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Mild and Moderate Extraction Methods to Assess Potentially Available Soil Organic Nitrogen

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ABSTRACT: The use of chemical methods to assess the soil organic nitrogen (N) potentially available to plants is not a common practice in Brazil. However, associated with others, this tool might improve efficiency in the use of waste and nitrogen fertilizers. In our study, chemical methods were tested to assess potentially available soil N in samples of 17 representative soils of the western plateau of the state of São Paulo (10 Oxisols and 7 Ultisols). Available soil N was extracted from the collected soil samples using moderate (ISNT-Illinois Soil Nitrogen Test) and mild (hot water and heated solutions of 2 mol L^{-1} KCl and 0.01 mol L^{-1} $CaCl_2$) extraction methods. The levels of potentially available N obtained from these chemical methods were correlated with dry matter (DM) and N uptake (N_{up}) by corn plants grown in pots in a greenhouse experiment carried out with the same 17 soil samples. The ISNT method showed the highest available N extraction capacity, whereas hot water showed the lowest capacity, followed closely by the hot 0.01 mol L^{-1} $CaCl_2$ solution. Despite the differences among the quantities of available N extracted, the methods correlated with each other and with DM and N_{up}, but the values from the ISNT method showed the lowest correlation with plant variables ($r_{DM} = 0.67^{**}$ and $r_{Nup} = 0.81^{**}$). Procedures of extraction with water or 0.01 mol L^{-1} $CaCl_2$ heated for 16 h, and 2 mol L^{-1} KCl heated for 4 h, resulted in similar correlation values (r) with plant DM and N_{up}. Thus, water ($r_{DM} = 0.77^{**}$ and $r_{Nup} = 0.90^{**}$) and 0.01 mol L^{-1} $CaCl_2$ ($r_{DM} = 0.82^{**}$ and $r_{Nup} = 0.93^{**}$) heated for 16 h can be recommended as the best options for N extraction. considering the possibility for predicting N availability, lower generation of waste, and lower cost of analysis.

Keywords: chemical analysis, organic matter, mineralization.

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INTRODUCTION

The quantity of soil organic N mineralized in a specific period of time may be estimated using short-term methods of soil incubation under aerobic or anaerobic conditions (Keeney and Bremner, 1966), or long-term methods under aerobic conditions (Stanford and Smith, 1972). The long-term method of Stanford and Smith (1972) under aerobic and controlled temperature and soil moisture conditions is the most used. This method allows estimation of potentially available N (N_0) in soils, which may be used as reference values in studies of chemical extractants for soil organic N. Nevertheless, this method might result in overestimated values of N_0 for plants due to sample handling and ideal temperature and moisture conditions during soil incubation (Sistani et al., 2008; Yagi et al., 2009). Thus, the use of N-uptake by plants as reference values of soil N availability to evaluate the efficacy of chemical methods is still a classic procedure, mainly because the presence of plants alters the rate of organic N mineralization in the soil. According to measurements taken by Herman et al. (2006), 9.2 mg kg^{-1} d^{-1} of mineral N was produced in the rhizosphere soil, whereas in the non-rhizosphere soil, the mineralization rate was 1 mg kg^{-1} d^{-1}.

Research on chemical methods to quantify potentially available organic N should result in methods suitable for routine procedures that are faster than incubation methods (Gianello and Bremner, 1986a).

Chemical methods as a tool to assess the soil N potentially available in agroecosystems has not yet been applied in Brazil. An efficient chemical method might improve the estimate of soil organic N mineralized in a specific period of time, and thus optimize the use of N fertilizers and organic residues. These methods are expected to closely estimate available soil N and, as much as possible, offer additional practical characteristics such as an easy, fast, low cost procedure with minimal residue production in the laboratory.

The amount of hydrolyzed organic N extracted by chemical methods depends on extraction intensity and might vary from less than 5 % to more than 50 % of total N. Methods that extract less than 5 % of total N are considered to have mild extraction intensity, and those that extract more than 35 % are considered to have strong extraction intensity (Ros et al., 2011a). Water and saline solutions are usually classified as mild extraction methods. In the case of saline solutions, extraction intensity will depend on the nature of the salt used, the concentration of the salt solution, the soil-solution ratio, the time period, and the temperature of extraction (Ros et al., 2009).

Moderate and mild extraction methods are used to evaluate the factors intensity and capacity of organic-N release (Gianello et al., 2000). Therefore, the use of chemical methods of moderate extraction intensity allows assessment of N bound to organic compounds resistant to biodegradation, whereas the methods of mild extraction intensity assess N bound to bioavailable compounds (Stanford, 1982). Among the methods of mild extraction intensity, the 2 mol L^{-1} KCl solution heated to 100 °C for 4 h (Gianello and Bremner, 1986a) has frequently been reported as a good index of N availability to plants (Oliveira, 1989; Gianello et al., 2000; Ros et al., 2011a; Velthof and Oenema, 2010).

There is little information in the literature about the use of hot water as an extraction method (Gregorich et al., 2003), despite its low cost and low residue generation, but Curtin et al. (2006) reported better results with hot water than with hot KCl solution.

Another alternative procedure was proposed by Houba et al. (1986) using 0.01 mol L^{-1} $CaCl_2$ solution at room temperature, which was considered an efficient method for extraction of potentially mineralizable organic N. However, Sharifi et al. (2007) tested this extraction procedure at room temperature in 153 soil samples, and the results did not correlate with the potentially mineralizable organic-N data obtained in an experiment of soil incubation at 25 °C over 24 weeks. Cordovil et al. (2007) corroborated the latter results; that is, the authors did not succeed when using $CaCl_2$ solution at room temperature, and argued that under such conditions organic-N is not extracted from the soil sample. However, it might be partially extracted using hot $CaCl_2$ solution, and this organic N fraction might represent potentially mineralizable N.

A chemical method for determination of N bound to amino sugars was proposed (Khan et al., 2001), which allows classification of soils into two groups: soils that respond to N fertilization and soils that do not respond. In this method, called the “Illinois Soil Nitrogen Test” (ISNT), soil samples are heated with NaOH solution in hermetically closed containers. Klapwyk et al. (2006), Sharifi et al. (2007), and Lawrence et al. (2009) obtained satisfactory results with the ISNT method, whereas Laboski et al. (2008) and Osterhaus et al. (2008) reported unsatisfactory results. In Brazil, Otto et al. (2013) compared the ISNT to other methods to estimate potentially mineralizable organic N in areas growing sugarcane and concluded that the ISNT method might improve the N recommendation for the crop.

We hypothesize that mild extraction of soil nitrogen is more selective to hydrolyze labile fractions of soil organic nitrogen, and consequently more efficient in predicting the potentially available nitrogen than moderate or intensive extraction methods. The objective of this study was to assess the efficiencies of chemical methods of moderate and mild extraction intensities for determination of potentially available organic N in soils of the state of São Paulo, Brazil.

MATERIALS AND METHODS

Samples were collected from 17 soils of the western plateau region of the state of São Paulo, Brazil, from the 0.00-0.20 m depth layer under forest areas (soils 1 and 5, Table 1) and cropped areas. The soil samples were air dried and passed through a 2 mm sieve. Afterwards, samples were analyzed in regard to particle size (Camargo et al., 2009) and chemical composition (Raij et al., 2001) (Table 1). Soil samples were analyzed to determine total N, according to Cantarella and Trivelin (2001a), and potentially available N using hot water, hot solutions of $CaCl_2$ and KCl, and the diffusion method (Illinois Soil Nitrogen Test - ISNT). The procedures are described below and all analyses were run in triplicate, using soil volume instead of soil weight. Additional adaptations of the methods are detailed for each case. A greenhouse experiment was carried out with the same soil samples using corn as test plants to evaluate accumulated N.

Hot water (HW-N)

This soil extraction procedure consisted of a combination of procedures adopted by Gianello and Bremner (1986a) (extraction with hot KCl) and by Curtin et al. (2006) (extraction with hot water). Soil samples of 4 cm^3 were transferred to digestion tubes and 30 mL of deionized water was added. Tubes were sealed with rubber stoppers fixed with adhesive tape, transferred to pre-heated digestion blocks at 80 °C, and heated for 16 h. Curtin et al. (2006) reported that maximum extraction occurs during the initial hours and for that reason, 2, 4, and 8 h of heating were also evaluated. After the heating period, the suspension was cooled, and a 10 mL aliquot sample was taken from each supernatant for distillation to determine the hydrolyzed NH_4^+-N, using MgO as an alkalizing agent. In distillates, determination of NH_4^+-N was made by titration with diluted H_2SO_4 solution, as described in Cantarella and Trivelin (2001b).

Hot 0.01 mol L^{-1} $CaCl_2$ solution ($CaCl_2$-N)

Extraction with $CaCl_2$ solution followed the same procedure used with hot water.

Hot 2 mol L^{-1} KCl solution (KCl-N)

The extraction procedure used followed Gianello and Bremner (1986a), except for soil weight; that is, 2.5 cm^3 of soil was used, instead of 3 g. The soil sample was transferred to digestion tubes and 20 mL of 2 mol L^{-1} KCl solution was added to each tube. Tubes were sealed with rubber stoppers fixed with adhesive tape, transferred to pre-heated digestion blocks at 100 °C, and heated for 4 h. After heating, tubes containing the samples were cooled under tap water and distilled and titrated as previously described.

Table 1. Classification and properties of soils studied in the greenhouse experiment and in laboratory analyses for potentially available organic nitrogen

Soil	Classification(1, 2)	pH($CaCl_2$)	OM	Total N	CEC	Sand	Clay
			g dm^{-3}	g kg^{-1}	mmol$_c$ dm^{-3}	——— g kg^{-1} ———	
1	*Latossolo Vermelho* (Rhodic Eutrudox)	6.2	87	3.91	149	210	620
2	Gleissolo (Typic Haplaquox)	4.2	63	2.27	189	240	650
3	*Latossolo Vermelho* (Rhodic Eutrudox)	5.2	50	2.55	109	170	700
4	*Argissolo Vermelho-Amarelo* (Arenic Hapludult)	5.1	36	1.84	79	860	100
5	*Latossolo Vermelho* (Typic Hapludox)	3.8	35	1.67	77	660	300
6	*Latossolo Vermelho* (Typic Hapludox)	5.3	35	1.63	79	590	380
7	*Latossolo Vermelho* (Typic Hapludox)	4.3	33	1.34	70	630	350
8	*Latossolo Vermelho* (Typic Hapludox)	5.1	32	1.34	75	570	380
9	*Latossolo Vermelho* (Typic Hapludox)	5.3	31	1.72	60	610	350
10	*Argissolo Vermelho-Amarelo* (Typic Kandiudult)	4.8	27	0.97	60	750	180
11	*Latossolo Vermelho* (Typic Hapludox)	5.3	27	1.39	70	630	350
12	*Latossolo Vermelho* (Typic Hapludox)	4.6	22	1.06	71	660	300
13	*Argissolo Vermelho-Amarelo* (Arenic Hapludult)	5.1	21	1.14	51	850	120
14	*Argissolo Vermelho-Amarelo* (Arenic Hapludult)	5.7	16	0.82	50	860	100
15	*Argissolo Vermelho-Amarelo* (Arenic Hapludult)	5.2	15	0.66	42	850	130
16	*Argissolo Vermelho-Amarelo* (Arenic Hapludult)	5.7	11	0.53	47	880	100
17	*Argissolo Vermelho-Amarelo* (Arenic Hapludult)	5.1	9	0.42	31	870	90

(1) According to Santos et al. (2013) and (2) Soil Survey Staff (1999).

Illinois Soil Nitrogen Test (ISNT-N)

The procedure used followed Khan et al. (2001) and adapted to the existing laboratory facilities. Soil samples of 1 cm^3 were transferred to 473 mL Mason jars with hermetic seals. A 50 mL beaker containing 5 mL of a mixture of H_3BO_3 + indicators was put inside each Mason jar (substituting the original Petri dish). The beaker was attached to the Mason jar lid by means of a plastic ring and screw. This system keeps the beaker suspended within the vessel when it is sealed. After that, 10 mL of 2 mol L^{-1} NaOH solution was added to the soil sample in the Mason jar and immediately sealed; the container was transferred to a water bath at 48-50 °C and heated for 5 h. Heating in a water bath was used instead of a hot plate, as recommended in the author's method, because a water bath provides greater temperature stability. At the end of the heating period, the beaker containing H_3BO_3 + indicators was removed from the Mason jar, the solution was diluted with 5 mL deionized water and titrated with standard 0.02 mol L^{-1} H_2SO_4 solution for determination of potentially available organic N (NH_4^+-N + amino-sugar-N).

Greenhouse trial

A pot experiment was carried out in the greenhouse, using corn as test plants grown in the same soil samples. A completely randomized experimental design was used, with 17 treatments (soil samples) and four replications. A 5.0 dm^3 volume of each soil was weighed and amended to adjust base saturation to at least 70 % and Mg to 8 $mmol_c$ dm^{-3}. After that, plastic pots were filled with the amended soil, moistened with deionized water up to 70 % of soil water retention capacity, and incubated for 20 days. One week after the beginning of incubation, the pots were treated with a nutrient solution free of N and Fe. Corn seeds were sown (DKB 390 hybrid) and thinned to five seedlings per pot. Throughout the experiment, the amount of water added was monitored by means of pot weighing and reposition of lost water to avoid lixiviation, maintaining soil moisture around 70 % of soil retention capacity. Corn plants were harvested 42 days after sowing, when plants were exhibiting visual symptoms of N deficiency in all treatments. Plants were cut at soil level, rinsed, and dried in a forced-air oven until constant weight to determine dry matter production (DM). After that, plants were ground and total N was determined, according to Carmo et al. (2000). Based on plant dry matter production and plant N concentrations, the quantity of N accumulated by plants per pot was calculated (N_{up}).

Statistical analysis

The program AgroEstat was used for statistical analysis (Barbosa and Maldonado Jr., 2015). Correlation analyses were run between methods, between methods and soil chemical properties, and between methods and DM and N_{up} by corn plants.

RESULTS AND DISCUSSION

The results indicated the following increasing order of extraction intensity (Table 2): water<$CaCl_2$<KCl<ISNT. With hot water and hot $CaCl_2$ solution (80 °C), the amount of N extracted stabilized at 8 h. On average, the $CaCl_2$ solution extracted 3 mg dm^{-3} more than the hot water. This difference persisted over all the periods studied (Figure 1). Although an 8-h extraction period might be enough to stabilize the process, the 16-h period is recommended in routine laboratory analyses for the sake of convenience because the extraction procedure may be run overnight, followed by extract distillation early in the morning, avoiding the need for extract conservation. The 16-h extraction period resulted in N quantities ranging from 1.3 to 25.3 mg dm^{-3} of HW-N, and 5.5 to 28.6 mg dm^{-3} of $CaCl_2$-N (Table 2), which represent 0.39 to 0.77 % and 0.43 to 1.78 % of total N, respectively, with average values from 0.55 to 0.96 %.

The average HW-N concentration values over 17 soil samples were 37 % lower than the $CaCl_2$-N values. In spite of that, high positive correlation values were found between the two extraction methods ($r = 0.95^{**}$, Table 3), suggesting that both methods extract N from the same pool with different intensities. It is supposed that the organic-N and NH_4^+-N extracted with hot water are derived from several organic

Table 2. Potentially available N concentrations in 17 soil samples subjected to organic N extraction using hot water (HW-N), hot solutions of 0.01 mol L^{-1} $CaCl_2$ ($CaCl_2$-N) and 2 mol L^{-1} KCl (KCl-N) under increasing heating periods, and the Illinois Soil Nitrogen Test (ISNT-N); corn plant dry matter production per pot (DM) and N uptake per pot (N_{up}) obtained in a greenhouse experiment with the same soil samples

Soil	HW-N				$CaCl_2$-N				KCl-N	ISNT-N	DM	N_{up}
	2 h	4 h	8 h	16 h	2 h	4 h	8 h	16 h				
	mg dm^{-3}										g per pot	mg per pot
1	10.1	9.7	17.2	25.3	10.8	11.1	27.6	28.6	51.5	316.9	75.5	393.1
2	2.6	5.7	6.7	9.1	8.8	8.7	10.7	9.9	24.4	144.8	29.6	84.4
3	5.4	6.8	11.9	13.8	9.3	9.5	11.4	14.2	39.1	203.6	55.3	198.1
4	5.5	8.7	11.5	10.1	10.5	10.9	11.7	15.1	29.1	92.1	78.5	269.2
5	4.3	7.3	9.5	9.2	7.2	8.7	13.0	14.1	21.4	86.5	47.5	188.0
6	3.8	4.6	8.9	8.5	6.8	8.9	9.8	10.1	22.9	72.5	42.3	131.1
7	2.8	6.4	9.0	9.0	5.9	7.3	12.0	10.7	21.9	67.1	50.5	172.1
8	2.5	6.2	5.7	6.0	5.8	7.8	9.6	11.3	17.8	70.3	39.6	114.4
9	3.7	5.2	8.0	8.8	6.5	9.5	11.3	12.1	21.8	77.5	40.0	100.6
10	3.6	4.6	4.6	4.2	4.9	7.2	9.3	10.8	16.5	54.2	37.7	98.8
11	3.1	3.9	5.6	5.3	4.4	7.5	9.8	10.4	22.9	72.4	51.2	137.5
12	4.0	1.5	4.1	4.0	5.7	9.3	9.8	6.3	14.7	44.1	20.5	52.6
13	2.1	2.8	6.0	4.6	5.9	8.1	7.4	6.7	17.6	44.5	43.2	126.8
14	1.5	3.0	4.9	4.6	4.6	8.2	7.4	7.7	13.7	30.7	32.7	80.1
15	1.5	2.0	2.6	2.3	3.8	5.1	7.3	6.5	11.5	21.4	26.1	68.8
16	0.7	1.9	2.3	1.9	2.3	4.6	5.1	5.6	10.4	19.7	21.2	67.4
17	0.4	1.5	0.9	1.3	3.0	4.5	5.6	5.5	8.8	16.8	18.5	44.8

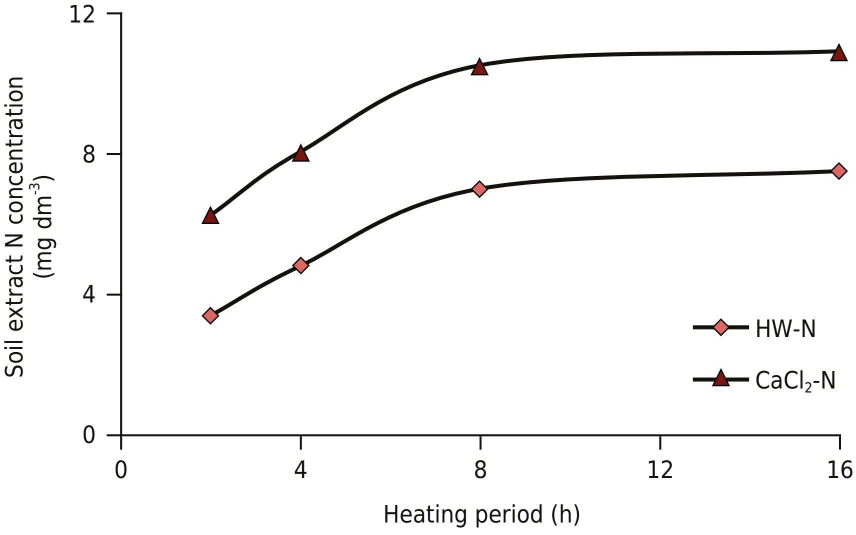

Figure 1. Average N concentrations in soil extracts of 17 soil samples using hot water (HW-N) and a hot solution of 0.01 mol L^{-1} $CaCl_2$ ($CaCl_2$-N) under increasing heating periods (2, 4, 8, and 16 h heating periods).

components, such as amino acids, proteins, amino sugars, and humic material, and, on average, 80 % of the total extracted is present in organic forms (Gregorich et al., 2003; Curtin et al., 2006). The remaining 20 % is the NH_4^+-N determined by distillation, and this mostly corresponds to the NH_4^+-N pre-existing in the soil plus hydrolyzed organic N, since admittedly very little organic N undergoes hydrolysis during distillation when the alkalinizing agent is MgO (Bremner and Keeney, 1966). The NH_4^+-N hydrolyzed is probably derived from labile organic N and is the result of hydrolysis of organic compounds, such as amino sugars (Gregorich et al., 2003). Therefore, pre-existent NH_4^+-N in the soil sample should be subtracted from the total N that is determined in order to calculate the hydrolyzed NH_4^+-N, which is the actual available soil organic N. However, such a procedure was not adopted in any of the extraction procedures evaluated because this effort does not improve the estimate of N availability, and, in addition, it represents additional work and cost of analysis (Jalil et al., 1996).

Assuming that the composition of the extracts with hot water and hot $CaCl_2$ solution are similar, the greater extraction intensity of the saline solution might be explained by the exchange of NH_4^+-N adsorbed on clays by Ca^{2+} from the solution, by the lower re-adsorption of NH_4^+-N hydrolyzed during the heating period by competition with Ca^{2+}, and also by the exchange of organic anions adsorbed by Cl^-. Generally, soil organic N present in protein molecules is adsorbed to the surfaces of solid particles in much greater amount than that which is free in the pore spaces, and the adsorbed fraction can be exchanged for inorganic anions such as Cl^-. Ions Cl^- have low affinity with clay solid particles and low exchange capacity with organic compounds, which results in low extraction capacity, less than 2 % of total soil N (Apple and Mengel, 1998), as shown in this study.

Table 3. Linear correlation coefficients (r) between the potentially available organic-N concentrations extracted by different extraction methods and plant dry matter production (DM), N uptake (N_{up}), soil organic matter (OM), soil total N, soil clay content, and pH values

Variable	KCl-N	ISNT-N	HW-N 2h	HW-N 4h	HW-N 8h	HW-N 16h	$CaCl_2$-N 2h	$CaCl_2$-N 4h	$CaCl_2$-N 8h	$CaCl_2$-N 16h	OM	Total-N	DM	N_{up}	Clay
ISNT-N	0.96**	-													
HW-N 2h	0.87**	0.90**	-												
HW-N 4h	0.83**	0.76**	0.79**	-											
HW-N 8h	0.94**	0.86**	0.91**	0.90**	-										
HW-N 16h	0.97**	0.96**	0.92**	0.84**	0.95**	-									
$CaCl_2$-N 2h	0.87**	0.81**	0.83**	0.86**	0.90**	0.85**	-								
$CaCl_2$-N 4h	0.76**	0.66**	0.81**	0.72**	0.83**	0.74**	0.89**	-							
$CaCl_2$-N 8h	0.88**	0.89**	0.93**	0.78**	0.87**	0.94**	0.73**	0.68**	-						
$CaCl_2$-N 16h	0.92**	0.90**	0.93**	0.87**	0.91**	0.95**	0.78**	0.69**	0.96**	-					
OM	0.91**	0.95**	0.83**	0.80**	0.84**	0.93**	0.85**	0.70**	0.88**	0.86**	-				
Total-N	0.97**	0.97**	0.90**	0.82**	0.92**	0.97**	0.89**	0.78**	0.90**	0.91**	0.97**	-			
DM	0.82**	0.67**	0.79**	0.87**	0.88**	0.77**	0.78**	0.72**	0.71**	0.82**	0.64**	0.73**	-		
N_{up}	0.90**	0.81**	0.89**	0.87**	0.93**	0.90**	0.79**	0.68**	0.88**	0.93**	0.76**	0.83**	0.93**	-	
Clay	0.74**	0.81**	0.59*	0.56*	0.63**	0.71**	0.64**	0.52*	0.60*	0.58*	0.84**	0.81**	0.34^{ns}	0.43^{ns}	-
pH	0.06^{ns}	0.25^{ns}	0.24^{ns}	0.20^{ns}	-0.05^{ns}	0.13^{ns}	0.23^{ns}	-0.04^{ns}	0.02^{ns}	0.20^{ns}	0.26^{ns}	0.24^{ns}	0.16^{ns}	0.17^{ns}	0.06^{ns}

Soil extract N concentrations obtained from: ISNT method = ISNT-N; hot water = HW-N; heated solution of 0.01 mol L^{-1} $CaCl_2$ = $CaCl_2$-N; and heated solution of 2 mol L^{-1} KCl = KCl-N. OM: soil organic matter; total-N: soil total-N; DM: corn dry matter production per pot; N_{up}: corn N uptake per pot. *, **, and ^{ns}: statistically significant at 0.05 and 0.01, and not significant, respectively.

The average values of KCl-N concentration over 17 soil samples ranged from 8.8 to 51.5 mg dm^{-3} of N (Table 2), which are similar to those reported for mineral soils in other studies (Gianello et al., 2000; Beauchamp et al., 2003; Velthof and Oenema, 2010). The KCl-N concentration values were 3.5 times higher than the values obtained from hot water and twice higher $CaCl_2$ values, and they represented 1.84 % of total N (average of 17 soil samples). Greater extraction capacity of (2 mol L^{-1}) KCl compared to (0.01 mol L^{-1}) $CaCl_2$ is explained by the higher KCl salt concentration (since the anion is the same in both saline solutions) and higher extraction temperature.

The ISNT-N concentration values (ranging from 16.8 to 316.9 mg dm^{-3}, Table 2) were lower than the values reported by other authors (Khan et al., 2001; Klapwyk et al., 2006; Sharifi et al., 2007; Lawrence et al., 2009). The average ISNT-N values over 17 soil samples were 10.9 times, 6.9 times, and 3.4 times higher than the HW-N, $CaCl_2$-N, and KCl-N values, respectively. It is known that the ISNT method extracts the organic N that corresponds to the hydrolysable N fraction in an alkaline medium, that is, an estimate of N bound to amino sugars (Mulvaney et al., 2001). In the present study, this ISNT-N fraction corresponded to 5.8 % (average) of total N, and this is within the 5-10 % range of N bound to amino sugars in relation to soil total N reported by Stevenson (1986). Furthermore, the 5.8 % obtained is close to the 7.9 % reported by Sharifi et al. (2007), but it is lower than the values of 13.5, 14.7, and 12.6 % reported by Marriot and Wander (2006), Laboski et al. (2008), and Osterhaus et al. (2008), respectively.

Positive correlations were found for N concentrations among all extraction methods (hot water, 0.01 mol L^{-1} $CaCl_2$, 2 mol L^{-1} KCl, and ISNT), that is, for all possible combinations between two methods, with r-values above 0.90. Regardless of the extraction method, the N concentrations obtained correlated with soil OM concentrations (Table 3). Correlation between organic N extracted by chemical methods and soil OM was expected since the objective of studying the extraction methods was in fact to obtain easily degradable chemical compounds that might represent a sub-fraction of the total soil OM (Apple and Mengel, 1998). Ros et al. (2011b), in a study involving soil incubation, also observed correlation between mineralized N and total organic C ($r = 0.69^{**}$). These authors suggested that systems for fertilizer recommendation that use an estimate of potentially available N should at least be supported by one soil variable that represents the size of the OM reservoir.

Since OM and total N are soil properties known to be in correlation, correlation between extracted N and total soil N was also expected, which was obtained in this study with correlation coefficient values (r) above 0.90 for all extraction methods (Table 3). Laboski et al. (2008) reported high correlation coefficient values between ISNT-N and soil OM concentrations and total soil N, and Beauchamp et al. (2003) suggested that the higher the extraction intensity of the method is, the higher the correlation with total N, which was not confirmed in the present study.

Soil OM and total N are relatively constant over time within the same management system, and extractable N depends on several edaphic and climatic factors. Beauchamp et al. (2003) argued that the humus-N contribution to the amount of extractable N might vary due to management practices in the field, such as plowing, no-till planting, and the use of green manure, as well as soil temperature and moisture. The authors observed correlation between KCl-N and soil total N only in the case of soils with incorporation of crop residues, and they concluded that the main KCl-N source was the crop residue added to the soil. Therefore, correlations between OM *vs* extractable N and total N *vs* extractable N may or may not exist, depending on the experimental conditions.

The results (Table 3) indicated that increasing N quantities in soil extracts were related to increasing soil clay contents, regardless of the extraction method used, but the correlation coefficient (r) values found were lower than those observed between soil extracted N and OM or total N (Table 3). Ros et al. (2011b) observed negative correlation between potentially available N and clay (-0.26**), which was attributed to greater OM stability in clayey soils, resulting in lower N extraction by hot water or saline solutions. Nevertheless, an opposite result might also be obtained, considering that greater exposure of hydrolysable organic compounds might occur during soil sample preparation, as in the present study, when soil samples were subjected to clay disaggregation.

No correlation was found between soil pH values and N concentrations in soil extracts (Table 3), in contrast with the results reported by Ros et al. (2011b), who observed negative correlation between these two variables ($r = -0.42^{***}$). According to these authors, soil OM explained 78 % of the potentially available N variation, while other variables, such as pH and clay content, explained only 8 % of the variation. For that reason, these variables are not included in mathematical models to predict N availability.

Positive correlations between soil extract N (HW-N and $CaCl_2$-N) concentrations and plant variables (DM and N_{up}) were observed (Table 3). Correlation coefficients (r) between HW-N *vs* DM and HW-N *vs* N_{up} ranged from 0.77 to 0.88 and from 0.87 to 0.93, respectively; r values between $CaCl_2$-N *vs* DM and $CaCl_2$-N *vs* N_{up} ranged from 0.71 to 0.82 and from 0.68 to 0.93, respectively. The correlation coefficients (r) varied randomly with the extraction time period for both extraction methods but, overall, higher r values were obtained from the 16-h extraction period (Table 3).

Positive correlations were also observed between KCl-N and plant variables (DM and N_{up}), with r values equal to 0.82 and 0.90, respectively (Table 3), results similar to those obtained by Gianello and Bremner (1986a,b), Oliveira (1989), Jalil et al. (1996), Campbell et al. (1997), and Velthof and Oenema (2010). Nyiraneza et al. (2012) concluded that KCl-N was the most efficient index for predicting potentially available N because it showed the highest r-values with plant DM and N_{up}.

For the ISNT method, the N results obtained from this procedure showed the lowest correlations between soil-extract N *vs* plant DM and N_{up} (r values = 0.67^{**} and 0.81^{**}, respectively). In this case, r values between soil N and N_{up} are not different among the methods (hot water, $CaCl_2$, KCl, or ISNT), but the r value between ISNT-N *vs* DM was lower than the r values obtained from the other extraction methods by the t test. Such results with ISNT are below those reported by Khan et al. (2001), Sharifi et al. (2007), and Klapwyk et al. (2006). According to Sharifi et al. (2007), the results obtained from ISNT were better than the ones with KCl in regard to correlation between soil N concentrations and potentially available N. The literature reported that N-amino sugars (the fraction that is extracted with ISNT) might be considered, overall, the labile fraction of soil organic N and it might be highly correlated with plant DM yield, N uptake (N_{up}), and response to N fertilization (Mulvaney and Khan, 2001; Barker et al., 2006). However, several authors disagree about ISNT ability to predict potentially available organic N (Laboski et al., 2008; Osterhaus et al., 2008) because this method may also extract a constant fraction of total soil N, which might not correspond to the most labile OM-N fraction. In Brazil, Otto et al. (2013) considered ISNT a promising index for estimating potential responses to N fertilization in sugarcane cropping areas. An alternative method to ISNT is direct distillation (Bushong et al., 2008; Roberts et al., 2009), which exhibited similar extraction capacity and high correlation coefficients with ISNT (Bushong et al., 2008; Roberts et al., 2009; Otto et al., 2013); in addition, direct distillation does not require the use of Mason Jar, a container not readily available in the Brazilian market and which might limit adoption of the method.

The success or failure of ISNT use in predicting labile N availability and crop response to N fertilization was related to soil texture. According to Nyiraneza et al. (2012), when only data of similar texture soils were separated and subjected to analysis of variance and correlation, higher correlation coefficients between ISNT-N or KCl-N and DM or N_{up} were obtained. In the present study, such procedure did not help to improve correlations, maybe because the calculus included a lower number of values.

No relationship was observed between extraction intensity and efficiency of the method in predicting N availability since all extraction methods, regardless of the extraction intensity, showed high correlation with DM and N_{up}, including soil OM and total N, which are considered intensive extraction methods for available N (Table 3). For a long period of time, the strength or intensity of the extraction method was considered an important characteristic in studies on determination of potentially available soil organic N because Kelley and Stevenson (1985) affirmed the existence of an inverse relationship between extraction intensity and selectivity to extract a specific fraction of soil N with a biological role. Until recently, it was supposed that this relationship existed and then an extensive analysis of published data on potentially available organic N revealed that there was no relationship between extraction intensity and method performance (Ros et al., 2011a). The same was observed in the present study; in fact, mild extraction methods (hot water and hot $CaCl_2$ and KCl solutions) best represented N availability. Therefore, these extraction methods are among the best and, based on compilation of data from 2,068 observations on chemical methods of N determination published in 218 scientific papers, the conclusion was that the best prognosis (57 % < R^2 < 74 %) was obtained from the hot solutions of $CaCl_2$, acid $KMnO_4$, $K_2Cr_2O_7$, water, and KCl. Nevertheless, organic N extracted from the soil by chemical methods, in this extensive evaluation, explained only 47 % of the variation of potentially available organic N determined in laboratory trials, a value not above the one obtained for total N (43 %). Both values were considered too low to provide a reliable fertilizer recommendation. Precise recommendations require R^2 values above 83 % (r = 0.91) (Ros et al., 2011a). Such requirements were met in the present study with the use of heated water for 8 h and a 0.01 mol L^{-1} $CaCl_2$ solution heated for 16 h (Table 3). Nevertheless, no significant difference was found between the two extraction procedures above and the 2 mol L^{-1} KCl solution (based on statistical comparison of r values by the t test). Furthermore, it should be noted that soil extracts obtained with hot water and $CaCl_2$ solution had the advantages of lower cost of analysis and fewer extract residues left in the laboratory.

CONCLUSIONS

The soil N extraction methods, namely the Illinois Soil Nitrogen Test, hot water, and 0.01 mol L^{-1} $CaCl_2$, and 2 mol L^{-1} KCl hot solutions, were efficient in estimating potentially available organic N to plants in the soils studied.

Considering the capability of the methods in estimating soil N availability, lower cost of analysis, and fewer extract residues left in the laboratory, the 16-h heated water and 16-h heated 0.01 mol L^{-1} $CaCl_2$ extraction methods are recommended.

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