AMMONIUM AND PHOSPHATE REACTIONS IN THE SOIL: EFFECT ON SOIL PHOSPHATASE ACTIVITY

by

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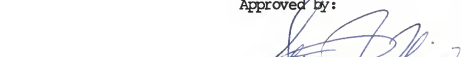

Major Professor

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INTRODUCTION

Little, if any, research has been done to study the activity of the enzyme phosphatase in soils treated with variable rates of fertilizer applications. Since much of the P content of soil is complexed in the soil organic matter fraction, the activity of soil phosphatase plays an important role in releasing nutrient phosphate to an available status for crop uptake.

Unpublished observations at Kansas State University show the dispersion of soil organic matter with application of ammoniacal N and ortho- and pyro-phosphates. We believe that dispersion of the soil organic matter will increase the release of organically bound phosphate by phosphatase, which in turn will affect an increase in plant available P.

The object of this study is to observe the effect of ammonium and ortho- and pyro-phosphate fertilization on soil phosphatase activity.

Literature Review

The study of soil enzymes has been a comparatively recent aspect of agricultural science. The development of soil enzyme studies was well reviewed by Skujins (1977). Though the discovery of 'abiontic' enzymes was established at the turn of the 20th century, only in the last 40-50 years has soil enzymology been seriously investigated and viewed as an integral soil process.

In 1899, A.F. Woods discovered that oxidizing enzymes, peridoxidases, existed in soils in the presence of decaying plant roots and other organic matter. For several years, the knowledge of enzyme activity was limited to catalase activity in soils to help explain the decomposition of organic residues and matter.

O.T. Rotini reported the activity of pyrophosphatase in soils in 1933. His work was re-evaluated, however, and it wasn't until 1942 that H.T. Rogers and his associates investigated the dephosphorylation of organic materials in soils, confirming the presence of phosphatases. In subsequent work, Rogers reported that phosphatase was exuded from the roots of corn and tomato plants, and this phenomena was later confirmed in a study of root physiology (Skujins, 1978).

Early investigations of phosphatase rested upon the analysis of orthophosphate released from substrate added to the soil. This did not take into account fixation of P by soil and thus results were often inaccurate. In 1955, Kroll and his associates developed a more accurate method of phosphatase assay that dertermined the release of phenol from phenolphosphate compounds. Similar methods of phosphatase assay were

developed in which the non-phosphate products of phosphate hydrolysis were determined. These included the use of -naphthylphosphate by Ramirez-Martinez in 1966 and glycerophosphate by Skujins in 1962. The development of the method using p-nitrophenylphosphate by Tabatabai and Bremner in 1969 is regarded as the most precise to date. (Skujins, 1978).

Phosphates in the Soil

Phosphates generally exist in the soil in three forms: as ionic phosphate in solution; precipitated as calcium, iron, or aluminum phosphates; or as organically bound P.

Considerable research is reported in the literature concerning the fate of inorganic P added to soils and its 'fixation' by soil minerals. Inorganic P (P_i) added to soils will quickly complex with metallic ions to form labile P compounds. Talibudeen (1981) reported that this complexation may occur in as short as a few hours after P application. With increasing time, more insoluble P compounds form that result in non-available soil P (Tisdale and Nelson, 1975; Brady 1974). The mechanisms of P sorption in soils are not entirely understood. It is known, however, that higher concentrations of P in solution result in increased P precipitation (Sample, Roper, and Racz, 1980), and may be observed as a 'continuum of precipitation, chemisorption, and adsorption to soils.'

Organic Phosphates

Because of the complexity of soil organic phosphates (SOP), comparatively few studies have been done to investigate their properties. From these studies, contrasting results abound as to the origin, amounts,

and types of organic P compounds in the soil.

Organic phosphates have been reported to comprise from 4% to 90% of the total soil P content, this depending on the soil types (Bartlett and Lewis, 1974; Cosgrove, 1967). Some reported values have included 50% (Thomas and Bowman, 1966), 50% to 66% (Bartlett and Lewis, 1974), 70% to 80% (Cosgrove, 1963), and from 3% to 52% (Tisdale and Nelson, 1975).

The origin of SOP has been established as the result of dead and decaying plant, animal, and microbial matter. G.L. Anderson (1975) reports that SOP results from the, "...vast amount of vegetation that undergoes decay." Cosgrove (1967), in contrast, states that organic P results from microbial utilization of inorganic and organic P in the soil. Along this line, C.V. Cole, et.al. (1977) indicate that for every organic P compound in the soil of plant origin, three organic P compounds are of microbial origin.

Regardless of origin, it is well known that as the organic matter content of the soil increases, in most cases, the SOP level increases also. (Appiah and Thomas, 1982; deHann and Zwerman, 1978). A C:P ratio of approximately 100:1 is the assumed mean ratio of soil carbon to SOP. This figure is highly variable with values as high as 500:1 and as low as 24:1 having been reported (Tisdale and Nelson, 1975).

Soil organic P may exist in several forms. Originally, nucleic acids and phospholipids were thought to account for most SOP (Brady, 1974; Wier and Black, 1966). These two forms actually constitute only 2.4% and 1.0% of the stabilized organic P of the soil (Black, 1968; Halstead and McKercher, 1975). Anderson (1980) writes that this low figure is due to the rapid hydrolysis of these P esters, yet nucleic acids and phospholipids enter the organic complex in greater amounts than

any organic P compound.

A large portion of the stabilized organic P fraction has been identified as various isomers of inositol-phosphate. Inositol is a cyclic, six-carbon compound saturated with a hydroxyl group on each carbon. Inositol phosphates will show any number of these hydroxyls replaced by a phosphate ion.

Myo-inositol hexaphosphate has been established as the most abundant isomer of this phosphate form (Anderson, 1975; Alexander, 1977; Black, 1968; Caldwell and Black, 1958a; Cosgrove, 1967, 1969; Greaves and Webley, 1969, Halstead and McKercher, 1975; and Spier and Ross, 1978). Caldwell and Black (1958b) reported a mean value of 17% of the total soil P content was myo-inositol hexaphosphate from 49 soils. For the same 49 soils, myo-inositol hexa-P constituted an average of 35% of the total organic P content. Several authors report the inositol P content to range from 50%-60% of the total SOP. (deHaan and Zwermann, 1978; Greaves and Webley, 1969; Spier and Ross, 1978).

Inositol phosphates originate from both plant and microbial populations. Caldwell and Black (1958a) hypothesized that approximately 46% of the soil inositol P originated from micro-organisms, the remainder attributed to plants. It is interesting to note that the most common form, myo-inositol hexa-P, has been identified only in plants when live biomass has been analyzed. (Alexander, 1977; Anderson, 1975; Cosgrove, 1969; Greaves and Webley, 1969; Halstead and McKercher, 1975; Moyer and Thomas, 1970).

A great portion of SOP exists as unidentifiable macromolecular P esters. These compounds may have molecular weights greater than 50,000 (Moyer and Thomas, 1970) and constitute 45%-60% of the total SOP content.

(Alexander, 1977; Anderson, 1975; Moyer and Thomas, 1970).

Soil Phosphatase

The phosphatase enzymes catalyze the hydrolysis of phosphate ions from organic P esters. The species of phosphatases have been classified into the following descriptive groups:

a) Phosphoric-monoester-hydrolases (E.C. 3.1.3) or phosphomonoestrases. These phosphatases cleave a single ortho-P ion from the ester group. Included are sugar phosphatases, nucleotidases, glycerophosphatase, and phytase (or inositol phosphatase). Phosphomonoesterases are subdivided into two groups: Alkaline phosphatases (E.C. 3.1.3.1) and Acid phosphatases (E.C. 3.1.3.2), these groups being prevalent in the associated pH environment.

b) Phosphoric-diester-hydrolases (E.C. 3.1.4) or phosphodiesterase. This enzyme hydrolyzes phosphate pairs from organic esters such as phospholipid, DNA, or RNA.

c) Phosphotriesterases (E.C. 3.1.5) hydrolyze phosphate groups from complex organo-phosphate compounds. This particular phosphatase is not common in soils.

d) Pyrophosphate phosphorylase (E.C. 3.6.1.1) or pyrophosphatase. Pyrophosphatase hydrolyzes inorganic pyrophosphatase to two ortho-phosphate ions.

Other phosphatases include enzymes acting on phosphoryl containing anhydrides and enzymes hydrolyzing P-N bonds (M.A. Tabatabai, 1982). The optimum activities of phosphatases differ markedly in relation to soil moisture, temperature, pH, soil type, and texture.

Origin of Phosphatases

As with the origin of soil organic phosphates, soil phosphatases are primarily synthesized in the living matter of plant and microbial populations and exuded into the soil solution or released upon death and decay (Alexander, 1977; Cassida, 1959; Cosgrove, 1967; Halstead and McKercher, 1975; Kuprevich and Scherbakova, 1966; Ladd, 1978; Ross, et.al., 1975; Rovira and McDougal, 1967; Smith, 1979; and Spier and Ross, 1978).

The contribution of the phosphatase enzyme by soil micro-organisms or by plant exudation has long been debated. Early researchers proposed that P mineralization was solely the result of microbial activity (Enwezor, 1967; van Diest and Black, 1959). However, the existence of free, extra-cellular enzymes has been well documented (Skujins, 1977). Abiontic phosphatases originate from root exudation and from the disruption of dead and decaying cellular matter (Cosgrove, 1967; Ladd, 1978, Ross, et.al., 1975; and Spier and Ross, 1978). Mineralization rates of organic P far in excess of that accounted for by microbial activity provides the best evidence in favor of abiontic phosphatase (Ramirez-Martinez, 1968). In support of this, O.L. Smith (1979) states that soil microbes synthesize far more enzymes than are required for metabolic activity.

The rhizosphere, the portion of the soil influenced by plant roots, exhibits the greatest amount of phosphatase activity. In this zone organic P is likely to be mineralized and become available for plant uptake. (Spier and Ross, 1978). Plant roots provide carbon-rich exudates from which microbial populations proliferate. (Alexander, 1977;

Appiah and Thomas, 1982). Along with increased numbers of microorganisms, levels of phosphatase activity are greater in the rhizosphere than in the bulk soil (Eivasi and Tabatabai, 1977; Irving and Cosgrove, 1976; Spier and Ross, 1978; Nannipieri, et.al., 1979).

Plant exudation of phosphatases has been well documented. Plants release phosphatase into the soil in response to P deficiencies (Kuprevich and Sherbakova, 1966; Halstead and McKercher, 1975; Wilde and Obe, 1966; and Skujins, 1967). Gilliam (1970) noted that hydrolysis of pyrophosphate is two to three times greater in the presence of P deficient wheat roots than in the presence of wheat roots not suffering such deficiencies. Subbarao, et.al. (1977), observed increased hydrolysis of pyro- and poly-phosphates in the presence of corn and soybean roots compared to chemical hydrolysis in pyro- and poly-phosphate hydroponic solution. Some phosphatases, such as pyro-phosphatase and acid phosphatase, exist on cell walls and root surfaces (Savant and Racz, 1980; Hasegawa, et.al., 1976).

Factors Influencing Phosphatase Activity

A wide variety of factors regulate the activity of soil phosphatase. These include soil organic matter content, soil pH, soil temperature, moisture, soil texture, and ionic type and concentration.

(i) Influence of Soil Organic Matter

Increases in the level of soil organic matter (SOM) generally increase the levels of soil organic P. Activities of acid and alkaline phosphatase correlate significantly to the organic C content of the soil (Juma and Tabatabai, 1976) and, correspondingly, enzyme activity

decreases with soil depth since organic matter level decreases.

Irving and Cosgrove (1976) recorded changes in phosphatase activity related to changes in the SOM content and microbial populations brought about by the presence of plants.

(ii) Influence of pH

The pH of the soil is dependent on several complex variables. Soil reaction changes according to the mineral make-up of the soil, base saturation, and the presence and decay of organic matter. Plant roots, in response to nutrient uptake, will often exude hydrogen and hydroxyl ions and change the pH level of the rhizosphere (Soon and Miller, 1977). Fertilizers may change the hydrogen ion content of the soil and contribute to acidity or alkalinity of the soil. Chemical fertilizers that change soil pH affect rates of enzymatic hydrolysis (Paw and Hughs, 1974).

Biological and biochemical activity is quite sensitive to changes in the soil pH. Species of phosphatases have different levels of pH that optimize rates of activity. Phosphomonoesterases show two pH optima, pH 4-6 (acid phosphatase) and pH 8-10 (alkaline phosphatase). Spier and Ross (1978) recorded an overall pH optima of 7 at which acid and alkaline and a 'neutral' phosphatase show the most combined activity. Saurez (1982) found a close relationship between the native pH of the soil and optimum pH of enzymatic activity. In contrast, Skujins (1978) states that the optimal pH for phosphatase activity occurs at pH 7.0 and not necessarily at the native soil pH.

Clark (1975) observed that in the presence of corn roots, maximum phosphatase activity was in the range of pH 3-7, where as with wheat

roots (Hasegawa, et.al., 1976) the optimal activity occured at pH 5.0. These findings suggest the dominance of acid phosphatase activity in the root zone. Juma and Tabatabai (1978) suggest that while there is limited information available concerning the effect of pH on the activity of phosphomonoesterases, the dominance of phosphatase type is due to the enzymes rate of synthesis and/or stabilization at certain pH levels.

The optimal rates of activity for pyrophosphatase and phosphodiesterase occur in more alkaline conditions. Pyrophosphatase showed optimal activity at pH 8.0 (Dick and Tabatabai, 1978). Using bis p-nitrophenyl phosphate as a substrate, Browman and Tabatabai (1978) recorded optimal phosphodiesterase activity at pH 8.0, though Eivasi and Tabatabai (1977) recorded optimal rates of activity for the same enzyme at pH 10.0 using a universal buffer.

(iii) Influence of Soil Temperature and Moisture

Like most chemical catalysts, phosphatase activity is influenced by temperature and moisture content of the existing medium. Soil phosphatase activity has been observed in a temperature range from below 0°C to approximately 60°C. Most types of soil phosphatases have temperature coefficient (Q_{10}) values of 1 to 2, that is, the rate of activity increases from 1 to 2 times for every increase of 10°C. The optimum temperature of highest activity falls between 50 and 60°C (Spier and Ross, 1978). At temperatures above 60°C, phosphatase starts to denature and activity declines. Clark (1975) observed that in corn roots, activity of phosphatase increased to approximately 40°C, declined, then rose again above 50°C. He postulated that this increase in activity may have been release of phosphatases due to cellular damage in the

roots. Skujins and McLaren (1968) wrote that phosphatase activity was present in tundra soils frozen for 9000 years. In cold soils, Rogers (1942) recorded small amounts of phosphatase activity as low as -20°C.

Adequate amounts of moisture must be present to insure optimum phosphatase activity (Gavrilova and Shimko, 1969). Spier and Ross (1978) note the distribution of acid and alkaline phosphatases was dependent upon soil moisture content. Generally, phosphatase activity correlates to the microbial population of the soil, and moisture levels for optimum microbial activity also optimize phosphatase activity in the soil. (Dalal, 1977; Cosgrove, 1977).

Sufficient literature to completely describe the effect of moisture on soil phosphatase activity is lacking. Periodic wetting and drying cycles increase P mineralization, yet drying of soils tends to significantly reduce phosphatase activity. Spier and Ross (1978) observed a 50-55% loss in phosphatase activity in air-dried soils. Similarly, Ramirez-Martinez (1968) reported enzyme activity losses of 30-40% in air-dried soils. This is in direct contrast to the work reported by Eivasi and Tabatabai (1977) in which the activity of acid phosphatase and phosphodiesterase increased after air-drying soils.

Influence of Soil Minerals

Soil phosphatase may be either activated or inhibited by many soil minerals. Juma and Tabatabai (1977) determined that at least twenty trace elements (in solution) inhibited acid and alkaline phosphatase to some degree. These included Hg^{2+}, As^{5+}, W^{6+}, Mo^{6+} (inhibited acid phosphatase more than 50%); As^{5+}, Ag^{+}, Cd^{2+}, and V^{4+} inhibited alkaline phosphatase at least 50%; and Cu^{+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Sn^{2+}, Ni^{2+}, Pb^{2+},

Fe^{2+}, Cr^{3+}, B^{3+}, Al^{3+}, and Se^{3+} all had some inhibitory effect on both enzymes. Concentrations of 25 $ug \cdot g^{-1}$ soil were more inhibitory than 2.5 $ug \cdot g^{-1}$ soil.

Hasegawa and Lynn (1976) found that Mg^{2+} and Ni^{2+} had no appreciable effect on phosphatase activity, yet activity was inhibited by Hg^{2+} and Fe^{3+}. Of further contrast, Clark (1975) reported 0.02 mM Mo^{2+} and 0.37 mM Al^{3+} inhibited phosphatase activity, and Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} had no inhibitory effect.

Some trace minerals may serve to act as enzyme activators for soil phosphatase. The presence of Mg^{2+} has been shown to specifically activate alkaline phosphatase, but only at high pH levels. Acid phosphatase is unaffected by Mg^{2+} but may depend upon Mn^{2+} to initiate activity (Burstone, 1962). In soils void of soluble Mg^{2+} and Mn^{2+} neither acid nor alkaline phosphatase show any activity (Bartlett and Lewis, 1973). It has been noted by several researchers that the presence of Ca^{2+} may be an inhibitor of phosphatases (Bartlett and Lewis, 1973; Dick and Tabatabai, 1978). The inhibiting effect of Ca^{2+} on phosphatase may help to explain the slow breakdown of polyphosphates in calcareous soils and that even in the most ideal of conditions, Ca^{2+} may retard the hydrolytic capacities of all phosphatases (Paw and Hughes, 1974).

Fertilizer

Fertilizers have varied effects on phosphatase activity. Most fertilizer salts have no inhibitory effect on phosphatase activity and, in fact, serve to increase the levels of activity. Stimulation of enzyme activity is likely due to increased plant growth and associated microbial populations (Ladd, 1978; Halstead and Sowden, 1968). Salts of NO_3^-,

NO_2^-, Cl^-, and SO_4^{2-} have no inhibitory effect on the enzyme activity (Juma and Tabatabai, 1977).

Contradictions concerning the effect additions of phosphate have on phosphatase activity appear in the literature. Some research indicates that the addition of phosphate to the soil stimulates microbial and phosphatase activity (Blair and Boland, 1978). However, Weir and Black (1966) strongly insist that increased P mineralization results from cultivation and that there is no evidence to account for increases in phosphatase activity due to P fertilization.

In biological organisms, inorganic P is the end product of phosphatase activity, and its accumulation serves to inhibit phosphatase activity. Increasing the levels of P in the soil also inhibit phosphatase activity as well as repressing phosphatase synthesis (Spier and Ross, 1978; Savant and Racz, 1965; Greenwood and Lewis,1977; Juma and Tabatabai, 1978). Spiers and McGill (1979) report that inhibition of phosphatase depends on the amount of P added and the soil organic matter content. As P is adsorbed by soil constituents, phosphatase activity increases. Phosphate concentrations of 0.55 mM or greater in the soil solution tend to repress activity, yet at lower concentrations (as in normal soil solutions) phosphatase activity is unaffected (Greenwood and Lewis, 1977).

To explain earlier reports of increased levels of phosphatase activity in high P soils, Anderson (1975) and Spiers and McGill(1979) indicate that the stimulation of plant and microorganism activity could account for increased levels of phosphatase activity. Currently, it is the consensus that there is an inverse relationship between phosphatase activity and P concentration in the soil (Skujins and McLaren, 1972;

Ramirez-Martinez, 1968).

Soil Texture

Soil texture, more specifically the clay content of soils, has a direct bearing on soil phosphatase activity. Like many soil constituents, phosphatases are subject to adsorption to clay particles (Anderson, 1975; Greaves and Webley, 1969; Kroll and Kramer, 1955; Kuprevich and Shcherbakova, 1966; Ladd, 1978; Makboul and Ottow, 1979; McLaren and Skujins, 1971; and Ramirez-Martinez, 1968). Adsorption of soil phosphatase to clay minerals may contribute to inhibition of its activity (Anderson, 1975; Bolt, 1978; Makboul and Ottow, 1979) as well as protecting it from hydrolysis by soil proteinases (Kroll and Kramer, 1955; Ladd, 1978). In contrast to this theory, R.G. Burns (1977) reported that adsorption of phosphatase to clays may actually enhance its activity by changing the configuration of the enzyme.

Several researchers have noted that the addition of montmorillonite clay to a soil will decrease phosphate mineralization and enzyme activity in general (Anderson, 1975; Greaves and Webley, 1969; Makboul and Ottow, 1979). In contrast, Ramirez-Martinez (1968) and McLaren (1978) state that phosphatases in soil are already adsorbed onto colloidal material and the addition of more montmorillonite will not change phosphatase activity. Kroll and Kramer (1955) observed there was no inhibition of phosphatase by clays if P mineralization was assayed by the release of phenol from phenolphosphate.

The concept that phosphatases are partially inactivated by clays was reinforced by Tabatabai and Bremner (1973) and Makboul and Ottow (1979). These findings report sorption of phosphatase to clay minerals and

subsequent increases in the K_m value for the enzyme. Makboul and Ottow postulate that this may be due to interlattice fixation of phosphatase in 2:1 clays. Increases in the V_{max} values were also observed by Makboul and Ottow. Results from Tabatabai and Bremner showed that shaking incubated samples decreased the K_m of soils while increasing the V_{max} value, presumably by increasing enzyme-substrate contact upon movement and release of phosphatase and substrate from sorption sites.

Michaelis-Menten Kinetics

In 1913, L. Michaelis and M. Menten developed a model to describe enzyme kinetics. In this model, V_{max} is the rate of enzyme activity at which all enzyme sites are saturated with substrate. At saturation, the rate of enzyme activity is maximum and velocity of the reaction is independent of the substrate concentration. The Michaelis constant, K_m, is the concentration of substrate at which half of the enzyme sites are filled, and velocity of the reaction approximates $V_{max}/2$. At substrate levels below the point at which Vmax is achieved, the rate of reaction is controlled by substrate concentration.

Mathematically, the model may be simply describe as

$$V = V_{max} * ([S]/[S]+K_m)$$

where V is the velocity of the reaction and [S] is the substrate concentration. Using the above equation, the fraction of sites filled can be calculated at any subtrate concentration once K_m has been determined. Generally, the smaller the K_m value, the greater the affinity of the enzyme for the substrate, and V_{max} may be achieved at

lower substrate concentrations (Stryer, 1975).

Soil phosphatase follows Michaelis-Menten kinetics. The K_m of soil phosphatases is affected by pH, ionic strength, temperature, and moisture levels (McLaren, 1978). Tabatabai and Bremner (1971) reported no correlation exists between K_m of soil phosphatase and pH, CEC, organic carbon, or the clay content of the soil.

While McLaren and Tabatabai and Bremner reported that soil phosphatase follows Michaelis-Menten kinetics linearly (using Lineweaver-Burke plots), Cervelli, et.al. (1973) also determined that soil phophatase followed Michaelis-Menten kinetics, but not without corrections. It was their findings that the substrate, p-nitrophenol phosphate, was adsorbed to the soil and thus the K_m derived was not valid. Their corrected values for K_m of phosphatase are smaller than those found in homogenous soil solutions and, in highly organic soils, approaches the K_m values for phosphatase found in plants and animals.

Irving and Cosgrove (1976) reported that calculation of soil phosphatase K_m values actually do not follow Michaelis-Menten kinetics when using p-nitrophenol phosphate as the substrate. Instead, they suggest that other researchers suppressed non-linear data, noting that the plot used by Tabatabai and Bremner to determine K_m, $[S]/v$ vs. $[S]$, and to determine the intercept (k_m/v_{max}). Their contention was that soil phosphatase reacts with organic compounds in the soil other than phenolic esters alone and thus the K_m values depicted by previous reports are actually not true representations.

Tabatabai and Bremner (1971) did undertake the task to report some representative K_m values of soils with the following results:

a) Phosphodiesterase, K_m of 1.26 mM to 2.02 mM (avg. 1.69 mM) using bis,

p-nitrophenolphosphate. V_{max} was 52-530 (avg. 303) ug P released gm^{-1}soil h^{-1}.

b) Pyrophosphatase, K_m of 20 mM to 51 mM (avg. 35 mM). V_{max} ranged from 26 to 166 (avg. 100) ug P released$\cdot gm^{-1}$soil$\cdot h^{-1}$. This high K_m may have been due to adsorption of pyrophosphate to colloidal material (Dick and Tabatabai, 1978).

c) Phosphomonoesterases, K_m ranged from 1.26 mM to 4.58 mM. No distinction was made between acid or alkaline phosphatases.

Summary

Soil phosphatase activity associates closely with organic matter content and dependends upon several regulating variables in the soil environment. The origin and range of activity of soil phosphatase and the role organic phosphate plays in plant nutrition is very complex and subject to debate.

It is established, however, that soil phosphatase has a primary role in the cycling of P through the soil system and its importance cannot be understated (Anderson, 1975). The role of phosphatase in releasing organically-complexed P to plant available forms should be considered alongside the realization of the importance of organic P as a nutrient source.

Our study will analyze the combined effects of changes in the soil environment brought about by ammonium and phosphate fertilization on changes in the phosphatase activity in the soil. Hopefully, our contribution to this body of knowledge will help better understand the role of phosphatase in the soil.

MATERIALS AND METHODS

The experiment was designed to study the effect of mono-ammonium phosphate or ammonium pyro-phosphate and ammoniacal N (as NH_4OH) on soil phosphatase activity. The experimental design was a randomized complete block of 20 fertilizer treatments and 7 incubation time periods, each replicated three times. The 3 replicates represent the whole plot and the 20 fertilizer treatments and 7 time periods represent the subplots. Each replicate was completed before the next one began.

Incubation Columns

Six Lucite sections each 5 cm long and 7 cm inner diameter were taped together to form an incubation column 30 cm tall. The bottom of the column was covered with filter paper and nylon screen held in place by an elastic band. A 0.15 cm hole was drilled into the third section from the bottom and a serum stopper inserted to serve as the injection port. The column is diagramed in Fig. 1.

Soil

The soil used in this study was a Kennebec silt loam, a fine silty, mixed, mesic Cumulic Hapludoll from Mosquito Creek bottomland 4.8 km southwest of Netawaka in northeast Kansas. The soil had a pH of 7.0, bulk density of 1.5 $g \cdot cm^{-3}$, and organic matter content of 19 $g\ kg^{-1}$.

Preparation of Soil Samples

The soil was air dried and sieved to 2 mm. It was then placed into

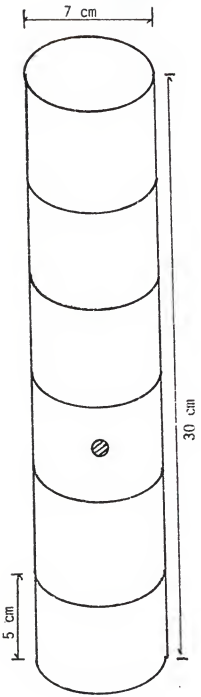

Fig. 1. Incubation Column
7 cm dia. x 30 cm

the incubation columns in 5 cm increments and tamped to a bulk density of 1.1 g cm^{-3} using a 1 kg weight. It was important that the entire surface was evenly tamped and the surface roughened before adding more soil to prevent density gradients at the interfaces.

After building a soil column 25.4 cm deep, the soil was moistened to approximately 19.5% w/w by adding 210 ml of deionized water. Glass wool was placed on top of the soil to retard drying. Columns equilibrated for 6 days to allow uniform distribution of the water. Columns were then incubated for 12 hours at 35°C prior to fertilizer injection.

Fertilization

Fertilizer treatments were prepared by combining either ortho-phosphate or pyro-phosphate with ammonium hydroxide and water as outlined in Table 1. A syringe and needle were used to inject the fertilizer mixture through the injection port into the center of the soil column. Fertilizer combinations were 0 $mg \cdot kg^{-1}$ phosphate, 300 and 600 $mg \cdot kg^{-1}$ ortho- or pyro-phosphate P combined with 0, 600, 1200, or 2400 $mg \cdot kg^{-1}$ NH_4-N (based on the weight of the center volume of the soil column) for a total of 20 treatment combinations. Prior to injection, ortho-phosphate treatments at the highest level of NH_4-N (2400 $mg \cdot kg^{-1}$) had to be heated at 35°C to resolubilize ammonium phosphate crystals that precipitated upon the addition of NH_4OH.

After fertilization, the soil columns were incubated for specified time periods at 35°C.

Leaching the Soil Samples

At the end of each incubation period the 5 cm section receiving the

Table 1. Treatment rates and formulations for individual soil samples.

Treatment †			Treatment solution			
MAP (mg P/kg)	APP (mg P/kg)	NH_4^+-N § (mg/kg)	1.55 M $NH_4H_2PO_4$ ml	0.78 M $(NH_4)_3HP_2O_7$ ml	13.8 M NH_4OH ml	H_2O ml
0	0	0	0	0	0	20
0	0	600	0	0	2.0	18
0	0	1200	0	0	4.0	16
0	0	2400	0	0	8.0	12
300	0	0	4.0	0	0	16
300	0	600	4.0	0	2.0	14
300	0	1200	4.0	0	4.0	12
300	0	2400	4.0	0	8.0	8
0	300	0	0	4.0	0	16
0	300	600	0	4.0	2.0	14
0	300	1200	0	4.0	4.0	12
0	300	2400	0	4.0	8.0	8
600	0	0	8.0	0	0	12
600	0	600	8.0	0	2.0	10
600	0	1200	8.0	0	4.0	8
600	0	2400	8.0	0	8.0	4
0	600	0	0	8.0	0	12
0	600	600	0	8.0	2.0	10
0	600	1200	0	8.0	4.0	8
0	600	2400	0	8.0	8.0	4

†Treatment rates of 600 mg/kg in a zone 7.0 cm in diameter at 61 cm spacings equate to 25 mg/kg uniform application. Rates were computed on the basis that treatments diffuse 7.6 cm both above and below the point of application.

§Ammonium hydroxide furnishes the nitrogen. In addition to that nitrogen, orthophosphate adds 135 mg/kg N using 300 mg/kg P and twice that at the higher P rate, and pyrophosphate adds 203 mg/kg N at the 300 mg/kg P rate and twice that at the higher P rate.

fertilizer treatment was removed to a 9 cm Buchner funnel fitted with a 111 m SPECTRA/MESH filter. Scraping the soil in a 'shaving' manner prevented large clumps of soil from dropping into the funnel.

Deionized water was added to the soil and allowed to sit for 2 minutes before suction (at $5.3 \cdot 10^4$ Pa) was applied for about 5 seconds. The filtrate was then readministered to the soil and suction resumed. The soil was kept constantly saturated until 250 ml of filtrate were collected.

The filtrate pH was determined and the leachate transfered to polyethylene storage bottles and stored at 4°C.

Leached Soil Samples

The pH of soil leachate was then determined. Next, the soil was subjected to drying at 35°C. When partially dry, the soil was broken apart, crushed, placed in plastic storage bags, and mixed thoroughly to homogenize the sample. The dried soil samples provided ease of handling and measurement, and proper mixing.

After air drying, pH was determined using a 1:1 soil to water ratio (by volume). The paste was allowed to stand 15 to 20 minutes before the pH was measured.

Ortho-Phosphate Determination

Because of the reported inhibition of ortho-phosphate on phosphatase activity, it was of interest to determine the ortho-phosphate concentrations of our soil and leachate samples.

Ortho-phosphate concentration in the leachate was determined using the method developed by Dick and Tabatabai (1977) with the following

modifications. Saurez (1980) suggested a 40-second waiting period between the addition of ammonium poly-molybdate reagent and the citrate-arsenite reagent. This period was designed to insure optimum phospho-molybdate complexing and color development. After 10 minutes, the samples were centrifuged at 12,000 rpm for 10 minutes to settle precipitated humic substances.

To determine ortho-phosphate concentrations in our soil samples, a modified version of the method by Dick and Tabatabai (1977) was employed. A 1.0 gm sample of dry soil was mixed with 10.0 ml of ascorbic acid (0.1M)-trichloroacetic acid (0.5M) for 30 seconds. The mixture was filtered through Whatman 42 qualitative ashless filter paper. An aliqout of filtrate containing 0 to 25 µg P (usually 0.5 to 1.0 ml) was transfered to a 25 ml volumetric flask and analyzed according to the procedure described by Dick and Tabatabai. This modification was developed due to the very high levels of ortho-P in the soil from the fertilizer treatments.

Analysis of Phosphatase Activity

Analysis of acid and alkaline phosphatase, phosphodiesterase, and pyrophosphatase activity was performed on all leachate and soil samples. Analysis of the leachate samples was usually performed within 72 hours after collection from the soil. Soil analysis was performed on air dried samples. Because treatments had differential rates of drying, when 1.0 gm samples were weighed for each enzyme assay, another sample was used to determine moisture content. Assay results were adjusted to activity per gram of oven-dried soil.

Acid and alkaline phosphatase activity was assayed according to the

procedure developed by Tabatabai and Bremner (1969) involving the hydrolysis of p-nitrophenol phosphate to p-nitrophenol and phosphate. Concentration of p-nitrophenol in solution (determined colorimetrically) correlates directly to the amount of acid or alkaline phosphatase activity. The standard curve of p-nitrophenol released ($mg \cdot kg^{-1} \cdot hr^{-1}$) vs. absorbance was used for the range from 0.065 to 0.66 absorbance units. Concentrations with absorbance readings greater than 0.66 were diluted with water to bring them within range of our standard curve.

Phosphodiesterase activity was assayed according to the procedure developed by Browman and Tabatabai (1978) involving the hydrolysis of bis p-nitrophenol phosphate to p-nitrophenol and phosphate. Spectrophotometric analysis of the p-nitrophenol released correlated directly to enzyme activity. An absorbance range between 0 and 0.66 units was used.

Pyrophosphatase activity was assayed by the method developed by Dick and Tabatabai (1978) involving the hydrolysis of inorganic pyro-phosphate to ortho-phosphate over a period of 5 hours at 37°C. Determination of ortho-phosphate released was performed by the method described by Dick and Tabatabai(1977) previously referenced.

Enzyme Kinetics

The Michaelis constant (K_m) was determined for acid and alkaline phosphatase, phosphodiesterase, and pyrophosphatase on our experimental soil. K_m and V_{max} for acid and alkaline phosphatase was determined according to the method used by Tabatabai and Bremner (1971) in which velocity (V) of the enzyme reaction is measured against substrate concentrations, [S], of 0, 1, 5, 10, 15, and 20 mM.

Determination of K_m and V_{max} for phosphodiesterase was performed in similar manner as that for acid and alkaline phosphatase. Concentrations of the substrate were 0, 0.2, 0.5, 1.0, 2.0, and 5.0 mM in the soil/substrate mixture.

Pyrophosphatase K_m and V_{max} were also determined in the manner described above. Concentrations of the substrate were 0, 10, 20, 30, 50, and 60 mM as used by Dick and Tabatabai (1978) in determination of pyrophosphatase kinetic parameters.

Samples for determining enzyme kinetic parameters were run in triplicate and the results analyzed by linear regression analysis. These data were plotted as [S]/V against [S] to illustrate the analysis. The y-intercept is equivalent to K_m/V_{max} and the slope of the regression is equivalent to $1/V_{max}$ (Tabatabai and Bremner, 1978).

Statistical Analysis

Analyses of variance, regression, and correlation analyses were performed on the collected data using the Statistical Analysis System software package.

RESULTS and DISCUSSION

In general, phosphatase activity decreased with increasing levels of ammonium and phosphate treatments and pH. This effect was more prominent in the soil analysis than in the leachate data. Phosphatase activity assays showed considerable variation in both leachate and soil samples. The large variability in the data resulted in low regression coefficients (R^2). Statistical analysis with $\alpha = .05$ and $\alpha = .20$ were reported.

Leachate phosphatase assay yielded very low enzyme activities. Soil samples showed significantly higher rates of activity than the leachate and the response of phosphatase to fertilizer treatment combinations can be observed.

Acid and Alkaline Phosphatase

Leachate acid phosphatase activity (Table 2) means from different fertilizer treatments were not significantly different at the 5% level, but differences were apparent at the 20% level. There is no apparent response to increasing the application level of ammonia nor to the addition of ortho- or pyro-phosphate P into the soil solution. Leachate phosphatase activity remained constant across the span of incubation times through all treatments. There is little correlation of leachate phosphatase to ammonia levels or ortho-phosphate concentrations in the leachate (Table 16).

Soil acid phosphatase activity (Table 3) is more responsive to treatment inputs. A significant ($\alpha = .05$) drop in enzyme activity with

Table 2. Leachate acid phosphatase activity for incubations periods 0 through 16 days. (mg P released·kg^{-1} soil·h^{-1})

TREATMENT AMM-N (mg/kg)	ORTHO-P (mg/kg)	PYRO-P (mg/kg)	TIME(DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05	LSD.20
0	0	0	5.5	10.0	6.6	6.6	4.0	8.3	5.6	8.6	5.4
600	0	0	4.4	6.6	17.8	15.8	8.4	13.4	9.4	15.2	9.6
1200	0	0	7.2	5.9	10.4	12.4	10.4	10.5	8.0	15.5	9.7
2400	0	0	5.6	6.9	15.5	12.9	4.6	11.9	9.9	11.9	7.4
0	300	0	9.5	11.0	12.5	12.4	4.6	10.7	6.4	9.9	6.2
600	300	0	6.8	3.8	12.5	17.8	4.3	9.2	7.1	12.3	7.7
1200	300	0	6.0	2.0	12.7	12.9	7.1	9.6	11.9	12.1	7.6
2400	300	0	3.6	8.0	7.9	14.2	6.1	9.9	10.4	12.2	7.6
0	0	300	6.7	9.7	4.1	5.2	11.4	10.5	8.6	10.2	6.4
600	0	300	5.1	4.8	9.4	13.7	3.6	10.9	6.3	10.9	6.8
1200	0	300	7.3	5.3	9.7	6.8	2.3	8.8	5.1	7.6	4.8
2400	0	300	5.8	9.5	5.6	12.9	4.1	8.1	4.0	8.4	5.3
0	600	0	11.9	6.9	16.8	10.9	6.3	8.9	7.1	14.3	8.9
600	600	0	3.0	5.6	15.5	6.9	7.6	15.5	6.6	14.3	9.0
1200	600	0	6.5	5.3	13.7	8.4	10.1	10.7	2.3	12.5	7.8
2400	600	0	5.8	18.3	8.1	21.5	14.5	14.1	6.6	17.0	10.6
0	0	600	12.8	6.2	8.6	8.4	8.6	5.8	5.3	10.8	6.8
600	0	600	10.1	5.1	5.1	10.7	9.4	15.8	5.3	15.5	9.7
1200	0	600	10.3	5.9	6.9	12.4	8.9	4.1	8.1	9.8	6.2
2400	0	600	15.0	4.6	4.8	8.4	11.7	8.5	1.8	11.5	7.2
		LSD.05	9.0	8.5	12.3	12.8	9.5	17.2	9.1		
		LSD.20	5.8	5.5	7.9	8.3	6.1	11.1	5.9		

Table 3. Soil acid phosphatase activity for incubation periods 0 through 16 days. (mg P released·kg^{-1}soil·h^{-1})

TREATMENT AMM-N (mg/kg)	ORTHO-P (mg/kg)	PYRO-P (mg/kg)	TIME (DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05	LSD.20
0	0	0	190.7	176.2	162.7	160.5	157.5	146.0	149.2	123.5	77.4
600	0	0	122.9	119.6	76.6	82.7	89.4	130.1	82.7	82.9	52.0
1200	0	0	144.4	106.2	85.3	58.1	62.3	79.6	57.8	57.2	36.2
2400	0	0	132.4	85.1	65.5	50.3	38.9	64.4	56.8	63.3	39.7
0	300	0	164.2	140.6	123.6	117.6	125.1	116.2	74.6	101.8	63.9
600	300	0	138.5	115.9	108.5	86.9	80.9	108.2	77.6	66.1	41.4
1200	300	0	99.9	98.3	90.2	71.3	63.8	80.4	77.5	0.0	38.2
2400	300	0	103.8	79.4	55.7	50.6	45.3	72.4	26.9	63.0	39.5
0	0	300	154.6	137.5	152.1	137.6	158.0	148.8	104.3	73.1	45.8
600	0	300	124.7	83.2	90.9	76.3	88.4	117.4	91.7	37.6	23.5
1200	0	300	125.7	68.6	86.0	64.7	58.2	59.9	65.8	57.5	36.0
2400	0	300	96.6	45.1	49.0	55.8	33.8	72.6	28.0	43.9	27.5
0	600	0	140.4	127.6	122.4	140.7	90.2	109.8	80.6	82.7	51.9
600	600	0	139.8	137.9	151.9	99.4	117.6	90.9	89.6	62.1	39.0
1200	600	0	113.1	76.9	96.3	54.7	62.7	64.3	53.6	50.1	31.4
2400	600	0	112.9	55.4	67.9	58.9	35.9	57.9	31.9	43.8	27.5
0	0	600	156.7	125.4	107.8	112.7	119.3	136.2	107.9	72.3	45.3
600	0	600	130.8	95.4	81.7	73.7	105.4	92.1	64.7	65.5	41.1
1200	0	600	111.8	99.1	78.7	56.4	50.6	63.6	41.0	55.4	34.7
2400	0	600	156.0	61.5	56.6	38.3	36.4	45.5	23.4	64.6	40.5
		LSD.05	56.6	71.8	59.6	52.3	84.2	68.0	57.4		
		LSD.20	36.5	46.3	38.4	33.7	54.3	43.8	37.0		

application of ammonia occurred across all levels of phosphate additions in the soil. Differing the rates of ammonium application (from 600 to 2400 $mg \cdot kg^{-1}$) within the 0 phosphate and 300 mg kg^{-1} ortho-phosphate P or pyro-phosphate P treatments did not significantly affect acid phosphatase activity. Figures 2, 3, 4, 5, and 6 illustrate this effect. Differences in enzyme activity due to ammonium levels occur at highest level of phosphate input. Phosphatase activity appears to decrease with increases in soil phosphate, especially at the 600 mg ortho-phosphate P kg^{-1} rate. Applications of pyro-phosphate had no significant effect on acid phosphatase activity.

Comparison of soil pH (Table 11) to acid phosphatase acitivity indicates decreases in the level of activity with increases of soil pH. Yet, as pH decreased to neutral or even acid values over time, acid phosphatase significantly decreased. This effect is opposite of what we might expect.

Ammonia demonstrates a more significant inhibition of acid phosphatase than ortho-phosphate. This may be due to inactivation of the enzyme or even possible denaturization of the protein structure from chemical modifications of the soil. Increasing the time of incubation showed significant loss of activity for all treatments except the control (0 fertilizer application).

Alkaline phosphatase showed very little activity in the leachate (Table 4), as did acid phosphatase activity, with few means demonstrating any significant differences. No pattern of response to either ammonium or phosphate treatments was apparent in the soil leachate. Again, enzyme activity did not change significantly with time. Correlation analysis shows no relationship of alkaline phosphatase activity to pH, ammonium,

Table 4. Leachate alkaline phosphatase activity for incubation periods 0 through 16 days. (mg P released·kg^{-1} soil·h^{-1})

TREATMENT AMM-N	ORTHO-P (mg/kg)	PYRO-P	TIME(DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05	LSD.20
0	0	0	5.3	2.5	7.3	3.8	3.6	8.6	3.6	11.3	7.0
600	0	0	1.3	8.7	7.6	8.8	3.1	8.9	6.1	11.1	7.0
1200	0	0	2.3	5.3	7.5	3.4	2.4	5.1	5.6	7.2	4.5
2400	0	0	4.8	3.1	5.6	3.0	3.4	6.3	6.9	8.9	5.6
0	300	0	2.6	5.3	14.8	2.4	6.2	7.4	1.5	11.8	7.4
600	300	0	7.7	4.8	6.1	7.9	4.1	9.6	6.4	12.0	7.5
1200	300	0	0.8	6.8	12.2	3.5	4.8	7.4	4.7	10.4	6.5
2400	300	0	5.0	5.8	5.4	1.5	2.3	8.9	9.4	8.2	5.2
0	0	300	8.4	8.5	4.5	3.5	7.8	5.3	4.3	14.3	9.0
600	0	300	5.1	11.7	5.6	1.0	8.7	6.9	7.0	12.0	7.5
1200	0	300	2.7	10.4	8.3	4.8	3.8	8.4	4.6	8.9	5.6
2400	0	300	1.5	2.8	9.8	4.1	4.1	5.1	7.2	8.5	5.3
0	600	0	0.8	21.8	4.6	1.5	5.7	1.5	5.9	20.9	13.1
600	600	0	6.1	20.6	11.2	1.8	5.4	10.3	6.9	24.9	15.6
1200	600	0	4.0	4.3	9.0	5.0	4.7	9.4	5.8	9.2	5.8
2400	600	0	6.1	3.5	4.6	7.4	8.9	1.8	6.8	9.0	5.7
0	0	600	2.8	10.1	10.0	9.9	2.5	5.3	5.9	10.8	6.7
600	0	600	3.8	11.7	6.2	0.0	8.3	7.8	10.4	12.9	8.1
1200	0	600	2.0	7.6	10.7	5.7	1.6	9.6	3.8	9.4	5.9
2400	0	600	5.8	8.9	2.9	4.5	6.1	5.8	4.6	13.3	8.3
		LSD.05	10.9	21.2	10.9	7.5	8.2	10.1	7.3		
		LSD.20	7.0	13.7	7.0	4.8	5.3	6.5	4.7		

Table 5. Soil alkaline phosphatase activity for incubation periods 0 through 16 days. (mg P released·kg^{-1}soil·h^{-1})

TREATMENT AMM-N	ORTHO-P (mg/kg)	PYRO-P	TIME (DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05	LSD.2
0	0	0	213.8	233.6	195.6	243.2	265.8	190.2	244.3	150.6	94.4
600	0	0	199.5	147.8	131.4	148.0	167.4	169.6	209.4	128.2	80.4
1200	0	0	211.6	130.1	107.6	88.0	128.0	109.1	139.6	97.7	61.1
2400	0	0	177.8	78.1	131.7	38.7	73.4	63.1	110.9	102.9	64.5
0	300	0	211.5	135.6	166.3	135.6	183.0	110.9	169.8	96.3	60.4
600	300	0	206.4	165.3	244.0	135.2	193.8	123.5	197.5	123.7	77.6
1200	300	0	175.1	135.8	162.0	82.1	163.4	113.1	164.0	41.4	59.2
2400			175.4	91.9	89.7	65.6	99.4	71.1	125.1	87.6	54.9
0	0	300	194.7	175.9	237.9	201.8	200.6	153.7	184.0	118.0	74.0
600	0	300	216.7	167.7	170.1	143.8	182.1	135.3	158.1	125.9	79.0
1200	0	300	194.0	146.5	174.3	113.6	153.0	113.0	130.4	128.3	80.4
2400	0	300	202.0	186.5	142.9	83.4	94.4	98.2	79.8	134.0	84.0
0	600	0	175.4	186.5	140.7	142.6	125.0	101.3	141.1	92.7	58.1
600	600	0	223.6	147.0	209.5	143.9	200.1	117.9	140.8	77.9	48.9
1200	600	0	195.9	134.5	169.0	95.5	147.4	93.6	119.5	60.3	37.8
2400	600	0	193.3	121.2	110.2	69.3	93.5	72.9	118.2	67.5	42.3
0	0	600	217.4	162.2	192.1	160.2	184.3	133.1	159.8	107.9	67.6
600	0	600	186.5	163.6	186.1	115.6	173.4	116.1	149.6	110.7	69.4
1200	0	600	220.8	110.2	105.2	103.8	100.9	112.6	133.5	78.4	49.2
2400	0	600	236.3	94.6	108.6	57.3	99.4	61.2	92.9	86.4	54.2
		LSD.05	168.9	97.0	96.2	89.2	55.0	82.0	71.1		
		LSD.20	108.9	62.5	62.0	57.5	35.4	52.8	45.8		

or ortho-P applications.

Soil alkaline phosphatase (Table 5) showed higher, but more variable, rates of activity than did acid phosphatase. This is reflected in the large LSD values, especially at the shorter incubation periods where no observable pattern in enzyme activity resulted from ammonia or phosphate application. The greatest drop in alkaline phosphatase activity is seen in samples treated with 0 $mg \cdot kg^{-1}$ phosphate, or 300 and 600 $mg \cdot kg^{-1}$ pyro-phosphate P, and between 0 to 2400 $mg \cdot kg^{-1}$ ammonium-N. Soils receiving no ammonia maintained a constant rate of activity over 16 days of incubation (Figures 7, 8, 9, 10, and 11).

In the absence of ammonium, ortho-phosphate significantly ($\alpha = .20$) restricts alkaline phosphatase activity 2 days or more after the initial injection of fertilizer treatment. Pyro-phosphate P (300 $mg \cdot kg^{-1}$ and 600 $mg \cdot kg^{-1}$) significantly reduced alkaline phosphatase activity after 0.5 days of incubation.

A higher level of alkaline phosphatase activity in our treated soil (relative to acid phosphatase) reflects the influence of high soil pH on the comparative rates of acid and alkaline phosphatase. When compared to the soil pH (Table 11), alkaline phosphatase activity decreased while the soil pH at the higher levels of ammonium application remained constant with time. The introduction of ammonia into the soil, while increasing pH, may possibly decrease enzyme activity by denaturization, increasing K_m and/or decreasing V_{max}, or changing substrate availability.

Enzyme Kinetics

Calculated values of K_m and V_{max}, respectively, are 28.0 mM and 534.7 μg P released g^{-1}soil h^{-1} for acid phosphatase (Fig. 27) and 5.8 mM and 383.1 μg P released g^{-1} h^{-1} for alkaline phosphatase (Fig. 28). The

K_m value of alkaline phosphatase is similar to that reported by Tabatabai and Bremner (1971) for 9 Iowa soils. The maximum rates of activity for acid phosphatase (190.7 $mg \cdot kg^{-1} \cdot h^{-1}$) is approximately 36% of V_{max}; however, the calculated V_{max} is questionable due to the poor fit of the data to linear regression. We should note that if we eliminate the values obtained for V using 5 mM substrate in our determination of V_{max} and K_m (Fig. 27), we calculate V_{max} as 354.3 g P released kg^{-1}soil h^{-1} and K_m as 14.0 mM. Maximum activity for alkaline phosphatase activity (265.8 $mg \cdot kg^{-1} \cdot h^{-1}$) was 80% of V_{max}.

Phosphodiesterase

Phosphodiesterase activity in the soil and leachate was noticeably less than for either acid or alkaline phosphatase. Leachate phosphodiesterase activity (Table 6) was very low with few significant differences between means. Despite its low level of activity, statistical analysis shows a comparatively strong relationship of phosphodiesterase activity to ammonia application. This may be indicative of the substrate's stability in the presence of ammonia and heat, not necessarily enzymatic hydrolysis.

Soil phosphodiesterase activity (Table 7) was substantially greater than that detected in the leachate. Increasing ammonia application levels in the absence of phosphates resulted in a decrease in enzyme activity (Fig. 12). After 2 days of incubation the decrease in activity due to ammonia levels is significant. Figures 13, 14, 15, and 16 illustrate how the level of phosphodiesterase activity in samples treated with ortho- and pyro-phosphates remained constant despite increased ammonia applications.

Table 6. Leachate phosphodiesterase activity for incubation periods 0 through 16 days. (mg P released·kg^{-1} soil·h^{-1})

TREATMENT AMM-N	ORTHO-P (mg/kg)	PYRO-P	TIME(DAYS) 0	0.5	1.0	2.0	4,0	8.0	16.0	LSD.05	LSD.20
0	0	0	1.1	0.4	0.1	0.0	0.3	0.3	0.0	1.3	0.8
600	0	0	0.4	0.0	1.7	0.8	0.7	0.7	0.5	1.6	1.0
1200	0	0	0.7	0.5	0.3	0.8	1.3	1.6	0.5	1.2	0.7
2400	0	0	1.1	0.8	1.6	0.4	0.8	4.0	0.0	2.3	1.4
0	300	0	0.5	0.3	0.3	0.0	0.0	0.0	0.3	0.6	0.4
600	300	0	0.8	2.1	0.3	0.3	0.8	0.5	0.5	1.7	1.0
1200	300	0	0.8	0.5	2.4	1.7	2.9	1.7	0.8	1.8	1.2
2400	300	0	1.1	0.8	1.7	1.1	3.3	3.7	3.2	1.9	1.2
0	0	300	0.8	0.0	0.3	0.0	0.1	0.3	0.5	1.2	0.7
600	0	300	0.4	1.9	1.2	0.0	0.3	0.7	0.4	1.2	0.8
1200	0	300	0.1	1.1	0.7	0.8	2.7	1.6	0.8	1.7	1.1
2400	0	300	0.7	1.1	1.2	0.5	2.8	3.1	0.8	1.8	1.2
0	600	0	0.8	0.3	0.3	0.1	0.5	0.4	0.8	1.1	0.7
600	600	0	1.1	0.8	1.1	0.5	0.7	0.5	0.0	1.5	0.9
1200	600	0	0.5	1.1	1.6	1.6	1.1	1.3	0.7	2.1	1.3
2400	600	0	0.7	0.3	1.1	1.1	2.0	2.7	1.2	1.5	0.9
0	0	600	1.2	0.3	0.3	0.4	0.1	0.3	0.9	1.1	0.7
600	0	600	0.8	0.5	1.1	1.1	0.8	0.8	0.3	1.5	0.9
1200	0	600	0.5	0.8	1.9	0.7	2.1	1.6	1.2	1.1	0.7
2400	0	600	1.2	0.8	1.1	0.3	1.2	5.1	1.3	2.1	1.3
		LSD.05	2.0	1.5	1.6	1.4	1.3	1.4	1.0		
		LSD.20	1.3	1.0	1.1	0.9	0.8	0.9	0.6		

Table 7. Soil phosphodiesterase activity for incubation periods 0 through 16 days. (mg P released·kg^{-1}soil·h^{-1})

TREATMENT AMM-N (mg/kg)	ORTHO-P (mg/kg)	PYRO-P (mg/kg)	TIME (DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05	LSD.20
0	0	0	49.4	54.4	45.1	52.1	63.9	56.5	52.2	27.3	17.1
600	0	0	47.9	49.4	43.0	44.9	51.1	46.1	37.7	13.0	8.2
1200	0	0	49.1	47.7	42.5	37.6	48.4	44.8	32.9	15.1	9.7
2400	0	0	48.2	37.3	42.4	23.2	35.6	32.1	25.9	15.5	9.7
0	300	0	36.1	39.7	34.8	29.6	33.8	25.8	23.4	12.8	8.0
600	300	0	35.1	50.6	35.9	35.3	39.8	26.8	23.4	13.9	8.7
1200	300	0	32.5	46.4	34.3	35.5	39.2	36.7	26.8	7.3	9.9
2400	300	0	31.1	40.1	30.7	23.8	30.2	28.0	18.9	15.8	9.9
0	0	300	33.9	37.0	41.5	39.1	39.5	39.8	22.8	13.4	8.4
600	0	300	37.3	39.9	32.3	35.5	37.0	39.8	28.8	13.2	8.3
1200	0	300	35.2	32.1	35.1	28.2	30.7	30.6	24.2	13.0	8.1
2400	0	300	33.6	30.6	28.6	24.5	30.8	26.8	18.0	12.2	7.7
0	600	0	31.3	28.0	19.3	16.5	18.6	15.5	11.7	7.9	5.0
600	600	0	33.5	44.6	35.8	27.9	28.4	23.7	15.7	13.0	8.2
1200	600	0	24.9	37.6	26.8	22.6	28.9	29.9	23.5	12.8	8.0
2400	600	0	21.1	30.0	25.4	26.1	28.3	21.7	19.1	11.2	7.0
0	0	600	26.7	27.4	31.0	29.6	31.7	29.8	19.2	11.3	7.1
600	0	600	27.6	29.2	28.1	26.5	29.6	24.8	23.3	10.2	6.4
1200	0	600	30.7	28.0	24.9	29.3	24.1	21.5	19.2	10.8	6.8
2400	0	600	26.3	23.5	23.9	23.6	25.4	15.7	14.2	11.8	7.5
		LSD.05	11.1	15.6	16.6	9.7	17.6	9.3	9.8		
		LSD.20	7.2	10.1	10.7	6.2	11.4	6.0	6.3		

Changes in phosphodiesterase activity are more pronounced in the presence of phosphate treatments. Browman and Tabatabai (1978) note that inorganic phosphate is an effective competitive inhibitor of phosphodiesterase, thus reductions in activity from increased fertilizer P applications are likely. Activity decreases with the addition of both ortho- and pyro-phosphate into the soil, though the decrease associated with increasing the P application from 300 mg kg^{-1} to 600 mg kg^{-1} is significant only at values of $\alpha = .20$ or greater. This change is consistent despite the different ammonia levels, suggesting that the application of ammonia has little influence on phosphodiesterase activity in the presence of phosphate P. These observations might also indicate the inhibiting influence of even low concentrations of ortho-phosphate in the soil. No discernable differences between phosphate species occurred.

Little decrease in the rate of activity with time was observed.

Enzyme Kinetics

K_m and V_{max} (Fig. 29) for phosphodiesterase are 3.64 mM and 218.8 µg P released g^{-1}soil h^{-1}, respectively. The value for K_m is slightly higher than that found by Browman and Tabatabai (1978) in Iowa soils. The comparatively low V_{max} may be indicative of a decreased occurrence of phosphodiesterase in the soil.

The low values of phosphodiesterase activity, compared to V_{max}, may stem from using a lower than optimum concentration of prepared substrate (bis p-nitrophenol phosphate) in the soil-substrate mixture. Using the Michaelis-Menten equation we can calculate the velocity, V, if we know K_m, V_{max}, and the substrate concentration in mixture. Our assay, using the method developed by Tabatabai and Browman, uses a substrate concentration of 1.0 mM.

$$V = (218.8 \text{ g } g^{-1} h^{-1})/(1 + 3.64/1)$$

$$V = 52.4 \text{ g P released } g^{-1} \text{soil } h^{-1}$$

The optimum reaction velocity we could expect using 1 mM substrate is 52.4 g P released g^{-1} soil h^{-1}, far below V_{max}. This value is close to the maximum rate of phosphodiesterase activity measured in our soil.

Pyrophosphatase Activity

Pyrophosphatase activity in the leachate (Table 8), like other phosphatases, was very low with no significant differences between means in most instances. An exception to this is in the leachate of samples receiving 600 mg ortho-P kg^{-1}. Noticeably higher rates of activity were observed at this P treatment level than for lesser P inputs. Accepting that concentrations greater than 0.55 mM inorganic P repress the enzyme, no increase of activity should occur at these high levels of P.

Soil pyrophosphatase activity (Table 9) was not siginificantly different for soils treated with 0 phosphate and pyro-phosphate. In soil treated with both levels of ortho-phosphate, rates of activity are highly variable and erratic. Patterns of activity are difficult to observe, if they exist at all, in these treatments. Significant changes in the enzyme activity over the times of incubation were not detectable.

Plotting the statistical regression of the data shows a wild flux of activity in samples treated with 600 mg·kg^{-1} ortho-phosphate (Fig. 20). When 0 phosphate or either level of pyro-phosphate P is added do we see consistent results depicting the negative effect of ammonia on pyrophosphatase activity (Figures 17, 19, and 21). It is possible that

Table 8. Leachate pyrophosphatase activity for incubation periods 0 through 16 days. (mg P released·kg^{-1} soil·h^{-1})

TREATMENT AMM-N	ORTHO-P (mg/kg)	PYRO-P	TIME(DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05	LSD.20
0	0	0	2.2	5.5	3.3	4.4	2.3	0.0	3.3	5.6	3.7
600	0	0	1.1	6.6	2.2	3.6	3.3	2.2	4.4	7.0	4.4
1200	0	0	5.5	7.7	2.2	6.6	1.1	1.1	4.4	6.9	4.3
2400	0	0	6.6	3.3	2.2	3.3	1.1	4.4	5.5	8.5	5.4
0	300	0	11.0	6.6	8.8	2.2	6.7	4.4	4.4	17.3	10.9
600	300	0	4.4	4.4	4.4	0.0	2.2	9.9	8.7	12.8	8.0
1200	300	0	8.8	8.8	6.6	12.1	0.0	4.4	2.2	13.2	8.3
2400	300	0	8.8	11.0	3.3	3.3	0.0	8.8	2.2	11.4	7.2
0	0	300	2.2	3.2	4.4	0.0	1.5	2.2	2.2	5.7	3.6
600	0	300	6.6	9.9	0.0	1.1	3.3	2.2	2.2	9.5	6.0
1200	0	300	2.2	0.0	0.0	2.2	0.0	2.2	0.0	4.4	2.7
2400	0	300	0.0	4.4	0.0	3.3	0.0	5.5	0.0	6.8	4.2
0	600	0	13.2	49.5	4.4	8.8	0.0	13.2	7.7	46.7	29.3
600	600	0	59.3	33.0	31.3	3.3	6.5	19.8	4.5	70.1	43.9
1200	600	0	0.0	28.6	11.0	12.1	11.2	14.3	5.5	22.0	13.8
2400	600	0	0.0	15.4	6.6	8.8	4.6	23.1	34.0	32.9	20.6
0	0	600	4.4	9.9	1.1	0.0	2.2	8.7	5.5	8.7	5.4
600	0	600	2.2	4.4	3.3	0.0	4.4	7.7	2.2	8.3	5.2
1200	0	600	7.7	4.4	0.0	1.1	2.2	7.7	1.1	8.7	5.5
2400	0	600	6.6	2.2	0.0	0.0	1.1	4.4	5.5	9.2	5.8
		LSD.05	38.7	28.2	15.1	12.1	7.7	11.3	16.9		
		LSD.20	25.0	18.2	9.8	7.8	5.0	7.2	10.9		

Table 9. Soil pyrophosphatase activity for incubation periods 0 through 16 days. (mg P released·kg^{-1}soil·h^{-1})

TREATMENT AMM-N	ORTHO-P (mg/kg)	PYRO-P	TIME (DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05	LSD.20
0	0	0	96.8	80.0	71.6	83.9	96.6	104.6	93.2	48.9	30.7
600	0	0	40.7	27.6	11.3	42.6	40.2	26.7	40.5	41.8	26.2
1200	0	0	38.1	18.3	26.9	24.0	49.8	29.7	32.1	28.7	18.4
2400	0	0	54.5	29.2	24.1	24.6	27.2	23.2	30.6	34.2	21.5
0	300	0	77.5	87.3	136.7	127.3	151.8	88.9	83.5	127.0	79.6
600	300	0	75.8	106.9	77.3	48.9	26.4	103.4	135.3	113.5	71.2
1200	300	0	29.0	110.9	65.5	52.7	97.1	41.8	69.2	35.4	86.4
2400	300	0	80.6	53.1	59.6	98.6	30.8	27.2	27.4	122.9	77.1
0	0	300	94.8	101.2	88.7	91.6	59.9	66.7	87.6	88.4	55.5
600	0	300	55.5	39.1	22.8	44.2	0.0	30.9	74.2	55.4	34.8
1200	0	300	80.4	20.9	17.4	36.6	41.5	44.5	38.0	82.6	51.8
2400	0	300	29.1	38.0	9.9	84.5	31.6	18.8	28.1	91.7	57.5
0	600	0	82.6	316.9	44.0	99.9	107.3	35.4	171.1	343.2	215.2
600	600	0	158.8	320.2	92.3	106.2	26.3	35.2	143.7	254.1	159.4
1200	600	0	0.0	129.5	19.3	165.1	83.1	41.4	233.9	102.8	184.8
2400	600	0	67.0	282.5	57.1	99.1	18.3	53.8	62.5	247.5	155.2
0	0	600	94.4	69.5	27.2	104.2	83.2	64.8	58.0	76.4	47.9
600	0	600	76.7	34.8	72.6	16.2	6.7	20.8	31.9	56.5	35.4
1200	0	600	68.7	14.8	0.5	80.6	4.5	4.4	34.4	62.1	39.0
2400	0	600	30.7	73.0	16.4	31.0	0.0	6.9	38.0	51.3	32.2
		LSD.05	74.4	267.2	78.5	141.1	98.5	63.6	138.1		
		LSD.20	47.9	172.2	50.6	90.7	63.5	41.0	89.0		

the highly eratic results seen by the ortho-phosphate P treated soils produced such high variability as account for the lack of significant difference seen in other data.

Enzyme Kinetics

Values of K_m and V_{max} (Fig. 30) calculated for soil pyrophosphatase activity are 19.22 mM and 100.1 µg P released g^{-1} soil h^{-1}, respectively. These values are similar to the K_m and V_{max} reported by Dick and Tabatabai(1977) who note that their high values for K_m may be due to the adsorption of pyrophosphate by soil minerals. The average V_{max} reported by them was 500, much higher than that found in our soil. The low V_{max} we observe may possibly reflect low concentrations of pyrophosphatase in the soil.

pH

Soil and leachate pH values responded similarly to fertilizer treatments (Tables 10 and 11). As might be expected, the influence of ammonium applied to the soil greatly affected the pH, causing the value in both leachate and soil to rise significantly. Regression analysis (Fig. 22) shows that the ammonium-N treatment may account for 80 to 90% of the change in soil pH. With no incubation, little difference in pH resulted from any ammonia-N applications. With increasing length of incubation pH decreased proportionatly to the level of ammonia-N applied.

A significant effect of phosphate's buffering capacity was noted in the soil pH data. The increase of pH due to ammonia-N with phosphate treatments was not as large as the increase in pH from ammonia without phosphate, but phosphate in the soil (both pyro- and ortho-P) does buffer the influence of ammonia on pH. The effect of phosphate on soil and

leachate pH is outlined in Tables 12 and 13. The data show that application of ortho-phosphate to the soil significantly reduced the pH increase in all ammonia-N treated soils. Only at time 0 did ortho-P fail to modify the increase in pH from application of 1200 and 2400 mg kg^{-1} ammonia-N.

Application of pyro-phosphate to the soil did not reduce the pH increase as much as applications of ortho-P. This effect is significant, however, for nearly all samples of leachate and soil treated with 0 and 600 mg kg^{-1} ammonia-N. In samples treated with higher levels of ammonia, pyro-P showed little effect on pH.

Changes in the leachate and soil pH from applications of ammonia-N and ortho- and pyro-phosphate P can be represented by the following multiple regression statements:

a) Soil

$$pH = 7.06+0.0021(N)-(5.4\cdot10^{-7})(N)^2-0.002(P) -(1.1\cdot10^{-6})(P)2-0.002(h). \quad (R^2 = .87)$$

$$pH = 7.06+0.0022(N)-(6.27\cdot10^{-7})(N)^2-0.0013(P-P) +(1.0\cdot10^{-6})(P-P)2- 0.0017(h). \quad (R^2 = .88)$$

b) Leachate

$$pH = 7.1+0.0034(N)-(8.97\cdot10^{-7})(N)^2-0.0022(P) +(9.7\cdot10^{-6})(P)2- 0.004 (h). \quad (R^2 = .89)$$

$$pH = 7.2+0.0035(N)-(9.3\cdot10^{-7})(N)^2-0.0013(P-P) +(1.4\cdot10^{-6})(P-P)2-0.004(h). \quad (R^2 = .90)$$

where N is mg kg^{-1} NH_4-N applied to the soil, P is mg kg^{-1} ortho-phosphate P applied, P-P is mg kg^{-1} pyro-phosphate P applied to the soil, and h is the time of incubation in hours. All variables are siginificant at $\alpha = 0.05$.

Table 10. Leachate pH values for incubation periods 0 through 16 days.

TREATMENT AMM-N	ORTHO-P (mg/kg)	PYRO-P	TIME(DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05
0	0	0	6.7	7.0	6.9	6.9	6.9	6.9	6.7	0.3
600	0	0	10.0	9.3	9.2	9.0	8.7	8.0	7.2	0.3
1200	0	0	10.3	9.9	9.6	9.5	8.9	8.7	8.4	0.3
2400	0	0	10.6	10.1	10.0	10.0	9.5	8.8	8.8	0.2
0	300	0	5.6	5.8	5.9	6.0	5.9	5.8	5.6	0.2
600	300	0	9.4	8.5	8.3	8.4	7.9	6.9	6.4	0.3
1200	300	0	10.1	9.5	9.3	9.1	8.7	8.5	7.9	0.3
2400	300	0	10.5	9.8	9.8	9.6	9.0	8.8	8.7	0.3
0	0	300	5.8	6.5	6.6	6.5	6.4	6.1	5.7	0.4
600	0	300	9.0	9.2	9.0	8.8	8.7	7.5	6.8	0.9
1200	0	300	10.0	9.7	9.5	9.4	8.9	8.7	8.2	0.3
2400	0	300	10.5	10.1	9.9	9.8	9.3	8.8	8.9	0.3
0	600	0	5.9	5.5	5.6	5.7	5.7	5.7	5.7	0.6
600	600	0	8.3	7.5	7.3	7.3	7.3	6.9	6.4	0.8
1200	600	0	9.8	9.0	8.6	8.4	8.4	7.7	7.4	0.4
2400	600	0	10.4	9.3	9.0	8.7	8.7	8.5	8.1	0.4
0	0	600	5.6	6.4	6.5	6.5	6.5	6.1	5.8	0.3
600	0	600	9.6	9.2	8.7	8.5	8.5	7.0	6.8	0.3
1200	0	600	10.2	9.6	9.4	8.8	8.8	8.6	8.0	0.3
2400	0	600	10.4	10.2	9.7	9.3	9.3	8.8	8.8	0.4
		LSD.05	0.8	0.2	0.2	0.3	0.4	0.2	0.3	

Table 11. Soil pH values for incubation periods 0 through 16 days.

TREATMENT AMM-N	ORTHO-P (mg/kg)	PYRO-P	TIME (DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05
0	0	0	7.1	7.0	6.9	7.0	6.9	7.0	7.1	0.4
600	0	0	8.4	8.6	8.6	8.6	8.1	7.3	7.3	0.4
1200	0	0	8.5	8.8	8.7	8.7	8.4	8.5	8.1	0.3
2400	0	0	8.5	8.7	8.6	8.7	8.5	8.5	8.6	0.2
0	300	0	6.5	6.2	6.2	6.1	6.1	6.0	5.9	0.5
600	300	0	8.2	7.9	7.8	7.7	7.1	6.7	6.1	0.5
1200	300	0	8.3	8.6	8.5	8.6	8.2	7.9	7.5	0.5
2400	300	0	8.5	8.6	8.7	8.5	8.4	8.4	8.2	0.4
0	0	300	6.6	6.5	6.7	6.4	6.5	6.1	6.0	0.3
600	0	300	8.1	8.1	8.5	8.1	8.1	7.0	6.9	0.4
1200	0	300	8.4	8.5	8.7	8.6	8.1	8.3	8.0	0.3
2400	0	300	8.5	8.6	8.5	8.5	7.8	8.3	8.3	0.3
0	600	0	6.4	6.1	6.1	6.0	6.0	5.9	5.9	0.3
600	600	0	7.3	7.1	7.0	7.2	6.7	6.5	5.9	0.4
1200	600	0	8.1	8.2	8.2	8.4	7.8	7.4	7.1	0.4
2400	600	0	8.2	8.4	8.3	8.3	8.2	8.1	7.9	0.3
0	0	600	6.7	6.5	6.6	6.5	6.8	6.3	6.1	0.3
600	0	600	8.0	7.9	7.8	7.9	7.9	7.0	6.8	0.4
1200	0	600	8.1	8.4	8.5	8.4	8.2	8.0	7.8	0.4
2400	0	600	8.2	8.5	8.4	8.5	8.3	8.1	8.2	0.4
		LSD.05	0.5	0.2	0.3	0.4	0.4	0.2	0.2	

Table 12. Leachate pH for incubation periods 0 through 16 days showing the effect of phosphate application.

AMM-N (mg/kg)	TIME (days)	PHOSPHATE SOURCE (mg/kg) 0	ORTHO-P 300	ORTHO-P 600	PYRO-P 300	PYRO-P 600	LSD.05
0	0	6.7	5.6	5.9	5.8	5.7	0.9
	0.5	7.0	5.8	5.5	6.5	6.4	0.2
	1.0	6.9	5.9	5.6	6.5	6.5	0.2
	2.0	6.9	6.0	5.7	6.5	6.6	0.2
	4.0	6.9	5.9	5.7	6.5	6.4	0.2
	8.0	6.8	5.8	5.7	6.1	6.1	0.2
	16.0	6.7	5.6	5.7	5.7	5.8	0.3
600	0	10.0	9.4	8.3	9.0	9.6	0.5
	0.5	9.3	8.5	7.4	9.2	9.2	0.2
	1.0	9.2	8.3	7.3	9.0	8.7	0.2
	2.0	9.0	8.4	7.8	8.8	8.8	0.4
	4.0	8.7	7.9	7.3	8.7	7.0	0.2
	8.0	8.0	6.9	6.9	7.5	7.0	0.2
	16.0	7.2	6.4	6.4	6.8	6.8	0.3
1200	0	10.3	10.1	9.8	10.0	10.2	0.5
	0.5	9.9	9.5	9.0	9.7	9.6	0.1
	1.0	9.6	9.3	8.6	9.5	9.4	0.3
	2.0	9.5	9.1	8.6	9.4	9.3	0.5
	4.0	8.9	8.7	8.4	8.9	8.8	0.4
	8.0	8.7	8.5	7.7	8.7	8.6	0.1
	16.0	8.4	7.9	7.4	8.2	8.0	0.3
2400	0	10.6	10.5	10.4	10.5	10.4	0.4
	0.5	10.1	9.8	9.3	10.1	10.2	0.4
	1.0	10.0	9.8	9.3	10.1	10.2	0.3
	2.0	9.9	9.6	9.0	9.9	9.7	0.2
	4.0	9.5	9.0	8.7	9.3	9.2	0.5
	8.0	8.8	8.8	8.5	8.8	8.8	0.1
	16.0	8.8	8.7	8.0	8.9	8.8	0.2

Table 13. Soil pH for incubation periods 0 through 16 days showing the effect of phosphate application.

AMM-N (mg/kg)	TIME (days)	PHOSPHATE SOURCE (mg/kg) 0	ORTHO-P 300	ORTHO-P 600	PYRO-P 300	PYRO-P 600	LSD.05
0	0	7.1	6.6	6.4	6.6	6.7	0.7
	0.5	7.0	6.2	6.1	6.4	6.5	0.3
	1.0	6.9	6.2	6.1	6.6	6.6	0.3
	2.0	7.0	6.0	6.0	6.4	6.3	0.3
	4.0	6.9	6.1	6.0	6.4	6.6	0.2
	8.0	7.0	6.0	5.9	6.1	6.3	0.2
	16.0	7.0	5.9	5.9	6.0	6.0	0.3
600	0	8.4	8.2	7.3	8.1	8.0	0.6
	0.5	8.6	7.8	7.1	8.1	7.9	0.2
	1.0	8.6	7.8	7.0	8.5	7.8	0.2
	2.0	8.6	7.7	7.2	8.1	7.9	0.6
	4.0	8.1	7.1	6.6	7.9	8.6	0.7
	8.0	7.3	6.7	6.5	7.0	7.0	0.2
	16.0	7.3	6.1	5.9	6.1	5.9	0.3
1200	0	8.5	8.3	8.1	8.4	8.1	0.5
	0.5	8.8	8.6	8.2	8.5	8.4	0.3
	1.0	8.7	8.5	8.2	8.7	8.5	0.3
	2.0	8.7	8.6	8.4	8.6	8.4	0.4
	4.0	8.4	8.2	7.8	8.4	8.3	0.3
	8.0	8.5	7.9	7.4	8.3	8.0	0.1
	16.0	8.1	7.5	7.1	8.0	7.8	0.4
2400	0	8.5	8.4	8.2	8.5	8.2	0.5
	0.5	8.7	8.6	8.4	8.6	8.5	0.2
	1.0	8.6	8.7	8.3	8.5	8.4	0.3
	2.0	8.7	8.5	8.3	8.5	8.5	0.3
	4.0	8.5	8.4	8.1	8.4	8.2	0.1
	8.0	8.5	8.4	8.1	8.2	8.1	0.2
	16.0	8.6	8.2	7.9	8.3	8.2	0.5

Ortho-phosphate Concentration

The ortho-P concentration in the soil and leachate may regulate phosphatase activity. Usually, the phosphate has an inhibitory effect on phosphatase activity. Introduction of ortho-P into the system would naturally increase the soil solution and soil extractable P levels. Due to the extraction process employed in this experiment, values of soil P may be higher than those evaluated using other means of P extraction. This is likely due to the higher concentration of acid solution used in our method compared to the methods of Bray and Kurtz, and Nelson (P availability indices listed by Olsen and Sommers, 1980).

Addition of ortho-phosphate (Table 14) significantly raised the concentration of solution P. The presence of ammonia reduced the degree of increase of solution ortho-phosphate concentration as ortho-P was added. The addition of pyro-phosphate also increased the ortho-P concentration of the leachate after 0.5 days by small but significant amounts.

With increasing time of incubation, there was a significant decrease in the concentration of P in the leachate. We may speculate that with increased time, soluble P was subject to mineral fixation in the soil. By the sixteenth day of incubation, the ortho-P levels of samples treated with equal amounts of P were similar in value regardless of the phosphate species applied.

Extractable P in the soil also increased following introduction of ortho-phosphate, though not so dramatically as that in the leachate (Table 15). Also noted was an increased level of ortho-P in samples treated with pyro-phosphate. Adding ammonia with phosphate applications

Table 14. Leachate ortho-phosphate concentrations for incubation periods 0 through 16 days. (mg P·kg^{-1} soil)

TREATMENT AMM-N	ORTHO-P (mg/kg)	PYRO-P	TIME(DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05	LSD.20
0	0	0	1.1	0.9	1.4	0.7	1.0	0.7	4.4	3.5	2.2
600	0	0	1.2	5.7	1.4	1.0	1.6	0.7	2.9	5.4	3.4
1200	0	0	1.2	6.6	1.7	1.3	2.3	2.5	2.6	5.8	3.6
2400	0	0	1.0	1.7	1.6	1.5	1.9	2.7	4.4	1.1	0.7
0	300	0	394.9	188.9	156.6	122.7	100.6	69.2	42.9	20.8	13.0
600	300	0	91.8	84.5	70.1	74.3	62.6	47.2	29.1	52.5	33.0
1200	300	0	65.1	66.7	46.7	96.6	63.1	73.1	42.3	49.8	31.2
2400	300	0	72.6	83.1	50.0	64.8	85.3	88.7	59.9	47.5	29.8
0	0	300	20.7	24.1	29.7	37.5	27.7	36.4	29.4	15.2	9.6
600	0	300	2.4	4.1	7.0	10.9	15.0	18.1	10.2	6.4	4.0
1200	0	300	3.0	3.6	8.9	11.1	20.1	41.7	26.2	9.6	6.0
2400	0	300	3.3	3.6	7.4	7.9	10.6	36.8	34.4	7.3	4.6
0	600	0	777.7	492.4	278.1	220.3	243.0	161.9	69.8	144.3	90.5
600	600	0	364.1	361.6	222.5	295.1	297.3	186.3	73.2	113.2	71.0
1200	600	0	390.8	369.5	205.0	306.1	290.5	219.3	77.2	87.0	54.5
2400	600	0	387.2	330.1	254.8	270.1	312.5	183.2	119.7	105.7	66.3
0	0	600	36.4	60.5	73.2	93.8	78.2	80.4	64.2	14.7	9.2
600	0	600	6.4	12.8	17.2	25.0	30.4	40.9	28.7	9.1	5.7
1200	0	600	7.0	8.5	6.0	14.7	35.2	74.4	42.1	15.7	9.8
2400	0	600	10.8	9.0	9.4	11.7	17.7	78.9	70.7	11.7	7.4
		LSD.05	59.1	45.9	65.1	62.3	45.3	38.6	43.1		
		LSD.20	38.1	29.6	42.0	40.1	29.2	24.9	27.8		

Table 15. Soil ortho-phosphate concentrations for incubation periods 0 through 16 days. ($mg \cdot kg^{-1}$)

TREATMENT AMM-N (mg/kg)	ORTHO-P (mg/kg)	PYRO-P (mg/kg)	TIME (DAYS) 0	0.5	1.0	2.0	4.0	8.0	16.0	LSD.05	LSD.20
0	0	0	144.0	131.7	145.8	136.7	174.4	120.0	121.8	70.8	44.4
600	0	0	183.4	168.2	201.4	147.3	184.7	147.0	161.0	62.8	39.4
1200	0	0	199.3	173.5	222.4	155.7	216.6	159.0	180.1	73.5	43.8
2400	0	0	173.8	163.5	220.7	174.1	233.7	163.6	208.2	69.8	43.8
0	300	0	306.5	210.0	195.3	216.1	248.8	199.6	252.5	187.0	117.3
600	300	0	374.3	257.7	280.3	215.2	248.2	224.8	206.6	144.4	90.5
1200	300	0	450.3	290.0	258.5	219.5	314.0	288.0	307.7	87.4	68.1
2400	300	0	352.7	293.8	267.3	282.6	363.2	275.0	286.9	118.7	74.4
0	0	300	261.8	203.3	229.8	148.6	216.3	169.1	159.2	136.0	85.3
600	0	300	302.4	268.1	201.4	212.7	83.7	195.9	200.1	146.6	92.0
1200	0	300	284.3	266.9	159.1	237.6	84.5	247.5	268.2	149.7	93.9
2400	0	300	275.8	255.9	237.8	244.1	278.8	255.7	272.5	64.7	40.6
0	600	0	210.9	320.6	256.8	230.0	340.6	306.6	358.2	160.4	100.5
600	600	0	349.9	550.0	312.7	270.0	398.9	350.9	350.2	220.6	138.3
1200	600	0	421.8	547.9	403.3	278.2	466.0	348.6	446.3	257.6	161.5
2400	600	0	440.3	615.8	395.7	309.9	421.4	391.7	427.8	255.0	159.9
0	0	600	197.1	208.6	205.6	159.6	313.1	167.3	204.8	114.4	71.8
600	0	600	298.2	259.4	226.6	230.3	313.2	216.6	237.7	93.0	58.3
1200	0	600	320.9	298.4	163.0	247.5	349.7	243.8	306.1	131.8	82.7
2400	0	600	301.5	317.2	243.2	254.8	355.5	287.5	329.7	93.0	58.3
		LSD.05	112.1	219.2	133.7	99.4	124.6	139.4	87.5		
		LSD.20	72.3	141.3	86.2	64.1	80.3	90.0	56.4		

seemed to increase the amount of extractable P, which was contrary to its effect in leachate.

With increasing time of incubation, soil extractable P levels held constant with 300 and 600 mg kg^{-1} ortho-phosphate P treatments. In samples treated with pyro-phosphate, the concentration of ortho-P increased up to 16 days, presumably reflecting the hydrolysis of pyro-phosphate ions.

Why the Loss of Phosphatase Activity?

Clearly, addition of ammonium and phosphate to soil reduces phosphatase activity. Several factors influence the degree which phosphatase activity decreases. Appiah and Thomas (1982) observed decreases in phosphatase activity resulting from P fertilization, reportedly due to enzyme inhibition as well as a decrease of its synthesis by microbes and plants. According to Juma and Tabatabai (1978), acid phosphatase is negatively correlated to soil pH and alkaline phosphatase activity is positively correlated to soil pH. The decreases in alkaline phosphatase activity we observed following ammonium application suggest that ammonium is an inhibitor of phosphatase activity, and that this inhibition is not entirely due to associated changes in the soil pH. Tomasiewicz and Henry (1982) also report that solubilization of soil organic matter relates more highly (R^2 = .94) to ammonia application rates than soil pH.

Exact mechanisms responsible for this reduction remain unidentified. Reduced phosphatase activity could result from changes in the enzyme kinetics, either K_m or V_{max}, which respond to pH and changes in the ionic strength of the soil solution. Abrupt pH changes in the enzyme's

environment may cause denaturization (breaking di-sulfide bonds or amino acid linkages which alter enzyme conformation) or by changing the charged state of the enzyme or substrate which lowers reaction velocity (Harper,1975).

Competitive inhibition may also decrease phosphatase activity, whereby the enzyme reacts with or complexes with substances other than substrate. Destruction of the enzyme may also result from changes in the ionic composition of the soil solution following the addition of our fertilizer treatments. Denaturization occuring from high salt concentrations in the soil ruptures weak ionic or non-polar bonds that maintain the enzyme's active configuration (Harper, 1975). The substrate itself may also become chemically altered and be rendered unavailable to enzymatic hydrolysis.

Sources of Error

The phosphatase assay procedures used were open to some sources of error which must by taken into account. J.L. Neal, el. al. (1981) showed that over-estimation of enzyme activity by p-nitrophenyl phosphate assay can occur as the result of heat-induced chemical hydrolysis of p-nitrophenyl phosphate instead of enzymatic activity. Irving and Cosgrove (1976) suggested that values from phosphatase analysis with p-nitrophenyl phosphate may require adjustment since more than one type of substrate may react with phosphatase in the soil.

The high degree of variability in our results may be the consequence of the analysis procedure used on such a complex system as that of soil enzymes. Tabatabai and Bremner (1971), in evaluation of soil enzyme kinetics, noted more consistent results when their samples were shaken

during the incubation period. Our samples were not shaken and may have experienced reduced rates of phosphatase activity since settling can decrease enzyme and substrate contact.

The variability our data show leads to uncertainty in the evaluation of the results beyond a level of 80% statistical confidence. Future assay of phosphatases (and soil enzymes in general) may possibly have better precision if precaution is taken in the experimental design and technique. These may include:

a) regular calibration of instrumentation and reagent measuring vessels. In this study, this may have contributed little error, as effort was maintained to keep all instruments and reagent dispensing devices well-calibrated.

b) fewer samples assayed at any one time. Large numbers in complicated, manual assays inadvertently lead to operator error.

c) shaking assay samples during incubation to maintain consistent homogenation of the soil-subtrate suspension for the duration of the incubation.

CONCLUSION

Analysis of the response of soil phosphatase activity to ammonium and phosphate fertilization represents an attempt to quantify a complex system. In our study of the effects of ammonium and phosphate applications on phosphatase activity, we tried to observe changes related not only to the direct influence of the fertilizer treatment, but also to time, soil pH, and ortho-P concentration of the soil.

Although very low levels of phosphatase activity were detected in the leachate, these values could nevertheless still account for a sizeable contribution of organic P to plant nutrition. None of the four phosphatases assayed showed response to the fertilizer treatment or were correlated well to the ortho-P content of the leachate. No statistical evidence of any pattern in changes of phosphatase activity was apparent.

While we were able to observe the dispersion of organic matter as a result of ammonia application to the soil, any release of organically bound phosphatase into the soil solution was masked by its probable inhibition from high pH, high levels of ortho-P, or increased concentration of ammonium. Our data does not allow interpretation of whether the enzymes are liberated from the organic complex due to ammonia application, but do indicate little, if any, effect on the phosphatase activity of the leachate.

Phosphatase activity of the soil, unlike the leachate, is affected by the additions of ammonium and phosphate. Acid and alkaline phosphatase activities showed a significant, negative response with application of ammonia-N. The effect of ammonium (and the corresponding

increase in soil pH) is especially evident for acid phosphatase as soil pH is moved from the enzyme's optimum level. Changes in acid and alkaline phosphatase activity from the influence of phosphate in the soil are not as large as the changes due to ammonia application, yet phosphate does have a restricting effect on acid and alkaline phosphatase activity.

Soil phosphodiesterase activity was influenced by ortho-P and pyro-P, decreasing with increasing phosphate application. The most profound effect came from the addition of 300 mg kg^{-1} of phosphate. This suggests that inhibition of phosphodiesterase occurs with soil phosphate concentrations lower than 300 mg P kg^{-1}soil. In accordance with this, Browman and Tabatabai (1978) noted that 0.05 mM ortho-phosphate acts as an effective inhibitor of phosphodiesterase activity. Phosphodiesterase activiy also reacted negatively to the presence of ammonia in the soil, however, this effect was less than that with phosphate.

Soil pyrophosphatase showed little response to addition of ammonium or phosphate to the soil. Pyrophosphatase activity is negatively influenced by the addition of ammonia, however, this observation is clear only in the absence of phosphate applications to the soil. Combinations of phosphate and ammonia in the soil did not consistently effect pyrophosphatase activity. While the addition of ortho-phosphate, a phosphatase inhibitor, produced some increased enzyme activity, the addition of pyro-phosphate, the substrate upon which pyrophosphatase reacts, did not increase any activity. Insight to this occurrence comes from the work of Dick and Tabatabai (1978) who noted that increased concentrations of pyro-phosphate actually inhibit the activity of pyrophosphatase.

Our study shows the inhibitory effects of ammonia-N and

ortho-phosphate on soil phosphatase activity. Soils treated with ammoniacal fertilizers showed large increases in pH, slowing the rate of enzyme activity significantly. Decreases in enzyme activity were most apparent in ammonia-N treated soils and correlate well to the presence of the ammonium ion. The increased presence of ortho-phosphate also served to significantly inhibit phosphatase activity.

Further investigation of the response of soil phosphatases to chemical soil ammendments is necessary to gain increased understanding of this complex subject. These results indicate that a variable response may be anticipated and certain reactions may be expected, these being:

i) a decrease in activity in response to ammonium (or ammonia) and phosphate applications, especially at elevated concentrations, and

ii) a decrease in activity from changes of soil pH from the level showing optimum activity.

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APPENDIX

Table 16a. Correlation coefficients for leachate phosphatase to leachate pH and ortho-P concentration.

	pH	ORTHO-P CONC
ACID PHOSPHATASE	-0.013	0.07
ALKALINE PHOSPHATASE	-0.07	0.06
PHOSPHODIESTERASE	0.24	-0.01
PYROPHOSPHATASE	-0.11	0.345
pH		-0.25

Table 16b. Correlation coefficients for soil phosphatase to soil pH and ortho-P concentration.

	pH	ORTHO-P CONC
ACID PHOSPHATASE	-0.386	-0.21
ALKALINE PHOSPHATASE	-0.262	-0.12
PHOSPHODIESTERASE	0.082	-0.30
PYROPHOSPHATASE	-0.25	0.36
pH		0.09

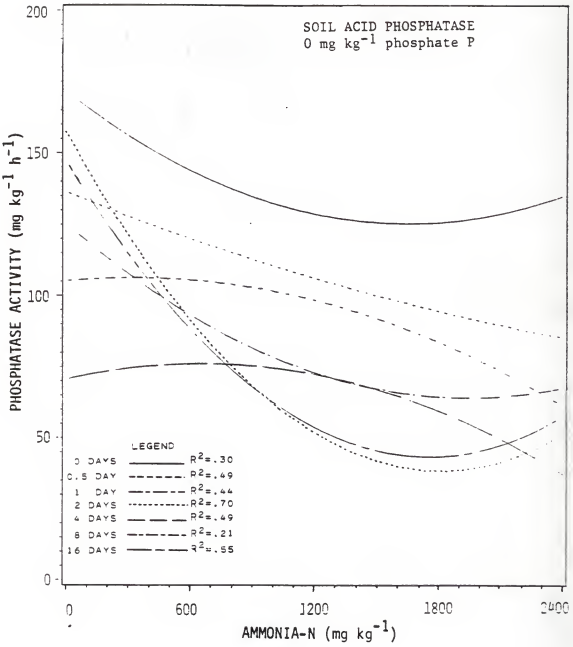

Fig. 2. Acid phosphatase activity vs. Ammonia-N rate with 0 mg kg^{-1} phosphate P applied.

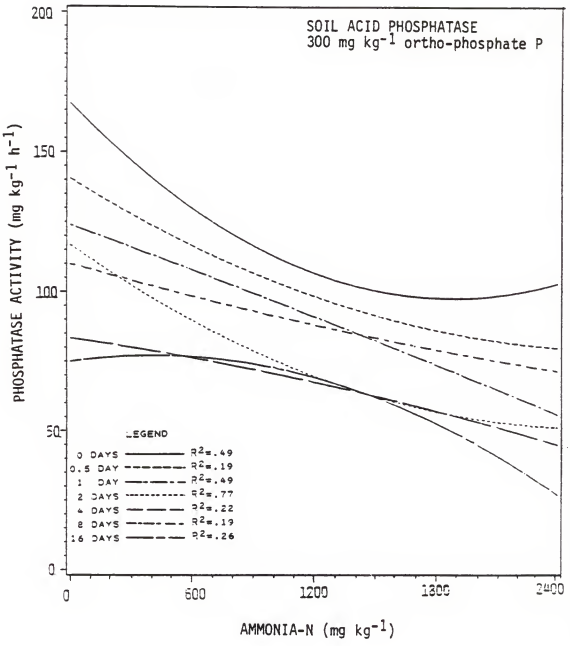

Fig. 3. Acid phosphatase activity vs. Ammonia-N rate with 300 mg kg-1 ortho-phosphate P applied.

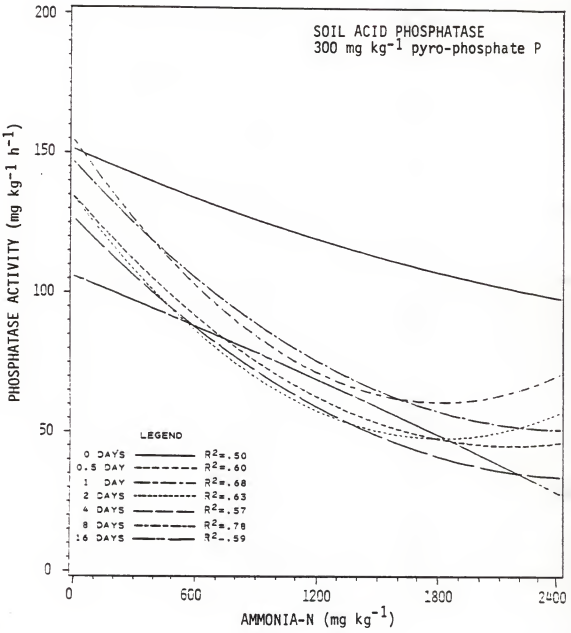

Fig. 4. Acid phosphatase activity vs. Ammonia-N rate with 300 mg kg^{-1} pyro-phosphate P applied.

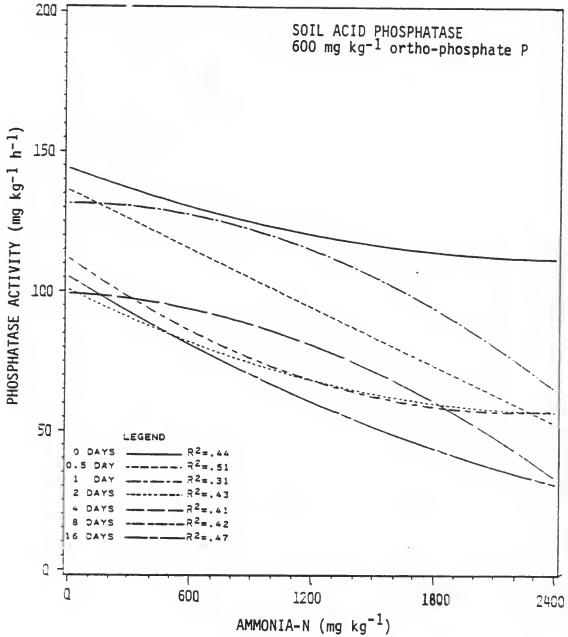

Fig. 5. Acid phosphatase activity vs. Ammonia-N rate with 600 mg kg^{-1} ortho-phosphate P applied.

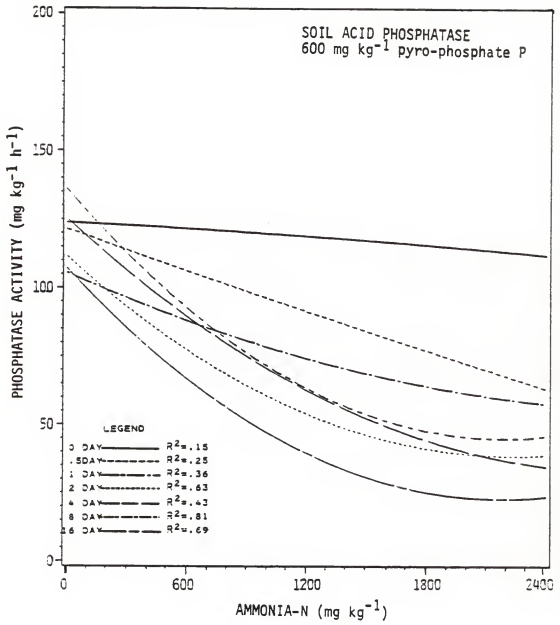

Fig. 6. Acid phosphatase activity vs. Ammonia-N rate with 600 mg kg^{-1} pyro-phosphate P applied.

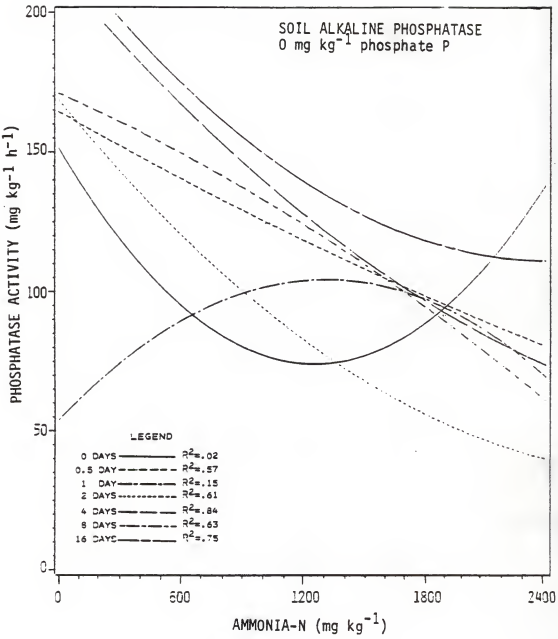

Fig. 7. Alkaline phosphatase activity vs. Ammonia-N rate with 0 mg kg^{-1} phosphate P applied.

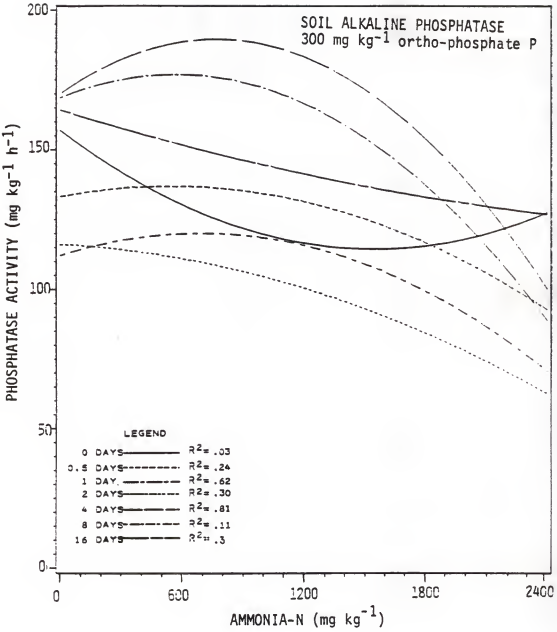

Fig. 8. Alkaline phosphatase activity vs. Ammonia-N rate with 300 mg kg^{-1} ortho-phosphate P applied.

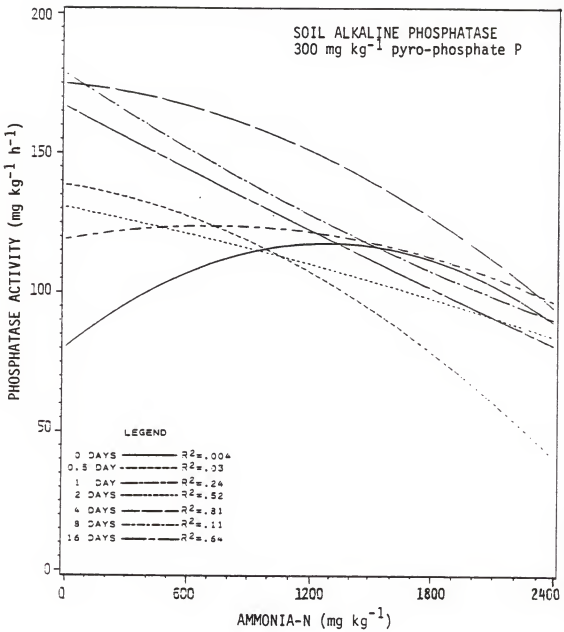

Fig. 9. Alkaline phosphatase activity vs. Ammonia-N rate with 300 mg kg^{-1} pyro-phosphate P applied.

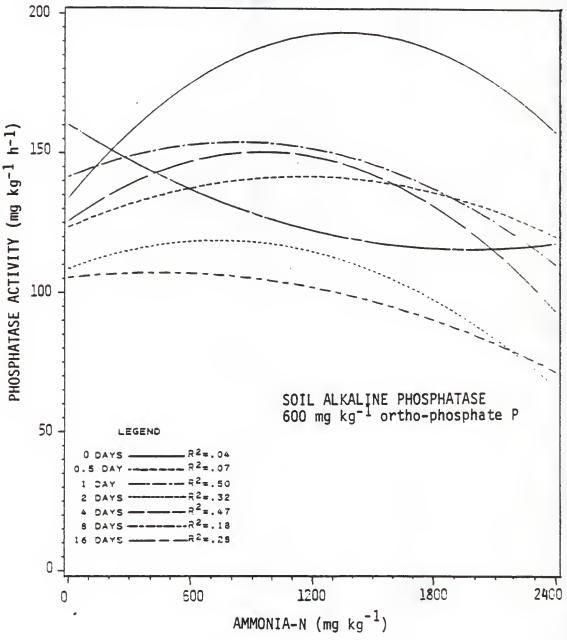

Fig. 10. Alkaline phosphatase activity vs. Ammonia-N rate with 600 mg kg^{-1} ortho-phosphate P applied.

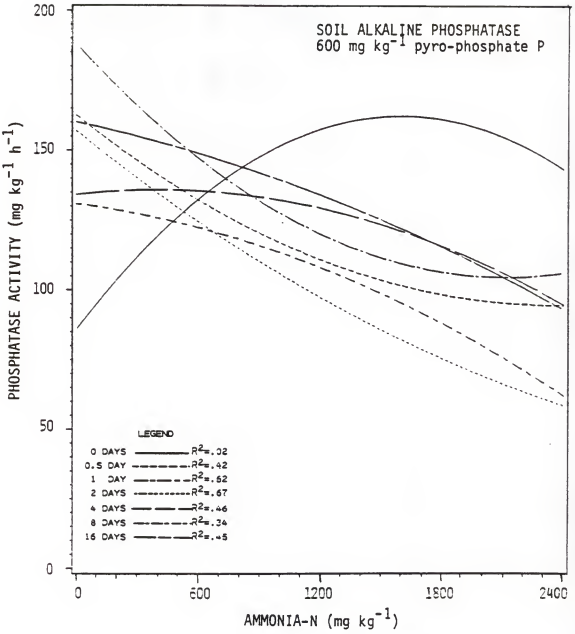

Fig. 11. Alkaline phosphatase activity vs. Ammonia-N rate with 600 mg kg^{-1} pyro-phosphate P applied.

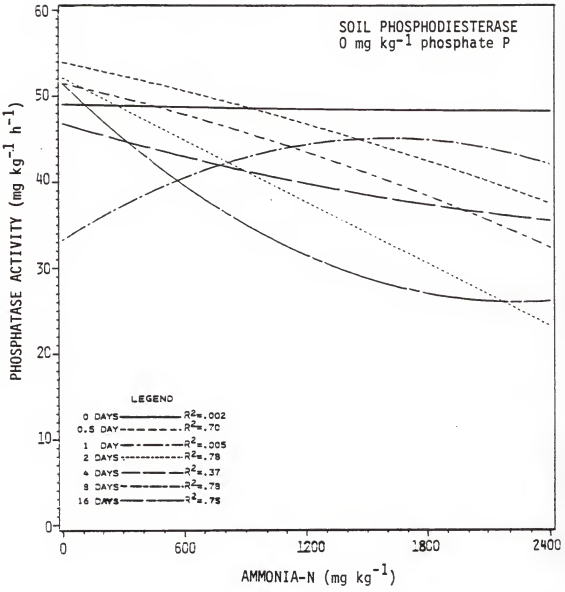

Fig. 12. Phosphodiesterase activity vs. Ammonia-N rate with 0 mg kg^{-1} phosphate P applied.

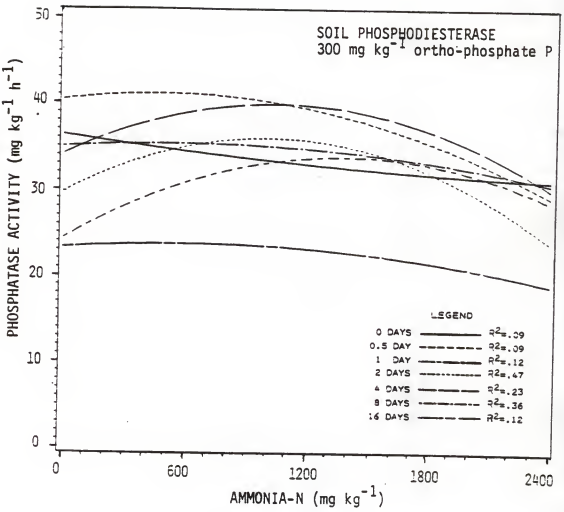

Fig. 13. Phosphodiesterase activity vs. Ammonia-N rate with 300 mg kg^{-1} ortho-phosphate P applied.

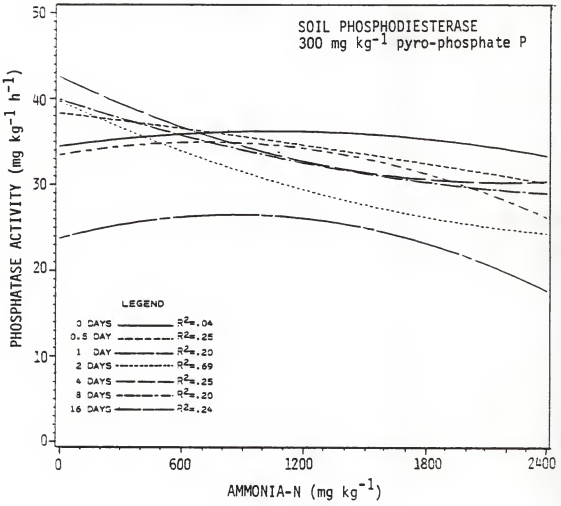

Fig. 14. Phosphodiesterase activity vs. Ammonia-N rate with 300 mg kg^{-1} pyro-phosphate P applied.

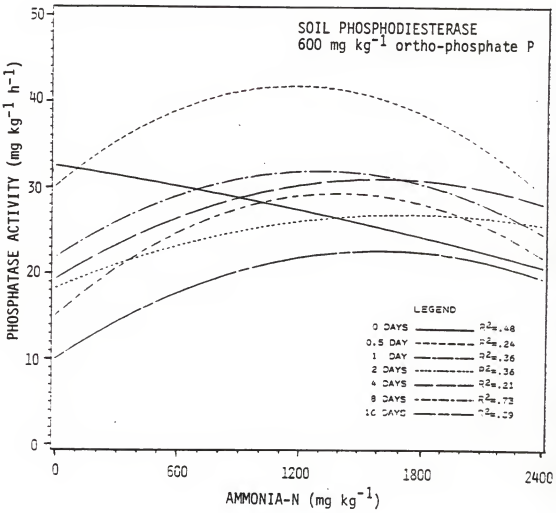

Fig. 15. Phosphodiesterase activity vs. Ammonia-N rate with 600 mg kg^{-1} ortho-phosphate P applied.

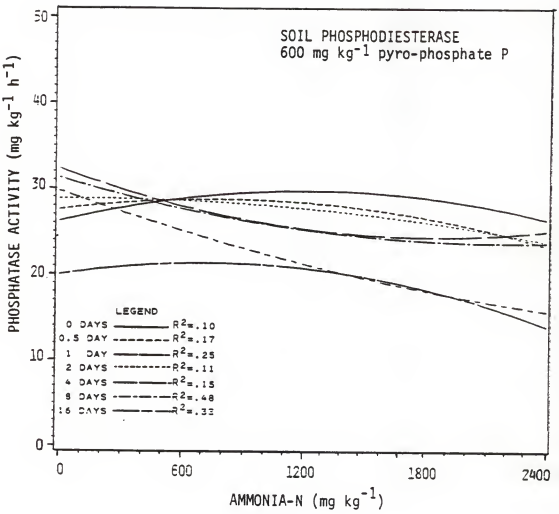

Fig. 16. Phosphodiesterase activity vs. Ammonia-N rate with 600 mg kg^{-1} pyro-phosphate P applied.

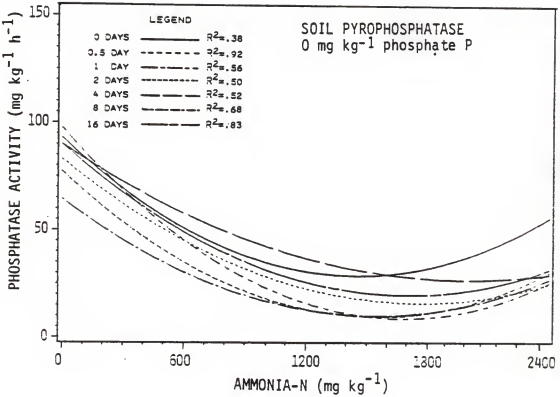

Fig. 17. Pyrophosphatase activity vs. Ammonia-N rate with 0 mg kg^{-1} phosphate P applied.

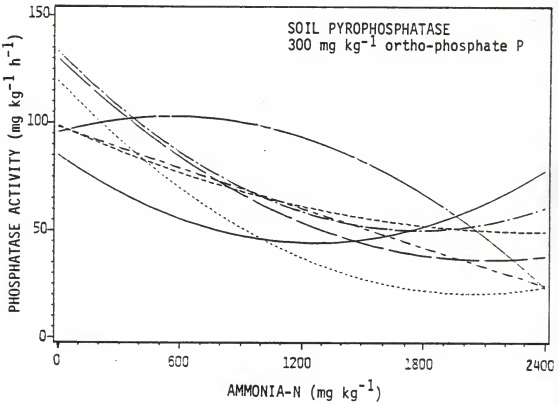

Fig. 18. Pyrophosphatase activity vs. Ammonia-N rate with 300 mg kg^{-1} ortho-phosphate P applied.

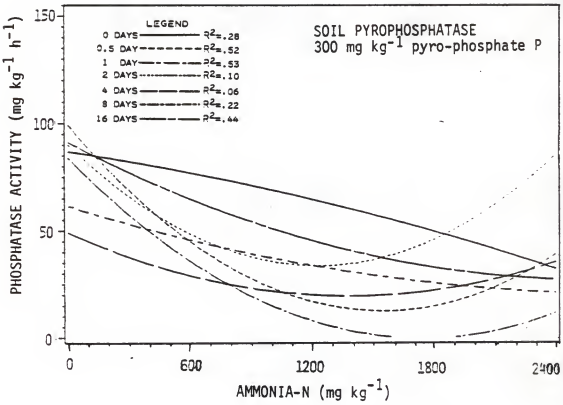

Fig. 19. Pyrophosphatase activity vs. Ammonia-N rate with 300 mg kg^{-1} pyro-phosphate P applied.

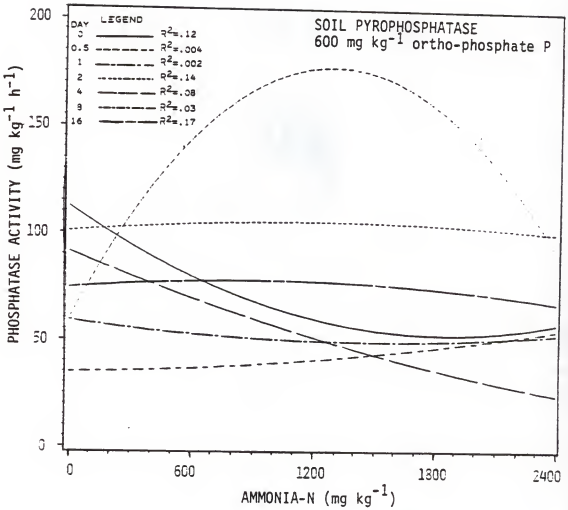

Fig. 20. Pyrophosphatase activity vs. Ammonia-N rate with 600 mg kg^{-1} ortho-phosphate applied.

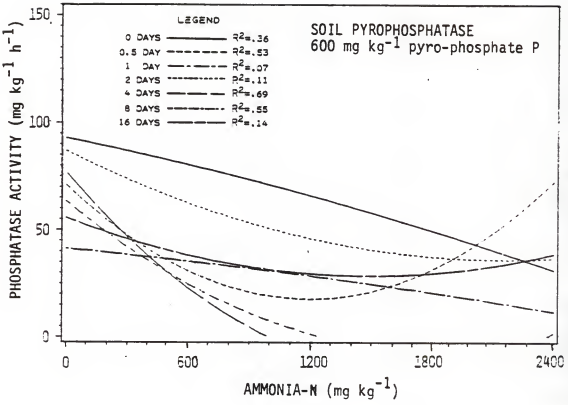

Fig. 21. Pyrophosphatase activity vs. Ammonia-N rate with 600 mg kg^{-1} pyro-phosphate P applied.

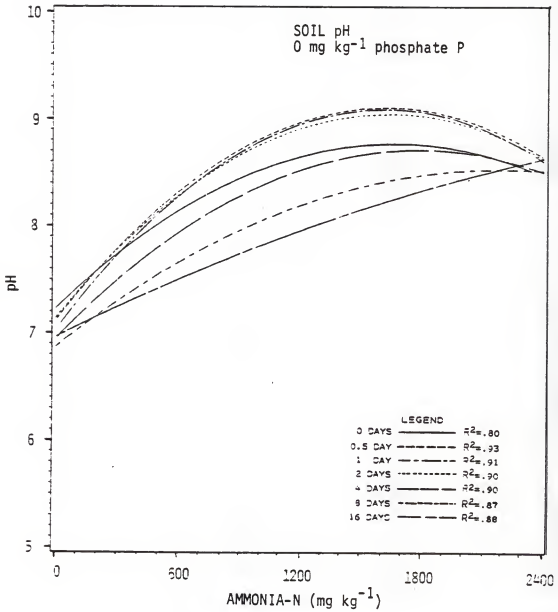

Fig. 22. Soil pH vs. Ammonia-N rate with 0 mg kg^{-1} phosphate P applied.

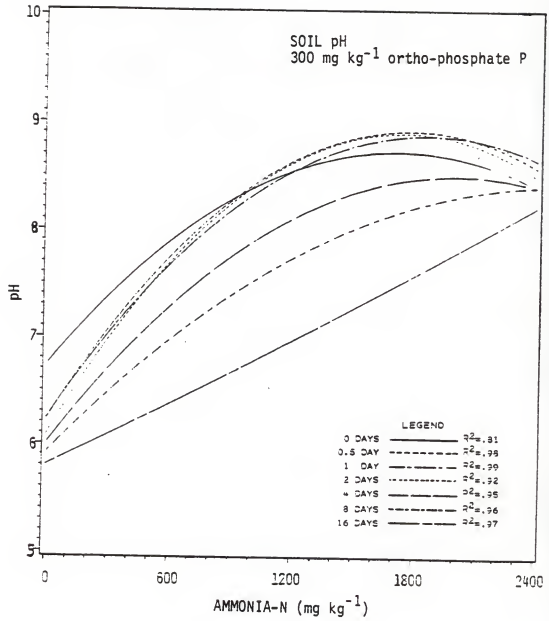

Fig. 23. Soil pH vs. Ammonia-N rate with 300 mg kg^{-1} ortho-phosphate P applied.

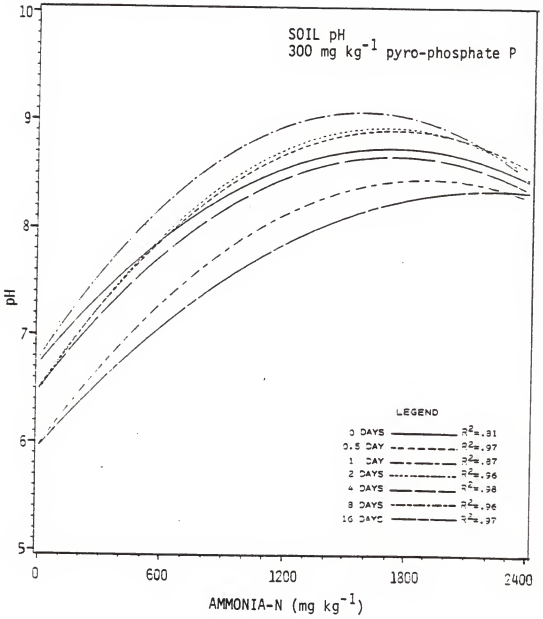

Fig. 24. Soil pH vs. Ammonia-N rate with 300 mg kg^{-1} pyro-phosphate P applied.

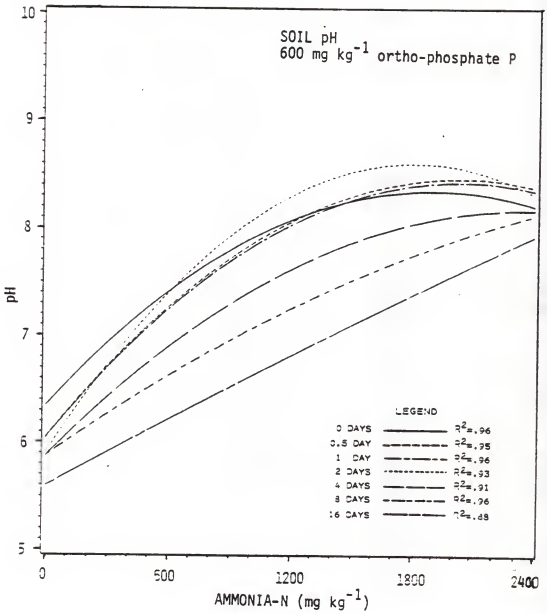

Fig. 25. Soil pH vs. Ammonia-N rate with 600 mg kg^{-1} ortho-phosphate P applied.

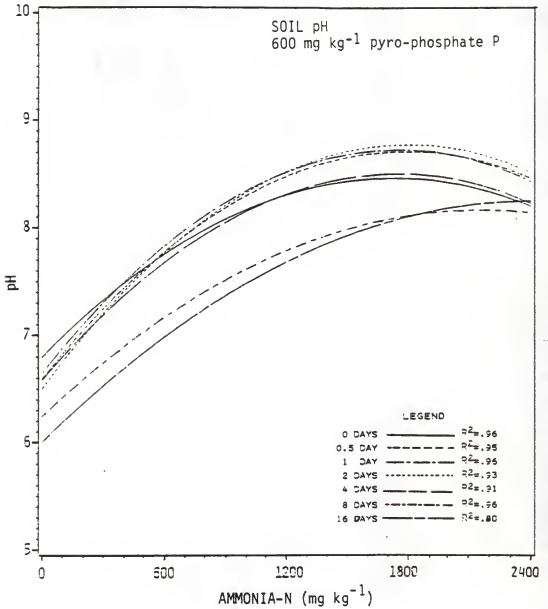

Fig. 26. Soil pH vs. Ammonia-N rate with 600 mg kg^{-1} pyro-phosphate P applied.

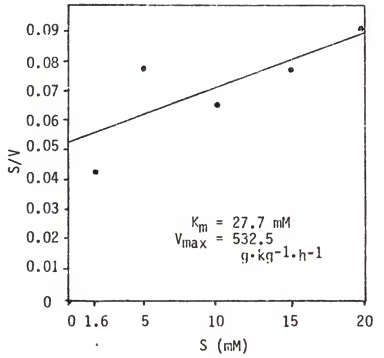

Fig. 27. Plot of S/V vs. S to determine K_m and V_{max} for soil acid phosphatase.

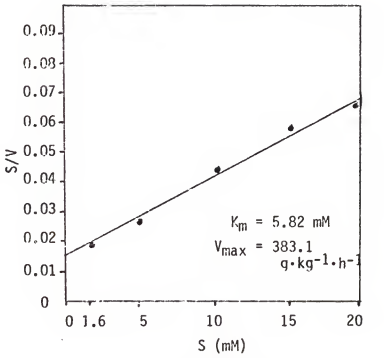

Fig. 28. Plot of S/V vs. S to determine K_m and V_{max} for soil alkaline phosphatase.

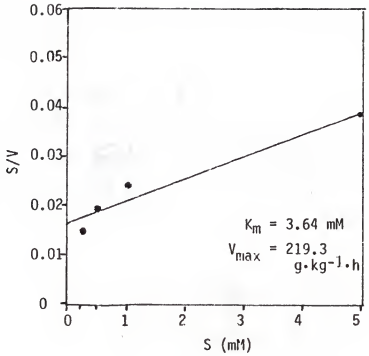

Fig. 29. Plot of S/V vs. S to determine K_m and V_{max} for phosphodiesterase.

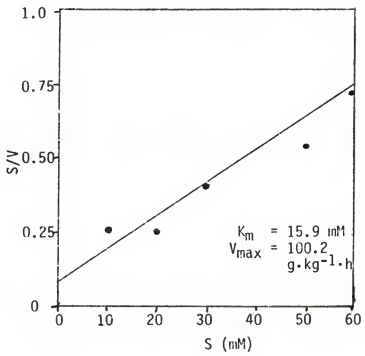

Fig. 30. Plot of S/V vs. S to determine K_m and V_{max} for pyrophosphatase.

VITA

Theodore Arlett Hartsig was born to Joseph and Barbara Hartsig on Oct. 4, 1956 in Mesa, Arizona. He is the fourth of seven children. In 1974, he graduated from high school at the American International School of Kabul, Afghanistan. In August of 1974, he enrolled at Northern Arizona University in Flagstaff, Arizona where he received his Bachelor of Science in Biology in December, 1978.

Mr. Hartsig worked for the U.S. Forest Service during 1979, and then became employed with W.L. Gore and Associates in research and development of medical products. In 1981, he came to Kansas State University to pursue his Master's degree in Agronomy. During that time he was employed as a graduate research assistant and a graduate teaching assistant under Dr. S.J. Thien.

Mr. Hartsig was married in 1980 to his wife, Rebecca. They have one daughter, Colleen, born April 4, 1984.

AMMONIUM AND PHOSPHATE REACTIONS IN THE SOIL: EFFECT ON SOIL PHOSPHATASE ACTIVITY

by

Theodore Arlett Hartsig
B.S. Northern Arizona University 1978

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

Kansas State University
Manhattan, Kansas

1984

Addition of ammoniacal fertilizers to soil disperses humic and fulvic acid from the soil organic matter complex. The object of our research was to investigate changes in the activity of soil phosphatases in response to ammonium and phosphate fertilizer treatments.

Treatment combinations of ammonium-N as NH_4OH (equivalent to 0, 600, 1200, and 2400 $mg \cdot kg^{-1}$ soil) and phosphate-P as mono-ammonium phosphate or ammonium pyro-phosphate (equivalent to 0, 300, and 600 $mg \cdot kg^{-1}$ soil) were incubated in soil columns for periods of 0 through 16 days. The samples were then analyzed for acid and alkaline phosphatase, phosphodiesterase, and pyrophosphatase activities in both the soil leachate and bulk soil in relation to the treatment combinations, pH, and the ortho-phosphate content of the soil.

Analysis of the data indicate a decrease in phosphatase activity with increasing fertilizer treatment levels. Leachate phosphatase activity was very low with no significant differences between means detected in response to addition of fertilizer treatments. Loss of soil acid and alkaline phosphatase activity was most strongly correlated to changes in the ammonium applications to the soil, with some loss attributed to pH change and ortho-P concentrations. In contrast, the activity of soil phosphodiesterase was more strongly correlated to the soil ortho-phosphate concentration. Soil pyrophosphatase activity showed no observable pattern in response to

the fertilizer treatments nor did it correlate well to soil pH, ortho-P concentration, or the ammonium content of the soil.

We may speculate as to whether it may be changes in the enzyme kinetics of phosphatase brought about by the fertilizer treatments, denaturization of the enzyme in the presence of high concentrations of ammonium, or changes in the availability of substrate. From our results, we may only be certain of decreases in phosphatase activity with application of ammonium and phosphate fertilizers to the soil.