PHOSPHATASE ACTIVITY, pH, AND PHOSPHORUS IN THE RHIZOSPHERE OF CORN

BY

GEORGE ERNEST BOERO

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Chapter 1

INTRODUCTION

The presence of a plant root changes the surrounding soil. In 1904, Hiltner introduced and defined the rhizosphere as the portion of soil influenced by the plant root. Rhizosphere soil differs microbiologically, chemically, and physically from bulk soil samples (Carson, 1971). Physically, the bulk density increases and moisture decreases. Microbes increase about ten fold and thrive on organic exudations and root debris. Chemically, oxygen is depleted and carbon dioxide increases. An imbalance of cation and anion uptake causes the root to release hydrogen (H^+) or hydroxyl (OH^-) ions, thus chemically changing the hydrogen ion activity (pH) and reduction-oxidation potential of the rhizosphere. This soil micro-environment, dominated by the presence of a root, is also the soil from which the root draws essential nutrients. The root, by influencing the adjacent soil, may be providing a survival mechanism by enhancing the nutrient availability in the rhizosphere. This becomes obviously useful in selecting or breeding a more efficient plant. Moreover, the dynamic differences in the rhizosphere soil versus the bulk soil must be realized in order to comprehend and analyze the nutrient potential of soils. Thus the conditions, magnitude, and significance of roots influencing their

adjacent soil warrants careful study and evaluation.

This study investigated the rhizosphere effect on plant availability and utilization of phosphate from organic and inorganic sources. The ability of the rhizosphere effect to control the solubility of inorganic phosphate is pH related. The release of phosphate from organic and poly-phosphate sources is dependent upon the soil enzyme phosphatase. The purpose of this study is to evaluate these two effects, rhizosphere pH and rhizosphere phosphatase, on the phosphorus nutrition of corn. Phosphatase and pH are independent rhizosphere effects and will be reported in separate chapters. However, the same sampling methods and soils were used for both the pH and phosphatase analyses and are covered in one common chapter.

The objective of this study is to quantify the rhizosphere effects of phosphatase and pH changes in the rhizosphere and then to evaluate these effects on the soil-phosphorus availability to corn.

Chapter 2

GENERAL METHODS

Introduction

The general methods of this investigation, rhizosphere sampling procedure, fumigation treatment, soils, and growing conditions are presented in this chapter. These techniques describe the common approach for collecting data and evaluating pH and phosphatase changes in the rhizosphere reported in later chapters.

Sampling the Rhizosphere

Because the rhizosphere is such a small zone of soil around a root, sampling becomes an inherent problem in rhizosphere studies. A perfect rhizosphere sample would include a complete soil sample, free of roots or root hairs, and taken from a known radius around an actively growing root from an undisturbed soil. This sampling technique has not yet been developed, and so rhizosphere studies lack common sampling techniques and distances. Various sampling methods have been reported along with different thickness of soil called the rhizosphere. This discussion describes previous methods and distances used to sample the rhizosphere and also the sampling device used in this study.

Previously reported sampling methods. A widely used method of sampling the rhizosphere (Katznelson, 1946; Riley and Barber, 1969, 1970, and 1971; and Smiley, 1974) employs shaking off and collecting soil adhering to roots. Riley and Barber (1969) called loosely adhering soil the rhizosphere (1-4 mm), and strongly adhering soil the rhizoplane (0-2 mm). These authors (Riley and Barber) in 1970 and 1971 added a new term, the rhizocylinder, meaning roots plus strongly adhering soil. This shaking method appears to be arbitrary because adhesion of soil particles to roots may vary due to soil texture, aggregate stability, moisture tension, or type of rooting system. Plus, the degree of shaking influences the accuracy and reproducibility of rhizosphere samples (Clark, 1947). Soon and Miller (1977a), using a rhizocylinder sample similar to Riley and Barber (1970, 1971), developed a centrifugal filter to force the soil solution around roots into a receptacle. They reported a rhizocylinder sample having a 2 to 2.5 mm diameter. The centrifuged solution was analyzed for pH and inorganic phosphate (Soon and Miller, 1977b). The soil adhering to roots is obviously influenced by the root, and in this sense is a sample of the rhizosphere.

Papavizas and Davey (1961) used a series of attached 3 mm diameter steel probes for taking a line of soil cores away from a tap root. Their study found increased microbial numbers due to root exudations extending out to 18 mm. Rovira (1953), not mentioning a method, indicated the rhizosphere effect was not more than 5 mm. Starkey (1931) enclosed roots in a 20 cm (diameter) cylinder of 30-mesh

wire screen. He sampled from inside the screen, next to the screen, 15 cm and 30 cm away from the screen and found R/S values (the number of rhizosphere organisms/the number of soil organisms) of 4.1, 1.9, 1.1 and 1 respectively.

Farr, Vaidyanathan, and Nye (1969) grew a single row of onion roots "sandwiched" between two pre-packed blocks of soil. The blocks were then removed from the line of roots, freeze-dried, and sliced 0.1 mm thick by a microtome. This system is most applicable for minimum soil depths and non-branching roots.

Some studies have tried to increase the root to soil ratio, characteristic of a rhizosphere, by growing many roots in a small container (Foy, Burns, Brown, and Fleming, 1965) or by adding small amounts of soil to a nutrient culture (Thompson and Black, 1970). Other studies have sampled directly under fibrous-turf roots (Goss and Gould, 1967) or have compared potted soil with and without plants present (Neal, 1973). Many reports extrapolate root-induced changes in nutrient solutions to rhizosphere phenomena (Clark and Brown, 1974; Ridge and Rovira, 1971; and Foy, Burns, Brown, and Fleming, 1965).

A concentric-ring sampling device. The sampling method employed in this study is a modified idea from Starkey (1931). Instead of using a wire screen, which roots reportedly penetrated, this method used a 43 um nylon fabric to confine corn roots in a 2 cm (diameter) central

core of soil. Radiating from the central root core are concentric rings of soil, 5 mm thick, also separated by nylon fabric. Each end of the concentric nylon cylinders are attached to a stainless steel frame (Figure 1), creating the divisions for four separable soil zones. The radial distances from the central root core follow:

Zone 1	0-5 mm
Zone 2	5-10 mm
Zone 3	10-15 mm
Zone 4	15-50 mm

There are three main advantages to this sampling device. One, soil samples, separated from roots by the nylon fabric, are free of root tissue; yet root exudates and solutions are able to move through the fabric. Two, the concentrated root effect of the central core on surrounding soil zones allows collection of large soil samples, 25 g, for performing many soil analyses. Three, soil samples are taken from a reproducible and known radius around roots. The major assumption with this sampling-ring device is that many roots in a central core simulate a macro-rhizosphere and will affect a large volume of soil similar to one root affecting a small rhizosphere of soil. The validity of this assumption may be easily questioned; however, the distinct advantages of this system and lack of uniform techniques in the literature were factors in deciding to use it.

After the nylon cylinders were attached to the support frame, the assembled device was inserted into a 70 cm (height) by 11 cm (diameter) column made from plastic pipe. Sieved, air-dried soil was

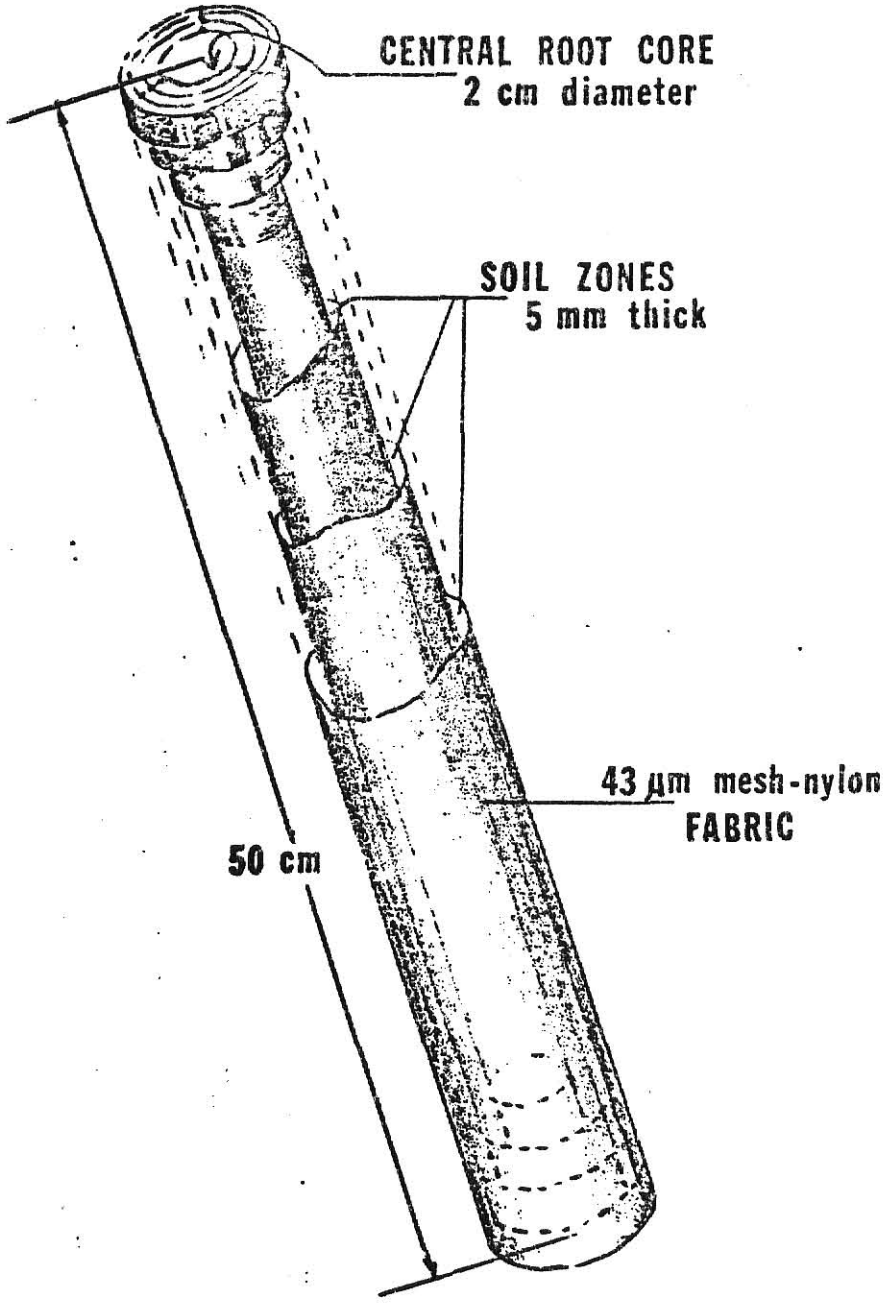

Figure 1. A concentric ring sampling device.

added and compacted to a bulk density of 1.25 g/cc. Water was then added to the 1/3 bar moisture tension. A plastic funnel, placed on top of the device, directed the roots of a germinated corn seed into the central core. Roots reached the bottom of the core in two weeks. The sampling device was then removed from the column and each ring of nylon was opened, yielding four 5 mm thick soil samples. Soil from the 2 cm central root core was hand-separated from roots and analyzed along with the soil-zone samples. Soil samples for each zone were collected, sieved and promptly analyzed for percent water, pH, and phosphatase activity. The moist samples were air-dried, sieved, and stored at room temperature for further phosphorus determinations.

Fumigation

Since the rhizosphere attracts an increased microflora, the rhizosphere effects either originate from the root, microbes, or both. The source of the effect, its magnitude, and its significance requires study. Classical examples of microbes contributing significant effects in the rhizosphere include mycorrhizae and Rhizobia. If microorganisms create or magnify a rhizosphere effect, then the feasibility of a microbial isolation and inoculum warrants study.

A fumigated treatment, aimed at eliminating or reducing microbes, was used to evaluate the contribution of a natural microflora to the rhizosphere effect. About 25 g of methylbromide, containing 2% chloropicrin (Dow MC2) was injected into the 11 cm (diameter) soil

columns, sealed at both ends with plexiglass tops. The soils initially contained 4,000 ppm MC2. Munnecke, Moore, and Abu-El-Haj (1971) found 2,400 ppm methylbromide for 24 hours eliminated all damping-off caused by Rhizoctonia and Phythium. After being sealed for 70 hours, the fumigant was purged out with filtered compressed air. Van Gundy, Munnecke, Bricker, and Minteer (1972), using smaller columns, showed 90% effective flushing after 70 minutes. However, flushing for 3 hours in this study was ineffective, causing immediate phytotoxicity. The columns were then covered with filter paper and incubated in a warm greenhouse (35°C) for 4 days to allow volatization of the residual fumigant.

Care was taken to reduce contamination of the columns after fumigation. Seeds, germinated in a 2% sodium hypochlorite solution (cholorox), were aseptically transferred to the treated soil columns. Realizing that keeping soil completely sterile was impossible, plate counts on nutrient agar checked the relative numbers of microbes present (Table 1). The fumigated treatment later was recolonized, but with less numbers than the non-fumigated treatment.

Soils

Three soils, selected to provide a wide range of properties, included: one, a Pachic Argiustoll, fine, montmorillonitic, mesic; two, a Fluventic Hapludoll, coarse-loamy, mixed, mesic; three, a Typic Hapludalf, loamy, mixed, mesic. These soils were selected

Table 1. Effect of fumigation on soil microbial numbers.

Sample	Microbial numbers					
	Fumigated			Non-fumigated		
	Ustoll	Udoll	Udalf	Ustoll	Udoll	Udalf
Before planting						
Soil	0	0	0	2.0×10^7	1.0×10^7	1.0×10^7
At harvest						
Rhizosphere	1.0×10^6	1.0×10^6	8.0×10^4	2.0×10^7	2.5×10^7	1.5×10^7
Soil	4.0×10^5	2.0×10^5	4.0×10^4	1.0×10^7	1.0×10^7	1.0×10^7
R/S value	2.5	5.0	2.0	2.0	2.5	1.5

from non-fertilized sites. Table 2 summarizes the nature of the soils used in this study. Most of the properties, especially pH, showed significant variation among soils. However, the Udoll and Udalf were proportionately similar in texture, cation exchange capacity (CEC), 1/3 bar moisture content, and available phosphorus (P). These similarities and differences allowed for comparisons and contrasts on the role of soil properties in modifying rhizosphere effects.

Soil samples were collected from the following locations. The Ustoll, Smolan series, was located on the K. S. U. Agronomy Farm 2,000 feet north of the intersection of Kimball and College Avenues (sec. 1, T. 10S., R. 9E). The Udalf and Udoll were collected from Pottawatomie County. The Udalf's location was 500 feet north from the southeast corner of the northwest quarter of sec. 11, T. 10S., R 8E. The Udoll was located 250 feet west of the southeast corner of the northwest quarter of sec. 12, T. 10S, R. 8E.

Growing Conditions

Selected for its high root to top ratio and phosphatase activity, a commercial hybrid, Pioneer 3195-A, was grown for two weeks in a fumigated and non-fumigated soil. In order to determine if growing time increased the rhizosphere effect, a non-fumigated treatment was allowed to grow for three weeks. Thus, this study included three separate trials with the three soils. The growth chamber conditions included a 16 hour photoperiod (30,000 lux) with a 30° -20°C diurnal

Table 2. Soil characteristics.

Characteristic	Method	Ustoll	Udoll	Udalf
Sand-Silt-Clay	Hydrometer	19%-53%-28%	55%-32%-12%	53%-38%-9%
Texture		Sicl	SL	SL
Moisture, 1/3 bar	Tension plate	26%	12%	10%
C. E. C.	Ca saturation	18.9% meq/100 g	9.9 meq/100 g	5.7 meq/100 g
Organic matter	Walkley-Black	2.3%	1.6%	0.6%
pH	Saturated paste	7.1	6.3	5.3
Available P	Bray's #1	14 ug/g	36 ug/g	38 ug/g
N as NH_4^+	Steam distillation	7.0 ug/g	4.2 ug/g	2.1 ug/g
N as NO_3^-	Steam distillation	4.2 ug/g	8.2 ug/g	0.7 ug/g
Vegetation	Observation	Brome grass	Native grass	Deciduous forest

cycle. The soil was not watered after the initial 1/3 bar conditions and no fertilizer was added.

Treatments showed differences in both total growth and root-shoot ratios (Table 3). Plants in the three week treatment produced the most dry matter. Fumigation treatment drastically reduced the dry matter production. Changes in dry-matter production with soils generally followed the decreasing order of Udoll, Ustoll, and Udalf.

Table 3. Growth characteristics of plants in three soils.

Treatment	Dry-matter production								
	Ustoll			Udoll			Udalf		
	Roots	Tops	Root-shoot ratio	Roots	Tops	Root-shoot ratio	Roots	Tops	Root-shoot ratio
	mg								
Two week	745	230	3.2	2,230	600	3.7	241	100	2.4
Fumigated	481	162	3.0	1,170	204	5.7	81	78	1.0
Three week	2,600	800	3.3	7,500	950	7.9	5,000	850	5.9

Chapter 3

PHOSPHATASE ACTIVITY IN SOIL ZONES NEAR ROOTS

Introduction

Phosphatase, phosphoric monoester hydrolase (EC 3.1.3), is responsible for enzymatically releasing orthophosphate from organic compounds. In soils, the product of the phosphatase reaction, inorganic phosphate, is available for plant utilization. Although often measured by p-nitrophenyl phosphate, soil phosphatases have a general specificity and act on many organic-phosphorus substrates including inositol hexaphosphate (phytic acid), phospholipids, nucleic acids, and pyrophosphates (Kuprevich and Scherbakova, 1971 and Dekker, 1977). Phosphatases have also been shown to release orthophosphate from recently developed high-analysis, polyphosphate fertilizers (Gilliam, 1970). By chemical hydrolysis, the liberation of phosphate from these compounds was extremely slow in soils (Subbarao, Ellis, Paulsen, and Paukstelis, 1977). For this reason phosphatase has received much attention. Most studies concerning root and microbe phosphatase were analyzed using soil-less cultures and have assessed the characteristics of root, microbe, and soil phosphatase separately. The objective of this study is to determine the significance of corn roots and associated rhizosphere microflora in enriching the indigenous

phosphatase of three different soils. This chapter, concerned with evaluating the rhizosphere effect of phosphatase includes a literature review, experimental methods, and results and discussions.

Phosphatase Literature Review

Nature of the enzyme. Many characteristics, including optimum pH, mode of action, inhibitors, adaptability, and kinetics are needed to define the nature of phosphatase.

Phosphatases are classified as acid or alkaline depending upon the optimum pH of their maximum activity. Most soil phosphatases are acid with a pH optimum at 6.5 (Tabatabai and Bremner, 1969). Alkaline phosphatases, having a pH optimum of 11, seem to have little significance to soils. However, Eivazi and Tabatabai (1977) found predominately acid phosphatase (assay pH 6.5) in acid soils and predominately alkaline phosphatase (assay pH 11) in alkaline soils. Skujins, Braal, and McLaren (1962) noted highest phosphatase activity when assayed at the actual soil pH. Another factor, distinguishing acid and alkaline phosphatase, is their inhibition by 0.5 M NaF. Alkaline phosphate, completely inhibited by NaF, contains metallic proteins, whereas acid phosphatase is only partially inhibited and thus contains non-metallic proteins (Hasewaga, Lynn, Brockbank, and James, 1976). Juma and Tabatabai (1977) reported 80% inhibition of alkaline phosphatase with 25 ug/g silver (Ag^+) and only 20% inhibition of alkaline phosphatase. Hasewaga et al. (1976) noted 100% inhibition

of five acid phosphatase isoenzymes with 0.5 M mercury choloride ($HgCl_2$). Neuman (1968) using specific substrates presented conclusive evidence showing acid and alkaline phosphatase act by different mechanisms.

Confirmed by oxygen isotopes (^{18}O), phosphatase splits the P-O bond with the addition of water (Barman, 1969). For this enzymatic reaction to occur, it appears the substrate must be soluble. Greaves and Webley (1969) showed the iron and aluminum salts of myo-inositol hexaphosphate to be only slightly hydrolyzed by bacteria. Greenwood and Lewis (1977) confirmed the same phenomena with soil yeasts.

The stability of phosphatase is well documented by Skujins and McLaren (1968), noticing comparable enzyme activity in soil samples stored for 60 years. The same communication reported traces of phosphatase activity preserved in 32,000 years of permafrost. Skujins (1967) noted an initial loss of enzyme activity with air drying, then negligible losses over prolonged storage periods. Eivazi and Tabatabai (1977) showed air drying slightly increased acid phosphatase and steam sterilization did not totally destroy it.

Phosphatase is an adaptive enzyme. Whether the enzyme is in the plant, microbe, or soil, it increases during orthophosphate stress (Ramirez-Martinez, 1968, and Bieleski, 1973). Greater phosphatase activity follows lowered phosphate levels. The fact that phosphatase indicates the phosphate availability of soils and plants has led researchers (Kuprevich and Shcherbakova, 1971) to suggest using

phosphatase as an agrochemical index correlated to phosphorus response. Soil phosphatase activity was a frequently selected factor for multiple regressions of microbial and biochemical characteristics on winter wheat yields (Verstrate and Voets, 1977). El-Fouly and Jung (1972) have positively correlated plant osmotic stress to increased phosphatase activity.

Most soil phosphatases follow Michaelis-Menten kinetics, that is, the more substrate added, the greater the velocity of the reaction. The unit increase in velocity (V) per unit increase in substrate (S) decreases exponentially until the ~~substrate~~ enzyme becomes saturated with respect to ~~enzyme~~ substrate. At this point the amount of enzyme is the rate limiting step, zero order kinetics apply, and the amount of enzyme is measured. Through graphical transformations of S and V, two values become unique in describing enzymes. One, is the theoretical maximum velocity (Vmax). Two, is the Michealis constant (Km), equaling the substrate concentration which produces half the Vmax (Neilands and Strumpf, 1953). Km values for soil phosphatase is the most unique and sensitive characterization of a soil sample (Thorton and McLaren, 1975). Changes in assay procedures, such as pH, buffer solution, shaking, temperature, ionic strength, and substrate preparations change the determined Km values (Cervelli, Nannipieri, Ceccanti, and Sequi, 1973; Irving and Cosgrove, 1976; Tabatabai and Bremner, 1971; and Brams and McLaren, 1974). For this reason comparisons of reported Km values are difficult (Eivazi and Tabatabai,

1977). However, typical Km values for soil phosphatase using p-nitrophenyl phosphate range from 1-6 mM and Vmax values range from 0.5 umol P hydrolyzed per gram of soil per hour.

Sources of the enzyme. Sources of phosphatase, able to attack the organic P pool, include microbial, plant, and free soil phosphatase.

Phosphatases originating from plants reach the soil by root secretion or leakage and by decomposition of plant residues. Plant phosphatases, associated with the cell wall of the vacular membrane, function to recycle organic P compounds in the plant. Phosphatases, also having metabolic functions, are associated with adenosine triphosphate (ATP) in the plasmallema and regulates phosporylation-dephosphoryiation reactions (Hall and Davie, 1971; Hall, 1969; and Bieleski and Johnson, 1972). Vakmistrov, Muchustina, Ismailov, Samvilova, and Tikhaya (1973) located a "transport" ATPase, activated by metallic cations, in cellular membranes. However, Bieleski (1973) noticing during P-stress most of the phosphatase was in roots and lower epidermis, suspected a migration of membrane-bound phosphatase to the cytoplasm. Hasewaga et al. (1976) characterized an isoenzyme of lower molecular weight, 28,000 versus 64,000, capable of greater movement. These movements and increased phosphatase activity during P-stress may indicate active root secretion to release soil phosphate from organic macromolecules (Chang and Bandurski, 1964 and Bieleski and Johnson, 1972) or increased metabolism of active

transport systems, using ATP at root membranes (Shaykh and Roberts, 1974; Hall and Davie, 1971; and Woolhouse, 1969). Vakmistrov et al. (1973) characterized ATPase in root membranes and not on the external root surface. Phosphatases are concentrated in areas of cellular decay, such as the petiole, root cap, sites of cellular differentiation, and points of lateral root initiation. Thus, one might suspect phosphatase exudation is solely due to root growth and abrasion (Shaykh and Roberts, 1974).

Whether exudated or actively secreted, roots are a significant source of phosphatase that can act on many substrates. Subbarao, Ellis, Paulsen, and Paukstelis (1977) reported 43 and 13 umoles of pyrophosphate hydrolyzed per hour per split root system of corn and soybeans, respectively. Using two Agrostis Tenuis root tips, Woolhouse (1969) reported 50 umol/hr for B-glycerophosphate, 20 umol/hr for inositol hexaphosphate, and 100 umol/hr for p-nitrophenyl phosphate. These values correlate to about 4 to 6 umol P released per mm^2 per hour. Ridge and Rovira (1971), Gilliam (1970), and Clark (1975) have reported values of similar magnitude.

Microbes, including bacteria (Greaves and Webley, 1965 and 1969), fungi (Casida, 1959), and yeasts (Greenwood and Lewis, 1977) not only release exocellular enzymes but also enrich the soil with phosphatase from microbial lysis and turnover. Since microbe populations turnover rapidly, ascertaining whether microbial phosphatases are exocellular or products of autolysis remains a problem (Skujins, 1967).

The significance of microbes contributing phosphatases to the soil at any one time is minimal; however, their cummulative effect may be important. A microbial culture of 10^9 cells, known to be high in phosphatase only released 3 umol P per 24 hours (Ridge and Rovira, 1971).

Mycorrhizal fungi's production of phosphatase has been qualified in pure culture (Theodorou, 1971) and on roots (Barlett and Lewis, 1973). Since these authors did not compare their findings to non-mycorrhizal roots the significance of this source of phosphatase was not quantified.

Some dichotomy exists over the role of rhizosphere organisms in contributing to the total phosphatase activity of roots. Gilliam (1970) using pyro- and tripolyphosphatase considered the effect of microbes negligible. Yet Savant and Racz (1972), using the same substrate, noticed 6 to 8 times more hydrolysis in non-sterile versus sterile cultures. Estermann and McLaren (1961) attributed 20% of the phosphatase activity in the rhizosphere to its microflora. Greaves and Webley (1965) accounted an increase of microbes which were capable of hydrolyzing organic P compounds in the rhizosphere. Ramirez-Martinez (1968) found corn roots absorbing phosphatase from bacterial lysates. However, Ridge and Rovira (1971) concluded:

> Most phosphatase activity of intact seedling roots was associated with the root surface and was not increased by microorganisms in the rhizoplane; in some experiments activity was significantly reduced by soil microorganisms.

Martin (1973), testing the effects of rhizosphere flora on hydrolysis

of inositol hexaphosphate, reported bacterial isolates or mixed rhizosphere flora had no effect on ^{32}P uptake by wheat. Part of the discrepancy reported in the literature may be due to the fact that Savant and Racz (1972), Estermann and McLaren (1961), and Saxena (1964) estimated phosphatase activity by the phosphate released, instead of by p-nitrophenol or ^{32}P techniques (Ridge and Rovira, 1971). In non-sterile conditions phosphate may be released from sources other than hydrolytic substrates.

Free soil phosphatase, once originating from plant or microbial sources, comprise a large enzyme activity. Associated with the clay and humus soil complex, most soil phosphatases are insoluble. It is interesting to speculate the similarities between membrane-bound phosphatase in plants and clay-humus complexed phosphatase in soils. Although Mortland and Giesking (1952) reduced phosphatase activity with increased cation exchange capacity of clay minerals, the complex of clay, humus, and enzyme makes a three dimensional macromolecule (McLaren, 1975). This stabilizes the enzyme and makes the soil a storehouse of enzyme activity independent of its original living organism. The activity of adsorbed enzymes, depending upon bonding of active sites, may be greater, lesser, or equal to soluble enzymes (McLaren and Packer, 1970).

Although assayed under ideal conditions such as optimum pH, temperature, and high levels of easily hydrolyzed substrate, the amounts of free soil phosphatase are reportedly large, ranging from

0.5-4 umol P released per gram of soil per hour. Ramirez-Martinez (1968) cautions that these values are inflated, not reflecting soil conditions. At these rates for 100 days, there is enough enzyme to potentially release 74,000 to 600,000 pounds of P per acre furrow slice (AFS). Yet soils average only 150 pounds per AFS of organic P (Brady, 1974). In relation to the high amounts of organic P and low availability, Black and Goring (1953) state:

> The hypothesis that appear to account most credibly for the gross behavior, however, are first that soil organic phosphorus exists in an inherently unreactive state, and second that soils normally have a low level of enzyme activity.

It appears that persistence of organic P in soils is due to its resistance to enzymatic attack; not low phosphatase levels.

The ecological role of phosphatases. The total scheme of phosphatases in relation to the plant-soil environment is uncertain. The fact that phosphatase activity increases and may become exocellular during P-stress implies the role of phosphatases as a mechanism for greater utilization of organic phosphorus. Based on this premise Woolhouse (1969), Bieleski and Johnson (1972), Chang and Bandurski (1964), and Neal (1973) consider phosphatase to be an adaptive enzyme, making phosphates more available. Woolhouse (1969) suggested using phosphatase to distinguish and select species better adapted to low phosphorus fertility. He also challenged plant geneticists to design a root enzyme system from molecular proteins for specific edaphic

conditions. Neal (1973) attributed the competitive advantage of invader grassland species to higher phosphatase levels and therefore a more competitive utilization of organic P. However, McLachlan (1976), testing the initial premise, showed the plants with the greatest yield, efficiency, and total uptake of P had the lowest root respiration and phosphatase activity. He concluded, ". . . efficiency of phosphorus use was affected more by morphology and rate of growth of the root than by root metabolic activity." Although Clark and Brown (1974) concluded that a high accumulation of P produced more phosphatase activity, their data showed the phosphorus-efficient plant (dry matter per unit P in tissue) had lower phosphatase activity.

Thompson and Black (1970b) added phosphatase to soils and recorded no decrease in natural organic phorphorus. The resistant organic P substrates (Martin, 1973) are probably saturated with a high level of enzymes. Reid and Bieleski (1970) found that increased phosphatase activity in plants, induced by P deficiency, did not cause a greater turnover of the phosphate-ester pool in plants. Thus, the role of increased phosphatase in making phosphate available is questionable and the phosphorus efficient plant does not appear to employ phosphatase as a mechanism for efficient P use.

Phosphatase Analytical Methods

Methods concerning the analysis of soil phosphatase include variety selection, the phosphatase assay procedure, the use of

dihydrogen p-nitrophenyl phosphate, selecting a substrate level, and measuring organic and available P.

Variety selection. Although Mossala (1976) reported trends but no significant difference among corn varieties' ability to hydrolyze pyrophosphates in nutrient solution, a test was conducted to select a commercial corn hybrid capable of enriching the soil phosphatase. In this experiment, the first trial measured the phosphatase activity in soil samples from pots growing a random selection of eighteen commercial corn crosses. The pots were autoclaved at 130°C and 1 bar pressure (gauge) to eliminate microorganisms and denature free soil phosphatase. Each pot was planted with five seeds excepting a control pot with no seeds. After two weeks of growing in an environmental chamber, roots and soil were separated, leaving a sieved-soil sample for phosphatase measurement. From this test six varieties showing the greatest difference in phosphatase enrichment were chosen for a second trial using the same procedure. The results of the second test (Table 4) showed all varieties significantly enriched the phosphatase activity above the control and two varieties, Pioneer 3195-A and Trojan TX5115-A, were significantly higher than the other hybrids in producing soil phosphatase. Pioneer 3195-A was selected for this study.

Phosphatase assay. The various methods used for estimating phosphatase activity measure products of enzymatic hydrolysis. Some methods measure the orthophosphate released from organic substrates such as inositol hexaphosphate (Greaves and Webley, 1969), B-glycerophosphate (Kuprevich and Shcherbakova, 1971), ATP (Chang

and Bandurski, 1964), and pyrophosphate (Savant and Racz, 1972). Martin (1973) supplied ^{32}P as inositol hexaphosphate and recorded $^{32}PO_4$ taken up by the plant. Subbarao et al. (1977) measured both the phosphate product and pyrophosphate substrate by nuclear magnetic resonnance (NMR) spectroscopy. Since soils are capable of fixing or

Table 4. Soil phosphatase activity of selected corn hybrids.

Hybrid	Mean phosphatase - umol/g soil/hour -
Pioneer 3195-A	0.943*
Trojan TXS115-A	0.871*
Funks 474W	0.643
NC + 59	0.629
Bojac X69	0.571
Century II 6710	0.557
Control	0.236*
LSD	0.139

*Significant at the 0.05 level

precipitating orthophosphate, the measurement of phosphate to indicate enzymatic hydrolysis in soils is less desirable. This prompted methods of measuring the organic moiety instead of the released phosphate. Kramer and Yerdei (1959) colorimetrically measured the phenol hydrolyzed from phenylphosphate. Ramirez-Martinez (1965) employed a fluorimetric technique using B-naphthyl phosphate.

Tabatabai and Bremner (1969) developed a soil phosphatase procedure using p-nitrophenyl phosphate, originally used to measure alkaline phosphatase of blood serum (Bessey, Lowry, and Brock, 1946). Phosphatase splits p-nitrophenyl phosphate (pNPP) into a phosphate ester and a p-nitrophenol (pNP) radical which develops a yellow color in dilute alkali (Figure 2). This color produces a visible absorbance peak at 410 nm and follows Beer's law by showing a linear relationship between the amount of color (absorbance) and concentration of pNP. The absorbance of the assay solution is compared to a standard curve made from plotting known concentrations of pNP, ranging from 5 to 50 uM, against their respective absorbance values (Figure 3). The enzyme activity, equaling the amount of hydrolyzed pNP, minus a blank with no soil, is usually expressed as umol per gram of soil per assay hour. Soil assays with pNPP are sensitive and precise, the developed color is stable for 24 hours, p-nitrophenol is recovered quantitatively from soils, chemical hydrolysis of pNPP is minimal, and the standard curve is reproducible (Tabatabai and Bremner, 1969).

This study employed a modification of Tabatabai and Bremner's (1969) method. One gram of soil, placed in a 50 ml volumetric flask was mixed with 3 ml of modified universal buffer, MUB, (Skujins et al., 1962) and prewarmed to 37°C in a water bath. Two ml of 62.5 umol/ml pNPP, buffered to pH 6.5, was added to start the reaction. After 55 minutes, 1 ml of 1N $CaCl_2$ was added to the assay solution and gently swirled. The reaction was stopped after 1 hour by bringing the flasks

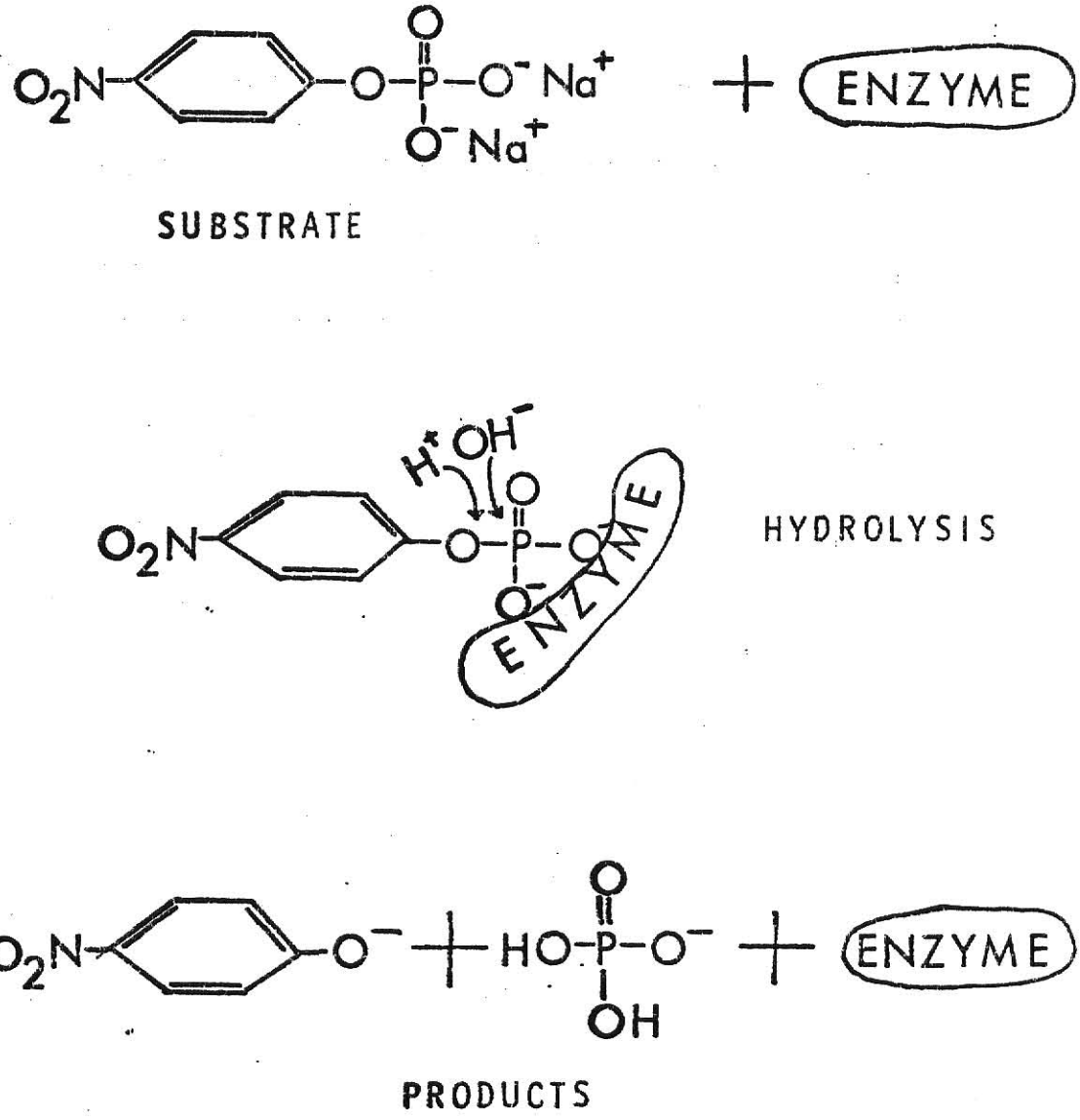

Figure 2. Enzymatic hydrolysis of p-nitrophenyl phosphate.

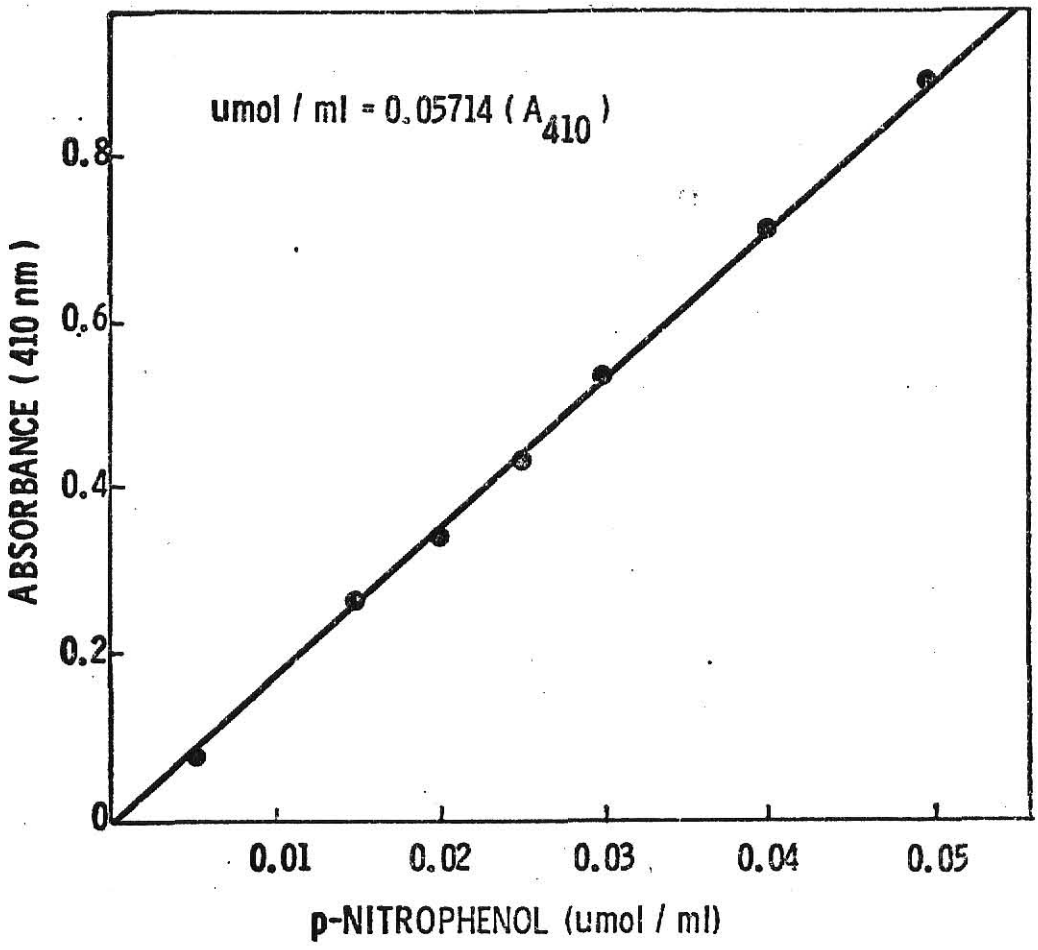

Figure 3. Standard curve for pNP.

up to 50 ml volume with 0.025N NaOH. This solution was then shaken thoroughly, filtered (Whatman #2), and the filtrate was measured for absorbance on a Bausch and Lomb Spectrophotometer 88. The addition of 1N $CaCl_2$ assured soil flocculation, thus aiding filtration and prohibiting color contamination from base-extracted organic matter. The addition of tuolene, suggested by Tabatabai and Bremner (1969) to plasmalyze and inhibit microbes, has been shown not to significantly change the assay results (Brams and McLaren, 1974; Irving and Cosgrove, 1976; Eivazi and Tabatabai, 1977) and was omitted in this procedure.

Uniform assay conditions, such as buffer pH, time, temperature, and substrate quality, were assumed constant in this study. However, there was more variation between assay groups than within an assay group using the same soil. For this reason, the author recommends measuring a standard enzyme source or a standard soil sample with each assay group. Adjusting values to a standard enzyme control would eliminate between group variation due to assay conditions.

Using dihydrogen p-nitrophenyl phosphate. All reported research with pNPP has used disodium p-nitrophenyl phosphate, tetrahydrate (molecular weight, 371). Suppliers of the substrate are Sigma Chemical Co., St. Louis, Mo. and Cal Biochem, Los Angeles, Ca. However, another product, dihydrogen p-nitrophenyl phosphate, anhydrous (molecular weight, 219) was listed by Eastman Organic Chemicals at a considerably lower price. The differences between the

two substrates, Eastman and Sigma, appeared to be the placement of hydrogen versus sodium ions on the phosphate ester and the additional waters of hydration. The disodium product (Sigma) was in acceptable pH form, but the dihydrogen product (Eastman) had a pH of 1.8 and required neutralization. This was done by adding 31.24 umol, or about 7 grams of dihydrogen pNPP, to a 500 ml volumetric flask containing 50 ml of sodium-saturated, cation-exchange resin, pH 8.0, (Dowex 50W, X-8) and was brought up to volume with MUB, pH 6.5. The solution, still acidic, was slowly neutralized to pH 6.5 with sodium bicarbonate powder. The cation-exchange resin was removed by filtering, cleaned, and recharged with sodium for further use. This method produced a highly buffered substrate of pH 6.5. This neutralized Eastman product gave high blank values, not previously observed in the Sigma product, and indicated about 3% free p-nitrophenol. High values of free p-nitrophenol indicated either chemical hydrolysis of pNPP during neutralization, or a contaminated product. These assumptions were tested by comparing ^{31}P-NMR spectrographs of the Eastman product, before and after neutralization, against the Sigma product as a standard. The spectrographs (Figure 4) qualitatively identified peaks for pNPP and free phosphate in the Eastman product. No free phosphate was found in the Sigma product. Quantitative comparisons of the areas under the curves indicated the same amount, about 7%, of free phosphate in Eastman samples before and after neutralization. This proved the Eastman product was contaminated (Paukstelis, personal communication). This was later confirmed by

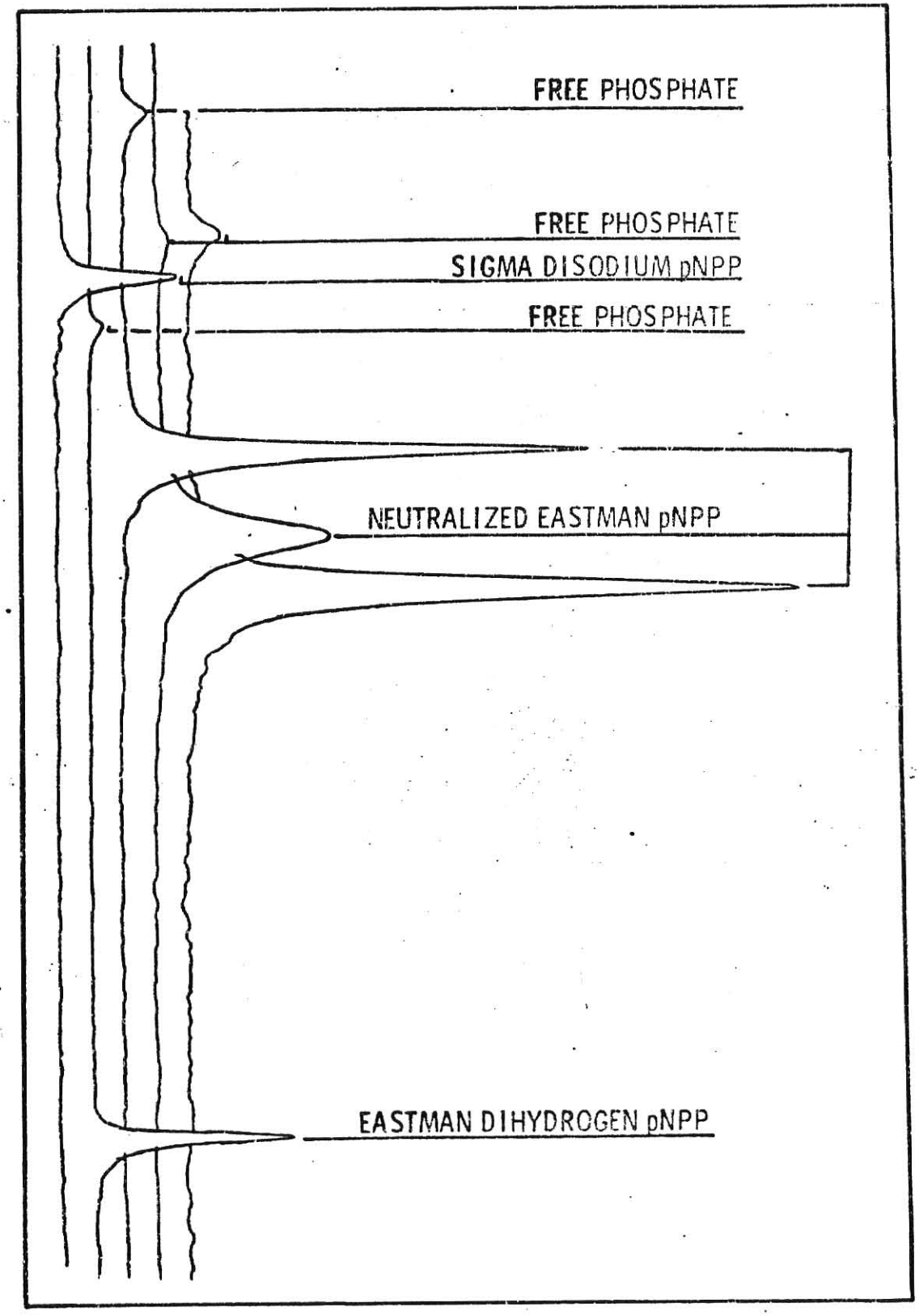

Figure 4. NMR spectrographs of pNPP substrates.

Eastman Product Specialist, Bill Lyman, who indicated a need to develop a new product more applicable to the standards of enzyme analysis. Phosphatase assays reported in this study used this neutralized-dihydrogen pNPP substrate. Comparisons of phosphatase activity in soils assayed with both Sigma and Eastman showed the two products to act similarly (correlation coefficient, r, equal to 0.91 at 0.01 significance) (Figure 5).

Selecting a level of substrate. Phosphatase activity and kinetics are determined largely by the concentration of the substrate. Various levels of substrate, reported in the literature, are summarized in Table 5. When the enzyme is saturated with substrate, zero order kinetics apply to the breakdown of substrate. But, if the enzyme is not saturated with substrate, then the amount of substrate controls the reaction rate, according to first order kinetics. During the zero order assay, the enzyme is the rate-limiting step and the measured reaction rate estimates the amount of enzyme and not the effect of substrate concentration on enzyme velocity. The substrate level used in this study was selected by measuring the reaction velocity (V) at various substrate concentrations (S), ranging from 1 to 62.5 umol/ml of pNPP (Figure 6). After 25 umol/ml, the effect of concentration on increasing the velocity was minimal in three soils: the 25 umol/ml was the selected substrate level for further phosphatase determinations.

Velocity, as a function of substrate concentration, graphically

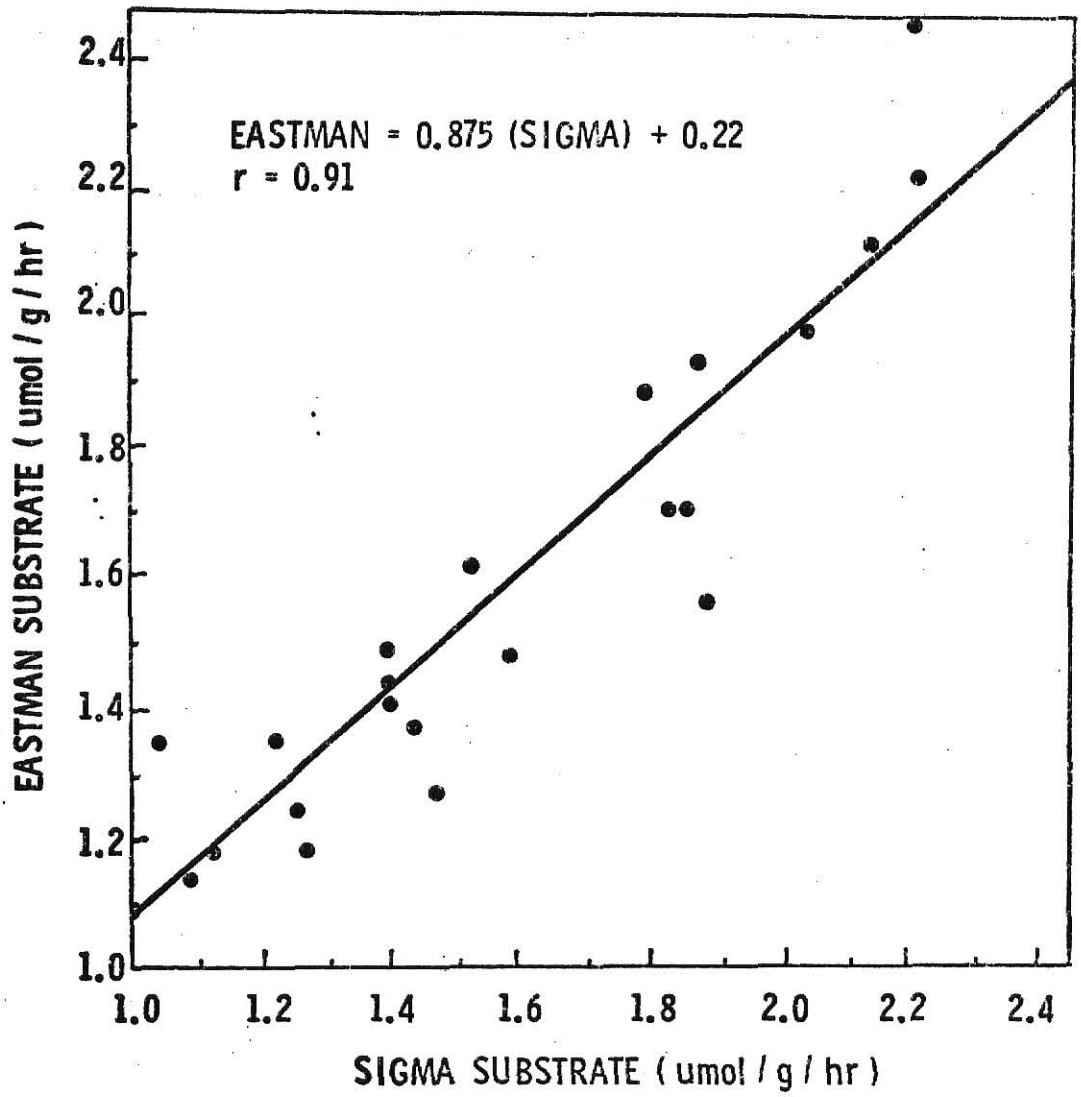

Figure 5. Correlation of Sigma and Eastman substrates.

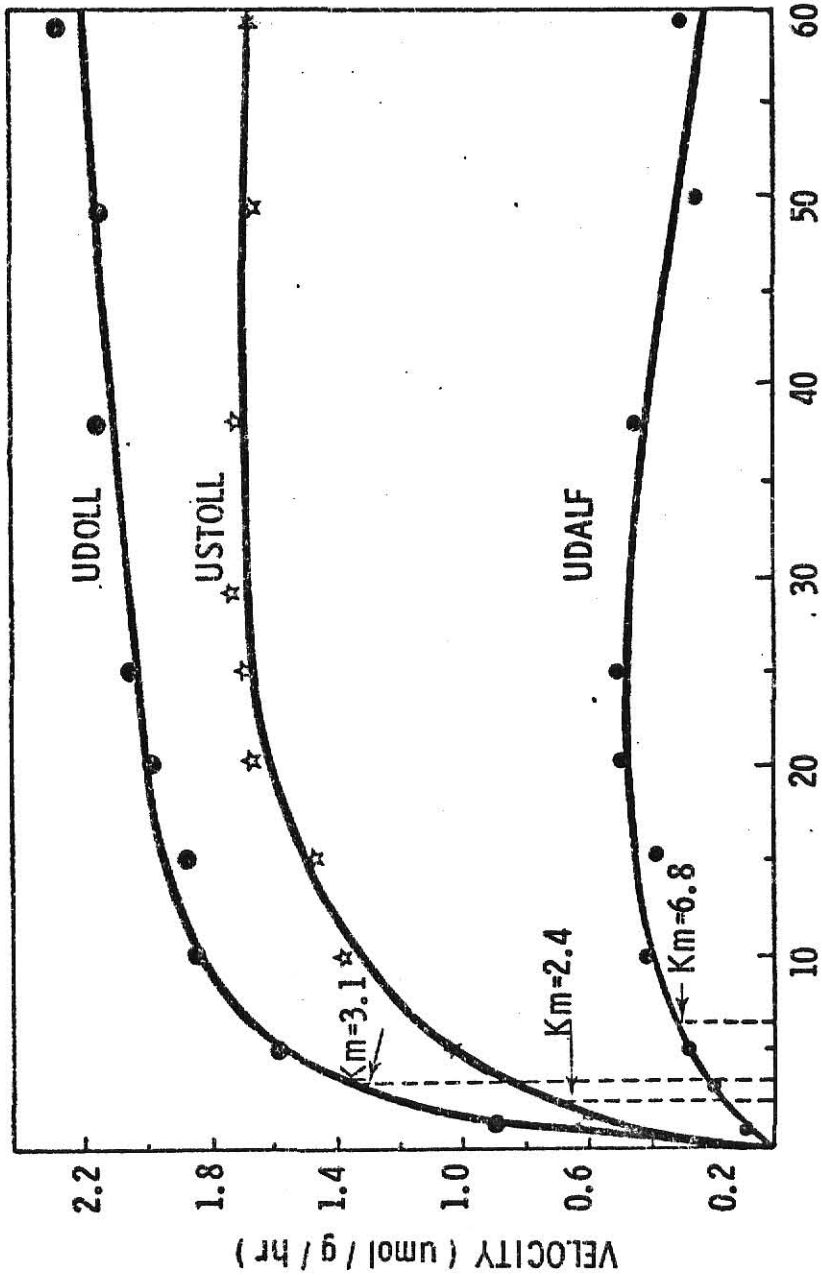

Figure 6. Effect of substrate concentration on velocity.

Table 5. Representative substrate levels reported in the literature.

Reference	System	Substrate (pNPP) Concentration	Amount
		umol/ml	umol
Tabatabai and Bremner, 1969	Soil	23	115
Brams and McLaren, 1974	Soil	0.1 to 3.0	2.8 to 84
Eivazi and Tabatabai, 1977	Soil	1 to 20	5 to 100
Woolhouse, 1969	Roots	1	1.5
McLachlan, 1976	Roots	0.033	0.825
Beileski and Johnson, 1972	Plants	2	20
Clark, 1975	Solution	0.1	20
Clark and Brown, 1974	Solution	0.022	26.8
Ridge and Rovira, 1971	Solution	0.90	26.9

Table 6. Phosphatase parameters of three soils.

Parameter	Ustoll	Udoll	Udalf
Km	2.36*	3.10*	6.81**
Vmax	1.72*	2.44*	0.46**

*, ** Significant at the 0.05 and 0.01 levels, respectively.

transformed as S/V versus S (Figure 7) provides unique kinetic information. The slope of this linear transformation equals 1/Vmax and the Y-intercept is Km/Vmax (White, Handler, and Smith, 1964). The Michaelis constant (Km) and the theoretical maximal velocity (Vmax) are determined by this plot. Computing the linear regression of S/V on S yielded the regression coefficient, B_1, equal to the slope and, therefore, 1/Vmax; B_o equals the Y intercept and, therefore Km/Vmax. The values of Km and Vmax, determined from this regression analysis, are presented in Table 6. Since B_1 (slope) and B_o (intercept) were both statistically significant at 95% probability, it was concluded the Km and Vmax values for each soil were significantly different. Km, having units of umol/ml, equals the substrate concentration required to produce half Vmax. If the substrate concentration is less than Km, then first order kinetics control the reaction velocity. Substrate concentrations greater than Km yield zero-order kinetics (McLaren, 1975). But to assure zero-order kinetics, White, Handler, and Smith (1964) conclude the substrate must be concentrated enough to produce velocities near Vmax. The substrate level in this study, 25 umol/ml, is larger than Km and produced velocities near Vmax.

Measuring organic and available phosphorus. Organic P was estimated by a modification of Olsen and Dean's (1965) ignition method. The difference in concentrated-hydrochloric-acid (HCl)-extracted P in non-ignited and ignited (240°C) soil samples represented the

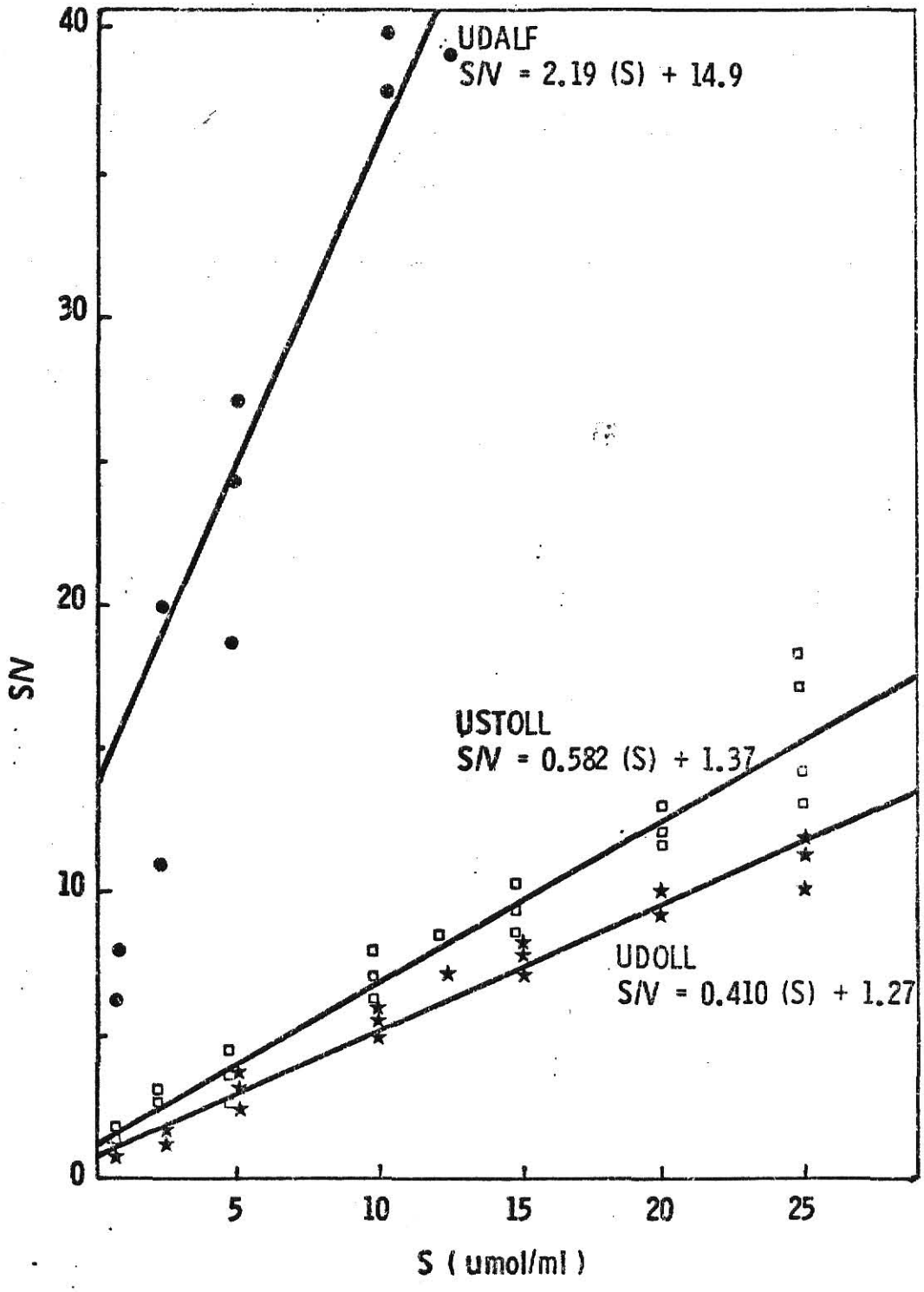

Figure 7. Regression of S/V on S.

organic phosphorus. One gram of soil was placed in a 250 ml volumetric flask with 10 ml of concentrated acid. After heating the soil and acid mixture to about 70°C, another 10 ml of concentrated HCl was added and allowed to react for 1 hour at room temperature. After bringing the flasks to 250 ml volume, the acidified soil particles flocculated and settled rapidly allowing a soil-free 10 ml aliquot for the vandomolybdate phosphorus determination (Jackson, 1970). A sample with no color reagent was used to correct any soil color extracted during the procedure.

Available phosphorus from one gram samples was extracted while shaking with 10 ml of Bray's #1 solution. The mixture was centrifuged and the supernatant filtered. From the filtered solution, a 5 ml aliquot was mixed with 2 ml of vandomolybdate reagent (Jackson, 1970) and measured for absorbance at 380 nm on a Beckman model 25 spectrophotometer.

Results and Discussion

Phosphatase activities, organic phosphorus, and available phosphorus were measured in the central root core and four succeeding soil zones. The results of three soils and three treatments includes phosphatase activity and phosphorus levels in the soil zones, the effect of fumigation on phosphatase and phosphorus, and the effect of growth period on phosphatase and phosphorus. Specific results and statistical significance for each soil zone and treatment are presented in Appendix 1.

Phosphatase activity and phosphorus near roots. In all treatments soil from the root core possessed significantly higher phosphatase than outer soil zones away from the roots. In most cases, the phosphatase in zone 1 (0-5 mm) was also significantly enriched over the outer zones (Appendix 1). The general trend was the closer the soil sample was to the root source, the higher soil phosphatase activity (Table 7). Averaging the phosphatase activity over the three treatments yielded 40%, 20%, and 17% enrichment of phosphatase activity from soil in the root core of the Udalf, Udoll, and Ustoll, respectively. Zone 1 (0-5 mm) increased the phosphatase by 15%, 5%, and 3% for the Ustoll, Udalf, and Udoll, respectively (Figure 8). Little or no differences in phosphatase were found in outer soil zones (5-50 mm). Even though each soil had widely different kinetic properties (Table 6), the phosphatase activity near roots exceded the calculated-theoretical Vmax for batch-soil samples by 40% to 50%. This signifies increased enzyme, probably due to the enrichment of phosphatase released from root sources.

Available phosphorus generally showed depletion patterns with the least available P in the root core and the most available P in outer soil zones. Trends in phosphorus depletion, due to plant uptake of P, showing a slight depression closer to roots masked any relationship between increased P availability and phosphatase activity (Table 7).

Changes in organic P were erratic and unpredictable. No trend in organic P levels appeared in the four soil zones. Thompson and Black

Table 7. Means averaged from three soils.

Treatment	Soil zone	Distance from root core (mm)	Phosphatase (umol/g/hr)	Available P (ug/g)	Organic P (ug/g)
Two week growth period					
	Root core	-	2.13	26.0	142
	Zone 1	0-5	1.90	26.8	170
	Zone 2	5-10	1.79	27.3	203
	Zone 3	10-15	1.81	27.6	125
	Zone 4	15-50	1.82	29.3	233
Three week growth period					
	Root core	-	2.21	25.4	163
	Zone 1	0-5	2.03	26.1	145
	Zone 2	5-10	1.86	27.5	175
	Zone 3	10-15	1.84	29.8	155
	Zone 4	15-50	1.86	30.2	127
Two week growth period, plus fumigation					
	Root core	-	0.81	33.6	155
	Zone 1	0-5	0.64	32.0	170
	Zone 2	5-10	0.62	31.1	155
	Zone 3	10-15	0.58	32.0	171
	Zone 4	15-50	0.59	30.4	156

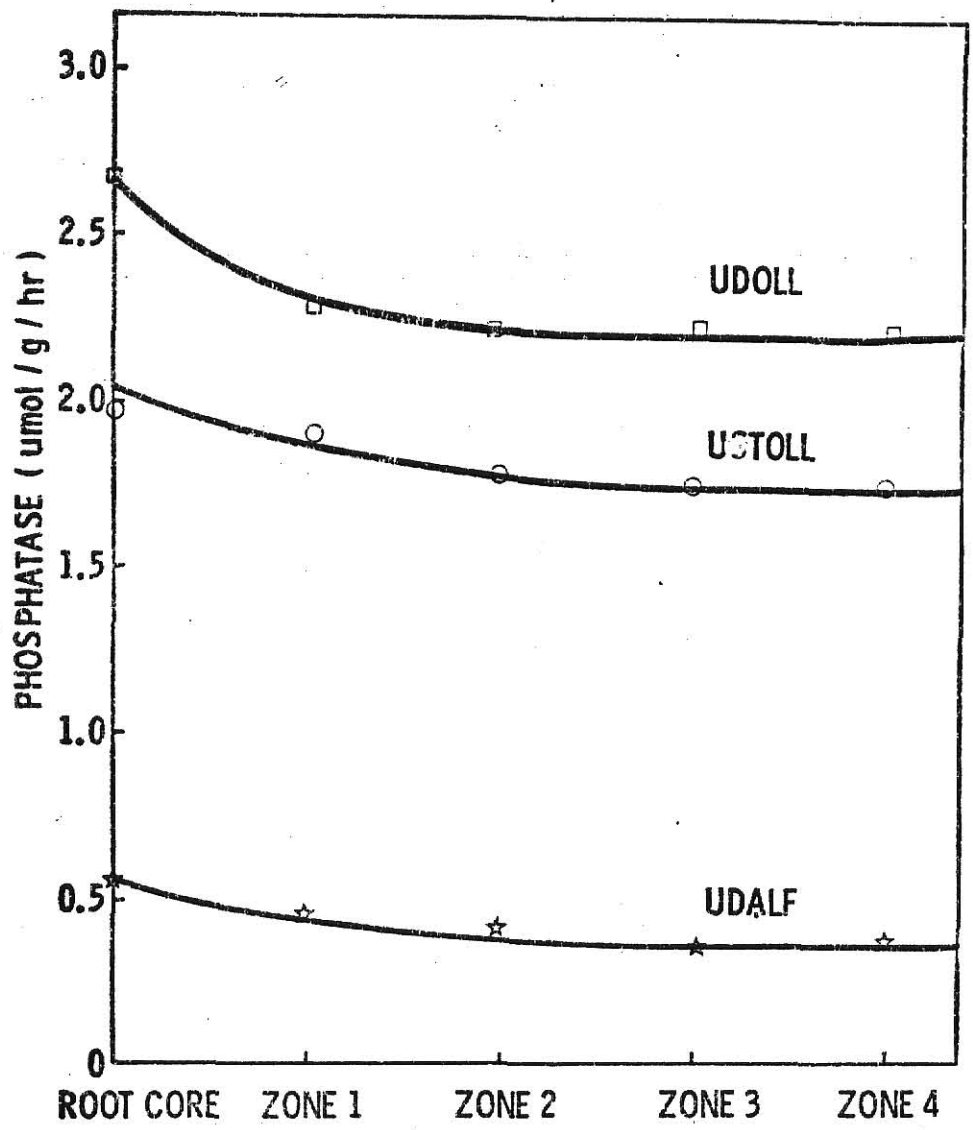

Figure 8. Change in phosphatase activity averaged over three treatments.

(1970a) showed depletions in organic P only after growing five successive corn plants for 7 days, each in solution tubes with small amounts of soil.

The effect of fumigation on phosphatase and phosphorus. The fumigation treatment decreased by half the phosphatase activity in all soil zones and available P was increased by 15% (Table 7). The exact mechanism of the changes caused by the fumigated treatment is uncertain. In this case, the decreased phosphatase activity may be due to the increased availability of phosphate. Fumigation with methyl bromide has reportedly changed the availability of soil nutrients. Rovira (1976) showed more P taken up by plants in fumigated soils.

The fumigation treatment was originally designed to evaluate the contribution of rhizosphere microorganisms to soil phosphatase activity. But since the fumigated treatment drastically changed other soil properties besides the microflora, it can not be used to conclusively assess changes in phosphatase due to microorganisms. Even though the phosphatase level in all zones was decreased by half, the same enrichment pattern was evident in the fumigated and non-fumigated treatments (Table 7). A statistical analysis proved no interaction ($\alpha = 0.05$) between phosphatase activity and treatments, meaning the same trends in phosphatase enrichment occurred in fumigated and non-fumigated treatments. This supports Ridge and Rovira's (1971) conclusions that roots alone or roots with decreased microflora are capable of increasing soil phosphatase.

The effect of growth period on phosphatase and phosphorus.

Although the three-week treatment produced three times the root and shoot dry matter (Table 3) of the two-week treatment, the phosphatase activities were very similar. In the two-week experiment, there appeared a slight decrease in phosphatase in either zone 1 or zone 2. The three-week treatment showed regular decrease in phosphatase from the root core to outer soil zones. Trends in organic and available P showed more variation in the two-week treatment than the three-week growth period. The negligible differences in phosphatase and phosphorus, for the growth periods may be because the active portions of the root were in the root core for the same amount of time. In the three-week treatment the mass of actively growing roots passed through the bottom of the sampling device.

Conclusions

Corn roots were able to enrich their adjacent soil with phosphatase enzyme. In a simulated macrorhizosphere, the soil in a central root core and soil 5 mm away from the roots were significantly increased with phosphatase above the indigenous soil phosphatase. However, this enrichment did not obviously change the soil organic phosphorus levels. This supports the work of Thompson and Black (1970b) who found added phosphatase did not change the organic P levels. Because the organic phosphorus in soils are both insoluble and resistant to enzyme attack (Jackman and Black, 1952), and probably saturated with indigenous

soil phosphatase, the rhizosphere effect of phosphatase enrichment has questionable significance in greater utilization of soil organic phosphorus. However, this enrichment of phosphatase showed the importance of plant roots in contributing to natural soil phosphatase. Even though a fumigated treatment changed other soil properties besides decreasing microbial numbers, the root still enriched the adjacent soil with phosphatase.

This work indicated that phosphatase enrichment by roots is a definite rhizosphere effect, but may not be a stress mechanism for releasing more phosphate from natural organic matter.

Chapter 4

CHANGES IN pH AND PHOSPHORUS IN SOIL ZONES NEAR ROOTS

Introduction

Soil pH controls the solubility and availability of mineral nutrients (Lindsey and Moreno, 1960 and Murrman and Peech, 1969). Considerable evidence (Smiley, 1974; Bagshaw, Vaidyanathan, and Nye, 1972; and Riley and Barber, 1971) shows the pH of the rhizosphere differs from the pH of bulk soil samples. The root-induced rhizosphere pH, not the bulk soil pH, then determines the chemical solubility and nutrient availability to the plant. Changing the pH and therefore changing the mineral-solubility equilibrium becomes important when considering plant-nutrient availability of calcium and phosphate and also mineral toxicity of heavy metals and aluminum (Foy, 1965). Rhizosphere pH differences, qualitatively and quantitatively changing the root microflora, influence the occurrence of root pathogens (Smiley, 1974b). Thus the plant root, creating a rhizosphere effect on its adjacent soil by altering the pH may be enhancing its survival by increasing nutrient availability. The purpose of this study was to quantify the pH changes in soil near roots and determine this rhizosphere effect on the phosphorus fractions of three soils. The discussion includes a review of literature, methods, results, and conclusions.

Literature Review of Rhizosphere pH Changes

This literature review analyzes the causes of rhizosphere pH changes and their influence on phosphate availability.

Mechanisms and amounts of rhizosphere pH changes. Rhizosphere pH changes are due to three factors; enrichment of carbon dioxide (CO_2) from root and microbe respiration, leakage of organic acids from the root into the rhizosphere, and imbalance of cation to anion uptake. The large amounts of CO_2 produced in the rhizosphere are easily hydrated to carbonic acids (Brady, 1974). CO_2 plays an important role in solubilizing minerals, especially when nitrate is the nitrogen (N) source (Bartlett, 1965). Along with CO_2, cellular abrasion, leakage, and autolysis of organic acids in roots and microbes lower the pH of the rhizosphere (Carson, 1974). The imbalance of cation to anion uptake causes a differential effect of lowering or raising the rhizosphere pH. If cations are taken up in excess of anions, the extra positive charges in the cell are balanced by a release of protons (H^+); thus lowering the pH. If more anions are taken up than cations, then a subsequent release of hydroxyl (OH^-) or bicarbonate (HCO_3^-) results in a higher pH (Kirby and Mengel, 1967). Dijkshoorn (1962) stated increased anion uptake caused greater organic-acid production to neutralize the internal alkaline effect.

The ionic forms of plant-available nitrogen (NO_3^- and NH_4^+) comprise a large portion of the ionic uptake. If one form is either

more available or preferentially taken up, the rhizosphere pH changes accordingly. When NH_4^+ is the N source, the pH drops; when NO_3^- is the N source the pH raises (Riley and Barber, 1971). Dijkshoorn (1962) hypothesized the alkaline effect of NO_3^- was due to OH^- release during nitrate reduction to amino groups. Thus, the effect of NH_4^+ and NO_3^- fertilization has received much attention. Riley and Barber (1971) recorded a change of 2 pH units between nitrate and ammonium fertilization. Bagshaw et al. (1972) measured a difference of 0.7 pH units at a distance of 5 mm in a sandy soil. Smiley (1974a) noted NH_4^+ fertilizer acidified a calcareous rhizosphere but did not change the pH of an acid rhizosphere. In an unfertilized rhizosphere pH remained unchanged Smiley (1974a) found less pH increases in the rhizospheres of dicots fertilized with NO_3^- and attributed this to transport of NO_3^- directly to the shoot in ionic form; whereas, NH_4^+ is assimilated in the roots of both monocots and dicots. He also correlated greater rhizosphere pH changes to root surfaces with lower cation exchange capacity.

The magnitude of rhizosphere pH changes is modified by the buffering capacity of soils. Farr et al. (1969), growing a row of single roots "sandwiched" between two blocks of soil reported no pH changes in clay soils. Bagshaw (1972), using the same system, recorded pH changes in a sandy soil. Smiley (1974a) significantly correlated the combination of organic carbon, percent clay, and base saturation to the magnitude of rhizosphere pH changes.

The influence of root-induced pH changes on phosphate availability. The fact that rhizosphere pH can differ from bulk soil pH and the possibility of adding a specific form of nitrogen fertilizer to differentially raise or lower the pH around roots, has directed a few studies to investigate an increase in phosphate solubility. Riley and Barber (1971) found significantly greater amounts of $H_2PO_4^-$ in the rhizocylinder fertilized with NH_4^+. The NO_3^- treatment on acid soils showed no increase in phosphate. Soon and Miller (1977a) confirmed this phenomena by finding the lowered rhizosphere pH caused by NH_4^+ increased phosphate, but the raised pH of the NO_3^- treatment freed less phosphate. Riley and Barber (1971) correlated the P in plant tissue to rhizocylinder pH and concluded the increased phosphorus uptake due to nitrogen fertilizers was due to root induced pH changes. However, Thien and McFee (1970) showed pretreatments of both NO_3^- and NH_4^+ stimulated uptake of P. Bagshaw et al. (1972) and Soon and Miller (1977) measured P desorption isotherms for bulk soils at various pH values. Both studies indicated the soluble phosphate at a specific pH near the root was higher than the predicted value from desorption isotherms. Bhatt, Nye, and Baldwin (1976) concluded, ". . . that pH changes in the rhizosphere cannot account sufficiently for the enhanced solubilization of soil P in this region."

Root-induced pH changes appear to be a response to the external soil media, and not a metabolic-survival function to make a more desirable pH. But, rhizosphere pH changes may play a partial role in solubilizing phosphates.

pH and Phosphorus Methods

The soils, treatments, and sampling methods of this study were identical to the phosphatase study and the analyses for pH and inorganic phosphorus were performed concurrently.

pH (saturated-paste method of Jackson, 1970) was measured using 5 gram samples from each soil zone. A Beckman 3500 digital pH meter was employed with a combination electrode.

An extraction with sulfuric acid (H_2SO_4) aimed at measuring calcium phosphates was added to the previously reported available-phosphorus test (Chapter 3). After extracting and centrifuging Bray's #1 solution, the remaining soil was mixed with 10 ml of 0.5 N H_2SO_4 for 10 minutes. A filtered 10 ml aliquot was mixed with 2 ml of vandomolybdate color reagent and measured for absorbance at 380 nm.

Results and Discussion

Different trends in the pH and phosphorus changes occurred in the fumigated versus the non-fumigated treatment (Table 8). This observation was substantiated by significant statistical interaction. This discussion concerns pH and phosphorus changes in the fumigated and non-fumigated treatment. A complete list of results is presented in Appendix 1.

Non-fumigated treatment. Trends show a significant rise in soil pH in the root core and zone 1 (0-5 mm). The outer zones (5-50 mm)

Table 8. Means averaged for three soils.

Treatment	Soil zone	Distance from root core	pH	H_2SO_4-P	Available P
		mm		ug/g	
Two week growth period					
	Root core	-	6.73	15.4	26.0
	Zone 1	0-5	6.63	15.0	26.8
	Zone 2	5-10	6.52	15.1	27.3
	Zone 3	10-15	6.49	15.8	27.6
	Zone 4	15-50	6.46	16.5	29.3
Three week growth period					
	Root core	-	6.44	15.6	25.4
	Zone 1	0-5	6.45	15.0	26.1
	Zone 2	5-10	6.40	16.1	27.5
	Zone 3	10-15	6.36	15.5	29.8
	Zone 4	15-50	6.30	14.8	30.2
Two week growth period, plus fumigation					
	Root core	-	5.87	15.3	33.6
	Zone 1	0-5	5.95	15.5	32.0
	Zone 2	5-10	5.92	15.5	32.0
	Zone 3	10-15	5.91	15.6	32.0
	Zone 4	15-50	5.98	16.6	30.4

showed a slight but even decrease in pH as the sample distance increased from the root core. The increased pH of 0.5 units in the root core represents a five-fold decrease in hydrogen ion activity. Since the soils were not fertilized, nitrate was the predominant source of nitrogen (Table 2). By supplying an excess of negative charges, the nitrate caused the root to ionically balance the charge by releasing OH^- and HCO_3^- ions. The Udalf and Udoll, pH of 5.5 and 6.3 respectively, and having a lower buffering capacity (C. E. C., Table 2) showed the greatest pH change. The Ustoll, pH 7.2, having the highest C. E. C., showed little pH change.

The average inorganic phosphorus tended to decrease in soil zones closest to the roots, thus representing depletion patterns and concentration gradients. Along with pH changes, the simultaneous uptake of P causing an equilibrium shift and replenishing the phosphate from mineral sources, tends to mask any effect on raised pH and phosphate availability. In some cases the increased pH corresponded to an increase in H_2SO_4- extracted phosphorus (H_2SO_4-P). However, H_2SO_4-P generally showed only slight changes.

Fumigated treatment. pH was reduced by more than 1 unit by the fumigation treatment (Appendix 1). The reason for the drastic pH change was not resolved. However, the lower pH corresponded with a 25% increase in available P for all zones and soils. Little change was noticed in H_2SO_4-P (Table 8).

Trends in root induced pH changes also changed in the fumigated treatment. Contrary to the non-fumigated treatments, pH values showed a decreasing trend as the sampling distance became closer to the root. The distance of root influence was still the same, being the root core and zone 1. If the fumigated treatment somehow released ammonium or the roots preferentially took up an excess of cations, then the decreasing trend in root-induced pH changes could be explained. These decreases corresponded with a considerable increase in available P, especially for the Ustoll having an initial pH of 7.3. The Ustoll, with decreasing pH trends also showed decreasing H_2SO_4-P trends.

Conclusions

Root-induced pH changes occurred in the central root core and zone 1 (0-5 mm). The direction of pH change was probably controlled by the external soil supplying an excess of cations or anions to the root. Supporting the work of Soon and Miller (1977b) and Smiley (1974a) this study showed when root induced pH values dropped, considerable phosphorus became available. However, when these pH values were raised to a more optimum pH, a corresponding increase in available P did not occur.

LITERATURE CITED

Bagshaw, R., L. V. Vaidyanathan, and P. H. Nye. 1972. The supply of nutrient ions by diffusion to plant roots in soil. VI. Effects of onion plant roots on pH and phosphate desorption characteristics in a sandy soil. Plant Soil. 37:627-639.

Barber, D. A. 1967. Influence of pH on uptake of phosphate by barley plants in sterile and non-sterile conditions. Nature 215:779-780.

Barlett, E. M. and D. H. Lewis. 1973. Surface phosphatase activity of mycorrhizal roots of beech. Soil Biol. Biochem. 5:249-257.

Bartlett, R. J. 1965. Importance of carbon dioxide in uptake of calcium by plants receiving only a nitrate source of nitrogen. Soil Sci. Soc. Amer. Proc. 29:555-557.

Barnam, Th. E. 1969. Enzyme Handbook. Springer-Verlay. New York.

Benians, G. J. and D. A. Barber. 1974. The uptake of phosphate by barley plants from soil under aseptic and non-sterile conditions. Soil Biol. and Biochem. 6:195-200.

Bessey, O. A., O. H. Lowry, and M. J. Brock. 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem. 164:321-329.

Bhatt, K. K. S., P. H. Nye, and J. P. Baldwin. 1976. Diffusion of phosphate to plant roots in soil. IV. The concentration distance profile in the rhizosphere of roots with root hairs in a low P soil. Plant and Soil. 44:63-72.

Bieleski, R. L. 1973. Phosphate pools, phosphate transport and phosphate availability. Ann. Rev. Plant Physiology. 24:225-252.

Bieleski, R. L. and P. N. Johnson. 1972. The external location of phosphatase activity in phosphorus deficient Spirodela oligorrhizza. Aust. J. Biol. Sci. 20:707-720.

Black, C. A., and C. A. I. Goring. 1953. Organic phosphorus in Soils. In (W. H. Pierre and A. G. Norman, eds.) Soil and Fertilizer Phosphorus. Agronomy IV. Academic Press, New York.

Brady, N. C. 1974. The Nature of Properties of Soils. Mac-Millan, New York. P. 469.

Brams, W. H. and A. D. McLaren. 1974. Phosphatase reactions in columns of soil. Soil Biol. and Biochem. 6:183-189.

Carson, E. W. (ed.). 1974. The Plant Root and Its Environment. University Press of Virginia. Charlottesville.

Casida, L. E. 1959. Phosphatase activity of some common soil fungi. Soil Sci. 87:305-310.

Cervelli, S., P. Nannipieri, B. Ceccanti, and P. Sequi. 1973. Michaelis constants of soil acid phosphatase. Soil Biol. and Biochem. 5:841-845.

Chang, W. and R. S. Bandurski. 1964. Exocellular enzymes of corn. Plant Physiology. 39:60-64.

Clark, F. E. 1947. Rhizosphere microflora as affected by soil moisture. Soil Sci. Soc. Amer. Proc. 12:239-242.

Clark. R. B. 1975. Characterization of phosphatase of intact maize roots. J. Agric. Food Chem. 23:458-460.

Clark, R. B. and J. C. Brown. 1974. Differential phosphorus uptake by phosphorus-stressed corn inbreds. Crop Sci. 14:505-508.

Dekker, L. A. 1977. Worthington Enzyme Manual. Worthington Biochemical Corp. Freehold, New Jersey. P. 145.

Dijkshoorn, W. 1962. Metabolic regulation of the alkaline effect of nitrate utilization in plants. Nature. 194:165-167.

Eivazi, F. and M. A. Tabatabai. 1977. Phosphatases in soils. Soil Biol. and Biochem. 9:167-172.

El-Fouly, M. M. and J. Jung. 1972. Activity of acid phosphatase in wheat seedlings associated with soil stress conditions. Plant Soil. 36:497-500.

Estermann, E. F. and A. D. McLaren. 1961. Contribution of rhizosphere organisms to the total capacity of plants to utilize organic nutrients. Plant Soil. 15:243-260.

Farr, E., L. V. Vaidyanathan, and P. H. Nye. 1969. Measurement of ionic concentration gradient in soil near roots. Soil Sci. 107:385-391.

Foy, C. D., G. R. Burns, J. C. Brown, and A. L. Fleming. 1965. Differential aluminium tolerance to two wheat varieties associated with plant-induced pH changes around their roots. Soil Soc. Amer. Proc. 29:64-67.

Gilliam, J. W. 1970. Hydrolysis of pyrophosphates by plant roots. Soil Sci. Soc. Amer. Proc. 34:83-86.

Goss, R. L. and C. J. Gould. 1967. Some interrelationships between fertility levels and ophiobolus patch disease in turfgrass. Agron. J. 59:149-151.

Greaves, M. P. and D. M. Webley. 1969. The hydrolysis of myoinositol hexaphosphate by soil microorganisms. Soil Biol. and Biochem. 1:37-43.

Greaves, M. P. and D. M. Webley. 1965. A study of the breakdown of organic phosphates by microorganisms from the root region of certain pasture grasses. J. Appl. Bact. 28:454-465.

Greenwood, A. J. and D. H. Lewis. 1977. Phosphatases and the utilization of inositol hexaphosphate by soil yeasts of the genus Cryptococcus. Soil Biol. and Biochem. 9:161:166.

Hall, J. L. 1969. Localization of cell surface adenosine triphosphatase. Planta. 85:105-107.

Hall, J. L. and C. A. M. Davie. 1971. Localization of acid hydrolases activity in Zea mays L. root tips. Ann. Bot. 35:849-855.

Hasewaga, Yoichi, K. R. Lynn, W. Brockbank, and James. 1976. Isolation and partial characterization of cytoplasmic and cell wall-bound acid phosphatases from wheat roots. Can. J. Bot. 54:1163-1169.

Hiltner, L. 1904. Meber neure Erfahrungen und Probleme auf dem Gebiet der Boden bakteriologie und unter besonderer Berucksichtigung der Grundingung und Broche. Arb. Deut. Landev. Ges. 98:59-78. (In Carson, 1974).

Irving, G. C. S. and D. J. Cosgrove. 1976. The kinetics of soil acid phosphatase. Soil Biol. and Biochem. 8:335-340.

Jackman, R. H. and C. A. Black. 1952. Hydrolysis of phytate phosphorus in soils. Soil Sci. 73:167-171.

Jackson, M. L. 1970. Soil Chemical Analysis. Prentice Hall. Englewood Cliffs. New Jersey.

Juma, N. G. and M. A. Tabatabai. 1977. Effects of trace elements on phosphatase activity in soils. Soil Sci. Soc. Amer. J. 41:343-346.

Katznelson, H. 1946. The "rhizosphere effect" of mangels on certain groups of soil microorganisms. Soil Sci. 62:343-354.

Kirby, E. A. and K. Mengel. 1967. Ionic balance in different tissues of the tomato plant in relation to nitrate, urea, and ammonium nutrition. Plant Phys. 42:6-14.

Kramer, M. and G. Yerdei. 1959. Application of the method of phosphatase activity determination in agricultural chemistry. Soviet Soil Sci. 9:1100-1103.

Kuprevich, V. F., and T. A. Shcherbakova. 1971. Soil Enzymes. Indian National Scientific Documentation Centre, New Delhi.

Lindsay, W. L. and E. G. Moreno. 1960. Phosphate phase equilibrium in soils. Soil Sci. Soc. Amer. Proc. 24:177-182.

Martin, J. K. 1973. The influence of rhizosphere microflora on the availability of ^{32}P myoinositol hexaphosphate, phosphorus to wheat., Soil Biol. and Biochem. 5:473-483.

McLachlan, K. D. 1976. Comparative phosphorus responses in plants to a range of available phosphorus situations. Aust. J. Agric. Res. 27:323-341.

McLaren, A. D. 1975. Soil as a system of humus and clay immobilized enzymes. Chem. Scripta. 8:97-99.

McLaren, A. D. and E. Packer. 1970. Some aspects of enzyme reactions in hetergenous systems. Advan. Enzmol. 33:245-308.

Metha, N. C., J. O. Legg, C. A. I. Goring, and C. A. Black. 1954. Determination of organic phosphorus in soils. I. Extraction Method. Soil Sci. Soc. Amer. Proc. 18:443-449.

Mortland, M. M. and J. E. Gieseking. 1952. The influence of clay minerals on the enzymatic hydrolysis or organic phosphorus compounds. Soil Sci. Soc. Amer. Proc. 16:10-13.

Mossala, M. 1976. Variations in phosphorus accumulation and polyphosphate hydrolysis by selected maize families (Zea mays L.), M. S. thesis. Kansas State University.

Munnecke, E., B. J. Moore, and F. Abu-El-Haj. 1971. Soil moisture effects on control of Phythium ultimum or rhizoctonia solani with methyl bromide. Phytopathology. 61:194-197.

Murrman, R. P. and M. Peech. 1969. Effect of pH on labile and soluble phosphate in soils. Soil Sci. Soc. Amer. Proc. 33:205-210.

Neal, J. L. 1973. Influence of selected grasses and forbs on soil phosphatase activity. Can. J. Soil Sci. 53:119-121.

Neilands and Stumpf. 1953. Outlines of Enzyme Chemistry. John Wiley & Sons, Inc. New York.

Neuman, H. 1968. Substrate selectivity in the action of alkaline, and acid phosphatase. J. Biol. Chem. 243:4671-4676.

Olsen, S. R. and L. A. Dean. 1965. Phosphorus. In (C. A. Black, ed.) Methods of Soil Analysis. Agronomy IX. American Society of Agronomy. Madison, Wis. P. 1038.

Papavizas, G. C. and C. B. Davey. 1961. Extent and nature of the rhizosphere of Lupinus. Plant Soil. 14:215-236.

Rovira, A. D. 1976. Studies on soil fumigation. I. Effects on ammonium, nitrate, and phosphate in soil and on the growth, nutrition, and yield of wheat. Soil Biol. and Biochem. 8:241-248.

Rovira, A. D. 1953. Some quantitative and qualitative aspects of the rhizosphere. Australian Conf. Soil Sci. 1:1-7. (In Carson, 1974).

Rovira, A. D. and G. D. Bowen. 1966. Phosphate incorporation by sterile and non-sterile plant roots. Aust. J. Biol. Sci. 19:1167-1169.

Ramirez-Martinez, J. R. 1968. Organic phosphorus mineralization and phosphatase activity in soils. Folia microbiol. 13:161-174.

Ramirez-Martinez, J. R. 1965. A method for the determination of soil phosphatase using Na-B-maphthylphosphate. Soil Biol. 4:12.

Reid, M. S. and R. L. Bieleski. 1970. Changes in phosphatase activity in phosphorus deficient Spirodela. Planta. 94:273-281.

Ridge, E. H. and A. D. Rovira. 1971. Phosphatase activity of intact young wheat roots under sterile and non-sterile conditions. New Phytol. 70:1017-1026.

Riley, D. and S. A. Barber. 1971. Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root-induced pH changes at the root-soil interface. Soil Sci. Soc. Amer. Proc. 35:301-306.

Riley, D. and S. A. Barber. 1970. Salt accumulation at the soybean (Glycine max.(L.) merr) root-soil interface. Soil Sci. Soc. Amer. Proc. 34:154-155.

Riley, D. and S. A. Barber. 1969. Bicarbonate accumulation and pH changes at the soybean (Glycine max L. Merr.) root soil interface. Soil Sci. Soc. Amer. Proc. 33:905-908.

Savant, N. K. and G. J. Racz. 1972. Hydrolysis of sodium pyrophosphate and tripolyphosphate by plant roots. Soil Sci. 113:18-22.

Saxena, S. N. 1964. Phytase activity of plant roots. J. Exp. Bot. 15:654.

Shaykh, M. M. and L. W. Roberts. 1974. Histochemical study in root apical meristems. Ann. Bot. 38:165-174.

Skujins, J. J. 1967. Enzymes in soil. In (A. D. McLaren and G. H. Peterson, eds.) Soil Biochemistry, Marcel-Dekker, New York. PP 317-414.

Skujins, J. J., L. Broal, and A. D. McLaren. 1962. Characterization of phosphatase in a terrestial soil sterilized with an electron beam. Enzymologia. 25:125-133.

Skujins, J. J. and A. D.McLaren. 1962. Persistence of enzymatic activity in stored and geologically preserved soils. Enzymologia. 34:213-225.

Smiley, R. W. 1974a. Rhizosphere pH as influenced by plants, soils, and nitrogen fertilizers. Soil Sci. Soc. Amer. Proc. 38:795-799.

Smiley, R. W. 1974b. Forms of nitrogen and the pH in the root zone, and their importance to root infections. In (G. W. Bruehl, ed.). Biology and Control of Soil-Borne Plant Pathogens. II. Amer. Phytopathol. Soc., St. Paul, Minn.

Soon, Y. K. and M. H. Miller. 1977a. A centrifugal filter technique for isolating rhizocylinder solution. Soil Sci. Soc. Amer. J. 41:143-144.

Soon, Y. K. and M. H. Miller. 1977b. Changes in the rhizosphere due to NH_4^+ and NO_3^- fertilization and phosphorus uptake by corn seedlings (Zea mays L.). Soil Sci. Soc. Amer. J. 41:77-80.

Starkey, R. L. 1931. Some influences of the development of higher plants upon microorganisms in the soil. IV. Influence of proximity to roots on abundance and activity of microorganisms. Soil Sci. 32:367-393.

Subbarao, Y. V., R. Ellis, Jr., G. M. Paulsen, and J. V. Paukstelis. 1977. Kinetics of pyro- and tripolyphosphate hydrolysis in the presence of corn and soybean roots as determined by NMR spectroscopy. Soil Sci. Soc. Amer. J. 41:316-319.

Tabatabai. M. A. and J. M. Bremner. 1969. The use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. and Biochem. 1:307-310.

Tabatabai, M. A. and J. M. Bremner. 1971. Michaelis constants of soil enzymes. Soil Biol. and Biochem. 3:317-323.

Theodorou, C. 1971. The phytase activity of the mycorrhizal fungus, Rhizopogon roseolus. Soil Biol. and Biochem. 3:89-90.

Thien, S. J. and W. W. McFee. 1970. Influence of nitrogen on phosphorus absorption and translocation in Zea mays. Soil Sci. Soc. Amer. Proc. 34:87-90.

Thompson, E. J. and C. A. Black. 1970a. Changes in extractable organic phosphorus in soil in the presence and absence of plants. II. Soil in a simulated rhizosphere. Plant Soil. 32:161-168.

Thompson, E. J. and C. A. Black. 1970b. Changes in extractable organic phosphorus in soil in the presence and absence of plants. III. Phosphatase effects. Plant Soil. 32:335-348.

Thorton, J. I. and A. D. McLaren. 1975. Enzymetic characterization of soil evidence. J. Forensic Sci. 20:674-692.

Vakhmistrov, D. B., N. E. Mishustina, U. N. Ismeilov, S. A. Samvilova, and N. I. Tikhaya. 1973. Certain characteristics of the surface phosphatase activity of barley roots. Soviet Plant Physiology. 20:142-1051.

Van Gundy, S. D., D. Munnecke, J. Bricker, and R. Minter. 1972. Response of meloidogyne incognita, Xiphinema index and Dorylaimus sp. to methyl bromide funigation. Phytopathology. 62:191-192.

Verstraete, W. and J. P. Voets. 1977. Soil microbial and biochemical characteristics in relation to soil management and fertility. Soil Biol. and Biochem. 9:253-258.

White, A., P. Handler, and E. L. Smith. 1964. Principles of Biochemistry. McGraw-Hill Book Co., New York. P. 219-234.

Woolhouse, H. H. W. 1969. Differences in the properties of the acid phosphatases of plant roots and their significance in the evolution of edaphic ecotypes. In (I. H. Rorison, ed.) Ecological Aspects of the Mineral Nutrition of Plants. Oxford. P. 357.

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APPENDIX I

Table A. Data for a two-week growth period.

Soil zone	Distance from root core	Phosphatase			Available P			Organic P			H_2SO_4-P			pH		
		Untoll	Udoll	Udalf	Untoll	Udoll	Udalf	Untoll	Udoll	Udalf	Untoll	Udoll	Udalf	Untoll	Udoll	Udalf
	mm	umol/g/hr			ug/g			ug/g			ug/g					
Root core	-	2.55	3.17	0.68	14.8	31.0	32.2	168	109	149	19.4	12.8	13.9	7.37	6.70	6.12
Zone 1	0-5	2.42	2.73	0.55	15.2	33.2	31.9	197	108	147	17.8	14.5	12.9	7.47	6.40	6.03
Zone 2	5-10	2.41	2.37	0.61	16.2	32.8	32.8	163	211	255	18.3	13.1	13.4	7.41	6.20	5.96
Zone 3	10-15	2.24	2.62	0.57	14.0	34.4	34.4	159	162	62	18.3	13.2	15.6	7.42	6.18	5.88
Zone 4	15-20	2.28	2.60	0.59	14.1	35.6	38.2	420	154	125	21.1	15.4	13.2	7.34	6.16	5.89
LSD .05		0.17	0.21	0.06	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.09	0.08

Table B. Data for a three-week growth period.

Soil zone	Distance from root core	Phosphatase			Available P			Organic P			H_2SO_4-P			pH		
		Untoll	Udoll	Udalf	Untoll	Udoll	Udalf	Untoll	Udoll	Udalf	Untoll	Udoll	Udalf	Untoll	Udoll	Udalf
	mm	umol/g/hr			ug/g			ug/g			ug/g					
Root core	-	2.66	3.37	0.60	13.6	31.3	31.4	184	211	86	19.2	14.3	13.4	7.26	6.43	5.64
Zone 1	0-5	2.52	2.95	0.62	13.4	34.0	30.9	159	192	93	19.0	14.0	12.2	7.24	6.23	5.99
Zone 2	5-10	2.21	2.84	0.53	13.4	36.4	32.8	228	211	86	19.4	14.4	14.6	7.15	6.03	6.03
Zone 3	10-15	2.19	2.83	0.56	14.5	39.6	35.2	220	178	69	19.0	14.5	12.8	7.17	6.00	5.92
Zone 4	15-20	2.28	2.74	0.58	14.4	38.2	38.0	201	150	31	18.0	14.2	12.4	7.10	5.96	5.84
LSD .05		0.15	0.23	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.03	0.03	0.02

Table C. Data for a fumigated, two-week growth period.

Soil zone	Distance from root core	Phosphatase			Available P			Organic P			H_2SO_4-P			pH		
		Untoll	Udoll	Udalf	Untoll	Udoll	Udalf	Untoll	Udoll	Udalf	Untoll	Udoll	Udalf	Untoll	Udoll	Udalf
	mm	umol/g/hr			ug/g			ug/g			ug/g					
Root core	-	0.73	1.34	0.36	22.6	35.6	42.6	225	161	78	17.6	14.0	14.4	6.85	5.55	5.23
Zone 1	0-5	0.89	1.02	0.32	21.8	39.5	34.8	166	180	165	17.9	14.8	13.8	6.91	5.75	5.21
Zone 2	5-10	0.71	1.15	0.60	19.5	39.2	36.5	175	218	75	18.2	15.5	12.8	6.93	5.69	5.15
Zone 3	10-15	0.68	0.99	0.06	19.4	38.0	38.9	201	185	128	19.2	15.0	12.5	6.87	5.70	5.16
Zone 4	15-20	0.65	1.11	0.10	18.9	37.5	34.8	131	247	111	20.5	16.6	12.9	6.95	5.74	5.24
LSD .05		0.14	0.15	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.05	0.06

PHOSPHATASE ACTIVITY, pH, AND PHOSPHORUS IN THE RHIZOSPHERE OF CORN

BY

GEORGE ERNEST BOERO

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San Luis Obispo, California

AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

A rhizosphere sampling device was developed to quantify the effects of phosphatase, pH, and phosphorus in the rhizosphere of corn. Soil phosphatase was enriched up to 40% by corn roots in the central core and 5 mm away from this central root source. The increased phosphatase near roots did not correspondingly decrease the organic phosphorus levels during the time of this experiment. Available phosphorus generally decreased in soil samples closer to roots. The root-induced pH change differed 0.5 units between the root core and outer soil zones. When the root-induced pH change was lowered, available phosphorus tended to increase.