et al., 2010). Similarly, no correlations were observed between pH and N_{mac}, N_0 or N_{an} at any of the sampling depths in this study (Table 4).

Soil-size fractions (sand, silt and clay) were found to be reliable predictors of mineralizable N in the soil (Table 4). There was a highly negative correlation between sand content and N_{mac}, N_0 and N_{an}, with R values of 0.99, 0.99 and 0.97, respectively, for the 0-20 cm layer. In the 20-40 cm layer, the sand content was negatively correlated with all mineralizable N

Table 3. Pre-existing NH_4^+-N in soil samples, mineralized N obtained by short-term anaerobic incubation (N_{an}) and the percentage ratio of pre-existing NH_4^+-N to mineralized N (NH_4^+/N_{an}) at three soil depths (mean ± standard deviation)

Variable	NH_4^+	N_{an}	NH_4^+/N_{an}
	mg kg^{-1}		%
		0-20 cm	
RE1	10.8 ± 1.4	42.6 ± 1.1	25.5 ± 3.4
TE1	5.9 ± 2.0	34.0 ± 1.7	17.3 ± 6.3
TH1	8.8 ± 3.1	25.6 ± 1.8	35.0 ± 15.2
TK1	5.1 ± 1.9	24.8 ± 2.4	21.5 ± 10.0
TH2	6.1 ± 1.3	21.8 ± 2.2	27.9 ± 3.1
Mean	7.4 ± 2.4	29.8 ± 8.5	25.5 ± 6.7
		20-40 cm	
RE1	7.2 ± 0.3	21.3 ± 1.3	34.0 ± 1.0
TE1	6.0 ± 1.4	13.4 ± 2.1	46.1 ± 12.3
TH1	7.4 ± 2.3	12.3 ± 1.5	59.5 ± 11.6
TK1	5.7 ± 1.8	11.1 ± 0.9	50.9 ± 14.1
TH2	4.8 ± 2.1	9.8 ± 0.7	49.5 ± 24.0
Mean	6.2 ± 1.1	13.6 ± 4.5	48.0 ± 9.2
		40-60 cm	
RE1	5.7 ± 1.3	9.0 ± 2.5	65.0 ± 20.1
TE1	2.6 ± 1.1	8.1 ± 2.2	34.2 ± 16.3
TH1	4.9 ± 1.3	7.4 ± 1.8	67.3 ± 8.1
TK1	2.5 ± 0.3	3.5 ± 0.4	70.9 ± 12.4
TH2	3.3 ± 1.5	6.1 ± 1.3	52.8 ± 17.7
Mean	3.8 ± 1.4	6.8 ± 2.1	58.0 ± 14.9

parameters. A negative correlation between sand content and N_{an} in the 40-60 cm layer was verified. Clay showed highly positive correlations with the amounts of N_{mac}, N_0 and N_{an} in the 0-20 cm layer and highly positive correlations with N_{mac} and N_{an} at the 20-40 and 40-60 cm layers, respectively. Silt content was positively correlated with N_{an} in the surface layer and with N_{mac}, N_0 and N_{an} in the 20-40 cm layer. In soils with high clay content, organo-mineral complexes may form between clay and SOM (Kaiser & Guggenberger, 2000; Bayer et al., 2002), thereby stocking organic N (Mengel, 1996) and increasing mineralization potential. Conversely, sandy soils tend to exhibit lower amounts of mineralized N over time (Yagi et al., 2009), possibly due to reduced SOM content and reduced formation of organo-mineral complexes.

At all three sampling depths, SOC was not correlated with any of the parameters used to estimate the mineralizable N in the soil (N_{mac}, N_0 and N_{an}) (Table 4). The lack of a correlation may be attributable to particular characteristics of the TH1 soil. Although its profile indicated a high SOC content (Table 1), it exhibited low net N mineralization rates, possibly due to the high C/N ratio in the soil. At the same time, the N re-immobilization process by microbiota, which has been found to occur in assays involving aerobic and anaerobic soil incubation (Wang et al., 2001), may have been significant in the TH1 soil. Several studies have reported a positive correlation between SOC and the indexes for estimating mineralizable N (Narteh & Sahrawat, 1997; Soon et al., 2007; Dessureault-Rompré et al., 2010), although one study reported a lack of correlation between these factors (Cantarella et al., 1994).

STN was positively correlated with N_{mac}, with R values of 0.88 and 0.93, for the 0-20 and 20-40 cm layers, respectively (Table 4). A correlation between STN and N_0 was also identified for the 20-40 cm layer (R = 0.90). No correlations were found for the deepest sampling depth (40-60 cm), possibly due to the low microbial activity and STN content in this soil layer. Positive correlations between STN and biological incubation parameters in the surface layer (0-20 cm) were also obtained by Rhoden et al. (2006), Schomberg et al. (2009) and Dessureault-Rompré et al. (2010). These results confirm that STN is a more reliable index than SOC for predicting net N mineralization rates, as previously reported by Yagi et al. (2009) in a study of 22 soils from the São Paulo State, Brazil.

The C/N ratio in the soil did not correlate with N_{mac}, N_0 or N_{an} at any of the soil depths (Table 4). Although the C/N ratio of organic materials is often used in mathematical models to predict the availability of soil N (Manzoni et al., 2008), the ratio of SOC to STN was not a suitable index in this study. Considering the changes affecting SOM, which include the incorporation of fresh organic matter to the formation of more stable humified fractions, the soil N mineralization may have been affected primarily by the degree of SOM decomposition and the lability of existing organic compounds. The C/N ratios in the soil ranged from 13 to 20, which are similar to the values of 15 to 19 found by Graham et al. (2002) in a long-term sugarcane experiment in South Africa but higher than the values of 10 to 12 typically reported in the literature (Schlesinger, 1995). The incorporation of crop residues with high C/N ratios into the soil after unburned harvesting may underlie these differences.

The mineralized NH_4^+-N under short-term anaerobic incubation (N_{an}) was highly correlated with the parameters used for estimating potentially mineralizable N obtained by long-term aerobic incubation (Table 5). N_{an} was positively correlated with N_{mac} at the 0-20 and 20-40 cm layers, with R values of 0.95 ($p \leq 0.05$) and 0.99 ($p \leq 0.001$), respectively. The same trend and similar values were identified for the correlations between N_{an} and N_0 at the 0-20 and 20-40 cm layers. Although Curtin & McCallum (2004) reported that the correlation between short-term anaerobic incubation and long-term aerobic incubation is weak, most studies have reported a high degree of correlation between these two methods (Soon et al., 2007; Yagi et al., 2009).

Table 4. Correlation coefficients (R) between soil properties and the following indexes: accumulated mineralized N under aerobic incubation (N_{mac}), potentially mineralizable N (N_0) and mineralized N under anaerobic incubation (N_{an})

Variable[(1)]	0-20 cm			20-40 cm			40-60 cm		
	N_{mac}	N_0	N_{an}	N_{mac}	N_0	N_{an}	N_{mac}	N_0	N_{an}
pH	-0.47	-0.39	-0.28	0.07	0.17	0.12	0.35	0.81	-0.28
Sand	-0.99***	-0.99**	-0.97**	-0.96*	-0.93*	-0.93*	-0.80	-0.80	-0.93*
Silt	0.79	0.73	0.90*	0.93*	0.96**	0.97**	0.45	0.75	0.63
Clay	0.97**	0.99**	0.90*	0.88*	0.84	0.83	0.86	0.77	0.96**
SOC	0.75	0.68	0.52	0.64	0.58	0.54	0.56	0.08	0.58
STN	0.88*	0.81	0.70	0.93*	0.90*	0.87	0.73	0.38	0.82
C/N	-0.83	-0.76	-0.81	-0.16	-0.21	-0.26	-0.23	-0.73	-0.24

[(1)] SOC: soil organic C; STN: soil total N; C/N: C/N ratio in the soil. ***, ** and *: $p \leq 0.001$; $p \leq 0.01$ and $p \leq 0.05$, respectively.

Table 5. Correlation coefficients (R) among the following indexes: the amount of pre-existing NH_4^+-N in the soil and the amount obtained after anaerobic incubation (considering or not considering the pre-existing NH_4^+-N), accumulated mineralized N under aerobic incubation (N_{mac}) and potentially mineralizable N (N_0)

Variable	0-20 cm		20-40 cm		40-60 cm	
	N_{mac}	N_0	N_{mac}	N_0	N_{mac}	N_0
Pre-existing NH_4^+-N	0.76	0.61	0.91*	0.86	0.33	0.41
Considering pre-existing NH_4^+-N	0.95*	0.95*	0.99***	0.99***	0.80	0.58
Not considering pre-existing NH_4^+-N	0.87	0.92*	0.97**	0.99**	0.62	0.33

***: $p \leq 0.001$; **: $p \leq 0.01$; $^*p \leq 0.05$.

Pre-existing NH_4^+-N in the soil was not a suitable index for predicting the mineralization of organic N over time because there were no correlations between this mineral N fraction and the N_{mac} and N_0 indexes, with the exception of the positive correlation with N_{mac} for the 20-40 cm layer ($R = 0.91$; $p \leq 0.05$) (Table 5). The inability to use KCl-extractable NH_4^+-N as an index for estimating mineralizable N may have been due to the high seasonal variation of this mineral N fraction, which is highly susceptible to microbial immobilization and absorption by plants. Additionally, the air-drying of samples prior to the determination of NH_4^+-N levels likely represented another contributing factor, as was previously discussed. Beyond these considerations, mineral N forms in the soil (NH_4^+-N and NO_3^--N) are not recommended as indicators of mineralizable N because they are restricted to the available N accumulated during a short period in the crop development cycle (St. Luce et al., 2011). However, there has been some success with preplant and presidedress NO_3^--N tests for corn in USA, which assess the need for supplemental nitrogen fertilizer based on the available NO_3^--N in the soil (St. Luce et al., 2011).

According to Keeney (1982), it is possible to disregard the determination of the levels of pre-existing NH_4^+-N that would normally be subtracted from the amount of mineralized NH_4^+-N in samples after short-term anaerobic incubation. To assess the validity of this assumption, correlations between the results of the aerobic incubation assay and the amounts of mineralized NH_4^+-N in the anaerobic incubation experiment, with and without the NH_4^+-N initially present in the soil, were assessed (Table 5). At all depths, subtracting pre-existing soil NH_4^+-N resulted in weaker correlations between N_{an} with N_{mac} and N_0 when compared including this fraction of mineral N. These results are similar to those of Yagi et al. (2009), who reported that the inclusion of pre-existing NH_4^+-N in the amounts of mineralized N increased the representation of the mineralization process and the availability of soil N for corn plants.

The results presented here provide support for the adoption of short-term anaerobic incubation in place of long-term aerobic incubation for predicting the levels of soil mineralizable N. The main advantage of the anaerobic incubation method is the shorter time requirement, which significantly reduces the number of required inorganic N determinations and, consequently, the cost associated with laboratory analyses. The possibility of eliminating the initial determination of pre-existing NH_4^+-N in the samples, a simplification that was actually found to increase the accuracy of the analysis, makes the anaerobic method even more practical.

The relevance of this study is more readily apparent in light of the increased emphasis on strategies for improving N fertilizer efficiency. Because several studies have demonstrated that soils are the main source of N to plants, the determination of mineralized N in SOM for fertilization management programs represents a critical step for increasing fertilizer-N efficiency in agricultural systems. Finally, the applicability of these findings may not be limited to soils under sugarcane because the differences in the physicochemical properties of the evaluated soils contributed more significantly to N mineralization than the crop itself.

CONCLUSIONS

1. Soil size fractions (sand, silt and clay) were highly correlated with indexes used for the prediction of mineralizable N from five soils cultivated with sugarcane.

2. The 0-40 cm layer was the best sampling depth for the estimation of soil mineralizable N primarily because of the higher STN content and SOM lability in this layer. However, the 40-60 cm soil layer had low net N mineralization in both incubation procedures.

3. In the 0-20 and 20-40 soil layers, correlations were observed between mineralized N following anaerobic incubation (N_{an}) and the indexes obtained by long-term aerobic incubation (N_{mac} and N_0). There were no correlations between N_{an}, N_{mac} and N_0 in the 40-60 cm layer.

4. The finding that the levels of pre-existing NH_4^+-N in the samples did not need to be determined prior

to anaerobic incubation further supports the utility and applicability of this method.

ACKNOWLEDGMENTS

The authors thank the São Paulo Research Foundation (FAPESP) for the scholarship granted to the first author (Process 2009/04691-2) and the National Council for Scientific and Technological Development (CNPq) for the financial support of the research project that includes the work presented here (Process 574982/2008-6). The authors would also like to acknowledge Guilherme Arf Torres and Maria Stefânia Cruanhes D'Andréa for their assistance with the laboratory work.

LITERATURE CITED

ADAMS, F. & MARTIN, J.B. Liming effect on nitrogen use efficiency. In: HAUCK, R.D., ed. Nitrogen in crop production. Madison, ASA/CSSA/SSSA, 1984. p.417-426.

ALVES, G.D.; SAMPAIO, E.V.S.B.; SALCEDO, I.H. & SILVA, V.M. Potencial de mineralização de N e C em vinte solos de Pernambuco. R. Bras. Ci. Solo, 23:245-256, 1999.

BARRIE, A. & PROSSER, S.J. Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In: BOUTTON, T.W. & YAMASAKI, S., eds. Mass spectrometry of soils. New York, Marcel Dekker, 1996. p.1-46.

BATTIE-LACLAU, P. & LACLAU, J.P. Growth of the whole root system for a plant crop of sugarcane under rainfed and irrigated environments in Brazil. Field Crop. Res., 114:351-360, 2009.

BAYER, C.; MIELNICZUK, J.; MARTIN-NETO, L. & ERNANI, P.R. Stocks and humification degree of organic matter fractions as affected by no-tillage on a subtropical soil. Plant Soil, 238:133-140, 2002.

BLAKE, G.R. & HARTGE, K.H. Bulk density. In: KLUTE, A., ed. Methods of soil analysis: Physical and mineralogical methods. 2.ed. Madison, ASA/SSSA, 1986. p.363-376. (Agronomy Series, 9)

BONDE, T.A.; SCHNÜRER, J. & ROSSWALL, T. Microbial biomass as a fraction of potentially mineralizable nitrogen in soils from long-term field experiments. Soil Biol. Biochem., 20:447-452, 1988.

CABRERA, M.L. & KISSEL, D.E. Evaluation of a method to predict nitrogen mineralized from soil organic matter under field conditions. Soil Sci. Soc. Am. J., 52:1027-1031, 1988.

CAMARGO, F.A.O.; GIANELLO, C. & VIDOR, C. Potencial de mineralização do nitrogênio em solos do Rio Grande do Sul. R. Bras. Ci. Solo, 21:575-579, 1997.

CAMPBELL, C.A.; MYERS, R.J.K. & WEIER, K.L. Potentially mineralizable nitrogen, decomposition rates and their relationship to temperature for five Queensland soils. Austr. J. Soil Res., 19:323-332, 1981.

CANTARELLA, H.; MATTOS JUNIOR, D. & RAIJ, B.van. Lime effect on soil N availability indexes as measured by plant uptake. Commun. Soil Sci. Plant Anal., 25:989-1006, 1994.

CANTARELLA, H. & TRIVELIN, P.C.O. Determinação do nitrogênio inorgânico em solo pelo método da destilação a vapor. In: RAIJ, B.van; ANDRADE, J.C.; CANTARELLA, H. & QUAGGIO, J.A., eds. Análise química para avaliação da fertilidade de solos tropicais. Campinas, Instituto Agronômico, 2001. p.271-276.

CASSMAN, K.G. & MUNNS, D.N. Nitrogen mineralization affected by soil moisture, temperature and depth. Soil Sci. Soc. Am. J., 44:1233-1237, 1980.

COALE, F.J.; SANCHEZ, C.A.; IZUNO, F.T. & BOTTCHER, A.B. Nutrient accumulation and removal by sugarcane grown on Everglades Histosols. Agron. J., 85:310-315, 1993.

CURTIN, D. & CAMPBELL, C.A. Mineralizable nitrogen. In: CARTER, M.R. & GREGORICH, E.G., eds. Soil sampling and methods of analysis. 2.ed. Boca Raton, CRC Press, 2007. p.599-606.

CURTIN, D. & McCALLUM, F.M. Biological and chemical assays to estimate nitrogen supplying power of soils with contrasting management histories. Austr. J. Soil Res., 42:37-746, 2004.

DESSUREAULT-ROMPRÉ, J.; ZEBARTH, B.J.; BURTON, D.L.; SHARIFI, M.; COOPER, J.; GRANT, C.A. & DRURY, C.F. Relationships among mineralizable soil nitrogen, soil properties, and climatic indices. Soil Sci. Soc. Am. J., 74:1218-12127, 2010.

DOURADO-NETO, D.; POWLSON, D.; ABU BAKAR, R.; BACCHI, O.O.S.; BASANTA, M.V.; THI CONG, P.; KEERTHISINGHE, G.; ISMAILI, M.; RAHMAN, S.M.; REICHARDT, K.; SAFWAT, M.S.A.; SANGAKKARA, R.; TIMM, L.C.; WANG, J.Y.; ZAGAL, E. & van KESSEL, C. Multiseason recoveries of organic and inorganic nitrogen-15 in tropical cropping systems. Soil Sci. Soc. Am. J., 74:139-152, 2010.

FRANCO, H.C.J.; OTTO, R.; FARONI, C.E.; VITTI, A.C.; OLIVEIRA, E.C.A. & TRIVELIN, P.C.O. Nitrogen in sugarcane derived from fertilizer under Brazilian field conditions. Field Crop. Res., 121:29-41, 2011.

GEE, G.W. & BAUDER, J.W. Particle-size analysis. In: KLUTE, A., ed. Methods of soil analysis: Physical and mineralogical methods. 2.ed. Madison, ASA/SSSA, 1986. p.383-411. (Agronomy Series, 9)

GIANELLO, C. & BREMNER, J.M. Comparison of chemical assessing potentially available organic nitrogen in soil. Commun. Soil Sci. Plant Anal., 17:215-236, 1986.

GRAHAM, M.H.; HAYNES, R.J. & MEYER, J.H. Soil organic matter content and quality: Effects of fertilizer applications, burning and trash retention on a long-term sugarcane experiment in South Africa. Soil Biol. Biochem., 34:93-102, 2002.

JOHNSON, D.W.; EDWARDS, N.T. & TODD, D.E. Nitrogen mineralization, immobilization, and nitrification following urea fertilization of a forest soil under field and laboratory conditions. Soil Sci. Soc. Am. J., 44:610-616, 1980.

KAISER, K. & GUGGENBERGER, G. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Org. Geochem., 31:711-725, 2000.

KEENEY, D.R. Nitrogen: availability indices. In: PAGE, A.L.; MILLER, R.H. & KEENEY, D.R., eds. Methods of soil analysis: Chemical and microbiological properties. 2.ed. Madison, ASA/SSSA, 1982. p.711-733.

KEENEY, D.R. & BREMNER, J.M. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agron. J., 58:498-503, 1966.

KHAN, S.A.; MULVANEY, R.L. & HOEFT, R.G. A simple soil test for detecting sites that are nonresponsive to nitrogen fertilization. Soil Sci. Soc. Am. J., 65:1751-1760, 2001.

LADD, I.N. & JACKSON, R.B. Biochemistry of ammonification. In: STEVENSON, F.J., ed. Nitrogen in agricultural soils. Madison, ASA/CSSA/SSSA, 1982. p.173-228.

MANZONI, S.; JACKSON, R.B.; TROFYMOW, J.A. & PORPORATO, A. The global stoichiometry of litter nitrogen mineralization. Science, 321:684-686, 2008.

MATAR, A.E.; BECK, D.P.; PALA, M. & GARABET, S. Nitrogen mineralization potentials of selected Mediterranean soils. Commun. Soil Sci. Plant Anal., 22:23-36, 1991.

MATTOS JUNIOR, D.; CANTARELLA, H. & RAIJ, B.van. Manuseio e conservação de amostras de solo para preservação do N inorgânico. R. Bras. Ci. Solo, 19:423-431, 1995.

MENGEL, K. Turnover of organic nitrogen in soil and its availability to crops. Plant Soil, 181:83-93, 1996.

NARTEH, L.T. & SAHRAWAT, K.L. Potentially mineralizable nitrogen in West African lowland rice soils. Geoderma, 76:145-154, 1997.

OADES, J.M.; VASSALLO, A.M.; WATERS, A.G. & WILSON, M.A. Characterization of organic matter in particle size and density fractions from a red-brown earth by solid state ^{13}C NMR. Austr. J. Soil Res., 25:71-82, 1987.

OLIVEIRA, E.C.A.; FREIRE, F.J.; OLIVEIRA, R.I.; FREIRE, M.B.G.S.; SIMÕES NETO, D.E. & SILVA, S.A.M. Extração e exportação de nutrientes por variedades de cana-de-açúcar cultivadas sob irrigação plena. R. Bras. Ci. Solo, 34:1343-1352, 2010.

PAUL, K.; BLACK, S. & CONYERS, M. Development of nitrogen mineralization gradients through surface soil depth and their influence on surface soil pH. Plant Soil, 234:239-246, 2001.

QUALLS, R.G. Biodegradability of humic substances and other fractions of decomposing leaf litter. Soil Sci. Soc. Am. J., 68:1705-1712, 2004.

RAIJ, B.van; ANDRADE, J.C.; CANTARELLA, H. & QUAGGIO, J.A., eds. Análise química para avaliação da fertilidade de solos tropicais. Campinas, Instituto Agronômico, 2001. 285p.

RHODEN, A.C.; SILVA, L.S.; CAMARGO, F.A.O.; BRITZKE, D. & BENEDETTI, E.L. Mineralização anaeróbia do nitrogênio em solos de várzea do Rio Grande do Sul. Ci. Rural, 36:1780-1787, 2006.

ROBERTS, T.L.; ROSS, W.J.; NORMAN, R.J.; SLATON, N.A. & WILSON JUNIOR, C.E. Predicting nitrogen fertilizer needs for rice in Arkansas using alkaline hydrolyzable-nitrogen. Soil Sci. Soc. Am. J., 75:1161-1171, 2011.

SAHRAWAT, K.L. Mineralization of soil organic matter under waterlogged conditions in relation to other properties of tropical rice soils. Austr. J. Soil Res., 21:133-138, 1983.

SAHRAWAT, K.L. & PONNAMPERUMA, F.N. Measurement of exchangeable NH_4^+ in tropical rice soils. Soil Sci. Soc. Am. J., 42:282-283, 1978.

SALCEDO, I.H.; SAMPAIO, E.V.S.B.; ALVES, G.D. Mineralização do carbono e do nitrogênio em solo cultivado com cana-de-açúcar. R. Bras. Ci. Solo, 9:33-38, 1985.

SCHLESINGER, W.H. An overview of the carbon cycle. In: LAL, R.; KIBBLE, J.; LEVINE, E. & STEWART, B.A., eds. Advances in soil science: Soils and global changes. Boca Raton, CRC Press, 1995. p.9-25.

SCHOMBERG, H.H.; WIETHOLTER, S.; GRIFFIN, T.S.; REEVES, D.W.; CABRERA, M.L.; FISHER, D.S.; ENDALE, D.M.; NOVAK, J.M.; BALKCOM, K.S.; KITCHEN, N.R.; LOCKE, M.A.; POTTER, K.N.; SCHWARTZ, R.C.; TRUMAN, C.C. & TYLER, D.D. Assessing indices for predicting potential nitrogen mineralization in soils under different management systems. Soil Sci. Soc. Am. J., 73:1575-1586, 2009.

SHARIFI, M.; ZEBARTH, B.J.; BURTON, D.L.; GRANT, C.A. & COOPER, J.M. Evaluation of some indices of potentially mineralizable nitrogen in soil. Soil Sci. Soc. Am. J., 71:1233-1239, 2007.

SMITH, D.M.; INMAN-BAMBER, N.G. & THORBURN, P.J. Growth and function of the sugarcane root system. Field Crop Res., 92:169-183, 2005.

SOIL SURVEY STAFF. Keys to soil taxonomy. 11.ed. Washington, USDA/Natural Resources Conservation Service, 2010. 344p.

SOON, Y.K.; HAQ, A. & ARSHAD, M.A. Sensitivity of nitrogen mineralization indicators to crop and soil management. Commum. Soil Sci. Plant Anal., 38:2029-2043, 2007.

ST. LUCE, M.S.; WHALEN, J.K.; ZIADI, N. & ZEBARTH, B. Nitrogen dynamics and indices to predict soil nitrogen supply in humid temperate soils. Adv. Agron., 112:55-102, 2011.

STANFORD, F. & SMITH, S.J. Nitrogen mineralization potentials of soil. Soil Sci. Soc. Am. Proc., 36:465-472, 1972.

STEVENSON, F.J. Humus chemistry: Genesis, composition and reactions. 2.ed. New York, John Wiley, 1994. 496p.

THORBURN, P.J.; BIGGS, J.S.; WEBSTER, A.J. & BIGGS, I.M. An improved way to determine nitrogen fertiliser requirements of sugarcane crops to meet global environmental challenges. Plant Soil, 339:51-67, 2011.

THORBURN, P.J.; DART, I.K.; BIGGS, I.M.; BAILLIE, C.P.; SMITH, M.A. & KEATING, B.A. The fate of nitrogen applied to sugarcane by trickle irrigation. Irrig. Sci., 22:201-209, 2003.

WANG, W.J.; CHALK, P.M.; CHEN, D. & SMITH, C.J. Nitrogen mineralization, immobilisation and loss, and their role in determining differences in net nitrogen production during waterlogged and aerobic incubation of soils. Soil Biol. Biochem., 33:1305-1315, 2001.

WARING, S.A. & BREMNER, J.M. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. Nature, 201:951-952, 1964.

YAGI, R.; FERREIRA, M.E.; CRUZ, M.C.P. & BARBOSA, J.C. Mineralização potencial e líquida de nitrogênio em solos. R. Bras. Ci. Solo, 33:385-394, 2009.