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Enzyme activities in a limed agricultural soil

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Abstract This study assessed the effect of eight lime application rates, with four field replications, on the activities of 14 enzymes involved in C, N, P, and S cycling in soils. The enzymes were assayed at their optimal pH values. The soil used was a Kenyon loam located at the Northeast Research Center in Nashua, Iowa. Lime was applied in 1984 at rates ranging from 0 to 17,920 kg effective calcium carbonate equivalent (ha^{-1}), and surface samples (0–15 cm) were taken after 7 years. Results showed that organic C and N were not significantly affected by lime application, whereas the soil pH was increased from 4.9 to 6.9. The activities of the following enzymes were assayed: α- and β-glucosidases, α- and β-galactosidases, amidase, arylamidase, urease, L-glutaminase, L-asparaginase, L-aspartase, acid and alkaline phosphatases, phosphodiesterase, and arylsulfatase. With the exception of acid phosphatase, which was significantly ($P<0.001$) but negatively correlated with soil pH ($r=-0.69$), the activities of all the other enzymes were significantly ($P<0.001$)and positively correlated with soil pH, with r values ranging from 0.53 for the activity of α-galactosidase to 0.89 for alkaline phosphatase and phosphodiesterase. The Δ activity/Δ pH values ranged from 4.4 to 38.5 for the activities of the glycosidases, from 1.0 to 107 for amidohydrolases and arylamidase, 97 for alkaline phosphatase, 39.4 for phosphodiesterase, and 11.2 for arylsulfatase. This value for acid phosphatase was -35.0. The results support the view that soil pH is an important indicator of soil health and quality.

Key words Soil quality · Glycosidases · Amidohydrolases · Arylamidase · Phosphatases

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Introduction

Application of lime to soils normally leads to significant increases in pH and, thus, in the chemical and biochemical reactions and in microbiological processes. Such treatments result in changes in the solubility of many chemical compounds and improvement in the environment of plant roots and development (Naftel 1965), increasing soil microbial biomass (Edmeades et al. 1981), including microbial dynamic and diversity, and, therefore, significant changes in enzyme activities (Zelles et al. 1987a,b, 1990; Bardgett and Leemans 1995).

Recent interest in defining soil quality has focused on identifying soil properties that affect soil health and quality (Doran et al. 1994). It has been proposed that measurement of changes in soil enzyme activities may provide a useful index of changes in soil quality (Dick 1992; Visser and Parkinson 1992). Previous studies with limed soils have focused mostly on the changes of the activity of acid phosphatases in forest soils, because of the positive correlation between phosphate availability and soil pH (Haynes and Swift 1988; Cepeda et al. 1991; Illmer and Schinner 1991). Even though pH is considered one of those important properties affecting soil health and quality, its role in modifying enzymatic reactions in soil has not been demonstrated clearly, i.e., with many enzymes involving a range of soil pH. It is important to obtain a complete assessment of soil enzyme activities that reflect the changes in soil metabolic processes by using different biochemical reactions involved in nutrient cycling in soils. Therefore, the objective of this study was to assess the effect of liming of an agricultural soil on the activities of 14 enzymes involved in C, N, S and P cycling in soils. All 14 enzymes were assayed at their optimal pH values. The activities of the following enzymes were studied: α- and β-glucosidases, α- and β-galactosidases, amidase, arylamidase, urease, L-glutaminase, L-asparaginase, L-aspartase, acid and alkaline phosphatases, phosphodiesterase and arylsulfatase.

Materials and methods

Experimental design

The soil used was a Kenyon loam (fine-loamy, mixed, mesic Typic Hapludoll). The experimental site was established in 1984 at the Northeast Research Center in Nashua, Iowa. Agricultural limestone from a local quarry was broadcast at the following rates: 0, 1120, 2240, 4480, 6720, 8960, 13,440, and 17,920 kg effective calcium carbonate equivalent (ECCE) ha^{-1}. Treatments were arranged in a randomized complete block design with four field replicates. The size of each field plot was 6×15 m. Corn (*Zea mays* L.) and soybean (*Glycine max* L.) were grown in alternate years, with periodic applications of fertilizers to maintain high nutrient levels of N, P, and K.

Sampling and laboratory analyses

Soil surface samples (0 to 15 cm) were taken after corn harvest from all the field replicates 7 years after lime application by pooling 6–8 core samples (7.6 cm diameter). The samples were air-dried for 48 h at room temperature (22 °C) and ground to pass a 2-mm sieve. Samples for chemical analyses such as organic C and organic N were ground to pass an 80-mesh (180 μm) sieve. Organic C was determined by the Mebius method (1960) and total N by a semimicro-Kjeldahl method (Bremner and Mulvaney 1982). The pH values were measured on the <2 mm soil samples by using a glass combination electrode (soil: water or 0.01 M $CaCl_2$ ratio, 1:2.5). The activities of the enzymes were assayed on the <2 mm air-dried samples at their optimal pH values in duplicates and one control, and are expressed on a moisture-free basis. Moisture was determined after drying at 105 °C for 48 h. The assay methods used are summarized in Table 1. For all data points reported in the figures, the differences between the laboratory duplicate were smaller than the point size.

Results and discussion

To demonstrate the effect of soil pH on enzyme activities, it is essential that the percentages of organic C and N remain constant. Results showed that this condition was satisfied, and soil pH was significantly increased after 7 years of the lime application, from 4.9 in the control to 6.9 in the plots treated with the highest lime application (Table 2).

Table 1 The methods used for assay of enzyme activities in soils

Class/EC number	Recommended name[a]	Reaction	Assay conditions Substrate[b]	Assay conditions Optimum pH
Glycosidases				
3.2.1.20	α-Glucosidase	Glucoside-R + H_2O → glucose + R-OH	*p*-Nitrophenyl-α-D-glucopyranoside (10 mM)	6.0
3.2.1.21	β-Glucosidase	Glucoside-R + H_2O → glucose + R-OH	*p*-Nitrophenyl-β-D-glucopyranoside (10 mM)	6.0
3.2.1.22	α-Galactosidase	Galactoside-R + H_2O → galactose + R-OH	p-Nitrophenyl-β-D-galactopyranoside (10 mM)	6.0
3.2.1.23	β-Galactosidase	Galactoside-R + H_2O → galactose + R-OH	p-Nitrophenyl-β-D-galactopyranoside (10 mM)	6.0
Amidohydrolases and arylamidase				
3.4.11.2	Arylamidase[c]	L-Leucine β-naphthylamine → L-leucine + β-naphthylamine	L-Leucine β-naphthylamine (2.0 mM)	8.0
3.5.1.1	L-Asparaginase	L-Asparagine + H_2O → L-aspartate + NH_3	L-Asparagine (50 mM)	10.0
3.5.1.2	L-Glutaminase	L-Glutamine + H_2O → L-glutamate + NH_3	L-Glutamine (50 mM)	10.0
3.5.1.4	Amidase	R-$CONH_2$ + H_2O → NH_3 + R-COOH	Formamide (50 mM)	8.5
3.5.1.5	Urease	Urea + H_2O → CO_2 + $2NH_3$	Urea (20 mM)	9.0
4.3.1.1	L-Aspartase[d]	L-Aspartate + H_2O → L-aspartic acid + NH_3	L-Aspartate (200 m*M*)	8.5
Phosphatases				
3.1.3.1	Alkaline phosphatase	RNa_2PO_4 + H_2O → R-OH + Na_2HPO_4	*p*-Nitrophenyl phosphate (10 mM)	11.0
3.1.3.2	Acid phosphatase	RNa_2PO_4 + H_2O → R-OH + Na_2HPO_4	*p*-Nitrophenyl phosphate (10 mM)	6.5
3.1.4.1	Phosphodiesterase	R_2NaHPO_4 + H_2O → R-OH + $RNaHPO_4$	*Bis*-*p*-Nitrophenyl phosphate (10 mM)	8.0
Sulfatase				
3.1.6.1	Arylsulfatase	$ROSO_3^-$ + H_2O → R-OH + H^+ + SO^{2-}_4	*p*-Nitrophenyl sulfate (10 m*M*)	5.8

[a] For the methods used, see Tabatabai (1994)
[b] Figures in parentheses are the substrate concentrations under assay conditions
[c] Acosta-Martinez and Tabatabai (1999)
[d] Senwo and Tabatabai (1996)

Table 2 Effect of lime application rates on selected soil chemical properties

Lime treatment	Chemical property		
	pH[a]	Organic C	Organic N
kg ECCE ha^{-1}		g kg^{-1} soil	g kg^{-1} soil
0	4.6–5.5 (4.9)[b]	14.2–15.6 (15.0)	1.2–1.4 (1.3)
1120	4.7–5.8 (5.1)	14.7–15.3 (15.0)	1.3–1.3 (1.3)
2240	5.1–5.8 (5.1)	14.8–17.2 (15.0)	1.2–1.4 (1.3)
4480	5.3–6.2 (5.7)	15.1–15.6 (15.3)	1.3–1.3 (1.3)
6720	6.1–6.7 (6.4)	14.7–15.8 (15.2)	1.2–1.4 (1.3)
8960	6.4–6.8 (6.6)	15.3–16.5 (15.8)	1.3–1.5 (1.4)
13440	6.2–6.9 (6.6)	14.9–16.0 (15.3)	1.3–1.4 (1.3)
17920	6.7–7.0 (6.9)	14.5–16.3 (15.1)	1.2–1.4 (1.3)
LSD$P<0.05$	0.5	NS	NS

[a] Soil:water or soil: 0.0 M $CaCl_2$ solution ratio, 1:2.5
[b] Values in parentheses are averages of soil samples from four replicate field plots

Activities of glycosidases

Glycosidases have been named according to the types of bond that they hydrolyze (Table 2). It is important to measure the activities of these enzymes in limed agricultural soils because this group of enzymes plays an important role in the degradation of organic C compounds (e.g., crop residues, biotechnology by-products, animal manure, sewage sludge) in soils (Ajwa and Tabatabai 1994; Martinez and Tabatabai 1997), and their hydrolysis products (sugars) are important energy sources for microorganisms in soils.

In this study, β-glucosidase was the most predominant of the soil glycosidases followed by β-galactosidase, α-galactosidase and α-glucosidase (Fig. 1). β-Glucosidase activity values ranged from 87 mg *p*-nitrophenol kg^{-1} soil h^{-1} in the control plots to 200 mg *p*-nitrophenol kg^{-1} soil h^{-1} in the treatments under the two highest lime application rates (13,440 and 17,920 kg ECCE ha^{-1}). The values of β-glucosidase activity were seven times greater than the values of the least predominant glycosidase (α-glucosidase). The predominance of β-glucosidase in soil has been well documented (Eivazi and Tabatabai 1988; Tabatabai 1994). The activities of the soil glycosidases were significantly correlated with soil pH. The correlation coefficient was the greatest for β-glucosidase ($r=0.87$, $P<0.001$), followed by β-galactosidase ($r=0.81$, $P<0.01$), α-glucosidase ($r=0.56$, $P<0.001$) and α-galactosidase ($r=0.53$, $P<0.05$). Other studies involving many soils reported no such correlation (Dick et al. 1988; Eivazi and Tabatabai 1990). Those studies, however, compared the activities of the glycosidases in different soils where the interaction of other intrinsic soil properties may have been involved.

The results of the current study demonstrate that the significant increases in the soil pH due to lime applications led not only to an increase in the activity of β-glucosidase but also an increase in the activity of the

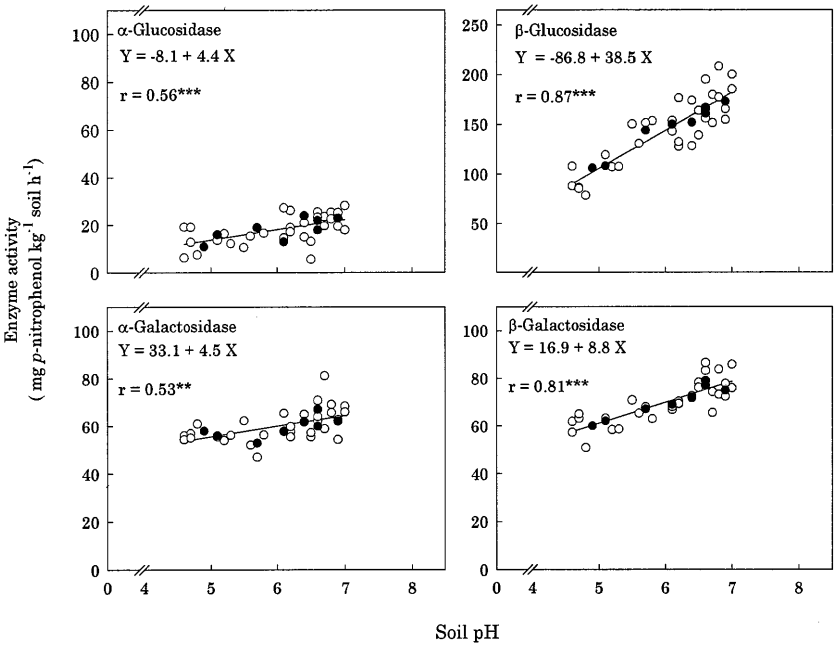

Fig. 1 Effect of soil pH on the activities of the glycosidases studied. *Open symbols* are results obtained for individual soil samples and *solid symbols* are averages obtained for the four field replicates. In calculating the regression equations, the results of the individual soil samples were used

less predominant soil glycosidases (α-glucosidase, and β- and α-galactosidase). To demonstrate the sensitivity of the activities of the glycosidases to changes to soil pH, we calculated the Δ activity/Δ pH ratios for the individual glycosidase activities (Table 3). The values were 38.5 for β-glucosidase, 8.8 for β-galactosidase, 4.5 for α-galactosidase, and 4.4 for α-glucosidase. Thus, β-glucosidase is the most sensitive to soil pH changes and should be a good biochemical indicator for measuring ecological changes resulting from soil acidification.

Activities of amidohydrolases and arylamidase

The assessment of the activities of the enzymes involved in N cycling in soils may provide more insight into the N mineralization cycle as each enzyme catalyzes a specific reaction (Tabatabai 1994). To our knowledge, little information is available on the effect of liming on the activities of those enzymes in soils. In addition to the well-known amidohydrolases, we studied the effect of liming on the activity of arylamidase, α-aminoacylpeptide hydrolase, in soils (Acosta-Martínez and Tabatabai 1999). Therefore, we studied the effect of liming on the activities of five enzymes involved in organic N hydrolysis in soils. Results showed that L-glutaminase activity was the most predominant of the N cycling enzymes, followed by the activities of amidase, L-asparaginase, arylamidase, urease and L-aspartase. The activity of L-glutaminase ranged from 160 in the control to 840 mg NH_4-N released kg^{-1} soil $2h^{-1}$ in the treatment under the highest lime application rate (17920 kg ECCE ha^{-1}). These results support the previous finding that most of the NH_4^+ released in soils is derived from the hydrolysis of amide (asparagine, aspartate and glutamine) residues in soil organic matter (Sowden 1958). The activities of these soil enzymes were significantly correlated ($P<0.001$) with soil pH (Fig. 2). L-Asparaginase showed the greatest correlation coefficient ($r=0.84$, $P<0.001$), followed by L-aspartase ($r=0.80$, $P<0.001$), arylamidase ($r=0.74$, $P<0.001$), L-glutaminase ($r=0.73$, $P<0.001$), urease ($r=0.71$, $P<0.001$), and amidase ($r=0.61$, $P<0.001$)

Table 3 Correlation coefficients (r) and slopes of linear relationships between soil pH and enzyme activities

Enzyme	Enzyme activity vs soil pH[a]	Δ Enzyme activity[b]/ Δ soil pH
	r	
Glycosidases		
α-Glucosidase	0.56***	4.4
β-Glucosidase	0.87***	38.5
α-Galactosidase	0.53**	4.5
β-Galactosidase	0.81***	8.8
Amidohydrolases and arylamidase		
Arylamidase	0.74***	9.0
L-Asparaginase	0.84***	15.8
L-Glutaminase	0.73***	107
Amidase	0.61***	14.4
Urease	0.71***	6.2
L-Aspartase	0.80***	1.0
Phosphatases		
Alkaline phosphatase	0.89***[c] (0.95***)[d]	97.0[c] (25.6)[d]
Acid phosphatase	–0.69***	–35.0
Phosphodiesterase	0.89***[c] (0.91***)[d]	39.4[c] (12.1)[d]
Sulfatase		
Arylsulfatase	0.66***	11.2

[a] $**P<0.01$, $***P<0.001$
[b] All activities are expressed in mg product released kg^{-1} soil h^{-1}
[c] Linear regression for soils with pH>6.0
[d] Second linear regression values

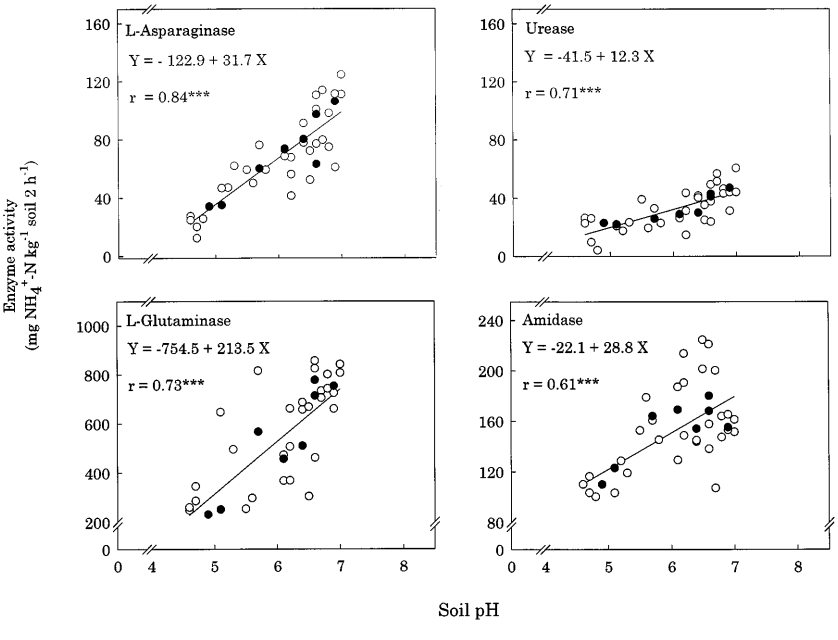

Fig. 2 Effect of soil pH on the activities of L-asparaginase, urease, L-glutaminase and amidase. For the symbols and regression equation, see the caption of Fig. 1

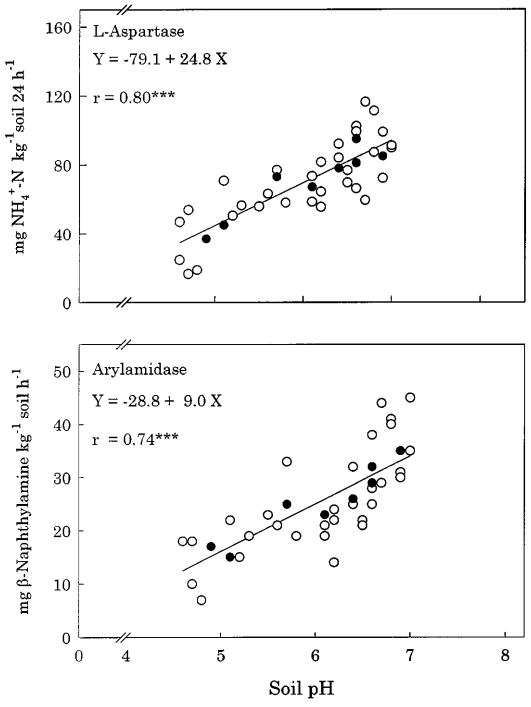

Fig. 3 Effect of soil pH on the activities of L-aspartase and arylamidase. For the symbols and regression equation, see the caption of Fig. 1

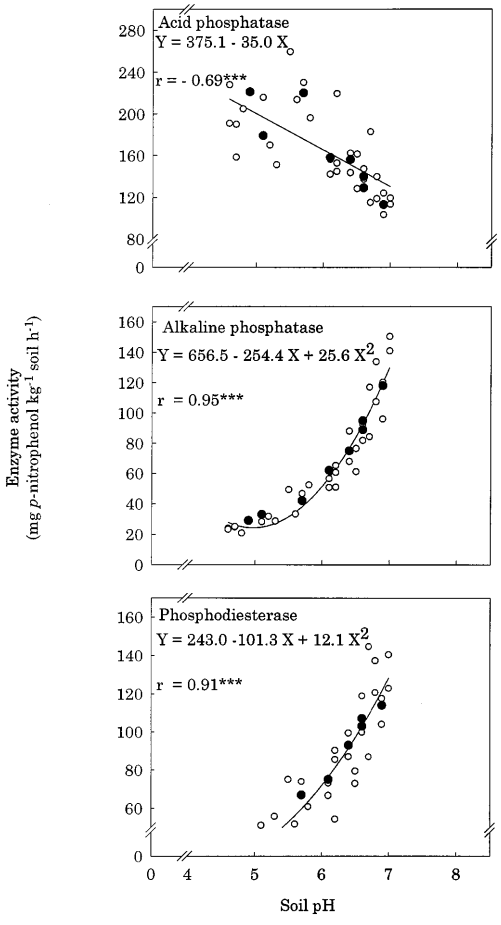

Fig. 4 Effect of soil pH on the activities of phosphatases. For the symbols and regression equation, see the caption of Fig. 1

(Figs. 2, 3). The Δ activity/Δ soil pH ratios were 107 for L-glutaminase, 15.8 for L-asparaginase, 14.4 for amidase, 9.0 for arylamidase, 6.2 for urease and 1.0 for L-aspartase. Results showed that L-glutaminase was not only the most sensitive enzyme to soil pH changes among those involved in N cycling but also the most sensitive enzyme among the 14 enzymes included in this study (Table 3).

Activities of phosphatases and arylsulfatase

Phosphatases is a general name for a broad group of enzymes that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Schmidt and Laskowski 1961). The study of this group of enzymes was important because the soil pH was significantly affected by the lime application, and because it has been suggested that the rates of synthesis, release, and stability of acid and alkaline phosphatases by soil microorganisms are dependent on the soil pH (Eivazi and Tabatabai 1977; Juma and Tabatabai 1977; Tabatabai 1994). Results showed that acid phosphatase was predominant in the control plots (pH 4.9) and that liming increased the activities of the alkaline phosphatase and phosphodiesterase (Fig. 4). These results support the findings of Juma and Tabatabai (1978) that acid phosphatase is predominant in acid soils and that alkaline phosphatase is predominant in alkaline soils. The correlation coefficient between the activities of acid phosphatase or alkaline phosphatase or phosphodiesterase and soil pH were -0.69, 0.89 and 0.89 respectively (each $P < 0.001$), (Table 3). The increase in the activity of alkaline phosphatase may demonstrate the effect of lime applications on the size of the soil microbial population as this enzyme is not present in higher plants, and, thus, its activity is derived totally from microorganisms (Dick et al. 1983; Juma and Tabatabai 1988a, b, c).

The different response of acid and alkaline phosphatases to lime application supports the previous findings that phosphatases are inducible enzymes and the intensity of their excretion by plant roots and microorganisms is determined by their requirement for orthophosphate, which is affected by soil pH (Skujins 1976). The Δ activity/Δ soil pH ratios for the phosphatases were 97 for alkaline phosphatase, 39.4 for phosphodiesterase, and -35.0 for acid phosphatase (Table 3).

Arylsulfatase is the enzyme involved in the hydrolysis of arylsulfate by fission of the O–S bond (Spencer 1958). This enzyme is believed to be involved in mineralization of ester sulfate in soils (Tabatabai 1994). The means of activity of this enzyme in the four field replicates ranged from 92 to 114 mg of *p*-nitrophenol released kg^{-1} soil h^{-1} in the control plots and at the high-

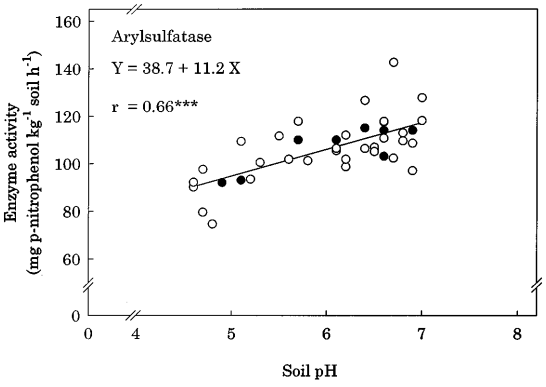

Fig. 5 Effect of soil pH on the arylsulfatase activity. For the symbols and regression equation, see the caption of Fig. 1

est rate of lime application, respectively. The activity of this enzyme was significantly correlated with soil pH ($r=0.66$, $P<0.001$) (Fig. 5). The Δ activity/Δ soil pH ratios for arylsulfatase was 11.2 (Table 3).

Conclusions

The significant increase in soil pH by lime applications may stimulate the microbial population and diversity, resulting in an increase in the soil enzyme activities and, thus, affecting nutrient cycling. With the exception of acid phosphatase activity, which decreased with increasing soil pH, the activities of the other 13 enzymes studied increased with increasing soil pH. The sensitivity of the enzyme activities to soil pH varied considerably. The order of the sensitivity of enzymes to soil pH was as follows: L-glutaminase > alkaline phosphatase > phosphodiesterase > β-glucosidase > acid phosphatase > L-asparaginase > amidase > arylsulfatase > arylamidase > β-galactosidase > urease > α-galactosidase > α-glucosidase > L-aspartase. The results provide information on important biochemical reactions that have potential as early and sensitive indicators to soil stress or health and quality.

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