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## Soil acid and alkaline phosphatase activity as pH adjustment indicators

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### Abstract

Soil fertility and crop production are affected by biological processes and these processes, including enzyme activities, are influenced by pH. We investigated the potential of using alkaline phosphatase (AlkP) and acid phosphatase (AcdP) activities, for determining the optimum soil pH for crop production and the amount of lime required to achieve this optimum. Five acid soils, which varied widely in selected properties, were treated with CaCO<sub>3</sub> at rates of 0, 0.2, 0.5, 1.0 and 2.0 × the soil's lime requirement needs. To remove soil variations in absolute enzyme activity values, an AlkP/AcdP activity ratio was used to test soil response. The ratios of AlkP/AcdP responded immediately to the changes in pH caused by CaCO<sub>3</sub> additions and an AlkP/AcdP ratio of approximately 0.5 divided soils into those with appropriate pH adjustment and those still needing additional lime treatment. However, incubation of the lime-treated soils for 67 days followed by treating the soils with organic amendments (which included finely ground chicken manure and alfalfa residues) increased the AlkP/AcdP ratios to approximately 3.0. For cropping systems that rely heavily on natural biological processes to maintain productivity, measuring the AlkP/AcdP ratio may be preferable to chemical approaches for evaluating effective soil pH and liming needs. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:* Soil enzymes; Soil liming; Lime test index; Soil pH; Acid and alkaline phosphatase

### 1. Introduction

Soil enzymes serve several important functions. They are intimately involved in the cycling of nutrients, effect fertilizer use efficiency, reflect the microbiological activity in soil and act as indicators of soil change. The focus of much of the soil enzyme research has been to develop methodologies for their measurement and to provide an understanding of their origin and the factors that affect their activity in soil. Several reviews have been published summarizing this work (Dick and Tabatabai, 1992; Ruggiero et al., 1996; Gianfreda and Bollag, 1996). However, little work has been done to actually develop methods or technologies that utilize soil enzymatic activity data as inputs into soil fertility management decisions. Dick and Tabatabai (1992) reviewed the current status of work related to the potential uses of soil enzyme measurements and provided examples or made suggestions of ways various soil enzymes may be used to provide practical benefits to agriculture. We have continued to research ways that directly relate soil enzyme activity to crop production, or some other natural ecosystem

or agroecosystem function, with the goal being the development of useful technologies that are more biologically than chemically based.

One of the most important soil properties is pH. It affects availability of nutrients, controls the composition and diversity of the microbial community, alters the equilibrium solid phase and impacts plant response. Soil pH also affects the activity of enzymes due to the pH sensitivity of amino acid functional groups that alter conformational and chemical changes of amino acids essential for binding and catalysis. The pH can also affect enzyme activity by influencing the concentration of inhibitors or activators in the soil solution and the effective concentration of the substrate.

Emphasis is increasingly being turned away from a strictly chemical approach for assessing soil fertility and other important soil qualities. Instead, biological approaches are being sought for assessing soil processes related to crop production, soil quality and overall soil sustainability (Dick, 1994). For cropping systems that rely heavily on natural biological processes to maintain productivity, a biological approach for measuring important soil functions, such as pH, may be preferable to chemical approaches.

The sensitivity of soil enzymes to pH should make it possible to evaluate the effective pH status of the soil by

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Table 1  
Selected properties and lime requirements of soils

Parameter	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
Original soil pH	3.3	4.7	5.0	5.4	6.6
Lime requirement (Mg CaCO <sub>3</sub> ha <sup>-1</sup> )	36.5	27.3	11.7	11.7	2.40
CaCO <sub>3</sub> applied to achieve pH 7 (g kg <sup>-1</sup> soil)	13.6	10.1	4.35	4.35	0.89
Organic matter content (%)	5.8	31.2	3.0	3.7	2.4
Cation exchange capacity (cmol kg <sup>-1</sup> soil)	43	53	14	25	7
Extractable nutrients (mg kg <sup>-1</sup> soil)					
P	9	9	15	33	143
K	85	94	121	238	97
Ca	2590	5350	660	2470	1130
Mg	89	<25	92	307	103
Particle size analyses (%)					
Clay	29.6	– <sup>a</sup>	16.9	42.2	6.1
Silt	53.5	–	58.5	40.5	18.3
Sand	16.9	–	24.6	17.3	75.6
Water content in soil at 0.5 bar tension (%)	22.8	78.5	29.2	29.8	13.5
Acid phosphatase activity (μg <i>p</i> -nitrophenol g <sup>-1</sup> soil h <sup>-1</sup> ) <sup>b</sup>	44.0	82.3	206	140	70.1
Alkaline phosphatase activity (μg <i>p</i> -nitrophenol g <sup>-1</sup> soil h <sup>-1</sup> ) <sup>b</sup>	5.0	28.1	10.5	19.7	34.6

<sup>a</sup> Organic soil containing almost no mineral material.

<sup>b</sup> Enzyme activity of soil prior to treatment.

the presence or the relative activity of certain soil enzymes. The term effective is purposely used because a biological approach for measuring the pH status of soil integrates all of the chemical, biological and mineralogical properties of the soil. For example, acid phosphatase activity (AcdP) was found predominantly in acid soils and alkaline phosphatase activity (AlkP) in neutral or alkaline soils (Eivazi and Tabatabai, 1977; Dick and Tabatabai, 1984). An adequate level of pH for crop growth, therefore, may be defined as a pH at which a proper AlkP/AcdP activity ratio occurs. Also, the liming of an acid soil may only be considered adequate if the AlkP activity or the AlkP/AcdP ratio is adjusted to some predetermined value. Research is needed to establish this quantitative value.

The concept of using AlkP and AcdP activities to assess effective soil pH was previously proposed by Dick and Tabatabai (1992). The purpose of this study was to compare the liming response of a soil, as determined by a classical chemical procedure, to an enzymatic response, i.e. the responses of the alkaline and acid phosphatases.

## 2. Materials and methods

### 2.1. Soils and soil properties

Five soils varying in pH, organic matter content, particle size distribution and extractable nutrients (Table 1) were

collected to a depth of 20 cm, brought to the laboratory, screened through a 2-mm sieve and air-dried. The soils were analyzed for pH using a glass electrode and a soil to water ratio of 1:2.5, lime test requirement (i.e. amount of lime needed to reach a pH of 7.0) using the SMP buffer method (McLean, 1982), organic matter content by loss after ignition (Nelson and Sommers, 1996), cation exchange capacity (Rhoades, 1982), extractable nutrients (Rhoades, 1982) including P using HCl and NH<sub>4</sub>F (Olsen and Sommers, 1982), and particle size analyses by the pipette method (Kilmer and Alexander, 1949).

### 2.2. Experimental procedures

Rates of lime (finely ground reagent grade CaCO<sub>3</sub>) were 0, 0.2, 0.5, 1.0 and 2.0 × the lime requirement (LR) of each soil (Table 1). There were three replicates of each treatment. The lime was mixed thoroughly with 500 g of air-dried soil and placed into 1 l plastic bottles. The percentage of water in each soil (Table 1) was adjusted to reflect the amount held against 0.5 bars of suction (Cassell and Klute, 1986). The bottles were placed on the lab bench for incubation (22–24°C).

Soils were analyzed after 0, 2, 7, 14, 21, 28, 38, 53 and 67 days of incubation. At each time, soil was removed from the incubation bottles and pH determined using a glass electrode (1:2.5, soil/water ratio). Water content was checked by removing approximately 5–10 g (wet weight) of soil and

Table 2  
Changes in pH when soils were treated with CaCO<sub>3</sub> and incubated

Lime rate (×) <sup>a</sup>	Incubation time (days)	Soil				
		1	2	3	4	5
0	0	3.3	4.7	5.0	5.4	6.6
	67	3.0	4.8	4.7	5.4	6.7
	95 <sup>b</sup>	3.2	– <sup>c</sup>	5.1	5.5	6.4
0.2	0	4.2	5.0	5.7	6.0	6.8
	67	3.7	4.9	5.5	5.8	6.8
	95	4.0	–	5.7	5.9	6.4
0.5	0	5.7	5.4	6.9	6.9	7.2
	67	5.0	5.2	6.4	6.6	6.9
	95	5.2	–	6.5	6.6	6.4
1.0	0	6.9	6.1	7.4	7.4	7.4
	67	7.1	5.8	7.4	7.4	7.1
	95	7.2	–	7.5	7.5	7.6
2.0	0	7.0	7.0	7.4	7.5	7.5
	67	7.4	6.8	7.5	7.6	7.4
	95	6.8	–	7.6	7.6	7.5

<sup>a</sup> Lime rates added to each soil and how they were determined is provided in Section 2.

<sup>b</sup> Values are means for soils also treated with alfalfa residues or chicken manure. Difference between these two amendments were not significantly ( $P \leq 0.05$ ) different.

<sup>c</sup> The organic soil was not amended with alfalfa residues or chicken manure.

weighing it before and after oven-drying at 105°C for 24 h. The soil was then allowed to aerate, water content adjusted (if required), the caps replaced and incubation continued.

Alkaline and acid phosphatase activities were determined on 1 g (wet weight) aliquots of the soil removed from the bottles at each sampling time according to the method of Tabatabai (1982). Activity of the enzymes is expressed on a soil dry weight basis by correcting for water content in the soil at the time the sample was removed from the incubation bottle and is given in units of  $\mu\text{g } p\text{-nitrophenol produced g}^{-1} \text{ soil h}^{-1}$ .

On day 67, all soils except Soil 2 (the organic soil) were removed from the bottles and mixed with either alfalfa residue or chicken manure (air-dried and ground to pass a sieve with 1 mm openings) at a rate of 2 g per 400 g soil. The soil was then treated with water to achieve the required content and the samples were incubated at 22–24°C. Alkaline and acid phosphatase activity were measured as previously described, 1, 14 and 28 days after treating the soil with the organic amendments.

Data from each individual soil were analyzed using analysis of variance (ANOVA) and when treatment effects were significant at the 0.05 level of probability, means were separated using the LSD<sub>0.05</sub> value.

### 3. Results

Addition of the CaCO<sub>3</sub> to the soils caused an immediate change in pH (Table 2). However, the changes were small for the 0.2 × LR rate application treatment. A pH of 7.0 was not obtained until the 1.0 × LR application rate which is what should occur since the lime requirement rates were based on the amount of lime each soil needed to achieve a target pH of 7.0. The exception was the organic soil (Soil 2) which required a 2.0 × LR rate to achieve a pH of 7.0. Organic soils are highly buffered against pH changes and the SMP buffer method used to determine the soil's lime requirement (McLean, 1982) was not developed for organic soils. Soil 5 had a pH of 6.4 or higher for all treatments and at all times. Incubation of the five soils with the CaCO<sub>3</sub> for up to 67 days resulted in little additional change in pH and only the 67-day results are shown in Table 2.

As soil pH increased, AlkP activity increased and AcdP activity decreased and Fig. 1 shows AlkP/AcdP ratios after 0 and 67 days of incubation. The soils responded rapidly to the addition of CaCO<sub>3</sub>, except for Soil 1. The almost immediate changes (i.e. the day 0 results) in the ratios indicate that AlkP is present in these soils but it is not expressed until after the pH of the soil itself is changed by addition of CaCO<sub>3</sub>. Incubating the soils, originally acid, in the alkaline buffer used to measure AlkP activity did not seem to allow this activity to be expressed. The reasons for this are not known. At the same time, the CaCO<sub>3</sub> addition inhibited AcdP activity as application rate increased.

The AcdP/AlkP ratios were significantly higher ( $P \leq$

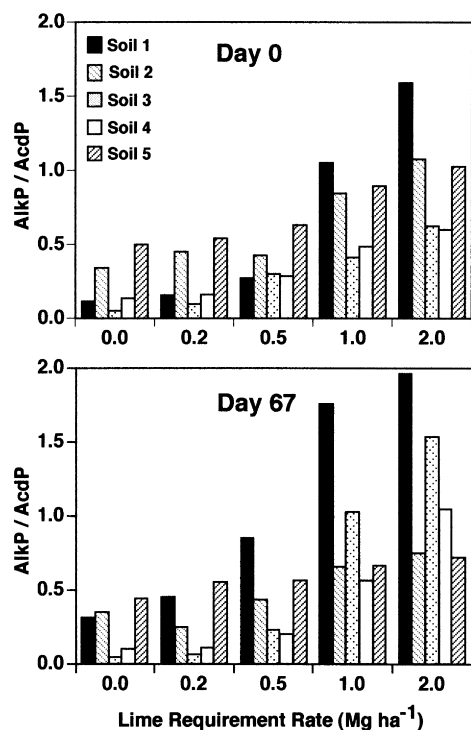


Fig. 1. Alkaline phosphatase/acid phosphatase (AlkP/AcdP) activity ratios at day 0 and day 67 after adjusting pH with CaCO<sub>3</sub> according to the lime requirement of each soil. LSD<sub>0.05</sub> values used for mean comparisons for Soils 1, 2, 3, 4 and 5 are 0.220, 0.137, 0.136, 0.084 and 0.087, respectively.

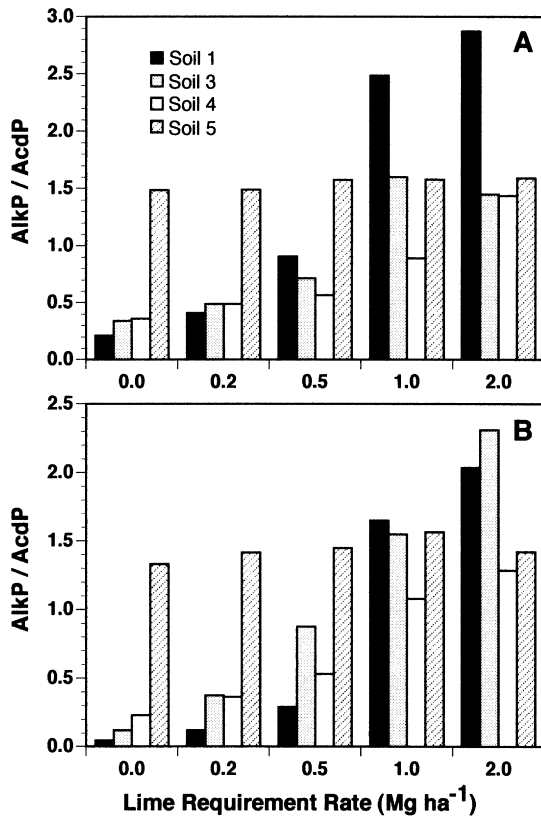


Fig. 2. Changes in alkaline phosphatase/acid phosphatase (AlkP/AcdP) activity ratios in soils amended with (A) chicken manure and (B) alfalfa residue after adjusting pH with CaCO<sub>3</sub> according to the lime requirement of each soil. LSD<sub>0.05</sub> values used for mean comparisons for Soils 1, 3, 4 and 5 are 0.277, 0.236, 0.215 and 0.341, respectively.

0.05) after 67 days of incubation compared to the day 0 ratios. Incubating the soils with CaCO<sub>3</sub> resulted in additional increases in AlkP activity and continued to decrease or cause inhibition of AcdP activity. This is not unexpected because the microbial population in the soil is sensitive to pH changes. Thus at day 67, the AlkP/AcdP ratios are a reflection of stabilized enzyme activity that exists in the soil and that of the newly formed microbial population that developed during the incubation period (Burns, 1982; Ruggiero et al., 1996).

Comparison of results in Table 2 and Fig. 1 indicates that, in general, a pH of approximately 6.0 was required to achieve an AlkP/AcdP ratio with a value greater than 0.5. This 0.5 ratio value separated soils with sufficient application of CaCO<sub>3</sub> and pH adjustment compared to those soils which still required additional pH adjustment. The one exception was Soil 2 where pH was sometimes below 6.0 but the AlkP/AcdP ratio remained at 0.5 or above. Soil 2 is an organic soil and such soils often can remain productive at more acid pH values than can mineral soils. This suggests that the enzymatic approach is probably a better method than the chemical one to determine the proper pH status of a soil, including high organic matter soils.

AlkP is thought to be primarily derived from microbial

sources and not from higher plant material (Tabatabai, 1994). Therefore, to stimulate microbial activity we decided to add ground alfalfa residue and chicken manure to soil and see what effect these organic additions have on the AlkP/AcdP ratios. Soil 2 was not used in this experiment because of the variability we described above. The addition of the alfalfa residues and chicken manure had little effect on the measured soil pH for the remaining four soils as the pH values for day 67 and day 95 were similar (Table 2). However, these organic additions made the AlkP/AcdP ratios even more sensitive to changes in soil pH with values of the ratios reaching almost to 3.0 (Fig. 2), whereas the highest value for the soils not treated with these organic amendments was approximately 1.9 (Fig. 1). Another observation was that Soil 5 now showed an enhanced AlkP/AcdP ratio even at the zero and 0.2 × LR rate. The 0.5 × LR rate also resulted in AlkP/AcdP ratios above 0.5 for Soils 3 and 4 when these soils were amended with chicken manure. Similarly, when alfalfa was added to the soils treated at the 0.5 × LR rate, the AlkP/AcdP ratios also approached the 0.5 ratio (Fig. 2). Assuming the 0.5 ratio level is the appropriate target ratio that reflects effective soil pH, as previously discussed, then this could be achieved with addition of lime at the 0.5 × LR rate combined with the application of an organic amendment. To confirm that the 0.5 ratio is, indeed, the proper AlkP/AcdP target ratio, field studies need to be conducted.

#### 4. Discussion

The question we were asking is how can soil enzymes be used to assess whether a soil has proper pH balance to provide for good crop growth? Traditionally, chemical methods are used for such purpose and many experiments have been conducted to relate laboratory results for testing lime requirements of a soil with crop responses (Sims, 1996). Our results indicated that the ratio of alkaline phosphatase activity to acid phosphatase activity (AlkP/AcdP) was also a sensitive indicator of soil pH status.

As with any soil fertility parameter, field tests are always required to relate laboratory results to field results. One such test would be to treat an acid soil, both in the laboratory and in the field, with different rates of lime and measure AlkP/AcdP activity before and after treatments. In addition yield response curves would be measured as affected by lime treatment and change in soil pH. The AlkP/AcdP ratio at which optimum yield was observed and the amount of lime added to the soil in the laboratory needed to achieve this ratio would be identified so that a correlation could be established. Additional experiments in other acid soils that vary in chemical and physical properties would provide a more robust test and determine how widely applicable this enzymatic approach may be for assessing soil pH responses and liming needs.

Our results suggest that an enzymatic approach may be

more accurate than chemical approaches in assessing effective soil pH, especially when soils are amended with organic materials. That is because the soil enzyme assays integrate soil chemical, soil physical and soil mineralogical parameters to express a single response. The AlkP/AcdP ratio is both rapid and also seems to be more accurate than current chemical methods for determining proper pH status or adjustment of soil. For example, when organic amendments were added to acid soils, the AlkP/AcdP ratio indicated that the need for CaCO<sub>3</sub> was reduced. It should also be possible to use the AlkP/AcdP ratio approach on air-dried or field moist soils since we are not concerned about absolute values but ratios of values. Finally, the enzymatic approach to pH assessment seems to be applicable to a wide variety of soils with different properties including wide variations in absolute values of individual phosphatase activity. Again this is due to the fact we are concerned with ratios, i.e. the relationship, between AlkP and AcdP activity.

More work is needed to develop and test other enzymes that may be useful reflectors or predictors of agronomic and environmental status and changes. In most cases, a comparative approach between enzyme activity values will be required because absolute values from soils that vary widely in their characteristics are often meaningless.

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