



Article

Evaluation of Alkaline Hydrolyzable Organic Nitrogen as an Index of Nitrogen Mineralization Potential of Some Coastal Savannah Soils of Ghana

Daniel E. Dodor ^{1,*} , Mohamed S. Kamara ^{1,2}, Abena Asamoah-Bediako ¹, Samuel G. K. Adiku ¹, Dilys S. MacCarthy ³ , Samuel K. Kumahor ¹ and Dora Neina ¹

¹ Department of Soil Science, University of Ghana, Legon, Accra GA-489-9979, Ghana

² Sierra Leone Agricultural Research Institute (SLARI), Freetown P.M.B 1313, Sierra Leone

³ Soil and Irrigation Research Centre, University of Ghana, Kpong EL-0633-5197, Ghana

* Correspondence: dedodor@ug.edu.gh; Tel.: +233-50-676-5852

Abstract: Numerous biological and chemical methods have been proposed over the years for estimating the nitrogen (N) mineralization capacity of soils; however, none of them has found general use in soil fertility testing. The efficacy of a recently proposed alkaline hydrolysis method for assessing N availability in soils compared with the standard long-term incubation technique for determining potentially available N was evaluated. The nitrogen mineralization of 12 surface soils incubated under aerobic conditions at 25 °C for 26 weeks was determined. Field-moist soils were direct-steam distilled with 1 M KOH or 1 M NaOH; the NH₃ released was trapped in boric acid, and its concentration was determined successively every 5 min for 40 min. The cumulative N mineralized or hydrolyzed was fitted to the first-order exponential equation to determine the potentially mineralizable N (N_0) and an analogous “potentially hydrolyzable N (N_{max})” for the soils. The flush of CO₂ (fCO_2) following the rewetting and incubation of air-dried soils under aerobic conditions for 3 days was also determined. The results showed that the N_{max} values differed considerably among the soils, indicating differences in the chemical nature and reactivity of the organic N content of the soils, and were significantly correlated with N_0 and fCO_2 values. The estimated N_{max} and N_0 values ranged from 105 to 371 mg N kg⁻¹ and 121 to 292 mg kg⁻¹, respectively. Based on the simple and inexpensive nature of the alkaline hydrolysis procedure, the reduction in the incubation time required to obtain N_0 (months to minutes), and the strong association between N_{max} and N_0 , we concluded that N_{max} is a good predictor of the biologically discrete and quantifiable labile pool of mineralizable soil organic N (ON), and the use of the alkaline hydrolyzable ON as a predictor of N_0 merits consideration for routine use in soil testing laboratories for estimating the N-supplying capacity of soils.

Keywords: nitrogen availability index; chemical index of N availability; alkaline hydrolyzable N; potentially hydrolyzable N; hydrolysis of organic N



Citation: Dodor, D.E.; Kamara, M.S.; Asamoah-Bediako, A.; Adiku, S.G.K.; MacCarthy, D.S.; Kumahor, S.K.; Neina, D. Evaluation of Alkaline Hydrolyzable Organic Nitrogen as an Index of Nitrogen Mineralization Potential of Some Coastal Savannah Soils of Ghana. *Nitrogen* **2022**, *3*, 652–662. <https://doi.org/10.3390/nitrogen3040043>

Academic Editor:
Jacynthe Dessureault-Rompré

Received: 20 October 2022
Accepted: 5 December 2022
Published: 16 December 2022

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Numerous biological and chemical methods have been proposed over the years for estimating the nitrogen (N) mineralization potential of soils [1]; however, none of these methods have been widely adopted in general soil fertility testing. The biologically based, long-term (>26 weeks) aerobic incubation procedure has been and continues to be the most widely used method for determining the potentially mineralizable N (N_0) and availability in soils [2]. Due to the impracticability of the long experimental duration, other short-term biologically based procedures, ranging from 7 to 28 days, have also been proposed [3–5]. Indeed, a satisfactory and highly predictive relationship between the amount of N mineralized using the standard long-term aerobic incubation technique and that observed with the short-term methods has been reported [6,7]. Recently, the flush of CO₂ (fCO_2) following the rewetting and incubation of air-dried soils at 25 °C for 3 days has been proposed as an

index of N_o , and significant correlation between fCO_2 and the net amount of N mineralized in a 24-day incubation at 25 °C has been reported [8].

Chemical extraction methods have been proposed as a rapid approach of estimating N mineralization in soils. These chemical methods include extraction of mineral N with hot water [9–11] or hot KCl [12], hydrolysis of organic N with strong acids (e.g., 1 M HCl [13]) or alkaline reagents (1 M NaOH [14]; 0.01 mM NaHCO₃ [11]), and direct-steam distillation of soils with pH 11.2 phosphate–borate buffer [15]. Although prone to some disadvantages [1], the chemical methods are proven to be fast and precise. Indeed, significant correlation between the amount of N extracted and/or hydrolyzed by the chemical methods and the N_o determined through the long-term biological incubation procedures has been reported [6,15–17].

Recently, a simple, rapid, and precise alkaline hydrolysis method for determining the chemical index of N_o of soils was proposed [18]. The method involves direct-steam distillation of field-moist soils with alkaline reagents (1 M KOH or NaOH); the NH₃ released is trapped in boric acid, and its concentration is determined successively every 5 min for a total of 40 min. Akin to the first-order reaction kinetics introduced by Stanford and Smith [2] to describe N mineralization in soils, Dodor and Tabatabai [18] used a non-linear exponential model to describe the kinetics of N hydrolysis with time and proposed the concept of “potentially hydrolyzable N (N_{max})” as a discrete and quantifiable labile N pool with a corresponding first-order hydrolysis rate constant (k_h) for soils.

The validity of the chemical indices of N mineralization potential is normally based on the degree of association with biological methods that simulate the microbial activity responsible for the mineralization of organic nitrogen (ON) in soils [1]. Therefore, an assessment of the efficacy of the proposed alkaline hydrolysis method compared to the long-term aerobic incubation, considered the most accurate predictor of the N-supplying capacity of soils, is imperative. This information could shed more light on the nature, pool size, and contribution of the hydrolyzable ON to the N mineralization potential of soils. In addition, the lability of soil organic carbon (SOC), as measured by fCO_2 in short-term incubation, has been shown to be a good proxy for the labile pool of N_o in soils [8]. However, the fCO_2 method has not been assessed against the alkaline hydrolysis method proposed by Dodor and Tabatabai [18]. Because the fCO_2 method is simple and rapid, it is of research interest to employ it as a surrogate procedure to validate new chemical tests of N availability in soils.

In the present study, we postulate that N_{max} is a discrete and definable portion of the labile and active N pool in soils, and that the alkaline hydrolysis technique could provide a reliable estimate of the N mineralization potential of soils. Therefore, the objective of this study was to assess the suitability of alkaline hydrolyzable ON as a chemical index of the mineralizable N pool in soils using the long-term incubation and the 3-day fCO_2 as benchmarks.

2. Materials and Methods

2.1. Soils and Characterization

This investigation was carried out using 12 soils sampled from fields with different management histories located at the University of Ghana Farms (latitude 5°66' N and longitude 00°19' E), Accra, Ghana. The area experiences a bi-modal rainfall pattern with average annual rainfall of approximately 1000 mm and a mean annual temperature of 27 °C. Nyigbenya and Toje series are classified according to the FAO-WRB criteria as Lixic Vetic Nitisol, while Adentan and Haatso series are classified as Haplic Lixisol and Endogleyic Lixisol, respectively [19]. The corresponding classifications based on the Soil Taxonomy criteria are Rhodic Kandustalf, Typic Kandustalf, and Kandic Haplustalf, respectively [19] (Table 1). On each field, an area of approximately 5 × 5 m was delineated, and six surface soil (0–20 cm) samples were randomly taken, passed through a 2.0-mm sieve, placed in polyethene bags, and transported to the laboratory. The soil samples were divided into two portions; one portion was air-dried at room temperature and used for the determination of physical and chemical properties, whilst the other portion was

refrigerated at 4 °C. A subsample of the refrigerated portion was used for the incubation and alkaline hydrolysis experiments.

Table 1. Physical and chemical properties of the soils used.

ID	Soil Series	Sand (g kg ⁻¹)	Silt (g kg ⁻¹)	Clay (g kg ⁻¹)	pH	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	Org-N (g kg ⁻¹)	fCO ₂ ^a (mg kg ⁻¹)
1	Nyigbenya	600	50	350	6.2	10.5	1.9	25.9	25.9	1.8	64
2	Nyigbenya	670	55	275	5.7	10.8	1.8	28.0	31.5	1.7	103
3	Nyigbenya	825	25	150	6.9	15.9	5.1	35.0	37.1	5.0	102
4	Toje	550	50	400	6.3	9.6	1.5	34.3	37.1	1.4	53
5	Toje	575	75	350	5.2	11.0	2.4	31.5	39.2	2.3	108
6	Toje	550	75	375	4.8	11.4	3.1	42.7	46.2	3.0	128
7	Adentan	650	75	275	5.6	5.7	1.7	20.1	24.5	1.7	45
8	Adentan	550	75	375	5.5	8.2	1.4	25.9	51.1	1.3	110
9	Adentan	725	25	250	5.0	9.5	3.5	22.4	33.6	3.4	131
10	Haatso	700	75	225	6.0	5.7	2.3	15.9	17.0	2.3	83
11	Haatso	755	75	175	6.3	6.6	2.4	16.8	23.1	2.4	119
12	Haatso	750	75	175	6.4	6.9	2.8	26.6	31.5	2.7	120

^a fCO₂ is the flush of CO₂ following rewetting and incubation of air-dried soils.

In the analyses reported in Table 1, pH was measured in a soil to water ratio of 1:1, bulk density was determined by the core sampler method [20], and particle size distribution was determined by the Bouyoucos hydrometer method [21]. The mineral N content of the soils was extracted with 2 M KCl, and a 20-mL aliquot of the extract was used for the determination of NH₄⁺-N and NO₃⁻-N by steam distillation [22]. Carbonates were removed from the soil before organic C determination by treating the soils with 2 M HCl and drying at 60 °C before the determination of the total C (TC) and N (TN) on <180-µm samples by dry combustion (LECO CNS Analyzer, LECO Corp., St. Joseph, MI, USA). The organic N (ON) content of the soils was determined by the differences between the TN and mineral N (sum of NH₄⁺-N and NO₃⁻-N). All laboratory analyses were conducted in triplicates.

2.2. Flush of CO₂ after Rewetting of Dried Soils

The procedure described by Franzluebbers et al. [8] was used to determine the fCO₂ when air-dried soils were rewetted and incubated under aerobic conditions for three days. Briefly, 100 g of air-dried soils was weighed into jars, moistened to 60% water holding capacity (WHC), sealed tightly, and incubated in the dark at 25 °C for three days. The amount of CO₂ released was adsorbed in a vial containing 30 mL of 1 M NaOH placed inside the jar. The concentration of CO₂ was determined by titration of the excess NaOH with 0.5 M HCl after precipitation of carbonates by 20 mL of 2 M BaCl₂ and addition of three drops of phenolphthalein indicator. Blank setups without soil were used to correct for background CO₂ concentration [23].

2.3. Long-Term Aerobic N Mineralization

The procedure described by Stanford and Smith [2] was used for the long-term N mineralization under aerobic conditions. Duplicate samples of a mixture of 75 g field-moist soil (on oven-dry basis) and 75 g acid-washed sand were placed in leaching tubes lined with nonabsorbent cotton wool at the bottom to avoid soil loss. The soils were incubated in a constant-temperature room (25 ± 1 °C) in the dark for 26 weeks. The samples were leached with 90 mL of 0.05 M CaCl₂ and 10 mL of N-free solution (0.002 M CaSO₄·2H₂O, 0.005 M Ca(H₂PO₄), 0.0025 M K₂SO₄, and 0.002 M MgSO₄) at 2, 4, 6, 8, 10, 12, 14, 18, 22, and 26 weeks after the commencement of incubation, and the NH₄⁺-N and NO₃⁻-N contents of the leachates were determined by steam distillation [22]. The moisture content of the soil was adjusted to 60% WHC every week. The NH₄⁺-N and NO₃⁻-N for each leaching date were summed to calculate the cumulative N mineralized.

2.4. Alkaline Hydrolysis of Organic N

The procedure described by Dodor and Tabatabai [18] was used for the alkaline hydrolysis of organic N. Briefly, 1 g of field-moist soil (on oven-dried basis) was placed in a distillation flask, and 20 mL of 1 M NaOH or KOH was added. The sample was direct-steam distilled, and the NH₃ released was captured in 5 mL of 5% boric acid, which was changed successively every 5 min for a total of 40 min. The amount of NH₃ in the distillate was determined by titrating with a 0.005 M H₂SO₄ standard solution [22]. The initial NH₄⁺-N present in the soils was subtracted from the results of the 5 min distillation.

All results reported for the alkaline hydrolysis were averages of triplicate analyses with the initial NH₄⁺-N present in the soils subtracted. The initial NH₄⁺-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 [24]. The moisture content of the soils was determined from the weight loss after drying at 105 °C for 48 h.

2.5. Modeling of N Mineralization and Hydrolysis

The cumulative N mineralized in the long-term aerobic incubation condition was fitted to the first-order kinetic equation [2]:

$$N_{min} = N_o[1 - \exp(-k_m t)] \quad (1)$$

where N_{min} (mg N kg⁻¹) is the cumulative amount of N mineralized at time, t (week); N_o (mg N kg⁻¹) is the potentially mineralizable N; and k_m (week⁻¹) is the first-order mineralization rate constant. Similarly, the cumulative amount of N hydrolyzed by the alkaline reagents was fitted to the nonlinear first-order model proposed by Dodor and Tabatabai [18]:

$$N_{hyd} = N_{max}[1 - \exp(-k_h t)] \quad (2)$$

where N_{hyd} (mg N kg⁻¹) is the cumulative amounts of N hydrolyzed in time, t (min); N_{max} (mg N kg⁻¹) is the potentially hydrolyzable N; and k_h (min⁻¹) is the first-order alkaline hydrolysis rate constant.

2.6. Statistical Analysis

The first-order kinetic parameters of N mineralization and hydrolysis were obtained using GraphPad Prism 5 (12th Edition). Analysis of variance was performed using Genstat 12th edition. Fisher's least significant difference (LSD) test was used to compare the means. We used SigmaPlot (v.13, Syntat Software, San Jose, CA, USA) for correlations among chemical and biological indices, N_o and N_{max} values. A significant level of $\alpha = 0.05$ was used in all cases.

3. Results

3.1. Flush of CO₂

The $f\text{CO}_2$ among the soils had values ranging from 45 to 131 mg CO₂-C kg⁻¹ soil (Table 1). Although $f\text{CO}_2$ was positively correlated with TC, TN, and ON, the correlations were not significant ($p > 0.05$). The correlations between $f\text{CO}_2$ and TC, TN, and ON were 0.201, 0.474, and 0.411, respectively.

3.2. Long-Term Aerobic N Mineralization

The patterns and cumulative amounts of N mineralized in four of the soils used are plotted in Figure 1a; the results for the other eight soils fall within those shown. In general, the patterns of N mineralization in the incubated soils were similar with the N release rate, increasing rapidly during the first 7 weeks of incubation followed by a slow phase later during the incubation, resulting in a near linear increase in cumulative N after approximately week 15 (Figure 1a). The total amount of N mineralized during the 26 weeks of incubation ranged from 120.8 to 291.8 mg kg⁻¹ soil (Figure 1b). Analysis of variance indicated that the total amount of N mineralized during the 26 weeks of incubation differed

significantly ($p < 0.05$) among the soils (Figure 1b). Expressed as a percentage of ON in the soils, the total amounts of N mineralized during the 26 weeks of incubation varied among the soils with values ranging from 4 to 13.6%.

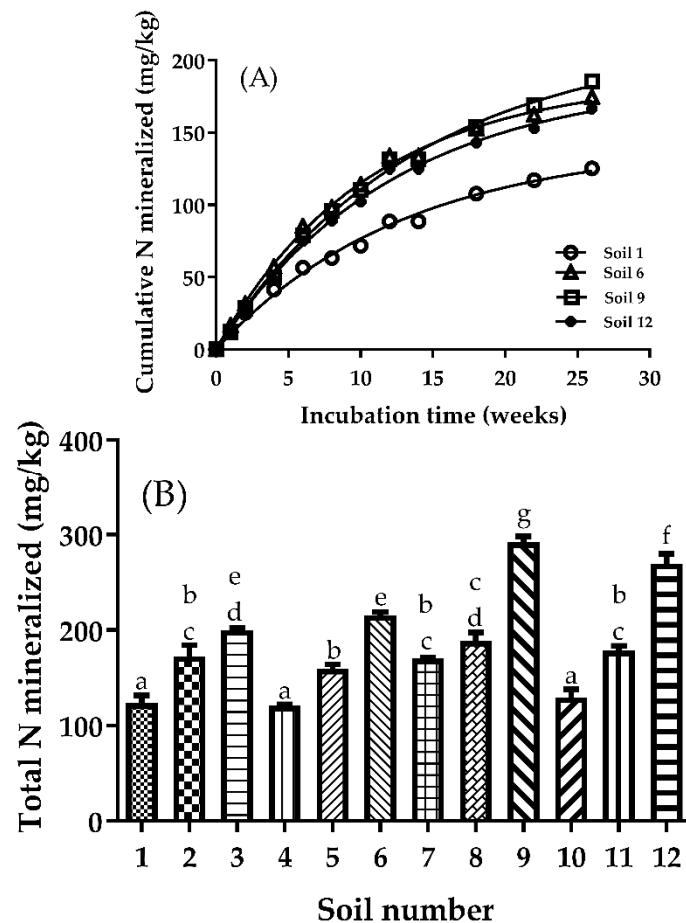


Figure 1. Cumulative amounts of N mineralized in four of the soils used in relation to incubation time (A), and net amounts of N mineralized in soils during incubation for 26 weeks at 25 °C (B). Error bars represent standard error of three replicates. Bars followed by the same letter are not significantly different at $p = 0.05$.

3.3. Alkaline Hydrolysis of Organic N

The cumulative amounts and patterns of alkaline hydrolysis of N with time of distillation in four of the soils used are shown in Figure 2a,b for KOH and NaOH, respectively; the results for the other soils fall within those shown. In general, the cumulative amounts of N hydrolyzed with time increased in a somewhat linear fashion during the first 15 min of distillation with the rate of N hydrolysis decreasing slowly as distillation progressed (Figure 2a,b). The total amounts of N hydrolyzed in the soils during the 40 min of distillation by the two reagents varied among the soils studied (Figure 2c,d), with the amounts ranging from 104.6–306.9 mg kg⁻¹ and 143.5–371.3 mg kg⁻¹ for NaOH and KOH, respectively. For each reagent, the amount of N hydrolyzed was significantly ($p < 0.05$) different among the soils (Figure 2c,d). Statistical analysis showed that the amount of N hydrolyzed with KOH was significantly ($p < 0.05$) higher than that with NaOH (Figure 2c,d). The total amounts of N hydrolyzed, expressed as a percentage of the ON contents of the soils, ranged between 3.9 to 15.1% for NaOH and between 4.6 and 15.9% for KOH.

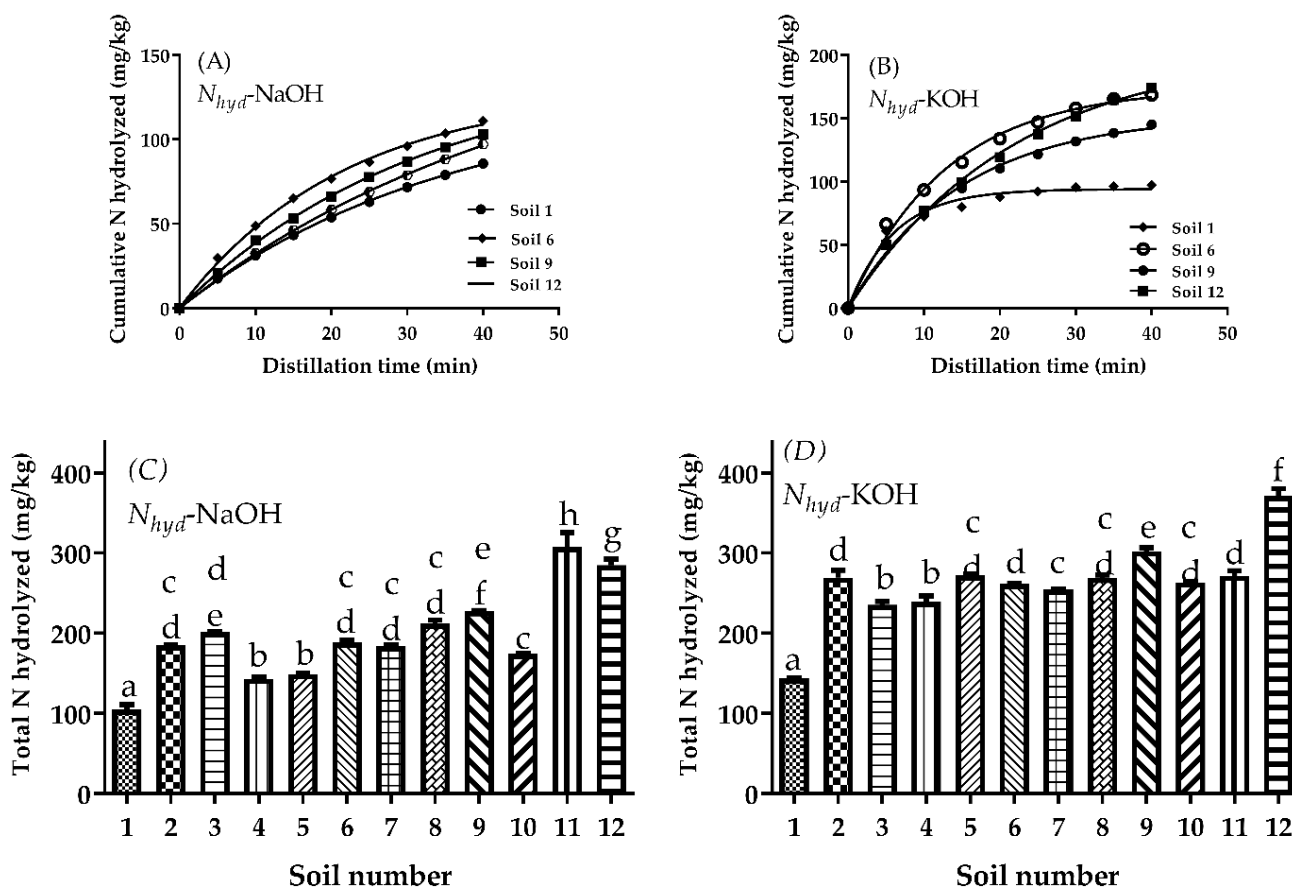


Figure 2. Cumulative N hydrolyzed with time of distillation using 1 M NaOH (A) and 1 M KOH (B) in four of the soils used. Total N hydrolyzed from the soils using 1 M NaOH (C), and 1 M KOH (D). Error bars represent standard error of three replicates. Within each reagent, bars followed by the same letter are not significantly different at $p = 0.05$.

3.4. Kinetic Parameter of N Mineralization and Hydrolysis

Application of the nonlinear regression analysis to calculate the kinetic parameters of N mineralization showed that convergence of the model occurred with 10 iterations or less, with R^2 values ranging between 0.998 and 0.999. The estimated N_o values of the soils studied varied from 127.2 to 215.7 mg kg⁻¹ soil (Table 2). The N_o values, expressed as a percentage of the ON, ranged from 4.0 to 13.6%. The k_m values ranged from 0.076 to 0.107 week⁻¹ (average = 0.088 week⁻¹). Analysis of variance indicated that the estimated N_o and k_m values differed significantly ($p < 0.05$) among the soils studied (Table 2).

The data for the alkaline hydrolysis of ON also fitted the exponential equation very well with all R^2 values greater than 0.995. The estimated N_{max} values for the alkaline reagents were significantly correlated with each other ($r = 0.868$; $p < 0.001$), with values ranging from 104.3 to 288.4 mg kg⁻¹ soil for NaOH (N_{max} -NaOH) and from 123.5 to 371.2 mg kg⁻¹ soil for KOH (N_{max} -KOH) (Table 2). Analysis of variance indicated that the estimated N_{max} -NaOH and N_{max} -KOH values differed significantly ($p < 0.05$) among the soils studied (Table 2).

Table 2. First-order kinetic parameters of N mineralization/hydrolysis for the soils †.

ID	Soil Series	N_{min}			$N_{hyd-NaOH}$			$N_{hyd-KOH}$		
		N_o	k_m	%NT	N_{max}	k_h	%NT	N_{max}	k_h	%NT
1	Nyigbenya	142.6	0.077	7.5	104.3	0.028	5.5	123.5	0.166	7.6
2	Nyigbenya	181.1	0.079	10.1	185.0	0.036	10.3	269.0	0.033	14.9
3	Nyigbenya	205.9	0.089	4.0	201.2	0.044	3.9	235.1	0.080	4.6
4	Toje	127.2	0.107	8.5	142.8	0.024	9.5	138.0	0.077	15.9
5	Toje	159.5	0.102	6.6	148.9	0.032	6.2	261.7	0.074	11.3
6	Toje	188.0	0.095	6.1	188.2	0.046	6.1	272.1	0.080	8.4
7	Adentan	163.1	0.076	9.6	183.9	0.038	10.8	254.3	0.067	15.0
8	Adentan	191.0	0.081	13.6	211.5	0.033	15.1	268.8	0.064	19.2
9	Adentan	215.7	0.072	5.3	227.5	0.031	6.5	302.1	0.057	8.6
10	Haatso	139.8	0.099	5.0	173.8	0.041	6.2	163.0	0.042	9.4
11	Haatso	158.4	0.096	6.6	204.6	0.022	8.5	271.1	0.061	11.3
12	Haatso	187.6	0.081	6.8	288.4	0.021	12.5	371.2	0.045	16.1
	LSD ($p < 0.05$)	3.3	0.001	0.8	10.5	0.001	0.5	8.5	0.002	0.6

† N_o , potentially mineralizable N (mg N kg^{-1} soil); k_m , first-order mineralization rate constant (week^{-1}); N_{max} , potentially hydrolyzable N (mg N kg^{-1} soil); k_h , first-order hydrolysis rate constant (min^{-1}); %NT, percent of total N mineralized or hydrolyzed (%).

When the N_{max} values were expressed as a percentage of ON content of the soils, the values varied from 3.9 to 15.1% and from 4.6 to 19.2% for N_{max} -NaOH and N_{max} -KOH, respectively. The k_h values varied from 0.021 to 0.046 min^{-1} (average = 0.033 min^{-1}) for NaOH hydrolysis and from 0.033 to 0.166 min^{-1} (average = 0.070 min^{-1}) for KOH hydrolysis. In general, the percentages of organic N contributing to N_o and N_{max} values followed similar trends in all the soils.

3.5. Relationship among Soil Properties, Estimated N_o , and N_{max} Values

The estimated N_{max} -NaOH and N_{max} -KOH values were positively and significantly ($p \leq 0.01$) correlated with the N_o values (Figure 3). Nonetheless, N_{max} -KOH correlated better with N_o compared to N_{max} -NaOH, and the ranges of the latter fitted better to the N_o values. Correlation coefficients between the estimated N_o values and ON, OC, TN, and $f\text{CO}_2$ were 0.534, 0.596 ($* p < 0.05$), 0.497, and 0.701 ($** p < 0.01$), respectively, and those between $f\text{CO}_2$ and the estimated N_{max} -KOH and N_{max} -NaOH values were 0.7112 ($** p < 0.01$) and 0.614 ($* p < 0.05$), respectively.

Further examination of the model parameters showed that, although the alkaline hydrolysis method was rapid, the percent of TN hydrolyzed and that mineralized in the biological method were comparable (Table 2). For the long-term incubation method, the percent of TN mineralized ranged from 4.0 to 13.6%, which compares very well with the 3.9–15.1% and 4.6–19.2% for hydrolysis with NaOH and KOH, respectively. Furthermore, despite the short duration of the alkaline hydrolysis method, the amount of N hydrolyzed also compared very well with that mineralized with the biological method. Whereas the N_o values ranged from 127.2 to 215.7 mg N kg^{-1} soil for the incubation period of 26 weeks, the N_{max} -NaOH and N_{max} -KOH values were 104.3–288.4 and 123.5–371.2 mg N kg^{-1} soil, respectively, for a distillation time of 40 min.

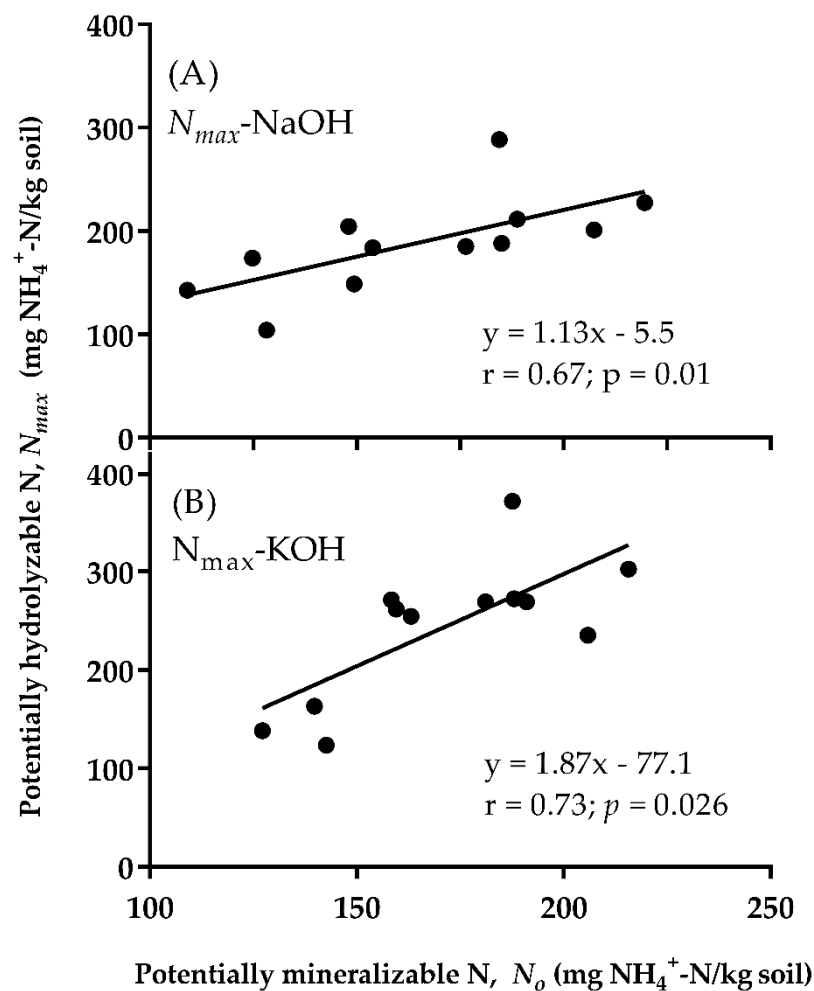


Figure 3. Relationship between potentially mineralizable N and potentially hydrolyzable N using, (A) 1 M NaOH, and (B) 1 M KOH.

4. Discussion

The positive but weak correlation between the N mineralization and OC contents of the soils may be due to the removal of soluble C critical for microbial growth and development during the periodic leaching. Similarly, the removal of a significant amount of soluble N during leaching [25] could result in lower N mineralization as a result of the shift in labile C and N stoichiometry. Other researchers have also reported an insignificant correlation between ON and N mineralization in soils and attributed it to the differences in the quality of the organic C and N content of the soils [18,26]. The results, however, contradict the reported close relationship among these parameters [27–29].

The very high R^2 values obtained when the cumulative N mineralization and hydrolysis data were fitted to the first-order exponential equation indicate that N mineralization in the soils conformed to the model proposed by Stanford and Smith [2] and are consistent with the results of other researchers [30,31]. The curvilinear pattern of cumulative N mineralization, which declined as incubation time progressed, could be attributed to the decreasing quantity of the easily mineralizable N pools in the soils. Our results, however, contradict other authors who reported that the mineralization of N in incubated soils increased linearly with time [32,33]. Similarly, the initially high amounts of N hydrolyzed by the alkaline reagents, which later dropped to an almost constant rate, can be attributed to the decline in the hydrolyzable ON in the soils.

The 26-week N_0 values reported in the present study are comparable to those reported in the literature. For example, Schomberg et al. [7] reported a range of 35 to 488 mg N kg⁻¹ soil, Sharifa et al. [34] reported a range of 54 to 197 mg N kg⁻¹ soil for 153 soil samples from 17 fields in Canada and the USA, and Jalil et al. [17] reported a range of 71 to 278 mg N kg⁻¹ soil. The average k_m of 0.088 week⁻¹ obtained in the present study is higher than the 0.054 week⁻¹ reported for 39 soils from North America [2] and can be attributed, perhaps, to the differences in soil properties such as the C/N ratios. Furthermore, the soils used in the present study are from a tropical climate where microbial activity and associated processes such as N mineralization are normally faster compared to their temperate counterparts. Different k_m values have, however, been reported in several studies [7,31].

The strong and positive correlation between the estimated N_{max} and N_0 values reported for the long-term incubation of soils of wide and varied properties indicates that the two procedures may be measuring the same pools of potentially mineralizable and available N. The strong relationship between fCO_2 and N_0 can be explained by the stoichiometric requirement for labile C and N by microbial populations [35]; whereby, soils with high fCO_2 , indicative of a high availability of labile SOC to drive microbial activity, also have a larger pool of labile and potentially mineralizable N. The results corroborate those of other workers showing that fCO_2 is closely related to the net N mineralization during a 24-day incubation [8]. Furthermore, previous studies using 13 uncultivated and unfertilized soils from Iowa, USA showed that the estimated N_{max} values related strongly to the amount of N mineralized in two weeks under aerobic and anaerobic incubation conditions at 30 °C, N released by 2 M KCl extraction at 80 °C for 20 h, and the initial NH₄⁺-N present in the soils [18].

An ideal chemical index for predicting potentially available N should meet the following criteria, among others: simple and rapid, inexpensive to encourage adoption, and amenable to high-throughput operations to give quick turn-around N application recommendations [36]. The alkaline hydrolysis method proposed by Dodor and Tabatabai [18] meets most of these requirements; as such, it could provide a very good proxy of the net N mineralization potential and N-supplying power of soils. Therefore, the strong positive correlation between the estimated N_{max} and N_0 values, coupled with the relationship with fCO_2 , lend strong support to our assertion that N_{max} could be used as a robust index of potential mineralizable N and supplying capacity of soils.

Although our study lacks information on the chemical nature of ON hydrolyzable by NaOH and KOH, it is generally known that the chemical hydrolysis of ON in soils is selective with regards to the bond being hydrolyzed. Gianello and Bremner [6] reported that only six out of the over 50 ON compounds known to occur in soils or soil hydrolysates released NH₄⁺-N in hot KCl. In the present study, we speculate that the NaOH and KOH hydrolyzable N are derived from exchangeable NH₄⁺, hexosamines in amino sugars, α-amino and amide-N (glutamine, asparagine, glucosamine, and galactosamine), and hydrolyzable unidentified N fractions [37–39]. These amine and amide bonds hydrolyzed by the alkaline reagents could be the same ones that were mineralized by the biological methods. This assertion is consistent with the results of Dodor and Tabatabai [40] who reported significant relationships between the estimated N_{max} values and the activities of arylamidase and amidohydrolases, especially amidase, and suggested that the alkaline reagents might be hydrolyzing mainly amide-N. The stronger relationship between N_{max} -KOH and N_0 may be attributed to KOH probably hydrolyzing more of the same ON pool as the biological methods, some of which may have originated from the compounds released from microbial cells.

5. Conclusions

The alkaline hydrolysis procedure is rapid, is precise, and reduces the long incubation time required to obtain N_0 (minutes compared to months). The estimated N_{max} values were highly correlated with N_0 during the 26 weeks of incubation as well as the fCO_2 , an indicator of overall soil biological activity. Based on the results from the present study, we

conclude that the method could provide an effective, time-efficient, and practical tool for the routine assessment of N mineralization potential in soils of varied properties. The use of alkaline hydrolyzable ON as a predictor of N_0 in soils, therefore, merits consideration for routine use in soil testing laboratories for estimating the N-supplying capacity of soils.

Author Contributions: Conceptualization, D.E.D.; methodology, D.E.D.; formal analysis, D.E.D. and S.G.K.A.; investigation, M.S.K., A.A.-B. and D.E.D.; resources, M.S.K., A.A.-B. and D.E.D.; data curation, M.S.K. and D.E.D.; writing—original draft preparation, D.E.D. and M.S.K.; writing—review and editing, D.E.D., M.S.K., S.G.K.A., D.S.M., S.K.K. and D.N.; supervision, D.E.D.; project administration, D.E.D. and S.G.K.A.; funding acquisition, D.E.D., S.G.K.A., D.S.M. and D.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are available upon reasonable request from the corresponding author.

Acknowledgments: This paper forms part of Mohamed S. Kamara's unpublished MPhil Thesis submitted to the University of Ghana, Legon, Ghana. The financial support by the German Academic Exchange Service (DAAD) to Mohamed S. Kamara and Abena Asamoah-Bediako for their MPhil and PhD studies, respectively, at the Department of Soil Science, University of Ghana, Legon, is acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bundy, L.G.; Meisinger, J.J. Nitrogen availability indices. In *Methods of Soil Analysis, Part 2—Microbiological and Biochemical Properties*; Weaver, R.W., Angle, J.S., Bottemley, P.S., Eds.; SSSA/ASA: Madison, WI, USA, 1994; pp. 951–984.
2. Stanford, G.; Smith, S.J. Nitrogen mineralization potential of soils. *Soil Sci. Soc. Am. Proc.* **1972**, *36*, 465–472. [[CrossRef](#)]
3. Waring, S.A.; Bremner, J.M. Ammonium Production in Soil under Waterlogged Conditions as an Index of Nitrogen Availability. *Nature* **1964**, *201*, 951–952. [[CrossRef](#)]
4. Keeney, D.R.; Bremner, J.M. Determination and Isotope-Ratio Analysis of Different Forms of Nitrogen in Soils: 6. Mineralizable Nitrogen. *Soil Sci. Soc. Am. J.* **1967**, *31*, 34–39. [[CrossRef](#)]
5. Stanford, G.; Carter, J.N.; Smith, S.J. Estimates of potentially mineralizable soil nitrogen based on short-term incubations. *Soil Sci. Soc. Am. Proc.* **1974**, *38*, 99–102. [[CrossRef](#)]
6. Gianello, C.; Bremner, J.M. A simple chemical method of assessing potentially available organic nitrogen in soils. *Commun. Soil Sci. Plant Anal.* **1986**, *17*, 195–214. [[CrossRef](#)]
7. Schomberg, H.H.; Wietholter, S.; Griffin, T.S.; Reeves, D.W.; Cabrera, M.L.; Fisher, D.S.; Kitchen, N.R. Assessing indices for predicting potential nitrogen mineralization in soils under different management systems. *Soil Sci. Soc. Am. J.* **2009**, *73*, 1575–1586. [[CrossRef](#)]
8. Franzluebbers, A.J.; Pershing, M.R.; Crozier, C.; Osmond, D.; Schroeder-Moreno, M. Soil-test biological activity with the flush of CO₂: I. C and N characteristics of soils in corn production. *Soil Sci. Soc. Am. J.* **2018**, *82*, 685–695. [[CrossRef](#)]
9. Jenkinson, D.S. Chemical tests for potentially available nitrogen in soil. *J. Sci. Food Agric.* **1968**, *19*, 160–168. [[CrossRef](#)]
10. Stanford, G. Extraction of soil organic nitrogen by autoclaving in water. II. A kinetic approach to estimating the NaOH-distillable fraction. *Soil Sci.* **1969**, *107*, 323–328. [[CrossRef](#)]
11. Curtin, D.; Bearea, M.H.; Lehto, K.; Tregurtha, C.; Qiua, W.; Tregurtha, R.; Peterson, M. Rapid Assays to Predict Nitrogen Mineralization Capacity of Agricultural Soils. *Soil Sci. Soc. Am. J.* **2017**, *81*, 979–991. [[CrossRef](#)]
12. Øien, A.; Selmer-Olsen, A.R. A laboratory method for evaluation of available nitrogen in soil. *Acta Agric. Scand.* **1980**, *30*, 149–156. [[CrossRef](#)]
13. Xu, J.M.; Cheng, H.H.; Koskinen, W.C.; Molina, J.A.E. Characterization of potentially bioreactive soil organic carbon and nitrogen by acid hydrolysis. *Nutr. Cycl. Agrosyst.* **1997**, *49*, 267–271. [[CrossRef](#)]
14. Stanford, G.; DeMar, W.H. Extraction of soil organic nitrogen by autoclaving in water. I. The NaOH-distillable fraction as an index of soil nitrogen availability. *Soil Sci.* **1969**, *109*, 203–205. [[CrossRef](#)]
15. Gianello, C.; Bremner, J.M. Comparison of chemical methods of assessing potentially available organic nitrogen in soil. *Comm. Soil Sci. Plant Anal.* **1986**, *17*, 215–236. [[CrossRef](#)]
16. Smith, K.A.; Li, S. Estimation of potentially mineralizable nitrogen in soil by KCl extraction: I. Comparison with pot experiments. *Plant Soils* **1993**, *157*, 167–174. [[CrossRef](#)]
17. Jalil, A.; Campbell, C.A.; Schoenau, J.; Henry, J.L.; Jame, Y.W.; Laford, G.P. Assessment of two chemical extraction methods as indexes of available nitrogen. *Soil Sci. Soc. Am. J.* **1996**, *60*, 1954–1960. [[CrossRef](#)]
18. Dodor, D.E.; Tabatabai, M.A. A simple alkaline hydrolysis method for estimating nitrogen mineralization potential of soils. *West Afr. J. Appl. Ecol.* **2019**, *27*, 16–31.

19. Eze, P.N. Characterization, Classification and Pedogenesis of Soils on a Legon Catena in the Accra Plains, Ghana. Ph.D. Thesis, University of Ghana, Legon, Ghana, 2008.
20. Blake, G.R.; Hartge, K.H. Bulk density. In *Methods of Soil Analysis, Part 1—Physical and Mineralogical Methods*; Klute, A., Ed.; SSSA/ASA: Madison, WI, USA, 1986; pp. 363–375.
21. Gee, G.W.; Bauder, J.W. Particle size analysis. In *Methods of Soil Analysis, Part 1—Physical and Mineralogical Methods*; Klute, A., Ed.; SSSA/ASA: Madison, WI, USA, 1986; pp. 383–411.
22. Mulvaney, R.L. Nitrogen–Inorganic Forms. In *Methods of Soil Analysis, Part 3—Chemical Methods*; Sparks, D.L., Ed.; SSSA/ASA: Madison, WI, USA, 1996; pp. 1123–1184.
23. Dodor, D.E.; Amanor, Y.J.; Attor, F.T.; Adjadeh, T.A.; Neina, D.; Miyittah, M. Co-application of biochar and cattle manure counteract positive priming of carbon mineralization in a sandy Soil. *Environ. Syst. Res.* **2018**, *7*, 5. [CrossRef]
24. Dodor, D.E. Enzyme Activities in Soils as Affected by Long-Term Cropping Systems. Ph.D. Dissertation, Iowa State University, Ames, IA, USA, 2002. Available online: <http://lib.dr.iastate.edu/rtd/990> (accessed on 8 November 2022).
25. Curtin, D.; Wen, G. Organic matter fractions contributing to soil nitrogen mineralization potential. *Soil Sci. Soc. Am. J.* **1999**, *63*, 410–415. [CrossRef]
26. Dodor, D.E.; Tabatabai, M.A. Arylamidase activity as an index of nitrogen mineralization in soils. *Comm. Soil Sci. Plant Anal.* **2007**, *38*, 2197–2207. [CrossRef]
27. Simard, R.R.; N'dayegamiye, A. Nitrogen mineralization potential of meadow soils. *Can. J. Soil Sci.* **1993**, *73*, 27–38. [CrossRef]
28. Serna, M.D.; Pomares, F. Evaluation of chemical indices of soil organic nitrogen availability in calcareous soils. *Soil Sci. Soc. Am. J.* **1992**, *56*, 1486–1491. [CrossRef]
29. Wang, W.; Smith, C.J.; Chalk, P.M.; Chen, D. Evaluation of chemical and physical indices of nitrogen mineralization capacity with an unequivocal reference. *Soil Sci. Soc. Am. J.* **2001**, *65*, 368–376. [CrossRef]
30. Griffin, G.F.; Laine, A.F. Nitrogen mineralization in soils previously amended with organic wastes. *Agron. J.* **1983**, *75*, 124–129. [CrossRef]
31. Wang, W.J.; Smith, C.J.; Chen, D. Towards a standardized procedure for determining the potentially mineralisable nitrogen. *Biol. Fertil. Soils* **2003**, *37*, 362–374. [CrossRef]
32. Tabatabai, M.A.; Al-Khafaji, A.A. Comparison of nitrogen and sulfur mineralization in soils. *Soil Sci. Soc. Am. Proc.* **1980**, *41*, 1000–1006. [CrossRef]
33. Addiscott, T.M. Kinetics and temperature relationships of mineralization and nitrification in Rothamsted soil with differing histories. *J. Soil Sci.* **1983**, *34*, 343–353. [CrossRef]
34. Sharifi, M.; Zebarth, B.J.; Burton, D.L.; Grant, C.A.; Cooper, J.M. Evaluation of some indices of potentially mineralizable nitrogen soils. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1233–1239. [CrossRef]
35. Dodor, D.E.; Amanor, Y.J.; Asamoah-Bediako, A.; MacCarthy, D.S.; Dovie, B.D. Kinetics of carbon mineralization and sequestration of sole and/or co-amended biochar and manure in a sandy soil. *Comm. Soil Sci. Plant Anal.* **2019**, *50*, 2593–2609. [CrossRef]
36. McDaniel, M.; Walters, D.; Bundy, L.; Li, X.; Drijber, R.A.; Sawyer, J.E.; Castellan, M.J.; Laboski, C.A.M.; Scharf, P.C.; Horwath, W.R. Combination of biological and chemical soil tests best predict maize nitrogen response. *Agron. J.* **2020**, *112*, 1263–1278. [CrossRef]
37. Sowden, F.J. The forms of nitrogen in the organic matter of different horizons of soil profiles. *Can. J. Soil Sci.* **1958**, *38*, 147–154. [CrossRef]
38. Stanford, G. Evaluation of ammonium release by alkaline permanganate extraction as an index of soil nitrogen availability. *Soil Sci.* **1978**, *126*, 244–253. [CrossRef]
39. Greenfield, L.G. The origin and nature of organic nitrogen in soil as assessed by acidic and alkaline hydrolysis. *Eur. J. Soil Sci.* **2001**, *52*, 575–583. [CrossRef]
40. Dodor, D.E.; Tabatabai, M.A. Alkaline hydrolyzable organic nitrogen as an index of nitrogen mineralization in soils: Relationship with activities of arylamidase and amidohydrolases. *Comm. Soil Sci. Plant Anal.* **2020**, *51*, 1757–1766. [CrossRef]