A Simple Alkaline Hydrolysis Method for Estimating Nitrogen Mineralization Potential of Soils

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Abstract

A simple, precise and rapid alkaline hydrolysis method for determining nitrogen (N) availability index of soils is described. It involves direct steam distillation of 1 g field-moist soil and 1 M KOH, NaOH, LiOH or phosphate-borate buffer (pH 11.8) and the amount of NH_4^+-N released trapped in boric acid and its concentration determined successively every 5 min for a total of 40 min. The cumulative N hydrolyzed was fitted to a hyperbolic equation to determine the maximum hydrolyzable N (N_{max}) and the time required to hydrolyze one-half of N_{max} (K_t) by linear regression of the transformed data. First-order equation was also used to estimate the potentially hydrolyzable N (N_o), hydrolysis rate constant (k) and the time required to hydrolyze one-half of N_o ($t_{1/2}$). Results showed that, for each soil and reagent, N_{max} and N_o values were similar, but differed significantly among soils, suggesting differences in the chemical nature or reactivity of organic N in the soils. In general, N_{max} and N_o values ranged from 401 to 1667 mg kg^{-1} soil and accounted for 12-56% of total organic N in the soils. The K_t values ranged between 15 and 30 min. Among the reagents tested, KOH and NaOH showed the best promise for estimating the total hydrolyzable organic N pool in the soils. The N_{max} and N_o values were significantly correlated with the amounts of N mineralized in two weeks under aerobic and anaerobic conditions at 30 ^oC, N released by 2 M KCl extraction at 80^oC for 20 h, and the initial NH_4^+-N present in the soils. We concluded that direct steam distillation of soils with 1M KOH or NaOH offer a quick and precise mean for estimating the potentially mineralizable organic N pool and availability index in soils.

Introduction

The use of extractable organic matter as an index of N availability has long been recognized, and considerable efforts have been expended to find specific component that correlates with N mineralization and plant uptake in the field. Stanford (1968) reported that soils contain appreciable amount of organic N (23-66%) extractable with boiling aqueous solution of 0.01 M $CaCl_2$ or 0.5 M Na-pyrophosphate, and that the amount of distillable N in the extracts correlated highly with the capacities of soils to mineralize N under anaerobic incubation conditions. A similar relationship was found for the NaOH-distillable fraction obtained by autoclaving soils in 0.01 M $CaCl_2$ at 121^oC for 16 h (Stanford and DeMar, 1969). Using a trial-and-error procedure, Stanford (1969) derived a rate expression describing the distillable organic fractions in the 0.01 M $CaCl_2$ extract. The above results, however, suggested that the chemical nature (i.e., reactivity) of the source of the 0.01 M $CaCl_2$ distillable N derived by autoclaving did not differ among soils, as such, the utility of the derived reaction rate constants in defining soil N availability cannot be made. Besides, the method is too complicated for use in soil testing laboratories, as it requires two extractions to remove the NH_4^+-N present in the soil sample before autoclaving, and one to extract the NH_4^+-N present after autoclaving. Khan et al. (2001) reported that amino sugar N in soil hydrolysates could be used to discriminate among sites that are responsive from those non-responsive to N fertilization. However, this method is also complicated in that it requires hydrolyzing the soil organic N before analysis for amino sugars. Furthermore, the use of chemical indices of N availability has been limited because of the poor correlation with crop N uptake, probably due to inability to selectively release the fraction of soil organic N that is made available for plant growth by soil microorganisms. For these reasons, a new and innovative chemical approach to the problem is imperative, particularly because chemical methods are usually more rapid and precise than biological methods.

Modeling of N mineralization kinetics in soils usually involves prediction of an active fraction of the total organic N and a rate constant to describe the rate of mineralization. Parameters for a simple functional approach have been obtained from long-term laboratory incubation studies, where the net N mineralization under such conditions was observed to follow first-order kinetics (Stanford and Smith, 1972) and is approximated by the equation:

$$N_m = N_o[1 - e^{-kt}] \qquad [1]$$

where, N_m is the N mineralized in time *t*, N_o is the initial amount of substrate or the potentially mineralizable N, *k* is the rate constant. The parameters N_o and *k* are estimated by fitting a series of observations of Nm and t to the Eq. 1 above by iteration procedure. Empirically determined polynomials and parabolic functions have also been proposed to describe N mineralization in soils (Broadbent, 1986; Marion and Black, 1987). Although these empirically determined equations provide a better fit to the data compared to first-order models, no physical meaning has been attached to the regression coefficients. Few attempts have been made to establish linkages between the model pool definitions and measurable quantities either by devising advanced laboratory fractionation procedures to match measurable organic matter fractions with model pool definitions, or by revising model pool definitions to coincide with measurable quantities (Christensen, 1996). To date, an integrated knowledge of the adequacy of the above kinetic parameters in relation to the different biological and chemical indices for predicting total mineralizable soil N pool is lacking.

The rate of alkaline hydrolysis of soil organic N over time may be described by a rectangular hyperbola in the form of the Michaelis-Menten equation for describing enzyme kinetics (Michaelis and Menten, 1913). The amount of maximum hydrolysis (N_{max}) would be the total pool of organic N that can be hydrolyzed by chemical, biochemical or biological means. A mathematical expression can be used to describe the rate equation for the effect of time of distillation on the cumulative amount of organic N hydrolyzed as follows:

$$N_c = N_{max}\,(t)/[K_t + t] \qquad [2]$$

where, N_c would be the cumulative N hydrolyzed at time *t*, K_t the time required to hydrolyze one-half of N_{max}.

To define the structure of this kinetically derived parameters, it is important to relate them to other biological and chemical indices of N mineralization. This would assist in advancing knowledge on the nature of soil organic N and chemical index of the pool size of active N in soils. Therefore, the objectives of this study were to: (1) develop an alkaline hydrolysis procedure for estimating the potentially hydrolyzable N in soils, (2) determine the rate and kinetic parameters of the hydrolysis process, and (3) study the relationships among the kinetic parameters and selected chemical and biological indices of N mineralization in soils.

Materials and methods

Soils and their properties

The 13 soils used in this study were surface soils (0-15 cm) sampled from uncultivated fields in Iowa, USA. The samples were mixed and screened to pass a 2-mm mesh sieve and stored in double plastic bags at 4°C when not in use. Soil pH was determined in 1:2.5 water to soil suspensions. Total C and N were determined on <180 µm air-dried samples by dry combustion (LECO CHN analyzer, St Joseph, MI) and inorganic N by the steam distillation method (Mulvaney, 1996). Organic N was calculated from the difference between total N and inorganic N (sum of NH_4^+-N and NO_3^--N) values. Particle size distribution was determined by a pipette method (Kilmer and Alexander, 1949). Selected chemical and biochemical properties of the soils used are presented in Table 1.

Chemical and biological indices of soil N availability

The first chemical method used to measure N availability index in the soils [hereafter referred to as Hot-KCl N (HKN)] involves determination of the NH_4^+-N produced by heating field-moist soil and 40 mL of 2 *M* KCl in a polypropylene tube with screw a cap at 80 °C for 20 h (Øien and Selmer-Olsen, 1980). The second method [hereafter referred to as sodium-borate N (SBN)] involves determination of the NH_4^+-N produced by steam distillation of field-moist soil with 40 mL of 0.066 *M* sodium-borate buffer (pH = 11.5) for 8 min (Gianello and Bremner, 1986). The third chemical method [hereafter referred to as phosphate-borate N (PBN)] involves determination of NH_4+-N produced by steam distillation of field-moist soil with 40 mL of phosphate-borate buffer (pH = 11.2) for 8 min (Gianello and Bremner, 1986). In all procedures, 4 g of field-moist soil on oven-dried basis was used, and the NH_4^+-N produced was determined by steam distillation

TABLE 1
Selected chemical, biochemical and physical properties of the soils used

Soil	pH	Total N	Inorganic N		Organic N	Total C	Sand	Silt	Clay
			$NH4^+$-N	$NO3^-$-N					
		g kg^{-1}	------mg kg^{-1}------		---------------------g kg^{-1}---------------------				
Canisteo	7.5	3.8	1.88	7.08	3.79	37.7	318	407	275
Clarion (I)	5.0	3.7	4.62	9.68	3.69	21.1	356	409	235
Coland	5.8	3.6	2.46	9.82	3.59	29.7	160	553	287
Crippin	7.5	2.9	6.64	14.59	2.89	24.2	432	341	227
Harps	7.9	4.3	1.44	6.79	4.29	53.9	335	384	281
Nicollet	6.5	3.2	3.18	12.13	3.18	21.3	367	396	237
Okoboji	7.8	4.2	1.73	8.95	4.19	37.4	267	438	295
Storden	7.8	2.2	2.46	14.44	2.18	28.9	480	387	133
Terrill	6.9	2.7	2.74	13.14	2.68	20.8	533	320	147
Webster (I)	8.0	3.5	1.44	8.38	3.49	32.3	370	398	232
Clarion (II)	6.6	3.0	4.77	12.71	2.98	37.1	317	425	258
Grundy	7.4	1.9	1.59	27.87	1.87	21.2	25	685	290
Webster (II)	6.5	2.4	4.91	6.93	2.39	38.3	297	426	277

(Mulvaney, 1996).
The biological methods of N availability index used were short-term aerobic and anaerobic incubations. In the aerobic incubation, the procedure described by Keeney and Bremner (1967) was used. Briefly, 10 g of air-dried soil sample was mixed with 30 g of 30 – 60 mesh quartz sand, moistened with 6 ml of distilled water and incubated under aerobic condition at 30°C for 14 days. At the end of the incubation period, the inorganic N produced was extracted with 2 M KCI and the amount determined by steam distillation (Mulvaney, 1996). The method described by Waring and Bremner (1964) was used for the anaerobic incubation. The procedure involves determination of the amount of NH_4^+-N produced when 12.5 mL of distilled water was added to 5 g of air-dried or field-moist soil (on oven dried basis) in a test tube and incubated under anaerobic condition for 14 days. At the end of the incubation period, 12.5 mL of 4 M KCI was added and the amount of NH_4^+-N produced was determined by steam distillation (Mulvaney, 1996).
All results reported are averages of duplicate analyses, with the initial NH_4^+-N present in the soils subtracted. The initial NH_4^+-N was determined by steam distillation of 5 g field-moist soil (on oven dried basis) in 20 mL of 2 M KCl with MgO for 4 min. Moisture content of the soils were determined from the weight loss after drying at 105°C for 48 h.

Alkaline hydrolysis of organic N

The procedure for the alkaline hydrolysis of soil organic N is described in detail by Dodor (2002). Briefly, 1 g of field-moist soil (on oven-dried basis) was placed in a 200-mL distillation flask and treated with 20 mL of alkaline reagent (1 M NaOH, 1M KOH, 1M LiOH, or phosphate-borate buffer [PBB], pH 11.8). The flask was connected to a distillation apparatus and the distillate collected in 5 mL of boric acid, which was changed every 5 min for a total of 40 min. The NH_4^+-N in the distillate was determined by titration with 0.005 M H_2SO_4 (Mulvaney, 1996). The initial NH_4^+-N present was subtracted from the results of the 5 min distillation.

Nitrogen hydrolysis models

The cumulative amounts of N hydrolyzed by the alkaline reagents with time were plotted according to the Lineweaver-Burk linearization of equation [2] (Lineweaver-Burk, 1934):

$$\frac{1}{N_c} = \frac{1}{N_{max}} + \frac{K_t}{N_{max}}\frac{1}{t} \qquad [3]$$

where, N_c is cumulative N hydrolyzed (mg kg^{-1} soil) in time t (min), K_t is the time required to hydrolyze a quantity of N equals to one-half of the maximum hydrolyzable N, N_{max} (mg kg^{-1} soil). A plot of $1/N_c$ vs. $1/t$ yields an intercept = $1/N_{max}$ and a slope = K_t/N_{max} from which the values for N_{max} and Kt were estimated. The Hanes-Woolf linearization was derived by multiplying both sides of equation [3] by t (Hanes, 1932):

$$\frac{t}{N_c} = \frac{K_t}{N_{max}} + \frac{1}{N_{max}}t \qquad [4]$$

A plot of t/N_c vs. t yields an intercept = K_t/N_{max} and a slope = $1/N_{max}$. Cross-multiplying equation [2], rearranging and dividing by K_t yield Eadie-Hofstee linearization (Eadie, 1942; Hofstee, 1959):

$$Nc = N_{max} - K_t\frac{N_c}{t} \qquad [5]$$

A plot of N_c vs. N_c/t yields an intercept = N_{max} and a slope = - K_t.
The exponential equation proposed by Stanford and Smith (1972) was also used to estimate the potentially hydrolyzable N (N_o)

and the hydrolysis rate constant (*k*) in Eq. 1. The Marquardt option of NLIN, a non-linear curve-fitting procedure (SAS Institute, 1996) was used to estimate No and *k*. The time required to hydrolyze 50% of the *No* was calculated as:

$$t_{1/2} = \frac{ln2}{k} \quad [6]$$

Statistical analysis

The models were assessed with respect to precision of parameter estimates and fit to the experimental data as indicated by the residual mean squares. The statistical significance of the differences in residual between any two models was assessed by an F-test. The general linear model procedure in SAS (SAS Institute, 1996) was used for the analysis of variance, and means separation of the parameters was done by the least significant difference method. Simple linear regression analysis was used to quantify the relationships between model parameters and the amounts of N mineralized.

Results and discussion

Chemical and biological indices of N mineralization

The amounts of N mineralized by the various mineralization indices are shown in Table 2. Generally, the percentages of organic N mineralized were very small (< 1%; Tables 1 and 2), with the amount of N mineralized by the chemical and biological indices evaluated being positively but insignificantly correlated with the organic C and N contents of the soils (data not shown). This finding contradicts the reported close correlation between N mineralization and total N and organic C of soils from Saskatchewan and Iowa (Simard and N'dayegamiye, 1993; Dodor and Tabatabai, 2007), but agrees with the results of Groot and Houba (1995) who reported a poor correlation between N mineralization

TABLE 2
Amounts of nitrogen mineralized by the chemical and biological methods

Soil	Chemical method[a]			Biological method		
	PBN	SBN	HKN	Aerobic	Anaerobic	
					moist soil	dry soil
	mg kg^{-1} soil					
Canisteo	36.2	7.4	30.6	13.8	6.4	31.2
Clarion (I)	34.8	8.2	19.2	13.0	6.7	54.4
Coland	16.7	6.9	29.4	6.1	18.7	91.0
Crippin	23.7	5.2	22.2	4.7	3.4	37.2
Harps	16.8	7.4	25.3	2.9	3.5	47.4
Nicollet	30.2	8.2	25.3	20.8	6.1	44.6
Okoboji	40.2	10.6	29.8	15.6	9.7	49.3
Storden	21.4	6.4	23.7	26.1	9.5	48.9
Terrill	28.9	5.7	21.2	5.2	7.7	63.0
Webster (I)	24.7	3.4	24.9	16.7	10.7	79.0
Clarion (II)	37.9	10.3	38.8	34.2	12.8	80.1
Grundy	33.8	2.8	23.1	25.4	2.0	19.9
Webster (II)	46.2	10.8	44.9	31.1	16.3	85.2

[a]PBN = phosphate-borate N; SBN = sodium-borate N; HKN = hot KCl N.

rates and soils organic matter and N contents. Among the chemical indices, PBB hydrolyzed the greatest amount of N, followed by hot-KCl. This trend reflects the degree of strength of the reagents in hydrolyzing the $–NH_2$ bonds, with PBB hydrolyzing more organic N due to the large difference between the initial pH of the soils (pH ≤ 8.0, Table 1) and that of the buffer (pH = 11.2).

The amounts of N mineralized under anaerobic condition when air-dried soils were used were at least six times greater than those mineralized in field-moist soils (Table 2). Air drying has been shown to increase the amount of mineral N produced compared with field-moist soils and this increase was positively correlated with the length of time the air-dried soil sample was stored prior to incubation (Keeney and Bremner, 1967; Stanford and Legg, 1968; Cabrera, 1993). The increase in N mineralization in the air-dried soils can be attributed to the rapid change in soil water potential associated with rewetting, causing microbes to undergo osmotic shock, and thus induce microbial cell lysis (Van Gestel et al., 1992) or release of intracellular solutes (Halverson et al., 2000). The remaining microbes mineralize these labile N substrates, yielding a pulse of N. Alternatively, drying and rewetting may cause disruption of soil aggregates, releasing hitherto protected and unavailable soluble substrates from microbial biomass for rapid mineralization by the microbial community (Kieft et al., 1987; Lundquist et al., 1999; Dodor et al., 2018; Dodor et al., 2019).

Contrary to our previous results with 51 surface soils from the North Central Region of the United States (Dodor and Tabatabai, 2007), generally, greater amounts of N were mineralized under anaerobic incubation conditions when air-dried soils were used compared to that obtained with the selected chemical indices (Table 2). This difference in the pattern of N mineralization is probably due to the differences in the nature and quantity of organic matter present, as well as soil texture, climate and possibly management history. The soils used in the present study had greater organic C contents and were sampled from uncultivated fields, whereas those used by Dodor and Tabatabai (2007) were from cultivated fields with low organic C contents. The results of these two studies suggest that there is variation in the pattern of the impact of air-drying and rewetting cycle on N mineralization in soils.

Evaluation of chemical indices of N availability are normally based on the relative degree of correlation with biological methods. The chemical indices of N mineralization evaluated were positively and significantly auto-correlated ($r \geq 0.56$; $P \leq 0.05$; Table 3), with HKN being significantly correlated with both PBN and SBN ($r = 0.56$ and 0.66, respectively; Table 3). The correlation analysis indicated that HKN was also positively and significantly correlated with the three biological indices of N mineralization evaluated in the present study ($r \geq 0.56$; $P \leq 0.05$; Table 3), as well as the initial inorganic N present in the soils ($r = 0.70$; $P \leq 0.01$; Table 3). The positive and significant correlation between HKN and the biological indices of N mineralization suggests that it (HKN) can be used to predict N availability in soils. Working with 51 soils from six different agroecological zones of the North Central Region of the USA, Dodor and Tabatabai (2007) also reported that HKN was the best predictor of N mineralization in soils compared with PBN or SBN. Similarly, other workers have also concluded that HKN

TABLE 3
Pearson correlation coefficients (r) indicating association between chemical and biological methods of N mineralization

Nitrogen mineralization index	AAM	AAD	AB	HKN	PBN	SBN	IN
Anaerobic - moist soil (AAM)	1.000						
Anaerobic - dry soil (AAD)	0.890	1.000					
Aerobic (AB)	0.280	0.143	1.000				
Hot KCl (HKN)	0.649	0.557	0.585	1.000			
Phosphate-borate N (PBN)	0.126	0.003	0.606	0.561	1.000		
Sodium-borate N (SBN)	0.448	0.346	0.310	0.664	0.560	1.000	
Initial $NH4^+$-N (IN)	0.348	0.271	0.653	0.697	0.667	0.644	1.000

r-values greater than 0.55, 0.68, or 0.80 are significant at 0.5%, 0.1% or 0.01%, respectively.

was superior in predicting N uptake by plants compared to PBN method (Jalil et al., 1996; Curtin et al., 1998).

Patterns of organic N hydrolysis

The patterns of cumulative mean N hydrolyzed by the four alkaline reagents with time of distillation in four of the soils used are shown in Figure 1. The shapes, trends and patterns for the other soils are similar and fall between those shown. The graphs show that there was an initial high rate of organic N hydrolysis, which declined to a low constant rate after 20 min of steam distillation. The standard deviation of the method ranged between 0.2 and 2.5, indicating the proposed method is reproducible and can be standardized readily. Generally, the total amount of N hydrolyzed

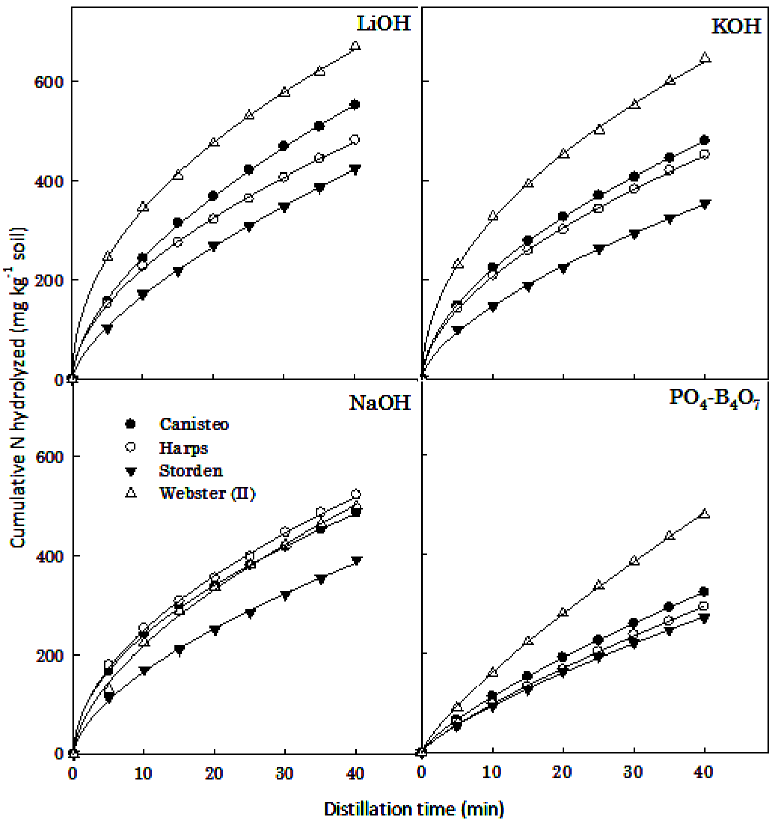

Fig. 1 Relationship between cumulative amount of N hydrolyzed and distillation time for four of the soils used

from the soils using NaOH, KOH or LiOH exceeded those obtained with PBB (Table 4). This order of magnitude of the amounts of NH_4^+-N released followed the same trend in all the soils, with LiOH hydrolyzing slightly greater amounts of N compared with NaOH and KOH. The total amounts of N hydrolyzed during the 40 min of steam distillation, expressed as a percentage of organic N in the soils, are also presented in Table 4. Analysis of variance indicated that the percentages of organic N hydrolyzed by the four alkaline reagents differed significantly among soils. This finding suggests differences in the chemical reactivity of organic N in the soils and contradicts Stanford and DeMar (1969) who found no difference in the nature of the organic matter extractable by 0.01 M $CaCl_2$ at 121 °C for 16 h.

Kinetics of organic N hydrolysis

The three transformations of the Michaelis-Menten equation applied to the cumulative amounts of organic N hydrolyzed by KOH with distillation time is shown in Figure 2 for four of the soils used. The plot for the other three reagents and soils showed similar fit to the three transformations of the Michaelis-Menten equation. The results indicated that the cumulative amounts of N hydrolyzed were adequately described by the linear transformations of the data, with *r*-values ranging between 0.96 and 1.0. The Lineweaver-Burk transformation, however, gave the best plot with the least residual mean square values (Fig. 2). Because linearization of the Michaelis-Menten equation tends to bias the regression analysis and subsequent estimated parameters, comparison of parameters obtained from the various transformations could be misleading. Work in enzyme chemistry has shown that the double reciprocal plots produce straight line regressions, but the parameter estimates may differ from the other transformations of the hyperbolic equation (Segel, 1975). This is because each transformation gives different weight to the error in the variables (Dowd and Rigg, 1965), which normally results in

TABLE 4
Total amount of NH_4^+-N hydrolyzed by the alkaline reagents as a percentage of total organic N in the soils

Soils	LiOH		KOH		NaOH		PO_4-B_4O_7	
	NH_4^+-N	% TN[a]	NH_4^+-N	% TN[a]	NH_4^+-N	% TN[a]	NH_4^+-N	% TN[a]
	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	
Canisteo	553	14.6	480	12.7	486	12.8	324	8.6
Clarion (I)	489	13.2	447	12.1	510	13.8	364	9.9
Coland	543	15.1	477	13.3	566	15.8	379	10.6
Crippin	461	15.9	407	14.1	443	15.3	336	11.6
Harps	482	11.2	452	10.5	522	12.2	295	6.9
Nicollet	470	14.8	403	12.7	443	13.9	320	10.1
Okoboji	559	13.3	441	10.5	509	12.1	305	7.3
Storden	424	19.5	355	16.3	392	18.0	273	12.5
Terrill	462	17.2	356	13.3	473	17.6	318	11.8
Webster (I)	543	15.6	452	13.0	502	14.4	337	9.7
Clarion (II)	686	23.0	603	20.2	611	20.5	457	15.3
Grundy	508	27.2	458	24.5	449	24.0	414	22.1
Webster (II)	670	28.0	646	27.0	500	20.9	480	20.1

[a] Percent of total organic N hydrolyzed

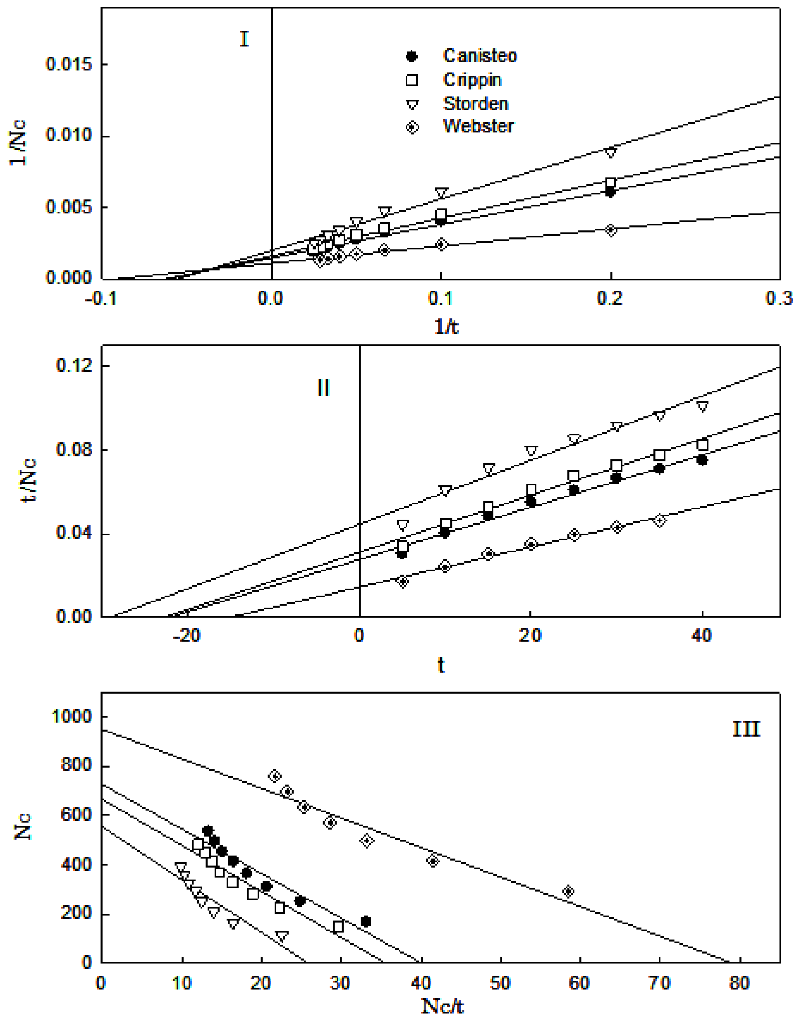

Fig. 2 Lineweaver-Burk (I), Hanes-Woolf (II), and Eadie-Hofstee (III) plots of the Michaelis-Menten equation for cumulative N hydrolyzed with time using KOH. t = distillation time (min), Nc = cumulative N hydrolyzed

variation in the estimated constants.

The calculated kinetic parameters of hydrolysis of soil organic N by the four reagents used are presented in Tables 5 – 8 for PBB, KOH, NaOH, and LiOH, respectively. Regression analysis showed that the N_{max} values calculated by using the three linear transformations of the Michaelis-Menten equation were similar and significantly correlated with each other across all soils, except those of the Lineweaver-Burk plot for PBB, which showed very few nonsignificant *r*-values (data not shown). When two reagents are hydrolyzing organic N from the same pool, it is expected that the actual amounts hydrolyzed will be very strongly correlated, but the gradient of the relationship needs not necessarily be unity. Thus, the autocorrelation among the N_{max} values observed in the present study suggests that the reagents might be hydrolyzing N from the same pool.

The concept of potentially mineralizable N (N_o) and its associated rate constant (*k*) describing the course of long-term (30 weeks) aerobic incubation introduced by Stanford and Smith (1972) has been the most widely used model in N mineralization studies. For comparison, the N_o values calculated for the chemical hydrolysis by the reagents used are also presented in Tables 5 – 8. The results indicate that the N_o and N_{max} values followed similar trend, with the two estimates being statistically similar across all soils. Determination of the most suitable mathematical equation and most appropriate method for calculating the values of the parameters in the equation describing the net N mineralization in soils showed that *No* and K_t determined by nonlinear least square yielded the best fit to the data for labeled soil and had the lowest root mean square error (Juma et al., 1984). These authors also indicated that linear regression of $1/N_c$ on $1/t$ yielded N_{max} and K_t values which were markedly different from those obtained with the N_c vs. N_c/t and t/N_c vs. t transformations. Considering the reagents individually, analysis of variance indicated that estimated N_{max} and N_o values differed significantly among soils (Tables 5 – 8), suggesting differences in the chemical reactivity of soil organic N that was hydrolyzed by the reagents. This finding

TABLE 5
Calculated kinetic parameters for phosphate-borate buffer hydrolysis

Soil	Lineweaver-Burk[a]			Hanes-Wolf[a]			Eadie-Hofstee[a]			First Order[a]			
	N_{max}	%TN	K_t	N_{max}	%TN	K_t	N_{max}	%TN	K_t	N_o	%TN	k	$t_{1/2}$
Canisteo	588	16	40	769	20	57	674	18	48	534	14	0.0230	30
Clarion (I)	909	25	62	909	25	64	912	25	63	616	17	0.0219	32
Coland	833	23	51	909	25	59	868	24	55	640	18	0.0229	30
Crippin	1250	43	110	1250	43	111	1260	44	111	817	28	0.0135	51
Harps	526	12	40	714	17	61	608	14	48	551	13	0.0190	37
Nicollet	714	22	53	769	24	58	762	24	57	498	16	0.0252	28
Okoboji	1250	30	124	1250	30	126	1275	30	128	777	19	0.0128	54
Storden	556	26	47	667	31	60	623	29	55	456	21	0.0227	31
Terrill	625	23	44	714	27	54	677	25	49	505	19	0.0240	29
Webster (I)	625	18	39	714	20	48	690	20	45	504	14	0.0272	25
Clarion (II)	1667	56	108	1667	56	109	1693	57	110	1101	37	0.0142	49
Grundy	769	41	42	1000	53	60	896	48	51	715	38	0.0212	33
Webster (II)	1000	42	50	1250	52	67	1184	50	62	861	36	0.0202	34

[a]N_{max} and N_o are expressed in mg NH_4 kg^{-1} soil 40 min^{-1}; K_t, $t_{1/2}$ in min; k in min^{-1}; %TN is percentage of total organic N

TABLE 6
Calculated kinetic parameters for KOH hydrolysis

Soil	Lineweaver-Burk[a]			Hanes-Wolf[a]			Eadie-Hofstee[a]			First Order[a]			
	N_{max}	%TN	K_t	N_{max}	%TN	K_t	N_{max}	%TN	K_t	N_o	%TN	k	$t_{1/2}$
Canisteo	667	18	16	769	20	21	725	19	18	532	14	0.0511	14
Clarion (I)	588	16	14	714	19	21	640	17	16	484	13	0.0531	13
Coland	714	20	14	769	21	18	734	20	15	501	14	0.0611	11
Crippin	625	22	17	714	25	22	665	23	19	459	16	0.0491	14
Harps	667	16	15	769	18	21	701	16	17	504	12	0.0497	14
Nicollet	526	17	14	667	21	21	573	18	16	439	14	0.0520	13
Okoboji	625	15	17	714	17	22	682	16	19	490	12	0.0510	14
Storden	500	23	18	667	31	30	553	25	21	438	20	0.0386	18
Terrill	500	19	18	588	22	22	527	20	18	401	15	0.0489	14
Webster (I)	588	17	13	714	20	19	641	18	15	482	14	0.0572	12
Clarion (II)	1000	34	14	1000	34	16	957	32	14	622	21	0.0660	11
Grundy	714	38	15	833	45	22	749	40	17	514	27	0.0483	14
Webster (II)	909	38	11	1111	46	17	996	42	13	670	28	0.0625	11

[a]N_{max} and N_o are expressed in mg NH_4^+-N kg^{-1} soil 40 min^{-1}; K_t, $t_{1/2}$ in min; k in min^{-1}; %TN is percentage of total organic N

TABLE 7
Calculated kinetic parameters for NaOH hydrolysis

Soil	Lineweaver-Burk[a]			Hanes-Wolf[a]			Eadie-Hofstee[a]			First Order[a]			
	N_{max}	%TN	K_t	N_{max}	%TN	K_t	N_{max}	%TN	K_t	N_o	%TN	k	$t_{1/2}$
Canisteo	667	18	13	769	20	18	685	18	14	511	13	0.0601	12
Clarion (I)	667	18	12	769	21	17	720	19	14	536	15	0.0617	11
Coland	769	21	11	909	25	17	811	23	12	586	16	0.0638	11
Crippin	667	23	15	769	27	21	700	24	17	486	17	0.0538	13
Harps	714	17	12	909	21	20	768	18	14	564	13	0.0556	12
Nicollet	588	18	13	714	22	19	630	20	15	467	15	0.0591	12
Okoboji	667	16	12	769	18	17	696	17	13	532	13	0.0641	11
Storden	558	26	17	667	31	26	591	27	20	464	21	0.0416	17
Terrill	625	23	14	769	29	22	676	25	16	526	20	0.0493	14
Webster (I)	667	19	12	769	22	17	683	20	13	521	15	0.0624	11
Clarion (II)	833	28	9	1000	34	13	887	30	10	607	20	0.0780	9
Grundy	667	36	12	769	41	17	682	36	13	464	25	0.0634	11
Webster (II)	1000	42	25	1000	42	26	989	41	25	605	25	0.0748	9

[a]N_{max} and N_o are expressed in mg NH_4^+-N kg^{-1} soil 40 min^{-1}; K_t, $t_{1/2}$ in min; k in min^{-1}; %TN is percentage of total organic N

TABLE 8
Calculated kinetic parameters for LiOH hydrolysis

Soil	Lineweaver-Burk[a]			Hanes-Wolf[a]			Eadie-Hofstee[a]			First Order[a]			
	N_{max}	%TN	K_t	N_{max}	%TN	K_t	N_{max}	%TN	K_t	N_o	%TN	k	$t_{1/2}$
Canisteo	833	22	19	1000	26	26	900	24	22	643	17	0.0460	15
Clarion (I)	625	17	13	769	21	21	687	19	16	538	15	0.0506	14
Coland	769	21	12	909	25	18	794	22	14	573	16	0.0589	12
Crippin	714	25	17	833	29	23	740	26	18	517	18	0.0492	14
Harps	714	17	15	833	19	22	745	17	17	535	12	0.0502	14
Nicollet	625	20	14	769	24	22	680	21	17	529	17	0.0482	14
Okoboji	909	22	23	1111	27	32	699	17	26	697	17	0.0381	18
Storden	714	33	26	833	38	34	780	36	30	554	25	0.0343	20
Terrill	714	27	22	909	34	32	787	29	26	570	21	0.0365	19
Webster (I)	769	22	18	909	26	24	848	24	21	631	18	0.0451	15
Clarion (II)	1000	34	11	1111	37	16	1037	35	12	709	24	0.0644	11
Grundy	833	45	19	1000	53	27	920	49	23	606	32	0.0418	17
Webster (II)	1000	42	11	1111	46	16	1022	43	12	686	29	0.0654	11

[a]N_{max} and N_o are expressed in mg NH_4^+-N kg^{-1} soil 40 min^{-1}; K_t, $t_{1/2}$ in min; k in min^{-1}; %TN is percentage of total organic N

is significant because studies to compare the distribution of organic N in different soils or among soils under different management practices, have generally indicated that, regardless of soil type, cropping or cultivation, there was little variation in the distribution of hydrolyzable soil organic N (Stevenson, 1957; Meints and Peterson, 1977).

The estimated N_{max} and N_o values as percentages of the total organic N in the soils ranged from 12 to 57%, with majority of the values below 25% (Tables 5 – 8). These values are lower than those reported by Stanford (1968), but greater than the amounts extracted with dilute acidic permanganate solution (0.1 M $KMnO_4$ in 0.1 or 0.5 M H_2SO_4) (Juma et al., 1984). The calculated time required to hydrolyze 50% of the N_{max}, K_t using the three linear transformation procedures as well as that for N_o, $t_{½}$ obtained from the non-linear regression showed that PBB required the longest time, with values for the other three reagents being generally similar (Tables 5 – 8). The K_t and $t_{½}$ values for PBB ranged between 39 – 128 and 28 - 54 min, respectively, whereas those for KOH, NaOH, and LiOH were all below 35 min. This trend in K_t and $t_{½}$ values are apparently due to the strength of the reagents, with PBB being the weakest and so showed a comparatively slow kinetic rate. As expected, the K_t and $t_{½}$ values were positively and significantly correlated with N_{max} and N_o, respectively, suggesting that larger organic N pools require longer time for mineralization. Applying the same mathematical principles above, Juma et al. (1984) calculated K_t values for 51 Saskatchewan soils to be between 7.3 and 45.8 weeks, while Stanford and Smith (1972) found the time required to mineralize one-half of N_o, $t_{1/2}$ in 31 soils to be 12.8 ± 2.2 weeks.

Kinetic parameters of organic N hydrolysis and N mineralization indices

Although estimated N_{max} and N_o values from the models were positively correlated with the amounts of N mineralized with the biological indices, most of the *r*-values were not significant (data not shown). However, among the chemical indices of N availability evaluated, HKN was significantly correlated with all N_{max} and N_o values (Fig. 3). Because the HKN has been thoroughly evaluated and shown to be a reliable index of N availability to plants (Selmer-Olsen et al., 1981; Jalil et al., 1996; Curtin et al., 1998), the results suggest that the alkaline hydrolysis procedure presented herein can be used as a chemical

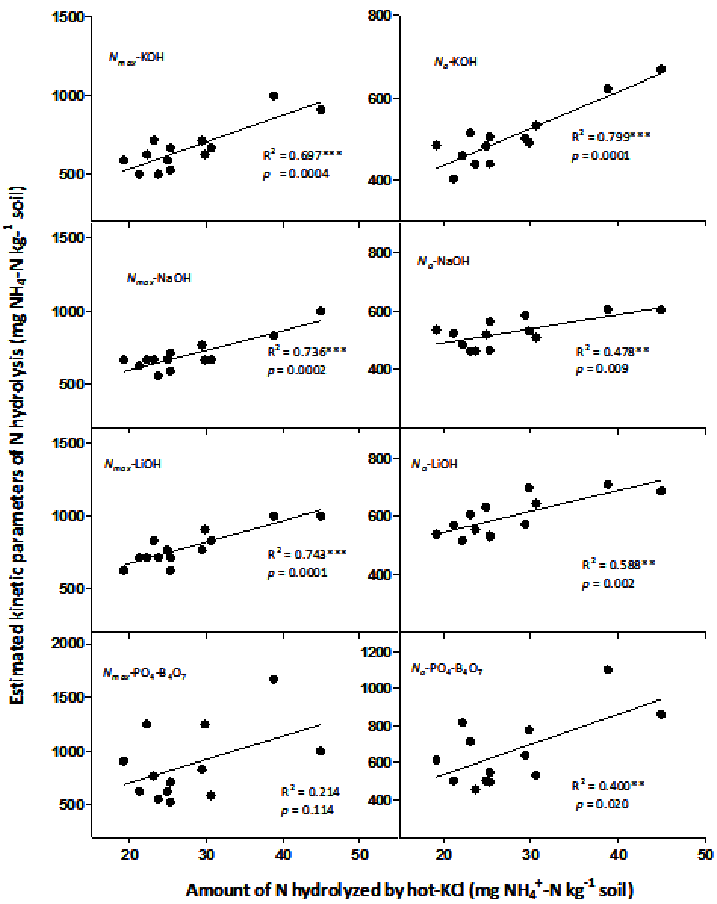

Fig. 3 Relationship between kinetic parameters of alkaline hydrolysis (N_{max} and N_a) and the amount of N hydrolyzed by hot-KCL

index of N mineralization in soils.

Among the four reagents used, the N_{max} and N_o values calculated from hydrolysis with KOH or NaOH, regardless of the transformation, were superiorly correlated with the amount of N hydrolyzed with hot-KCl, and the least correlations were with those obtained with PBB (Fig. 3; Table 3). This suggests that either 1 M KOH or NaOH can be used to evaluate the chemical index of N mineralization in soils. However, a critical evaluation of the data indicated that N_{max} and N_o values estimated from organic N hydrolysis with 1 M KOH were also positively and significantly correlated with the initial amount of NH_4^+-N present in the soils ($r \geq 0.73^{**}$; $P < 0.01$), suggesting that KOH is the best reagent for evaluating N availability index by chemical hydrolysis methods described in this paper.

It is generally accepted that the amount of NH_4^+-N produced from mineralization of organic N determines the fertility status of a soil in terms of supplying inorganic N for plant uptake. As noted above, HKN was positively and significantly correlated with the biological indices of N mineralization. Therefore, the positive and significant correlation between KOH hydrolyzable N and HKN, coupled with the relationships with initial mineral N present in the soils, gives an additional strong credence to our assertion that KOH hydrolysis can be used as a reliable chemical index of N mineralization in soils.

Mechanism of organic N hydrolysis

Evidently, the removal of soil organic N by successive alkaline hydrolysis represents a gradual and somewhat selective dissolution of N forms susceptible to mineralization. In the absence of specific knowledge of the nature of the soil organic substances giving rise to the hydrolyzable N by the reagents, however, little can be said regarding the mechanisms involved in the hydrolysis and the chemical alterations. Nevertheless, the source of distillable N and other forms of N hydrolyzed by the reagents can be surmised from other historical studies oriented towards making such identifications, i.e., hydrolysis of -NH_2 functional groups in soil organic matter.

Organic matter hydrolysis showed that nitrogenous constituents of microbial origin including previously living organisms disrupted by heating and the high pH, and products of microbial synthesis not yet fully incorporated into the fraction of difficulty decomposable organic matter could be the probable source (Bremner, 1965, Sowden, 1958; Sowden, 1968). It is also probable that partial destruction of certain amino acids during alkaline hydrolysis involving strong bases, such as NaOH and KOH, may give rise to hydrolyzable N. Studies by Juma et al. (1984) showed that acid hydrolysis extracted 72% of the total N, and amino acid accounted for 32% of the N, whereas NH_4^+-N released on hydrolysis accounted for 20%.

Conclusions

This study investigated an alkaline hydrolysis method for determining the total N potentially hydrolyzable in soils. It involves the determination of the NH_4^+-N produced by direct steam distillation of 1 g field-moist soil and 1M KOH, NaOH, LiOH or with phosphate-borate buffer (pH 11.8) successively every 5 min for 40 min. Calculated total hydrolyzable N (N_{max} or N_o) values differed among soils, ranging from 401 to 1667 mg kg^{-1} soil and accounted for 12-56% of organic N in the soils.

The N_{max} or N_o values obtained with KOH and NaOH were significantly correlated with the values obtained by using selected biological and chemical indices of N mineralization in soils. The method offers a quick and precise means to assess the potentially mineralizable organic N pool and availability in soils.

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