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Alkaline Hydrolyzable Organic Nitrogen as an Index of Nitrogen Mineralization in Soils: Relationship with Activities of Arylamidase and Amidohydrolases

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ABSTRACT

This study investigated the relationship between a recently proposed alkaline hydrolysis method for estimating the chemical index of nitrogen (N) mineralization potential of soils and the activities of arylamidase and four amidohydrolases involved in hydrolysis of organic N (ON) in soils. Nitrogen mineralization was studied in 13 soils from uncultivated fields in Iowa, USA, by direct steam distillation of 1 g field-most soil treated with 1 M KOH or 1 M NaOH. The distillate was collected in boric acids, 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. The cumulative amounts of N hydrolyzed were fitted to the first-order exponential equation to determine the "potentially hydrolyzable N (N_{max})" for the soils. The activities of arylamidase, L-asparaginase, L-glutaminase, amidase, and L-aspartase were assayed at their optimal pH values. Results showed that estimated N_{max} values were strongly correlated with the activities of arylamidase and amidohydrolases. The activities of arylamidase and the amidohydrolases were significantly correlated, indicating that the activities of the two groups of enzymes are coupled in mineralization of ON in soils. Based on the specificity of enzyme reactions and the strong relationship between estimated N_{max} values and the activities of arylamidase and amidohydrolases, we concluded that similar amide-N bonds were susceptible to enzymatic and alkaline hydrolysis, and that alkaline hydrolyzable ON can be used as an index of N mineralization in soils.

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Soil organic N; chemical index of available N; alkaline hydrolysis; potentially hydrolyzable N; soil enzymes

Introduction

A major challenge facing farmers and agricultural researchers today is a satisfactory method for predicting nitrogen (N) mineralization potential of soils. The biological methods involving estimation of the mineral N produced when soil is incubated under conditions that promote mineralization of soil organic N (SON) have gained considerable acceptance (Bundy and Meisinger 1994; Stanford 1982). However, an incubation period of more than 16 weeks is usually necessary to ensure that all potentially available N has been mineralized (Bundy and Meisinger 1994; Stanford 1982). For this reason, a chemical approach to the problem is attractive, particularly because chemical methods are usually more rapid and precise than biological methods (Bundy and Meisinger 1994; Gianello and Bremner 1986; OSien and Selmer-Olsen 1980; Smith and Li 1993).

Recently, a simple and precise alkaline hydrolysis method for evaluating the chemical index of potentially mineralizable N in soils has been proposed (Dodor and Tabatabai 2019). The procedure involves direct steam distillation of 1 g field-most soil treated with 1 M KOH or 1 M NaOH. The

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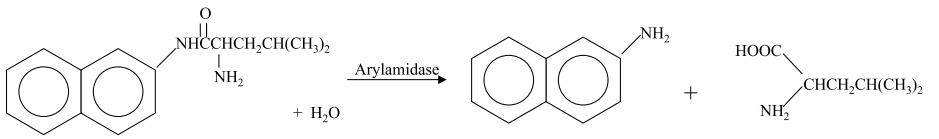
distillate was collected in boric acids, which was changed every 5 min for a total of 40 min. The NH_4^+-N in the distillate was determined by titration with 0.005 M H_2SO_4. The authors used the first-order reaction kinetics proposed by Stanford and Smith (1972) for determining the potentially mineralizable N (N_o) of soils, to derive an analogous "potentially hydrolyzable N (N_{max})" and hydrolysis rate constant (k_h) for selected soils from Iowa, USA (Dodor and Tabatabai 2019). To define the structure of the derived kinetic parameters of the hydrolysis process, it is important to relate them to biochemical processes responsible for N mineralization in soils.

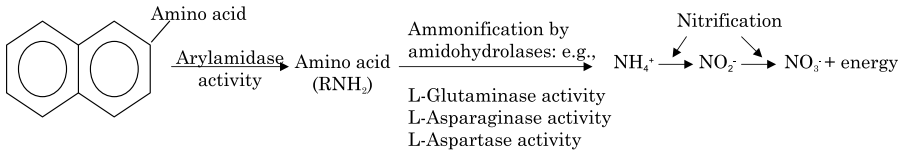
Enzymes are central to microbial transformation of organic N (ON) in soils, therefore, activities of specific enzymes have been evaluated as possible indices of N mineralization in soils (Burket and Dick 1998; Burton and McGill 1992; Deng, Moore, and Tabatabai 2000; Dodor and Tabatabai 2002, 2003, 2007; Ekenler and Tabatabai 2004a). Results from these studies have shown that N mineralization involves a sequence of microbial and enzymatic activities and that enzymatic conversion of ON substrates to NH_4^+ is the rate-limiting step in N mineralization in soils (Burket and Dick 1998; Burton and McGill 1992; Deng, Moore, and Tabatabai 2000; Dodor and Tabatabai 2002, 2003, 2007). The importance of arylamidase [EC 3.4.11.2] and amidohydrolases as the enzymes involved in sequential mineralization of SON have been indicated (Acosta-Martinez and Tabatabai 2000a; Deng and Tabatabai 2000; Dodor and Tabatabai 2002, 2007; Senwo and Tabatabai 1996; Tabatabai 1994).

Arylamidase is the enzyme that catalyzes the hydrolysis of N-terminal amino acid from arylamides according to the reaction using L-leucine as the amino acid moiety (Acosta-Martinez and Tabatabai, 2000a):



L-Leucine β-naphthylamide β-Naphthylamine Leucine

The activity of arylamidase has been suggested as one of the initial reaction-limiting steps in N mineralization in soils according to the reaction (Acosta-Martinez and Tabatabai 2000b; Dodor and Tabatabai 2002, 2007):



The amino acids released from arylamides in soil organic matter (SOM) by the action of arylamidase serves as substrate for ammonification by one of several specific amidohydrolases, depending on the amino acid moiety released.

The amidohydrolases L-asparaginase (EC 3.5.1.1), L-glutaminase (3.5.1.2), amidase (EC 3.5.1.4), and L-aspartase (EC 4.3.1.1) are the enzymes that catalyze the hydrolysis of C-N bond other than peptide bonds in linear amides (Sewno and Tabatabai 1996; Tabatabai 1994). These enzymes play major roles in N mineralization because it is known that acid hydrolysis (6 M HCl) of soils releases large proportion (15–25%) of N as NH_4^+-N, a portion of which is derived from the hydrolysis of amino acids in linear amides such as asparagine and glutaminase residues in SOM (Sowden 1958). Arylamidase and amidohydrolases are widely distributed in nature; however, they are believed to be

primarily of microbial origin (Acosta-Martinez and Tabatabai 2000a; Senwo and Tabatabai 1996; Tabatabai 1994). The activities of arylamidase and amidohydrolases have been shown to be significantly correlated with the amounts of N mineralized under aerobic incubation condition at 30°C for 20 weeks (Deng and Tabatabai 2000; Dodor and Tabatabai 2002, 2003, 2007).

To advance knowledge on the nature and the pool size of the alkaline hydrolyzable ON in soils, the relationship between the derived kinetic parameters of the hydrolysis procedure and the activities of enzymes involved in mineralization of SON needs to be studied. This information is needed for better assessment of N fertilizer needs of crops (Deng, Moore, and Tabatabai 2000). The criteria for choosing enzyme assays to achieve these objectives should be based on their relevance to N cycling in soils. Furthermore, should the slope of the correlation between N mineralization and the activity of the enzyme be unity, it can be inferred that the two are hydrolyzing the same bond and the known specificity of enzymes can then allow deductions to be made regarding the bond being hydrolyzed by the reagents.

In the present study, we hypothesize that there is a strong association between the amounts of N released by alkaline hydrolysis of SON and the activities of arylamidase and amidohydrolases, which suggest that the bonds that are susceptible to enzymatic and alkaline hydrolysis are similar. Therefore, the objective of this study was to assess the relationship between the amounts of N hydrolyzed and the kinetic parameters of alkaline hydrolysis of ON and the activities of arylamidase and amidohydrolases involved in N cycling in soils.

Materials and methods

Soils sampling and analysis

The 13 samples used were surface soils (0–15 cm) taken from uncultivated and unfertilized fields in Iowa, USA, to include wide ranges of chemical, physical, and biological properties. On each field, an area of 5 by 5 m was marked, and six cores of surface soils were taken, mixed thoroughly, and transported to the laboratory. The soil samples were passed through a 2-mm sieve and stored under field-moist conditions at 4°C when not in use. Portion of the soils was air-dried at room temperature and used for the determination of routine physical and chemical properties.

In the analyzes reported in Table 1, soil pH was determined on the <2-mm mesh air-dried samples by using a combination glass electrode (soil:water ratio of 1:2.5). Particles size distribution was determined by the pipette method (Kilmar and Alexander 1949). Total C (TC) and N (TN) were determined on the <180 μm air-dried samples by dry combustion using LECO CHN 600 analyzer (St Joseph, MI) and inorganic N (NH_4^+-N and NO_3^-N) by steam distillation (Mulvaney 1996). Organic N was calculated from the difference between TN and inorganic N (sum of NH_4^+-N and NO_3^-N)

Table 1. Selected chemical, biochemical, and physical properties of the soils.

Soil	Classification	pH	Total N	NH_4^+-N	NO_3^-N	Org N	Total C	C_{mic} [a]	N_{mic} [a]	Clay	Sand
			g kg^{-1}	mg kg^{-1}		g kg^{-1}		mg kg^{-1}		g kg^{-1}	
Canisteo	Typic Endoaquolls	7.5	3.8	1.9	7.1	3.8	37.7	527	40.3	275	318
Clarion (I)	Typic Hapludolls	5.0	3.7	4.6	9.7	3.7	21.1	335	13.2	235	356
Coland	Cumulic Endoaquolls	5.8	3.6	2.5	9.8	3.6	29.7	420	21.0	287	160
Crippin	Aquic Pachic Hapludolls	7.5	2.9	6.6	14.6	2.9	24.2	422	32.1	227	432
Harps	Typic Calciaquolls	7.9	4.3	1.4	6.8	4.3	53.9	273	37.8	281	335
Nicollet	Aquic Hapludolls	6.5	3.2	3.2	12.1	3.2	21.3	279	21.8	237	367
Okoboji	Vertic Endoaquolls	7.8	4.2	1.7	9.0	4.2	37.4	659	43.5	295	267
Storden	Typic Eutrudepts	7.8	2.2	2.5	14.4	2.2	28.9	167	22.0	133	480
Terrill	Cumulic Hapludolls	6.9	2.7	2.7	13.1	2.7	20.8	254	20.9	147	533
Webster (I)	Typic Endoaquolls	8.0	3.5	1.4	8.4	3.5	32.3	553	41.2	232	370
Clarion (II)	Typic Hapludolls	6.6	3.0	4.8	12.7	3.0	37.1	nd	nd	258	317
Grundy	Aquertic Argiudolls	7.4	1.9	1.6	27.9	1.9	21.2	nd	nd	290	25
Webster (II)	Typic Endoaquolls	6.5	2.4	4.9	6.9	2.4	38.3	nd	nd	277	297

[a] C_{mic} and N_{mic}, microbial biomass C and N, respectively. Nd = not determined.

values. Microbial biomass C (C_{mic}) was determined by the chloroform-fumigation-extraction method described by Vance, Brookes, and Jenkinson (1987), and microbial biomass N (N_{mic}) by the chloroform-fumigation-incubation method described by Horwath and Paul (1994).

Alkaline hydrolysis of SON

The alkaline hydrolysis of SON was determined as described by Dodor and Tabatabai (2019). Briefly, 1 g field-moist soil (on oven-dried basis) was placed in a 200-mL distillation flask and treated with 20 mL of 1 M NaOH or KOH. The flask was connected to the distillation unit and the distillate collected in 5 mL of boric acids, 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). A nonlinear regression model was used to estimate the potentially hydrolyzable N, N_{max} (mg N kg^{-1} soil) and the first-order hydrolysis rate constant, k_h (min^{-1}) using the following equation (Dodor and Tabatabai 2019):

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

where N_{hyd} (mg N kg^{-1} soil) is the cumulative amounts of N hydrolyzed at a specific time, t (min). All results reported for the alkaline hydrolysis were 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 (Dodor 2002). Moisture content of the soils was determined from the weight loss after drying at 105°C for 48 h. The amounts of N hydrolyzed by the alkaline reagents as well as estimated N_{max} values have been reported previously (Dodor and Tabatabai 2019).

Assay of the activities of arylamidase and amidohydrolases

The method described by Acosta-Martinez and Tabatabai (2000a) was used to assay the activity of arylamidase. The procedure involves quantitative extraction and colorimetric determination of the β-naphthylamine produced when 1 g of soil is incubated with 3 ml of 0.1 mM THAM [tris-(hydroximethyl)aminomethane] buffer (pH 8.0) and 1 ml of 8.0 mM L-leucine β-naphthylamide hydrochloride at 37°C for 1 h. The extracted β-naphthylamine is converted to an azo compound by reacting with *p*-dimethylaminocinnamaldehyde and the absorbance measured at 540 nm.

The assay methods for determining the activities of the amidohydrolases are described by Tabatabai (1994) and Senwo and Tabatabai (1996). The methods are based on the determination of NH_4^+-N released when soil is incubated with THAM buffer, L-asparagine, L-glutamine, L-aspartic acid, or formamide, and toluene at 37°C for 2 h (for asparaginase, glutaminase and amidase) and 24 h (for aspartase). The NH_4^+-N released was determined by treating the incubated soil samples with 2 M KCl

Table 2. The methods used for the assay of the activities of arylamidase and amidohydrolases.

Recommended name	EC number	Reaction	Substrate concentration[a]	Optimum pH	Reference
Arylamidase	3.4.11.2	L-Leucine β-naphthylamide → L-Leucine + β-naphthylamine	L-Leucine β-naphthylamide (2.0 mM)	8	Acosta-Martinez and Tabatabai (2000a)
L-Asparaginase	3.5.1.1	L-Asparagine + H_2O → L-aspartate + NH_3	L-Asparagine (50 mM)	10	Tabatabai (1994)
L-Glutaminase	3.5.1.2	L-Glutamine + H_2O → L-glutamate + NH_3	L-Glutamine (50 mM)	10	Tabatabai (1994)
Amidase	3.5.1.4	R-$CONH_2$ + H_2O → NH_3 + R-COOH	Formamide (50 mM)	8.5	Tabatabai (1994)
L-Aspartase	4.3.1.1	L-Aspartase + H_2O → Fumarate + NH_3	L-Aspartase (200 mM)	8.5	Senwo and Tabatabai (1996)

[a]Figures in parentheses are the substrate concentrations under assay conditions.

containing Ag_2SO_4 (to stop enzyme activity) and steam distillation of an aliquot of the resulting soil suspension with MgO for 4 min (Mulvaney 1996).

The activities of arylamidase and amidohydrolases were assayed on <2 mm field-moist samples at the optimal pH values in duplicates and one control and are expressed on a moisture-free basis. Substrates were added to the controls after incubation and termination of the enzyme reactions, and moisture content was determined from weight loss after drying at 105°C for 48 h. The methods used for the assay of the activities of the enzymes are summarized in Table 2.

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the differences in enzyme activities among the soils. Duncan's multiple range tests were performed to show a significant difference between the means. Differences were considered significant at the $P < .05$ level. Regression analysis was used to evaluate the relationship between enzyme activities and estimated N_{max} values. All statistical analyses were conducted using the general linear model procedures in SAS system (SAS Institute 1996).

Results

Arylamidase and amidohydrolases activities and N mineralization

Arylamidase activity was significantly ($P \leq 0.05$) different among the soils used, with values varying from 30 to 143 mg β-naphthylamide kg^{-1} soil h^{-1} (Table 3). Similarly, the activities of the

Table 3. Activities of arylamidase and amidohydrolases in the soils.

	Enzyme activity[a]				
Soil	Arylamidase	L-Asparaginase	L-Glutaminase	Amidase	L-Aspartase
Canisteo	143 d	76 g	359 def	189 c	22 g
Clarion (I)	132 d	19 a	207 a	142 a	6 a
Coland	57 bc	53 e	327 bcde	241 e	18 f
Crippin	49 b	34 bc	196 a	226 de	9 c
Harps	59 bc	52 e	351 def	181 bc	13 d
Nicollet	68 c	32 e	313 bcd	176 bc	10 c
Okoboji	72 c	46 d	346 cde	195 c	10 c
Storden	30 a	43 d	300 bcd	217 d	10 c
Terrill	68 c	40 cd	284 bc	158 ab	10 c
Webster (I)	70 c	63 f	378 ef	173 bc	16 e
Clarion (II)	196 e	84 h	446 g	388 f	22 g
Grundy	70 c	36 bc	274 b	186 c	8 b
Webster (II)	142 d	76 g	409 fg	381 f	18 f

[a]Except for arylamidase activity which is expressed in mg β-naphthylamine kg^{-1} soil h^{-1}, all other enzyme activities are expressed in mg NH_4^+-N kg^{-1} soil h^{-1}. Within each column, values followed by the same letter(s) are not significantly different at the $P < 0.05$ level.

Table 4. Correlation among the activities of arylamidase and amidohydrolases.

Enzyme activity[a]	Amidase	Glutaminase	Asparaginase	Aspartase
		Correlation coefficient (r)[b]		
Amidase	–			
Glutaminase	0.62	–		
Asparaginase	0.70	0.89	–	
Aspartase	0.63	0.81	0.94	–
Arylamidase	0.58	0.50	0.59	0.59

[a]Except for arylamidase activity which is expressed in mg β-naphthylamine kg^{-1} soil h^{-1}, all other enzyme activities are expressed in mg NH_4^+-N kg^{-1} soil h^{-1}.
[b]*r*-values greater than 0.55, 0.68, or 0.80 are significant at $P < 0.5\%$, $P < 0.1\%$ or $P < 0.01\%$, respectively.

amidohydrolases were significantly ($P \leq 0.05$) different among the soils used, with glutaminase activity being the dominant of the four enzymes studied, followed by amidase, asparaginase, and aspartase (Table 3). The activities of the amidohydrolases ranged from 196 to 446 mg N kg^{-1} soil h^{-1}, 142–388 mg N kg^{-1} soil h^{-1}, 19–76 mg N kg^{-1} soil $h^{-1,}$ and 6–22 mg N kg^{-1} soil h^{-1} for glutaminase, amidase, asparaginase, and aspartase, respectively (Table 3). The activities of the amidohydrolases were significantly and positively correlated with each other (Table 4). Except for glutaminase, the activities of arylamidase and amidohydrolases were positively and significantly correlated (Table 4). The activities of glutaminase and asparaginase were positive and significantly correlated with TC and N_{mic}; the activities of arylamidase and the rest of the amidohydrolases were positively but insignificantly correlated with TC, TN, ON and C_{mic} contents of the soils. The activities of arylamidase and amidohydrolases were negatively correlated with the NH_4^+-N and NO_3^-N contents of the soils.

Kinetic parameters of ON hydrolysis and activities of the enzymes

The estimated N_{max} values for KOH (N_{max}-KOH) and NaOH (N_{max}-NaOH) were positively and significantly correlated with the activities of the amidohydrolases, with amidase being the most highly correlated with the kinetic parameters of the alkaline hydrolysis process (Figure 1). In general, estimated N_{max}-KOH values were superiorly correlated with the activities of the amidohydrolases studied compared with N_{max}-NaOH (Figure 1). The r values between N_{max}-KOH and amidohydrolases were 0.84 ($P \leq 0.001$), 0.66 ($P \leq 0.05$), 0.66 ($P \leq 0.05$), and 0.74 ($P \leq 0.01$) for amidase, glutaminase, aspartase and asparaginase, respectively, and that for the relationship between N_{max}-NaOH and the amidohydrolases were 0.64 ($P \leq 0.05$), 0.60 ($P \leq 0.05$), 0.62 ($P \leq 0.05$) and 0.61 ($P \leq 0.05$), respectively (Figure 1). Similarly, the activity of arylamidase was positively and significantly ($P \leq 0.05$) correlated with N_{max}-KOH and N_{max}-NaOH, with the correlation with N_{max}-KOH being superior compared to N_{max}-NaOH; the r values were 0.75 ($P \leq 0.01$) and 0.58 ($P \leq 0.05$) for N_{max}-KOH and N_{max}-NaOH, respectively.

Discussion

The dominance of glutaminase activity among the amidohydrolases studied is consistent with the results of other authors who reported greater glutaminase activity compared with the activities of other enzymes involved in the hydrolysis of ON compounds in soils (Acosta-Martinez and Tabatabai 2000b; Dodor and Tabatabai 2003; Ekenler and Tabatabai 2004a). This trend in the activities of the amidohydrolases is supported by studies on acid hydrolysis of humic preparations which have shown that 7–13% of the total N in soils can be present in the form of amide-N, and that most of the NH_4^+-N released from hydrolysis of organic matter was from glutamic and aspartic acid-N derived from glutamine and asparagine (Bremner 1955; Sowden 1958). The positive and significant correlation among the activities of the amidohydrolases suggests that they are hydrolyzing the same amide bonds in SON. The results of the present study agree with those of other authors who reported close relationship among the activities of amidohydrolases in soils (Deng, Moore, and Tabatabai 2000; Dodor and Tabatabai 2003; Tabatabai, Ekenler, and Senwo 2010).

The observed negative correlation between the activities of arylamidase and amidohydrolases and the NH_4^+-N and NO_3^-N content of the soils can be attributed to negative feedback repression. Similar observation was made by Dick, Rasmussen, and Kerle (1988) who showed that increasing the rate of ammonia-based N fertilizer decreased the activities of amidase and urease, both enzymes are involved in N cycle. The authors hypothesized feedback mechanisms as the mean by which the production of the enzymes was suppressed. McCarty, Shogren, and Bremner (1992) later showed that NH_4^+ represses urease activity, but that the effect was indirect due to the by-products of NH_4^+ assimilation by microoganisms.

It is known that arylamidase catalyzes the hydrolysis of N-terminal amino acids from arylamides to release amino acids, which are then hydrolyzed by one of a number of specific amidohydrolases, depending on the amino acid moiety released, to produce NH_4^+ (Acosta-Martinez and Tabatabai

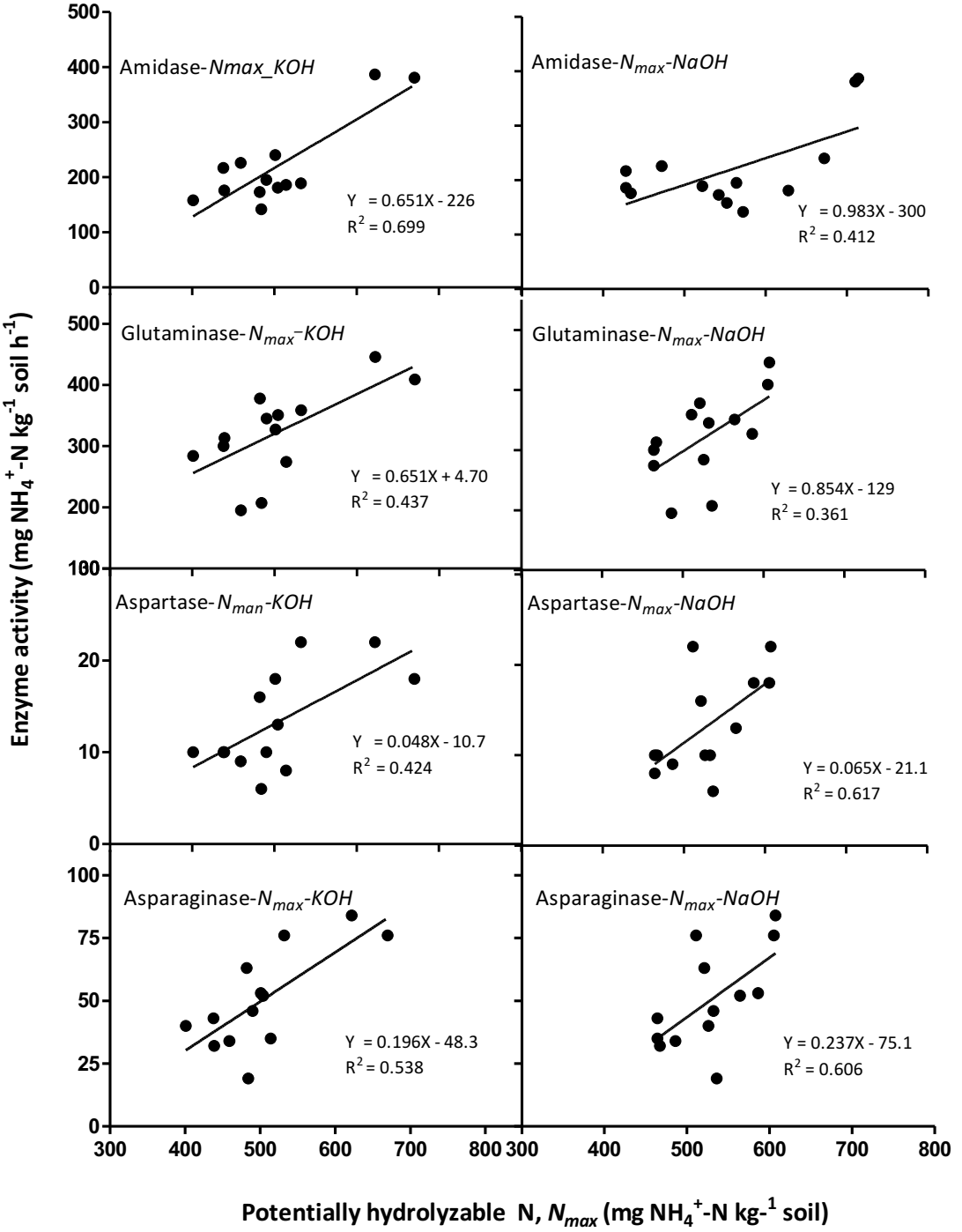


Figure 1. Relationship between activities of amidohydrolases and the estimated alkaline hydrolyzable N contents of the soils. R^2-values greater than 0.303, 0.462, and 0.640 are significant at $P < 0.5$, $P < 0.01$, and $P < 0.001$%, respectively.

2000b; Dodor and Tabatabai 2002, 2007). Therefore, the positive relationship between the activities of arylamidase and amidohydrolases indicates that the activities of the two groups of enzymes are coupled in hydrolyzing SON sequentially. This finding is significant because it has been suggested that the activities of arylamidase and amidohydrolases represent the soil's capacity to hydrolyze SON

to release inorganic N (Acosta-Martinez and Tabatabai 2000b; Dodor and Tabatabai 2002, 2003, 2007). Furthermore, previous studies have also suggested that the activities of arylamidase and amidohydrolases may be the rate-limiting factors in ON mineralization in soils (Cantarella and Tabatabai 1983; Dodor and Tabatabai 2002, 2007). The results of the present study suggest that the activities of arylamidase and amidohydrolases can be used to predict N mineralization in soils.

Nitrogen mineralization in soil is mediated by soil microorganisms through the action of enzymes in close association with microbial biomass (Dodor and Tabatabai 2002; Klose and Tabatabai 2000). Therefore, the significant relationship between estimated N_{max} values and the activities of the amidohydrolases, especially amidase, suggests that the alkaline reagents (NaOH and KOH) are hydrolyzing mainly amide-N. The results are consistent with studies on the characterization of active N pools in soils under different cropping systems showing that N mineralization is predominantly controlled by the activities of soil enzymes, including amidase, which plays an important role in N mineralization in soils (Deng, Moore, and Tabatabai 2000). Working with soils from different cropping systems and N fertilizer regimes, Dodor and Tabatabai (2007) and Tabatabai, Ekenler, and Senwo (2010) also reported a significant correlation between arylamidase activity and the amounts of N mineralized during 24 weeks of incubation at 30°C.

Other studies evaluating the relationship between indices of N mineralization and the activities of enzymes in soils from six agroecological zones of the north-central region of USA indicated that the amounts on N mineralized using various chemical and biological indices of plant-available N were positively and significantly correlated with the activity of N-acetyl-β-D-glucosaminidase, which catalyzes the hydrolysis of N-acetyl-β-D-glucosamine residues in chitooligosacharides in soils (Ekenler and Tabatabai 2004b). Results of the present study together with those of other authors (Burket and Dick 1998; Burton and McGill 1992; Zaman et al. 1999) suggest that mineralization of ON to release inorganic N is mediated by the activities of enzymes, including arylamidase and amidohydrolases.

The specific nature of ON amenable to hydrolysis by the alkaline reagents was not evaluated in the present study. Based on the specificity of enzyme reactions, however, the strong relationship between estimated N_{max} values and the activities of arylamidase and amidohydrolases suggests that similar N bonds were susceptible to enzymatic and alkaline hydrolysis. This supposition is supported by information on organic matter hydrolysis showing that majority of ON in soils is amides (Sowden 1958; Bremner, 1955). Furthermore, a variety of amino acid-associated linear and aryl-amides are believed to be present in SON (Stevenson, 1994), the mineralization or hydrolysis of which can release mineral N into soils.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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