

THE EFFECTS OF P FERTILIZER ADDITION ON P TRANSFORMATIONS ON HIGH-P
FIXING AND GRASSLAND SOILS

by

JOY PIERZYNSKI

B.S., Michigan State University, 1982
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Abstract

Although phosphorus (P) is an essential nutrient for the growth of plants, it is one of the most limiting nutrients in terms of availability as a high proportion of applied P rapidly transforms into insoluble forms with low solubility in soils. To further understand the fate of P applied to soils, two separate but related studies using three high P-fixing soil types each were used for which the objectives were to investigate the mobility, availability, and reaction products from two granular and one liquid P fertilizer alone or plus a fertilizer enhancement product. Energy dispersive spectroscopy showed a substantial amount of P remained in the granule following a 5-week incubation. At the end of the 35-day incubation period there was evidence that the fluid fertilizer was superior over the granular sources in terms of enhanced diffusion and extractability of P for three calcareous soils with varying levels of CaCO_3 . Phosphorus x-ray absorption near-edge structure (XANES) spectroscopy results in conjunction with resin-extractable P indicated a strong negative correlation between Ca-P solids formed and P extractability, suggesting that degree of Ca-P formation limits P solubility. For the three acidic P-fixing soils the results were complex. In two out of three acid soils, liquid P treatments diffused farther from the application point than the granular treatments. Phosphorus XANES results suggested that Fe-P or Al-P interactions control the overall P solubility. Integration of pH, resin extractable-P and XANES results suggested the P retention mechanism was either dominated by adsorption or precipitation depending on soil pH. More acidic soil conditions favored precipitation.

The objectives of the third study were to observe how long-term (14 years) addition of P with or without N influences the inorganic and organic P pools in a native grassland soil using sequential fractionation, XANES, and ^{31}P -nuclear magnetic resonance (NMR) spectroscopy.

The overall results suggested that P and N fertilization and associated changes in plant productivity induced significant changes in soil P pools such as Ca-P, phytic acid, monoesters, and residual forms of P. The addition of P alone induced formation of inorganic P forms while the addition of P and N induced transformation of residual P forms into more labile and/or organic P forms.

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Approved by:

Major Professor
Ganga Hettiarachchi

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Dedication

To my loving husband and children

Gary Pierzynski

Jeanne Pierzynski

Garrison Pierzynski

For all of your love, support, and patience

We must do the things we think we cannot do – Eleanor Roosevelt

Chapter 1 - Introduction

In many agriculture systems, phosphorus (P) is the second most limiting macronutrient in the soil behind nitrogen and is a nutrient every living organism needs to survive. This macronutrient is a component in all cell membranes, RNA, DNA, and aids in the transfer of energy within the cell among its many functions. All sources of P for such a diverse group of uses ultimately come only from the soil. The average total P concentration in soil is 600 mg kg^{-1} . Despite the soil containing high concentrations of total P, soil P availability is limited. This element is slowly solubilized from apatite minerals, which is the primary source of all P fertilizers.

For commercial crop production, the slow solubilization of P for plant uptake is not an option. Differences in P sources added to the soil can determine both how far P diffuses or moves into the surrounding soil and its plant availability. For phosphorus granules added to soils, diffusion is opposite the hygroscopic flow of water toward the P granule. The particular area, or zone immediately adjacent to the fertilizer granule becomes saturated with solubilizing P fertilizer. As a result, P precipitates with aluminum (Al), iron (Fe), or calcium (Ca) depending on their availability in soil. As the remaining fertilizer diffuses beyond the immediate precipitated area P becomes sorbed onto mineral surfaces where it can be somewhat easily removed and equilibrated relatively fast with soil solution as available P is taken up by plant roots. The P sorption phenomenon, which is the transfer of orthophosphate from the soil solution to new or existing solid phases, is the result of a variety of mechanisms. Adsorption mechanisms include electrostatic attraction of orthophosphate, as well as ligand exchange on pH dependent charge sites. Sorption can also be the result of isomorphic substitution as well as precipitation of new

solid phases. However, for soil P, ligand exchange or chemisorption and precipitation are the major mechanisms of P sorption.

Many soils used for agricultural production around the world exhibit a high P fixation behavior. The degree of soil weathering as well as soil pH can produce very different mineralogies resulting in increased fixation. Soils found near the equator are highly weathered and acidic as a result of the high annual rainfall that leaches minerals from the soil profile, leaving behind high amounts of Fe and Al oxy-hydroxides, or hydrous oxides. Increased surface area, a decrease in pH and non-crystallinity makes them highly reactive thereby exerting a greater influence on soil chemical properties such as P fixation. Soils containing increased calcium carbonates from sediments or parent material generally have a high pH and are found in more arid environments which result in a slow weathering process. There are significant periods of soil drying between rainfall events. Phosphorus fertilizers added to these soils react with the calcium carbonate and form a series of calcium phosphates that may eventually weather into hydroxyapatite. The development of phosphorus fertilizer enhancement products (FEP) has been proposed as a means to potentially increase P availability and increase crop production in the high P-fixing soils. The proposed mode of action of FEP is the reduction in available Ca, Fe or Al in soil solution by complexation, thereby reducing P interactions with these elements.

Native tallgrass prairie is an ecosystem inherently low in available plant nutrients including phosphorus. These grassland soils are low in plant available P as there are limited P additions such as recycling of P in biomass, ash deposition following burning, dust additions and weathering of primary minerals to the system. As a result, the vegetation in this environment uses numerous strategies to obtain P needed for growth. An important source of orthophosphate for plant uptake is the solubilization of immobile P by arbuscular mycorrhizae (AM) fungi.

Through symbiotic relationships, the fungi colonize roots of vascular plants and extend hyphae into the soil. Although microscopic, the fungi produce some of the largest biomass in grassland soils. The extensive hyphae result in an effective increase in the root surface area of the host plant with which the plant can absorb water and nutrients. The P and N are translocated from the soil inorganic and organic sources to the plant hosts. Other methods for obtaining P by the plant include acidification with root exudates within the rhizosphere zone influencing the pH and microbial activity that promotes the solubilization of nutrients. Also, root exudates can facilitate the chelation of metal ions allowing for the uptake and transfer of P by AM and plant roots to the plant. Lastly, the imbalance of P chemical equilibria within the soil as a result of plant uptake of P promotes desorption of P and the transfer of P into the soil solution. The complex relationship between plants and other organisms in the native grassland influences above and belowground ecosystem processes.

Three separate studies were initiated to look at the effect of fertilizer P in different systems. Two studies involved using different P sources in high P-fixing soils and studying their effects after a five-week laboratory incubation period. Soils were selected that had low plant available P, low or high soil pH that may have contained minerals that strongly adsorb P, and that had not been fertilized. The soils sampled for the first study (Chapter 3) were an Andisol, Oxisol, and Ultisol, and all were acidic. Soils sampled for the second study (Chapter 4) were all calcareous; an Entisol, Inceptisol, and a Mollisol. The third study investigated the effect of long-term P and N fertilization on soil P cycling in an unplowed, native grassland system. The study utilized an ongoing P and N fertilization experiment to gain a better understanding of the fate of P fertilizers in prairie soils and to contribute to the overall knowledge and understanding of the P nutrient cycle in this ecosystem.

The objectives of the P fertilizer research on the high P-fixing soil were to understand mobility and availability of various P fertilizer sources in three major acidic soil types (Chapter 3) and three different calcareous soils with varying levels of CaCO₃ (Chapter 4) considered to have high P fixation capacity using a well-controlled, laboratory-based incubation experiment, and to identify possible P reaction products formed within and around fertilizer granules or droplets that may contribute to decreased P efficiency. The objectives were achieved through the use of laboratory-based methods including P mobility using total P extractions, lability measurements using resin-extractable P, the use of scanning electron microscopy and energy dispersive spectroscopy for reaction products formed in incubated P fertilizer granules and the use of x-ray absorption near edge spectroscopy (XANES) for direct identification of P reaction products in soils.

The objectives of the P fertilizer research on the native grassland soil (Chapter 5) were to determine how P and N fertilizer additions influence the forms of P in a grassland soil system utilizing a variety of approaches including soil P sequential fractionation for inorganic and organic P pools, XANES for direct P speciation, and ³¹P-nuclear magnetic resonance to identify organic P pools. We hypothesized that the addition of P and/or N fertilizers would affect both labile and non-labile forms of inorganic and organic P through a combined effect of soil chemical processes and changes to plant community structure and productivity.

Chapter 2 - Review of Literature

Introduction

Phosphorus (P) is a soil macronutrient and is vital to healthy plant growth. As a plant nutrient, P is essential in converting the sun's energy into chemical energy and is important in the transport of energy to every cell in the plant. It is a necessary nutrient for root growth and development, stalk strength, flowering and the reproduction process, and is essential in seed or grain development and for timely crop maturity. Phosphorus is an essential element in all living cells as well.

Plant available soil P is a limitation to maximum productivity in production agriculture. Inorganic P fertilizer is often used to bridge the gap between total P in soils and available P for crops. When P fertilizer is applied, a small portion is taken up by the plants as orthophosphate during the growing season and the remainder slowly becomes unavailable by transformations into insoluble compounds either by precipitation into secondary P solid phases, sorption onto mineral or clay surfaces, or immobilized by soil microorganisms into the soil organic fraction. This necessitates either frequent or heavy applications of P fertilizer to maintain crop productivity, either of which is costly and can result in P runoff or leaching losses.

In unfertilized grassland soils, the availability of P for plant availability comes primarily from the slow weathering of rock and soil minerals. Soils may contain a high concentration of total P but be very limited in P availability for plant uptake. Native grassland soils are an example of this. The total P concentration in prairie soil may be high; however, it is the slow dissolution of P from the weathering of apatite minerals into the soil solution that distributes P into different P pools. In solution, orthophosphate can be taken up by plants, sorbed onto soil particles, precipitated as secondary solid phases, or immobilized by soil microorganisms. In

addition, cycling of P by plants can slowly increase soil total P concentration in the surface horizon as the deep roots of grassland plants take up P from deeper soil horizons for growth and development and then depositing that P at the soil surface after the plants die. Grasses in the grassland ecosystem have adapted to low P availability through a variety of mechanisms including root exudates that increase soil P availability and symbiotic mycorrhizal associations with plants.

Soil Phosphorus Cycle

The soil P cycle is a description or depiction of P pools, inputs and outputs, and associated inorganic, organic and microbial P processes and transformations occurring within a soil system (Fig. 2.1). Phosphorus exists within various pools of varying solubility, including very labile forms, forms which are sorbed or present as discrete solid phases or as part of soil organic matter. The average total P concentration in soil is approximately 600 ppm (Lindsay, 1979). However, orthophosphate in the soil solution is present at very low concentration, between 0.003 to 0.3 mg/L, and is replenished from the solid phase P pools to sustain plant growth (Havlin et al., 2005). The overall P cycle is quite complex and P within the system is never static nor in equilibrium. It is influenced by plants, microorganisms present in the soil, climate, temperature, moisture, and parent material (Pierzynski et al., 2005).

Phosphorus inputs to the soil system influence or promote P chemical reactions and transformations. Inputs include the addition of fertilizers, either inorganic P fertilizers (H_2PO_4^- , HPO_4^{2-}) or organic based by-products such as composted manure, as well as decomposing plant residue. In addition, above and below ground decaying plant residue release P back to the soil in organic forms which are eventually released as inorganic P. In agriculture systems, not all P added is returned to the soil. Phosphorus contained in biomass or grain is removed resulting in a

loss of P from the soil P cycle. Other P outputs include run-off of sediment-bound P, particulate and soluble organic P, and leaching of P through the soil to ground water or into tile lines and eventually into open or surface waters (Sharpley et al., 2015). In all, P inputs and outputs greatly influence sorption, precipitation and immobilization of P in different pools, particularly as they relate to the soil gaining or losing P.

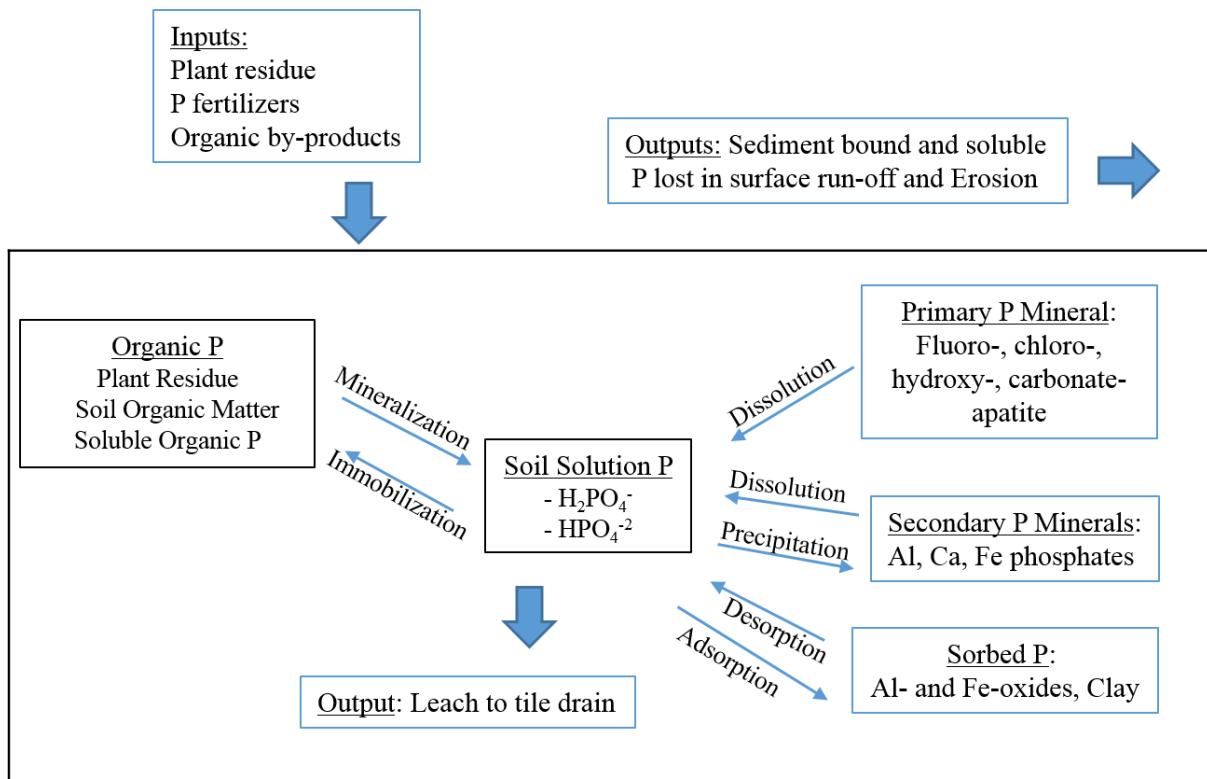


Figure 2.1 The soil phosphorus cycle

In an ecosystem such as native grasslands where there are no P fertilizer additions, soil solution P most likely comes from the dissolution of primary P minerals such as the apatite, which maintain very low P concentration in solution (Walker and Syers, 1976). Native grassland soil can be inherently low in plant available P as there are limited P additions. Additions include recycling of P in biomass and ash following burning, dust additions, and weathering of primary minerals in the system. Normal practices would not have us adding P fertilizer to native grassland soils. However, phosphorus fertilizers are sometimes to forage grasses (Turner, 2003).

Within soils, the principal primary P mineral is apatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{X})$ where the X represents Cl^- , CO_3^{2-} , F^- or OH^- with fluoroapatite being most important (Fig. 2.1) (Pierzynski et al., 2005). The dissolution reactions of apatite are a significant component of the P cycle. As apatite minerals slowly dissolve, inorganic P is brought into solution as orthophosphate such as H_2PO_4^- or HPO_4^{2-} , dependent on soil pH. In temperate soils, orthophosphate is available for plant uptake or can be removed from the soil solution through chemical processes. Available P can precipitate with cations such as Fe, Al, Ca and Mn to form secondary minerals. Phosphorus can also sorb onto clay or organic matter surfaces, or onto Fe or Al oxides. Soluble P may be immobilized and used by soil microorganisms or plants for growth.

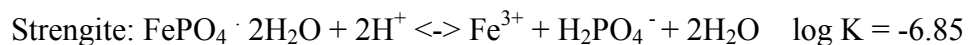
In highly weathered soil environments, apatite as well as the basic cations have been slowly leached through the profile, decreasing the soil pH and leaving behind the Fe and Al mineral forms where solution P can precipitate with free Al or Fe, or become strongly sorbed to Fe and Al oxy-hydroxide and hydrous oxide minerals resulting in very low P plant availability (Sanchez and Uehara, 1980). In calcareous soils, dissolution of P can be minimal and added P is rapidly precipitated with free Ca, Fe, and Al (Montalvo et al., 2014).

Phosphorus sorption-desorption processes occur along the open edges of clay minerals or on the surfaces of Fe and Al oxy-hydroxide solid phases in temperate or acid soils, or on carbonates and Fe or Al oxides in calcareous soils (Fig. 2.1). This P pool represents the most labile or available P that can contribute to soil solution P as P is removed by plant uptake. The adsorption reactions of P onto surfaces may be mono- or bidentate, as inner-sphere complexes, or as an outer-sphere complex. The adsorption reaction results in the displacement of OH⁻ or H₂O (Essington, 2003). As orthophosphate is removed from the soil solution, such as in plant uptake, adsorbed or sorbed P is released to re-equilibrate with soil solution P.

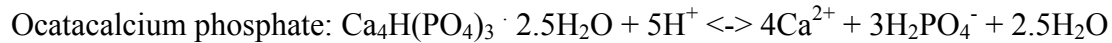
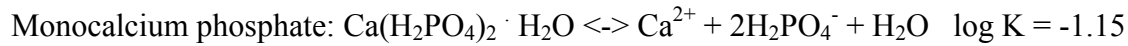
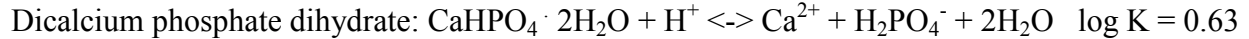
In acidic soils P is sorbed onto Al silicates such as kaolinite, hydroxide minerals such as gibbsite (Al(OH)₃) or iron oxide-hydroxide (Fe(OH)₃), oxides such as alumina, or hydrous ferric oxyhydroxides such as ferrihydrite (Fe₅HO₈•4H₂O). It is sorbed tightly enough that very little P is desorbed into the soil solution. In calcareous soils, P sorbs onto minerals similar to those in acid soils but to a lesser extent with goethite, alumina, gibbsite, and (Fe³⁺)₂O₃•0.5H₂O (Parfitt, 1978).

Secondary P mineral precipitation-dissolution processes occur with P and Fe, Al, or Ca slowly over time in temperate soils as a continuation of the sorption-process component to form amorphous or crystalline solid phases.

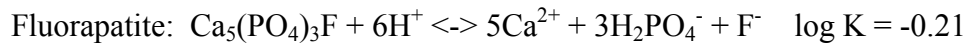
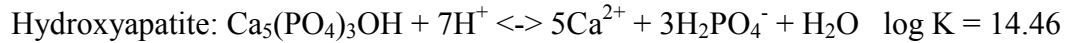
In acid soils important secondary P solid-phase reactions with their equilibrium constants include (Lindsay, 1979):



In calcareous soils calcium P species dominate:



$$\log K = 11.76$$



There is some question as to whether apatite minerals actually form as secondary solid phases in soils because apatite needs heat and pressure in order to form; when we find apatite in soil it is usually from parent material rather than a secondary mineral formation process (Moreno et al., 1960; Bell and Black, 1970; Fixen et al., 1983;).

Soil organic matter in soil is a large reservoir of P and can be an important source for plant uptake. Animal waste is often applied as a source of plant nutrients, including P, for crop production and contains both inorganic and organic forms of P. Condrón et al. (2005) reported that, depending upon the soil and its composition, the average total P concentration in soil may vary between 100 – 3000 mg kg⁻¹ with 30 to 65% of total P concentration being organic P. This wide range of organic P results in highly variable organic P contributions to the soil system.

Soil solution P can be immobilized by microorganisms and incorporated into soil organic matter. General organic P pools include microbial biomass, plant biomass, decaying plant residues, and soil organic matter. Some specific and important organic P forms include inositol P, nucleic acids, and phospholipids (Turner et al., 2003). Soil organic P forms within the soil microbial biomass include labile orthophosphate monoesters and ortho-diester, organic polyphosphates, and phosphonates. The stable organic matter includes organic P forms such as

the higher inositol phosphates, phosphonates and phosphate esters (Condrón et al., 2005). Soil organic P in the active soil organic fraction contains organic polyphosphates as well as orthophosphate diesters and labile orthophosphate monoesters. Grassland soils, with increased organic matter content, may contain up to 90% of the total P as organic P rather than inorganic P (Harrison, 1987). Plant biomass on the grassland soils constitutes the largest contributor to the organic P soil cycle (Tiessen et al., 1984).

When organic P is mineralized or transformed by various extracellular phosphatase enzymes to inorganic P it becomes plant available or may be immobilized once again by microorganisms. Alkaline and acid phosphatases help facilitate the cleavage of ester bonds, such as those found in diesters and monoesters (Alexander, 1977).

Phosphorus fertilizers

Inorganic P fertilizers originate from deposits of raw rock phosphate material. Rock phosphate deposits have been found and mined in many locations around the world including the United States, China, Russia, with the largest deposit to date in Morocco. The natural deposits exist in several forms: igneous rock, sedimentary deposits, and bat-guano deposits. The P in igneous deposits tends to be of apatite crystal found within igneous deposits which are relatively unreactive and the deposits tend to be of a lower grade, while the sedimentary deposits are more the more reactive and of higher grade rock-phosphate deposits.

The manufacture of P fertilizer begins with phosphate rock being acidulated with sulfuric acid to produce phosphoric acid with a gypsum by-product. This phosphoric acid, or green, wet-process acid product, is the basis for the common phosphate fertilizers (Leikam and Achorn, 2005; Havlin, 2005). A number of additional clarification and purification steps are used in

processing phosphoric acid that make this product more valuable and applicable for the fertilizer industry.

The original inorganic fertilizer was normal or single superphosphate (SSP) produced by taking finely ground phosphate rock and acidulating with sulfuric acid. The P compound in SSP is $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (monocalcium phosphate, MCP) and also contains gypsum. After completion of a denning and curing process the SSP is crushed for improved handling and use. Growers spread this P fertilizer as a granular material. This P fertilizer contains around 20% P_2O_5 (8.9% P) as well as a small percentage of sulfur. For many years SSP was considered a valuable and important P fertilizer product at least through the 1950s. Due to the transportation costs, SSP use has declined as other higher analysis P fertilizers are preferred. However, it is still in production and use in different areas of the world (Leikam and Achorn, 2005).

Triple super phosphate (TSP) is produced with an increased P content of up to 46% P_2O_5 (20.1% P) depending on where the phosphate rock was mined and the quality of sulfuric acid used during production. Triple super phosphate replaced SSP within the US during the 1950s and through the 1960s. Growers could use this fertilizer in either its granular or liquid form; however, the granular form was used to blend with other fertilizer materials for field application. Similar to SSP, the P compound in TSP is $\text{Ca}(\text{H}_2\text{PO}_4)_2$, but there is no gypsum present. Use of this fertilizer has decreased in recent years due to a need for higher grade phosphoric acid as compared to the granular ammonium phosphates.

The ammonium phosphate fertilizers began to become popular during the 1960s and eventually became the most popular P fertilizers used in the US by the end of the 20th century. These groups of fertilizers are easy to produce and have a high P_2O_5 content (Leikam and Achorn, 2005). Ammonia can be added to wet-process phosphoric acid to produce either

granular monoammonium phosphate (MAP: $\text{NH}_4\text{H}_2\text{PO}_4$) or diammonium phosphate (DAP: $(\text{NH}_4)_2\text{H}_2\text{PO}_4$) fertilizer or fluid ammonium polyphosphate (APP: $(\text{NH}_4)_3\text{HP}_2\text{O}_7$). The fluid ammonium polyphosphate is produced by reacting high grade phosphoric acid with ammonia. The drying or dehydration caused by the reaction between the acid and ammonia produces pyrophosphoric acid by combining together two or more orthophosphoric acid ions with the loss of a water molecule for every two H_2PO_4^- ions (Leikam and Achorn, 2005). This results in a ratio of 75:25 polyphosphate to orthophosphate in the finished liquid fertilizer. The presence of the polyphosphates allows for a higher P content in the liquid fertilizer as compared to liquids with only orthophosphate. Growers mix APP with other liquid fertilizers, such as urea and ammonium nitrate (UAN), or potassium sulfate (K_2SO_4), which are suitable for a variety of application techniques.

Processes responsible for Phosphorus fixation

Phosphorus is considered one of the most limiting soil macronutrients and is essential in numerous physiologic processes for optimal crop growth and development (Smil, 1999; Vance, 2001). In addition to P deficiency in plants, low P availability in agricultural soil reduces the efficiency of use for other essential plant nutrients. When P fertilizer is incorporated into soils, fertilizer P undergoes a series of transformations that reduces plant availability over time, even over a growing season. Heavy or frequent P fertilizer applications may be necessary to maintain adequate crop growth and yield (Pierzynski, 1991). A gradual decrease in P availability over time is an issue in agricultural soils regardless of the soil type, location, or the crop grown; however, it becomes a significant problem in areas where crops are grown on very acidic or calcareous soils. Phosphorus fertilizer added to soils with these characteristics are rapidly transformed into insoluble P forms, either adsorbed onto the surface of minerals or precipitated

as secondary solid phases. The process is collectively termed P fixation. The mechanisms behind increased P fixation in soils are complex and dependent on soil pH, climate, and soil mineralogy (Sanchez and Uehara, 1980; Lombi et al., 2004; Sample et. al., 1980).

The geographic distribution of high P-fixing soils is widespread. There are hundreds of millions of hectares of soils considered to have high P-fixing capacity that are in agriculture production around the world (FAO, 2010). With the addition of P fertilizer to these soils P participates in any number of soil reactions upon dissolution. The sorption phenomenon is the result of a variety of mechanisms including adsorption (sometimes called chemisorption) and precipitation reactions. Adsorption mechanisms include both inner-sphere and outer-sphere complexes with a soil solid through electrostatic attraction or ligand exchange mechanisms at pH-dependent charge sites (Essington, 2003).

Weathered, acidic soils present a unique challenge because these soils generally contain increased amounts of Fe and Al oxy-hydroxides, or hydrous oxides of Al or Fe. The adsorption or chemisorption reaction is a mechanism responsible for retaining anions such as PO_4^- onto the weathered, acid soil non-crystalline surfaces. Weathered acidic soils tend to have more pH-dependent surface charge sites that show strong pH-dependent P adsorption behavior. Adsorption is favored at a low pH when the adsorption sites are protonated and have a positive charge. Violante and Pigna (2002) found that P sorption capacity increases with increasing surface area of soil minerals.

Weathered, acidic soils have 1:1 clay minerals such as kaolinite and poorly crystalline minerals such as amorphous gibbsite or amorphous iron hydroxide with increased surface area. With the addition of P fertilizer, hydroxide ions are released from the Fe and Al oxides surfaces upon chemisorption of dissolved P (Stoop, 1983). For kaolinite, similar or comparable reactions

take place primarily at the crystal edges. When the functional group from the surface of the poorly crystalline mineral reacts with an orthophosphate anion from the dissolving P fertilizer, a complex between the anion and the mineral surface is formed. Two types of surface complexing exists: inner-sphere, and outer sphere. Outer-sphere complexes have a water molecule between the orthophosphate anion and mineral surface. These are generally electrostatic bonds. This adsorbed orthophosphate is easily desorbed or displaced from the surface by other ions in solution. Then, inner-sphere complexes do not have a water molecule between the orthophosphate anion and the mineral surface. A direct M-O-P bond is formed where M=the metal cation in the mineral. When the orthophosphate anion is retained through the inner-sphere mechanism it is more tightly bound and stable and is not easily released by into soil solution. In identifying the strength of the inner-sphere bond, monodentate-mononuclear is the term used to characterizes one type of inner-sphere complexation. Monodentate indicates a single M-O-P bond has formed. Bidentate complexes have an M-O-P-O-M configuration. This configuration can be mononuclear or binuclear (Essington, 2003).

Evidence of P-fixation behavior in acidic soils has been well documented (Arai and Sparks, 2007; Montalvo et al., 2013; Lindsay et al., 1959; Sanchez and Uehara, 1980; Stoop, 1983). Hedley and McLaughlin (2005) found that the addition of P fertilizer causes primarily the adsorption of P onto Fe and Al oxides near the fertilizer application. It greatly reduced the release of dissolved P fertilizer into the surrounding soil, and in turn, decreased the potential plant availability of applied fertilizer P.

Another type of acidic soil capable of P fixation are soils developed from volcanic parent material higher in amorphous materials such as allophone and imogolite (Zehetner et al. 2003). In moist climates the solution silica may be leached and the soils are left with these very reactive

amorphous materials that have a high surface area and a low pH. These materials can be very reactive with anions such as orthophosphates (Zehetner and Miller, 2006; Nieuwenhuysen et al., 2000).

Precipitation reactions in the weathered, acidic soils occur between PO_4^- and cations of Al, Fe, and Mn. The lower the soil pH, the greater the solution concentration of these ions. This complexation reaction is found in more deeply acidic soils and add to the P fixing nature of weathered soils (Pierzynski, 1990; Veith and Sposito, 1977; Boyle and Lindsay, 1985).

Soils containing high calcium carbonate (CaCO_3) frequently have high P fixing capacity and present a unique challenge in terms of agriculture production. Generally, these soils are located in more arid environments and have a high pH as a result of slower weathering processes. Producing crops with adequate yields is a challenge in such soils because with the addition of P fertilizers generally results in a high degree of insoluble Ca-P precipitation, and adsorption of P onto Fe and Al minerals, which reduces P fertilizer dissolution into the surrounding soil, thereby diminishing plant availability of P (Sample et al., 1980; Lombi et al., 2004; Hettiarachchi et al., 2006). Despite the abundance of Ca available for precipitation with P, the adsorption mechanism is important for P retention in such soils (Hesterberg, 2010).

The precipitation reactions occur between the free calcium in the soil solution and the added dissolving P fertilizer. The precipitated Ca-P species generally have a low solubility. Lindsay (1979) suggested that P fertilizer added to calcareous soils formed reaction products similar to dicalcium phosphate dehydrate (DCPD) or octacalcium (OCP)-like phosphate minerals, and given enough time, the formation of insoluble hydroxyapatite-like minerals. Freeman et al. (1981) found similar results in that fertilizer P addition led to the formation of Ca-

P and precipitated species such as DCPD and OCP in addition to adsorbed species, suggesting there was greatly decreased potential P plant availability in the soil environment.

In addition to Ca solid phases, Fe and Al oxides have an important role in sequestering P in calcareous soils. Holford and Mattingly (1975) utilizing bonding energies using a two-surface Langmuir equation, found that high-energy P adsorption capacities were related more to hydrous oxides, such as dithionite-soluble Fe, providing principle sites for adsorption in calcareous soils. Lower energy adsorption capacities were more correlated with the total surface areas of calcium carbonate. Low energy adsorption capacity was not related to pH, dithionite-soluble Fe or percent CaCO₃. Similarly, Ryan et al. (1985) found that CBD extractable Fe was associated with a longer term decrease in extractable P along with Ca-P solid phases in calcareous Mediterranean soils. Iron oxides were responsible for sorption reactions on amorphous surfaces with a gradual crystallization of various forms of Fe-P over time.

In alkaline, non-calcareous soils, P fixation is not expected to be significant because adsorption is minimal due to the pH-dependent charged sites being deprotonated and negatively charged. Also, precipitation would be limited because the cations present are not cations that form insoluble P minerals. However, Bertrand et al. (2003) did determine that amorphous Fe and Al oxides influenced P sorption behavior in alkaline, non-calcareous soils. Further, Lombi et al. (2004) found that the availability of liquid fertilizers as compared to granular fertilizers were not greater in alkaline, non-calcareous soil, whereas liquid fertilizers were significantly greater in alkaline calcareous soils.

On high P-fixing Oxisols in Brazil, Yost et al. (1981) found that P uptake by corn over time, as measured by yield, was greater with deep banding of P fertilizer compared to broadcast application. However, this difference was not apparent at very high P applications. Kar et al.

(2012) investigated whether banding of P fertilizer might reduce soil and P fertilizer contact thereby reducing the formation of P reaction products via sorption or precipitation mechanisms.

Another approach to potentially increasing P availability by minimizing soil P fixation have been the use of P fertilizer enhancement products (FEP). There are fertilizer enhancement products that stimulate microbial growth with a subsequent release of enzymes, which releases additional organic P from the soil which can increase fertilizer nutrient uptake by crops. Several products naturally increase plant hormones that may result in enhanced plant growth and development by increased nutrient uptake. There are products manufactured containing exclusive technologies, such as Huma Gro®. Another, a group of humic acid-based soil amendment products have been created to reduce stresses on crops. By improving soil structure the UltraMate SG® product allows crops to efficiently utilize fertilizer applied N, P and micro-nutrients.

A number of field studies on different soils using P fertilizer at planting containing another (FEP) have been summarized by Tindall (2007). This FEP is a long-chain carbon compound composed of maleic-itaconic acid copolymers with high cation exchange capacities (CEC) which complex multivalent cations in close proximity to the point of application that normally would adsorb or precipitate with P anions thus decreasing P availability. Independent field studies reportedly show mixed results although increased yield and P use efficiency was demonstrated at some sites. The product can be utilized at any soil pH. In a field trial on a calcareous soil in Idaho, Hopkins (2013) broadcast applied and incorporated MAP fertilizer at planting with or without an FEP for Russet Burbank potatoes. The MAP + FEP addition resulted in greater petiole P concentrations and total yield increase in all but one site.

Identification of Phosphorus fertilizer reaction products

The identification of P fertilizer reaction products or solid P phases in P fertilized soils is very important because it can provide a mechanistic understanding behind P fixation in high P-fixing soils. Identifying the reaction product in these soils potentially allows a better understanding soil P bioavailability and will lead to increased P fertilizer-use efficiency at the field scale with resulting economic and environmental benefits.

There are two general approaches to identifying P fertilizer reaction products in soils, indirect and direct. The indirect methods include sequential extraction methods which are operationally defined soil P fractions determined using a series of different chemical extractants to sequentially separate total soil P into fractions extractability and solubility. Lastly, solubility equilibria, a method whereby the composition of an aqueous solution assumed to be in equilibrium with a soil is compared to that expected for a solution in equilibrium with a known P solid, is used to infer the presence of particular P solids in that soil (Lindsay, 1979).

Direct methods for identification of P reaction products exist as well. Most are forms of spectroscopy that use the interactions between P atoms and electromagnetic radiation to directly identify individual P solids. These include ^{31}P -NMR, scanning electron microscopy-energy dispersive spectroscopy, x-ray diffraction (XRD), and x-ray absorption techniques which utilize synchrotron radiation. Synchrotron offers a direct characterization of inorganic and organic solid-phase P species in soils using synchrotron x-ray at an atomic level and has been found useful in determining forms of P speciation with a high degree of spatial resolution. This method has been in wide-spread use in many areas of soil science for a number of years. X-ray diffraction uses the diffraction of a focused x-ray beam by atoms arranged in planes within a crystal to identify crystalline minerals in soils (Harris and White, 2008).

With the addition of P fertilizer to soils, reactions are formed that impact P availability. Research on reaction products in soils go back more than 50 years. Kittrick and Jackson (1956) showed that crystalline phosphates formed by reacting kaolinite clays or hydrous oxides with P. X-ray diffraction and electron microscopy were used as the direct methods.

Lindsay and Stephenson (1958a) showed that partially dissolved, monocalcium phosphate monohydrate (MCP) produced a very acidic solution and, when it was reacted with soil over 17 days, formed dicalcium phosphate dehydrate (DCPD) as determined using solubility equilibria. Over time, the solution composition transformed, dissolving a portion of the DCPD and precipitating anhydrous DCP with some undissolved MCP remaining. Further, they found that the band placement of MCP in a Hartsell fine sandy loam soil induced a number of processes. Reduced water vapor pressure within the band caused soil water to move toward the band. The concentrated P fertilizer solution moved in the opposite direction from the fertilizer band as a wetting front into the drier soil, in the process dissolving Fe, Al, and Mn. The cations of Fe, Al, Ca and Mn were available for precipitation with P as the solution reacted with the soil, increasing the pH to that of the surrounding soil. As a follow-up Lindsay and Stephenson (1958b) identified by microscopy and XRD crystalline Fe, Al, and Ca phosphates along with unidentified, amorphous precipitates. These studies were done with one acidic (pH 4.6) and one calcareous soil (pH 7.6). Moreno et al. (1960) using solubility equilibria found DCPD as a reaction product upon addition of MCP to an acidic Hartsells fine sandy loam and Fe and Al hydrous oxides for reaction times at 20 days or less.

Racz and Soper (1967) reacted concentrated phosphate solutions with 22 different soils of varying Ca and Mg contents and characterized the precipitates formed after a 21-day incubation period using XRD. For soils with a higher proportion of Ca than Mg, DCPD and OCP were

found, whereas soils richer in Mg contained tri-magnesium phosphate tetrahydrate. None of the soils were calcareous. Bell and Black (1970), using petrographic and XRD methods and on slightly acidic to slightly alkaline soils, found primarily DCPD after addition of MCP, MAP, and DAP. However, evidence of mixed Ca- Mg- NH₄ phosphates was also identified. Over time, the soil reaction products transformed to either DCPD or to OCP.

Freeman and Rowell (1981) adsorbed phosphate onto calcite and identified calcium phosphates with scanning electron microscopy. Solubility equilibria studies suggested the solids were calcium phosphate and OCP. X-ray diffraction data showed the existence of both OCP and DCP with DCP predominating.

In a long-term field study in which TSP was added to calcareous soil under alfalfa production, solubility equilibria suggested OCP and β -tricalcium phosphate (β -TCP) were controlling P solubility (Fixen et al., 1983). Pierzynski et al. (1990a; 1990b; 1990c) combined equilibria studies with scanning transmission electron microscopy to study P solid phases in excessively fertilized soils. Solubility equilibria suggested calcium phosphate controlled P solubility for soils with a pH > 7 and amorphous Al-P solids controlled P solubility for soils with pH < 7. Scanning electron microscopy work confirmed the presence of discrete P-enriched solids with a mixed-cation composition at all soil pH levels.

Hesterberg et al. (1999) determined that XANES spectroscopy could distinguish between varieties of P solid phases thought to form in soils and identified the presence of calcium phosphates in soils with a pH of 7.6. Sato et al. (2005) studied P solid phases in soils amended with poultry manure for varying periods of time using XANES. Phosphorus in unamended acidic soils was associated with Fe compounds such as strengite and Fe-oxides. Shortly after manure amendment, DCPD and amorphous calcium phosphates were found, which transformed to less

soluble β -TCP over time. Ajiboye et al. (2008) used X-ray absorption near edge structure to identify P species in two calcareous soils amended with either MAP or various organic amendments. Solid phase adsorbed P was dominant in both soils and evidence of hydroxyapatite (HA) and β -TCP were also found.

Beauchemin et al. (2003), studied P speciation using XANES spectroscopy in slightly acidic or alkaline soils in Quebec, CA., that had received a variety of P amendments. They found P adsorbed onto Fe- or Al-oxide minerals, were found in all soils, with a greater amount in the slightly acidic soils. Calcium P species were also found in all soils with no relationship to soil pH. Liu et al. (2014a) studied group of Ultisols (Soil Survey Staff, 2004) in China, with a range of 4.2 to 5.5 pH, under cultivation. The results were found to have P associated with Fe and soluble Ca by XANES, and the most heavily fertilized soils were found to have HA.

Direct methods such as ^{31}P NMR and XANES can also be used to characterize organic P in soils with the use of ^{31}P NMR most commonly applied to NaOH-EDTA extracts of the soil. The organic P species typically identified include inositol hexakisphosphate (IHP), orthophosphate monoester (Mono), orthophosphate diesters (Di), β -glycerophosphate, and phosphocholine, among others (Liu et al., 2014b; Li et al., 2013). However, the NaOH-EDTA extract doesn't remove all organic P from the soil and therefore this approach inherently underestimates organic P species (Cade-Menun and Liu, 2014).

Effect of Phosphorus Source on Soil P

Increasing P use efficiency in P limiting soils has been a continued topic of interest. A number of studies employed soil-P fertilizer incubations in petri dishes. These studies examined a wide range of P sources and soils, and employed a variety of experimental approaches to assess P mobility and lability, and P fertilizer reaction products. Soil is added to the dish, P fertilizer is

applied to the center, and soil is sampled in concentric rings centered on the P fertilizer application point. The soil samples are subject to a selection of chemical and mineralogical characterization procedures.

The benefits of P supplied in fluid forms over conventional granular products demonstrated in field studies on highly calcareous soils in South Australia have been explored at the laboratory scale through x-ray, spectroscopic and laboratory-based chemical analyses (Lombi et al., 2004; 2006). Incubation results suggest the use of technical grade monoammonium phosphate liquid fertilizer (TG-MAP) on highly calcareous soils allowed for greater diffusion of P from the point of application and increased lability as compared to granular monoammonium phosphate (MAP) fertilizer partly due to a reduction in the formation of Ca-P precipitates. Their XRD and microanalysis energy dispersive X-ray examination of MAP granules incubated in the highly calcareous soil suggested a greater percentage of fertilizer P remained within the granule at the end of the incubation period as compared to the P fluid fertilizer. Lombi et al. (2004) initiated a second laboratory-based study to examine mobility, solubility and lability of P fertilizers on highly calcareous soils and one alkaline non-calcareous soil, whereby they included a variety of granular (MAP, DAP and TSP) and fluid (TG-MAP, phosphoric acid and polyphosphate (APP) P fertilizers. Their results suggested the granular P fertilizers had lower dissolution and diffusion from the point of application, as compared to fluid P fertilizers regardless of soil type. However, P fertilizer diffusion, solubility and lability were significantly greater with the P fluid fertilizers as compared to the granular P in all calcareous soils but not with the non-calcareous alkaline soil. For the greater P fluid mobility and lability in the calcareous soils it was suggested that P fixation processes were reduced compared to any of the granular P materials.

Hettiarachchi et al. (2006) found that the form of P fertilizer added to calcareous soil greatly influenced diffusion and lability away from the point of application. X-ray computed microtomography was employed to look at differences in soil moisture over time with either a granular MAP or liquid TG-MAP injected into a comparable volume of soil. For MAP granules, the hygroscopic water movement toward the granule decreased P diffusion resulting in increased P precipitates immediately surrounding the granule. However, with the addition of the P fluid fertilizer to the soil there was not a decrease or restriction in P diffusion and this likely reduced precipitation of P fertilizer. The reduced release of dissolved granular P fertilizer into the surrounding soil implies decreased potential plant availability of applied fertilizer P.

Montalvo et al. (2014) initiated a laboratory-based soil incubation experiment examining mobility, solubility and lability of different granular (calcium phosphate and ammonium phosphate) and liquid (polyphosphate and ammonium phosphate) P fertilizer sources on acidic Oxisols and Andisols, both high P-fixing soils. It was discovered that although the fluid P fertilizer diffused farther from the application point in the Oxisols and Andisols as compared to the granular materials, it did not necessarily increase P solubility and availability in these soils; rather, there was more P availability with the P granular fertilizers in these soils as compared to liquid P fertilizer forms. They suggested that perhaps strong adsorption rather than precipitation reactions limited P availability because spreading the fluid P fertilizer over a greater soil volume increased adsorption and reducing P availability.

Khatiwada et al. (2012) evaluated fertilizer reaction products under field conditions on a Smolan silt loam, pH 5.9, using MAP and technical grade (TG)-MAP deep banded or broadcast. Granular MAP produced Fe-P like forms whereas TG-MAP produced more adsorbed P-like forms at 5 weeks. After 6 months, all reaction products tended to move toward Ca-phosphates or

mixtures of Ca-, Fe-, and adsorbed phosphate-like forms. Resin extractable P was lower for broadcast than for deep banded and higher for liquid compared to granular fertilizers.

Knowledge Gaps

At this time a logical extension in the research would be to initiate a lab-based incubation study incorporating other common granular and fluid P fertilizers used in production agriculture, to include a wider range of soils and soil properties, and to expand the chemical and mineralogical characterization methods employed. In addition, resolving the P fixation problem with inexpensive, reasonable management strategies would go a long way in helping to economically increase agricultural production for growers farming these soils.

Thesis Aims and Objectives

The objectives of this research were to (1) understand the mobility and availability of various P fertilizer sources in three different acidic soils considered to have high P fixation capacity using a well-controlled, laboratory-based incubation experiment and to (2) identify possible P reaction products formed within and around fertilizer granules or droplets that may contribute to decreased P efficiency, and (3) examine possible soil chemical changes related to P diffusion, lability and speciation with both granular and liquid P fertilizers with or without an FEP capable of interacting with cations that may influence fertilizer P reaction products.

In a separate study on unfertilized grassland soils, similar questions were addressed under the scenario of the initiation of P fertilizer additions to the grassland ecosystem and continuation for 12 years. The objectives of this research were to determine how P and N fertilizer additions influenced the forms of P in a grassland soil system utilizing a variety of approaches including soil P sequential fractionation, XANES and ³¹P-NMR.

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Chapter 3 - Phosphorus Fertilizer Reactions in Three Acidic Soils with High Phosphorus Fixing Capacity

Abstract

Limited phosphorus (P) availability for plants in acidic soils is one key factor that limits crop production in many areas of the world. Fluid P fertilizer has enhanced diffusion and lability in calcareous soil compared to granular P fertilizers but these relationships have not been studied extensively in acid soils. Further, there is limited information on the possible mechanisms behind reduced P efficiency in acid soils that have a high P-fixing capacity. In this study, we investigated the mobility and availability of P from three fertilizers: monoammonium phosphate (MAP), diammonium phosphate (DAP), and ammonium polyphosphate (APP). In particular, we compared the effects of the fertilizers alone and enhanced with a fertilizer enhancement product (FEP) in three soils, an Oxisol, an Andisol, and an Ultisol, to examine associations between the P fertilizer reaction products formed, P mobility, and potentially labile P.

The soils were incubated for five weeks at 25° C in petri dishes that contained five replicates of each of six fertilizer treatments plus the respective unfertilized control soil. At the end of the incubation period, the petri dishes were disassembled by collecting soil samples at varying distances from the point of P application. Measurements included soil pH, total P, resin extractable P, scanning electron microscope-energy dispersive x-ray (SEM-EDS) analysis of the P granules, and P reaction products using x-ray absorption near-edge structure spectroscopy (XANES). The data show that P mobility with the granular fertilizer was limited in all three soils, but mobility was enhanced in the Ultisol and Andisol for the liquid P treatments. In general, the addition of FEP did not increase P mobility in the soils. The Oxisol showed that the P source had minimal effect on lability, measured using anion exchangeable resin strips and

calculating the percent resin P for each dish section, but in the Andisol, lability was significantly higher in the 0-7.5mm section with both liquid P treatments compared to the granular P treatments. For the Ultisol only there was generally greater lability for the granular P treatments compared to the liquid P treatments at 0-7.5 mm. In addition, lability was higher in the center section for APP + FEP when compared to APP. There was no direct FEP effect for the other soils. The speciation results indicate that P source and FEP had variable effects across the three soils in this study. The addition of P resulted in increases or decreases in the percentages of Fe- and Al- precipitated and adsorbed phosphate-like forms compared to the unfertilized control soils, but there was little evidence to suggest that P source influenced the speciation. The only apparent effect of FEP was an increase in Fe-P-like forms in the Oxisol and an increase in Fe-adsorbed P-like forms in the Ultisol for all P sources as compared to the unfertilized control soil. Speciation did influence P lability. For the Andisol, regardless of P source with and without the FEP, the fraction of P present as Fe-adsorbed P-like forms was positively correlated with P lability. Overall, this research suggests there was not a consistent benefit of using a fluid over a granular P source, or adding the FEP used in this research, on these acidic high P-fixing soils.

Introduction

When P fertilizers are added to soil it increases P availability for plant growth and production. However, availability of P may decrease over time. The gradual decrease in available P encourages additional or excessive applications of P fertilizer for continued crop productivity, which pose potential environmental risks and an associated increase in cost (Sharpley et al., 2015; Pierzynski et al., 2005). Decreased P availability is a concern in agricultural soils regardless of soil type or the location. However, it becomes most significant in agricultural areas where crops are grown on highly calcareous or acidic soils. Phosphorus fertilizer added to these groups of soils is rapidly transformed into insoluble P forms, either strongly adsorbed onto the surface of minerals or precipitated as secondary solid phases. The process is collectively termed P fixation.

Weathered, acidic soils present a unique challenge because many of these soils contain increased amounts of iron (Fe) and aluminum (Al) oxy-hydroxides, or hydrous oxides. The addition of P fertilizer causes the adsorption of P primarily onto the oxides near the fertilizer application point (Hedley and McLaughlin, 2005). This greatly reduces the release of dissolved P fertilizer into the surrounding soil, in turn decreasing the potential plant availability of applied fertilizer P. Another type of acidic soil capable of P fixation is soil developed from volcanic parent material that is higher in amorphous materials such as allophone and imogolite (Zehetner et al. 2003). In moist climates, the solution silicon may be leached, and the soils are left with very reactive material such as hydrous aluminum silicate clay mineraloid materials that have a high surface area with a low pH. These materials can be very reactive with anions such as orthophosphates (Zehetner and Miller, 2006; Nieuwenhuyse et al., 2000).

Evidence of P-fixation behavior in acidic soils is well documented (Arai and Sparks, 2007; Montalvo et al., 2013; Lindsay and Stephenson, 1959; Stoop, 1983). Sanchez and Uehara (1980) studied P adsorption and found a large increase in P adsorption in acid soils containing sesquioxides. Pierzynski et al. (1990) reported that amorphous P solid phases existed as distinct particles and coatings on other soil particles in soils that were excessively fertilized and classified as Alfisol and Entisols.

Phosphorus fixation in soil presents a challenge agriculturally. Different strategies have been explored and implemented for overcoming P fixation. Higher P fertilizer applications have been used to overcome the rapid decreases in plant P availability with low amounts of P added to high P fixing soils (Sanchez and Uehara, 1980). Younge and Plucknett (1966) fertilized a Hawaiian latosol with varying P rates before planting and had sustained crop yield increases only with the highest P treatment. The increased crop yield from the high initial P application extended over several years of cropping. On high P-fixing Oxisols in Brazil, Yost et al. (1981) found that P uptake by corn over time, as measured by yield, was greater with deep banding of P fertilizer compared to broadcast application. However, this difference was not apparent at very high P applications. Kar et al. (2012) investigated whether banding of P fertilizer might reduce soil and P fertilizer contact thereby reducing the formation of P reaction products via sorption or precipitation mechanisms. Using a combination of chemical analyses, a sequential extraction method to separate soil P into different fractions, anion exchange resin membrane probes to measure labile extractable soil P, and a synchrotron based technique to perform spatially-resolved P speciation. Subsequently, P fertilizer availability for plant uptake was increased using this method as compared to the broadcast application method.

The application of fluid P fertilizers to overcome the adsorption and precipitation reactions found with P granular fertilizers in high P fixing soils is another method to increase P use efficiency (McBeath et al., 2005). In contrast, Montalvo et al. (2014) found in a soil incubation experiment using two acidic high P-fixing Andisols and Oxisols, that although the fluid P fertilizer used in the dish experiment diffused farther than the P granular treatments, it was not increased P availability over the P granular in these soils. The lack of difference in P diffusion was explained in terms of soil adsorption strength. Phosphorus diffusion in the soils was negatively correlated with ammonium oxalate-extractable Fe and Al content. For both the P liquid and granular fertilizers, adsorption and diffusion were controlled by the concentration of Fe and Al poorly crystalline soil solids and its adsorptive capacity.

Phosphorus fertilizer enhancement products (FEP) have been proposed as a possible means to minimize P fixation, in order to maintain P availability for plant growth. Some of the popular products include humic or fulvic acid-based compounds. There are products manufactured containing exclusive technologies, such as from Huma Gro® that use carbon-rich molecules as nutrient carriers which enable efficient nutrient absorption, including P, for an increased plant growth. Lastly, there are several products that provide enhanced nutrient availability resulting in exceptional growth and development.

One widely used FEP is a long chain carbon compound composed of maleic-itaconic acid copolymer $(C_9H_6XO_8)_n$ (X= cation) marketed under the product name of Avail®. Due to its molecular structure, the product has a high cation exchange capacity (CEC) and complexes multivalent cations (Al, Ca, Fe) in direct proximity to the point of application that normally would precipitate or adsorb P anions thereby decreasing P plant availability (Karamanos and Puurveen, 2011). Tindall (2007) summarized a number of field studies and reported increased

crop yield on several different soils with the addition of P fertilizer containing a FEP applied at planting. Some studies have reported increased yield and P use efficiency employing this FEP (Dunn and Stevens, 2008). In contrast, other studies have not found a significant increase in P use efficiency, grain yield, or any significant interaction between P rate and use of this FEP (Karamanos and Puurveen, 2011; McGrath and Binford, 2012, Ward and Mengel, 2009; Degreyse et al., 2013).

An approach for evaluating P solid phase formation with the addition of P fertilizer to soils includes a form of absorption spectroscopy, x-ray absorption near edge structure (XANES). This synchrotron-based spectroscopy method offers a direct characterization of P in soils using synchrotron X-ray at an atomic level and is useful in determining forms of P speciation with a high degree of spatial resolution. Studies have used P K-edge XANES spectroscopy to examine P reaction products formed as related to P fertilizer sources, fertilizer placement, and the fate of inorganic and organic P in the environment in short term and long-term field studies (Beauchemin et al., 2003; Kar et al., 2012; Khatiwada et al., 2012; Ajiboye et al. 2007; Lombi et al., 2006). However, there has been limited research using XANES for direct speciation of P reaction products in acidic high P-fixing soils or in close proximity to P fertilizer additions in soils.

The objectives of this research were to (1) understand the mobility and availability of various P fertilizer sources in three different acidic soils considered to have high P fixation capacity using a well-controlled, laboratory-based incubation experiment and to (2) identify possible P reaction products formed within and around fertilizer granules or droplets that may contribute to decreased P efficiency, and (3) examine possible soil chemical changes related to P

diffusion, lability and speciation with both granular and liquid P fertilizers with and without an FEP capable of interacting with cations that may influence fertilizer P reaction products.

Materials & Methods

Three soils were sampled for the study, an Oxisol (Typic Hapludox) from Rondonopolis, Brazil (N 07° 09' 40''; W 54° 43' 10''), an Andisol (Humic Udivitrand) from the Cotacachi region in Ecuador (collected at an elevation approximately 2934 m at N 00° 20' 12.3''; W 78° 17' 22.5''), and an Ultisol (Humic Hapudult) from the Talawakele-Hatton Up Country wet zone region, Sri Lanka (collected at an elevation approximately 1350 m at N 06° 55'; E 80° 41'). All soils samples were collected at 0 to 15 cm depth and were air dried and sieved to < 2mm before being shipped from the place of origin.

The pH of the soil samples were measured in a 1:10 soil:water extract (Watson and Brown, 1998); Mehlich-3 P as described in Frank et al. (1998); cation exchange capacity (CEC) determined using a summation method of Chapman (1965); and total P, Al and Fe determined according to Zarcinas et al. (1996) modified to use a digestion block instead of microwave. Total Fe oxide (Fe_{CBD}) and citrate-bicarbonate-dithionite extractable Al (Al_{CBD}) were determined using the citrate-bicarbonate-dithionite (CBD) method of Loeppert and Inskeep (1996). Amorphous Fe and ammonium oxalate extractable Al were determined using the acid ammonium oxalate method of Loeppert and Inskeep (1996). This method is used to estimate an active Fe concentration in soils. Total organic carbon (TOC) determined using dry combustion method of Nelson et al. (1996) using soil ground and sieved to ≤150 μm and treated with 1N phosphoric acid to remove inorganic C. Direct combustion of soils was completed using a Carlo Erba C/N analyzer (Carlo Erba instruments, Milan, Italy).

Particle size analysis was determined using a combination of a modification of the pipette method by Kilmer and Alexander (1949) and method 3A-1 from the Soil Survey Laboratory Methods Manual (Soil Survey Laboratory Staff, 2004). Clay mineralogy was completed using K-25 and Mg-25 combinations (Harris and White, 2008). The X-ray Diffraction (XRD) analyses were performed on a PANalytical Empyrean Multi-Purpose X-Ray Diffractometer (Spectris Company, Surrey, United Kingdom) using a copper anode material and generator settings of 35 eV and 20 mA.

The maximum water holding capacity (MWHC) determination was made using the protocol from Jenkinson and Powlson (1976). Each soil was pre-moistened with 10% of the total distilled water needed for 60% MWHC. Soil was packed into petri dishes (87x11 mm) at a bulk density of 1.2 g cm^{-3} for the Oxisol, 1.04 g cm^{-3} for the Andisol, and 0.97 g cm^{-3} for the Ultisol. Preliminary work indicated that these bulk densities were ideal for completely filling the volume of the petri dish without unnecessary compression. There were seven treatments with five replications. Two additional sets of petri dishes were prepared for scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS) analysis. The remaining distilled water was added to bring the soil to 60% MWHC by carefully dripping the water on the soil packed in each plate. The plates were sealed using Parafilm M (Bemis Flexible Packaging, Neenah, WI), wrapped in aluminum foil and left to equilibrate over night at room temperature. The plates were unwrapped and the treatments introduced by placing the fertilizer just below the soil surface in the center of the dish and covering with soil as follows:

- (1) Unfertilized control soil sample
- (2) Monoammonium phosphate (MAP) granular (11-52-0; 11% N - 52% P_2O_5 - 0% K_2O by weight). A $43 \pm 0.05 \text{ mg}$, ($\sim 9.76 \text{ mg P}$) standard grade MAP granule

(3) Diammonium phosphate (DAP) granular (18-46-0; 18% N - 46% P₂O₅ - 0% K₂O by weight).

A 49 ± 0.05 mg, standard grade DAP granule

(4) Ammonium polyphosphate (APP) liquid (11-37-0; 11% N - 37% P₂O₅ - 0% K₂O by weight).

A 60.5 mg (49 µL), standard grade APP liquid was placed using an Eppendorf Reference 100 µL pipette (Eppendorf, Malaysia)

(5) MAP+FEP granular (11-52-0): A 43 ± 0.05 mg, standard grade MAP+FEP granule

(6) DAP+FEP granular (18-46-0): A 49 ± 0.05 mg standard grade DAP+FEP granule

(7) APP+FEP liquid (11-37-0): A 60.5 mg (49 µL), standard grade APP liquid was placed using an Eppendorf Reference 100 µL pipette (Eppendorf, Malaysia)

The FEP used in this research was a maleic-itaconic copolymer chain (C₉H₃O₈X)_n (X= cation) with a cation exchange capacity of approximately 1800 meq 100 g⁻¹ (Servi-Tech, 2009). Typical formulations of the pure compound are 400 g kg⁻¹ as a mixture with water. Granular fertilizers are infused with FEP at 2.5 g kg⁻¹ weight while the liquid fertilizer received 5 mL FEP L⁻¹. Its proposed mode of action is that the compound sequesters multivalent cations in the soil that would otherwise complex with orthophosphate diffusing from the fertilizer granule into the soil. Degryse et al. (2013) performed a potentiometric titration as part of the characterization of the FEP. The FEP had a zero negative charge at approximately pH 3 suggesting the point of zero charge of the material is ≤ pH 3.0.

All fertilizer treatments received equivalent amounts of P, approximately 9.8 mg P per petri plate. Nitrogen was balanced against DAP using finely ground commercial grade urea (46-0-0): MAP and MAP + FEP treatments, APP and APP + FEP treatments.

After all treatment preparations were complete, Petri dishes were sealed with Parafilm and wrapped in aluminum foil. Soils were incubated (Precision Low Temp Incubator, Waltham,

MA) in the dark at 25° C for 5 weeks. This particular incubation time was chosen because at five weeks root development would be limited and this is considered one of the critical P uptake periods for cereal crops (Hettiarachchi et al., 2010; Williams, 1948).

At the end of the 5-week incubation period, the plates were opened, and the soil was collected in four concentric rings: 0-7.5 mm, 7.5-13.75 mm, 13.75-25 mm, 25-43.5 mm, using metal cylinders that were pushed gently into the soil to the bottom of the plate, starting with the smallest cylinder in the center and carefully collecting all soil from the circular section (Lombi et al., 2004). Each concentric soil sample was placed in a separate plastic specimen container (Fisher Scientific, Waltham, MA) with the soil weight recorded. The sample was then fast dried immediately at 40° C (Fisher Scientific drying oven, Waltham, MA). After drying, the weight of the soil was recorded, and a lid was placed and closed on the specimen cup to reduce the chance of contamination. Two of the seven replicates from the granular treatments were utilized for scanning electron microscopy coupled with energy dispersive x-ray spectroscopy analysis. For these, the fertilizer granules were carefully removed using a small metal spatula and tweezers, placed in separate labeled containers, dried at 40° C. The samples were sealed and stored in the refrigerator until SEM-EDS analysis.

Aqua regia (1:3 volume to volume ratio of HNO₃:HCl) soil digestion was performed for total P analysis using a 0.25 g soil subsample from all 0-7.5 mm treatments and a 0.5 g subsample from the remaining three sections for all treatments. Each soil sample was placed in a separate 75 ml glass digestion tube containing 5 mL of aqua regia mixture and placed on a Foss Digester Unit (Fisher Scientific, Waltham, MA), slowly increasing the temperature from 75° C to 140° C until the acid mixture had evaporated to approximately 1.0 ml. The digested samples were cooled, diluted with 25 mL of 0.1% HNO₃ and filtered through a #42 Whatman filter paper.

Each filtered sample was analyzed for total P using inductively coupled plasma emission spectrometry (ICP-OES, Varian 720-ES, Santa Clara, CA). Since slight variations exist in the amount of P applied to each petri dish due to the variability in fertilizer granule composition, total P data were normalized by calculating percent P added (PPA) for each dish section for all treatments (Hettiarachchi et al., 2010) is defined as follows:

$$PPA = [(P_f)S_i \times M_i] / \sum_{i=1-4} [(P_f) S_i \times M_i] \times 100$$

Where i = dish section (1-4), $(P_f)S_i$ = the concentration of P fertilizer in each dish section, and M_i = the mass of soil in each dish section. $(P_f)S_i$ is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section. Total P concentrations are shown in Fig. 3.1, 3.2, and 3.3.

A 0.5 g soil subsample from all 0-7.5 mm treatments and 1.0 g subsample from the remaining three sections for all treatments were extracted for potential plant available P (labile P) using one 50 x 25 mm resin exchange strip (VWR International Ltd., Radnor, PA) for the 0-7.5 mm section and two strips for each one g sub-sample as described by Myers et al. (2005). The resulting filtered extract was analyzed according to the Murphy & Riley Method (Murphy and Riley, 1962) using a UV spectrophotometer (Beckman-Coulter, DU-800, Brea, CA). Since variations exist in the amount of P in each fertilizer granule and in an effort to minimize the effects due to granule variability that could be expected and difficult to control between P treatments, resin extractable P data were normalized by calculating percent resin P (PRP) for each dish section for all treatments as follows:

$$PRP_i = (\text{resin extractable } P_i / \text{total } P_i) \times 100$$

Where i = dish section (1-4), resin extractable P_i = the resin P concentration, and total P_i = total P concentration. Resin extractable P concentrations are shown in Fig. 3.4, 3.5, and 3.6.

To look for morphological changes within the granule, SEM analysis was performed on the original and P fertilizer granules incubated five weeks in the soils. After carefully removing residual P granule from the petri dish, the attached soil particles were carefully removed using a soft paint brush and tweezers. All fertilizer granules used for SEM- EDS were carefully fractured using a stainless steel blade, and mounted on an Al pin stub. Analysis of incubated fertilizer granules from the Andisol and Ultisol samples was performed using an FEI Nova Nano-SEM 430 with helix detector (Hillsboro, Oregon) with high resolution and low-vacuum capability. To enhance SEM image quality at low kV, a Schottky emitter which allows stable emission and high current modes, the immersion lens optimizes the collection of secondary electrons. These granules were not sputter coated as this instrument has a low vacuum capability. Analysis of the incubated granules from the Oxisol sample was performed on a Hitachi S-3500N SEM equipped with a model *S-6542* absorbed electron detector (Hitachi Science Systems, Japan) with an accelerating potential of 5 kV using an X-ray Detector-Link Pentafet 7021 (Oxford Instruments Microanalysis Limited, Bucks, England). To minimize static electrical surface charging these granules were sputter-coated with a 40% palladium (Pd) conductive coating four nm in thickness and allowed to dry before imaging.

The remaining P, Al, Fe and Ca in the incubated MAP and DAP granules were determined using another set of parallel incubation studies for the Andisol and Ultisol. The analysis was not performed for the Oxisol because of lack of sufficient soil sample. The

granules were digested in aqua regia and analyzed for total P, Al, Fe and Ca to access the mass of each element remaining in the granule after incubation.

X-ray absorption near edge structure analysis was performed on a composite sample of the 0-7.5 mm sections of the five replicates used for chemical analysis. The P K-edge XANES data were collected at two different synchrotron facilities. Oxisol and Andisol data collections were performed at Sector 9-BM-B, Advanced Photon Source (APS), Argonne National Laboratory, IL. The storage ring at the facility operates at 7 GeV with a maximum current of 100 mA. The energy range at Sector 9 is 2.1 to 23 keV. The beam line provides a focused x-ray beam for XANES as well as x-ray absorption fine structure (XAFS) experiments in an area of 500 μ m x 500 μ m. All spectra including the P pentoxide (P₂O₅) standard used for daily monochromator alignment were collected in fluorescence mode using a solid-state drift detector. The soils were ground to < 150 μ m using an agate mortar and pestle, and then a 4-mm size soil pellet was prepared from the soil using the KBr Quick Press Kit (International Crystal Laboratories, Garfield, NJ) and carefully glued onto a 33-mm Teflon disk using double-sided carbon tape (SPI Supplies, West Chester, PA) before placing on an Al sample holder for analysis (Fig. 3.7).

Six scans were collected per sample at a range 2.1 to 2.4 keV and 12 scans were collected for each control treatment soil using a three-step scan procedure. Due to beamline upgrades, sector 9 BMB at APS was not available so the Ultisol XANES data collection was performed at Canadian Light Source Inc., Soft X-ray Microcharacterization Beamline (SXRMB) 06B1-1, Saskatoon, Saskatchewan, Canada. The third generation storage ring at CLS operates at 2.9 GeV. The energy range at Sector 06B1-1 is 1.7 to 10 keV and the beam line provides a focused x-ray beam for XANES to 300 μ m x 300 μ m. All spectra including the phosphorus standard used for

the InSb (III) monochromatic alignment were collected in florescence mode. The composite replications from the 0-7.5 mm section for each treatment were used in the spectral collection. All soils were ground to < 150 mm using an agate mortar and pestle and evenly sprinkled onto double-sided carbon tape placed on a stainless steel sample holder in a vacuum chamber for analysis. Multiple scans were collected per sample at a range 2.1 to 2.2 keV, and 6 scans were collected for the control treatment soil. All data collected were analyzed using IFEFFIT-Athena software (Ravel and Newville, 2005). The scans of collected spectra were averaged for each treatment. The edge energy was calibrated, the fitting the pre-edge was subtracted by a linear function, the spectrum was normalized to a second-order polynomial.

All P standards were either prepared in the laboratory or purchased, and the pureness of each prepared standard was confirmed by using XRD. The following P standards were synthesized by Khatiwada et al. (2012): variscite ($\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$), brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), strengite-1d, strengite-3d ($\text{FePO}_4 \cdot 2\text{H}_2\text{O}$), PO_4 -goethite, PO_4 -alumina, and PO_4 -gibbsite. Apatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and vivianite $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$, University of Adelaide. Aluminum phosphate and Fe III-P spectra were obtained from K. Scheckel, EPA, Cincinnati, Ohio. The PO_4 -adsorbed to Al-saturated montmorillonite spectra were obtained from J. Prietzel, University of Technology, Munchen, Germany. The PO_4 -adsorbed kaolinite spectrum was obtained from D. Hesterberg, North Carolina State University. P spectra were collected at Synchrotron Light Research Institute, Nakhon Ratchasima, Thailand, beam line scientist Wantana Kllysuburn.

Spectra for the aligned standards were used to reconstruct the spectra of the unknown samples using a linear combination fitting (LCF) procedure that allowed for the determination of the relative proportion of the P solids in our samples. The Manceau Combo Method (Manceau et al., 2012) was also used to validate our data fitting procedure. The results closely matched the

LCF results for the three acidic soils, providing an added confidence on the P solid phases identified by LCF.

Statistical Analysis

Soils data were statistically analyzed using the PROC MIXED Procedure in SAS (SAS 9.3, 2011). The experimental design was a complete randomized design (CRD). Data were analyzed using ANOVA with P fertilizer treatment as the main treatment, and dish sections as subplot treatments. The Tukey Pairwise Method was used for a comparison of all treatments at a 0.05 level of significance.

Results and Discussion

Select Chemical and Physical Properties of Soils

Properties and chemical characteristics of the soils are provided in Table 3.1. Initial soil pH ranged from 3.9 in the Oxisol to 5.9 in the Andisol. Total Fe oxide (Fe_{CBD}) and citrate-bicarbonate-dithionite extractable Al (Al_{CBD}) varied widely across the soils while amorphous Fe (Fe_{OX}) and ammonium-oxalate extractable Al (Al_{OX}) were more consistent. Mehlich-3 P concentrations for the Oxisol and Andisol were low and suggest that P fertilization would be needed for crop production whereas the Ultisol would be less P limited. Total organic C levels were as high as 6.0%. In addition, the Ultisol contained a greater amount of amorphous Fe. Kaolinite was present in all soils.

Soil pH

The influence of P treatments on soil pH was different for each soil (Table 3.2). The soil pH after incubation was a result of the net effect of a variety of factors. The initial pH of the P fertilizer itself or a saturated solution of the fertilizer can range widely and there is an initial increase in pH produced by urea hydrolysis, both of which can have a residual effect.

Nitrification causes acidification and pH may be lower wherever ammoniacal forms of N diffuse. The release of hydroxide groups from the adsorption of P via inner-sphere/chemisorption mechanisms will increase soil pH. Lastly, the final pH may be the result of increases or decreases in pH due to precipitation reactions, and the pH buffering capacity of the soil. Further studies would be needed to clarify the specific processes or mechanisms that play a role in determining the final pH. For example, the following reactions demonstrate how adsorption or precipitation reactions can influence pH:

Adsorption reaction forming monodendate complex as shown in equation 3.1 (McBride, 1994):



S = Fe, Al

A⁽ⁿ⁻¹⁾ = anion of charge -n

OH⁻ = reactive metal hydroxyl group

Precipitation reaction for amorphous variscite (Eq. 3.2) (Veith and Sposito, 1977):



For the Oxisol, which had an initial pH of 3.9, pH was significantly higher after P addition regardless of P source, as compared to the control soil in the first two petri dish sections. APP and DAP + FEP significantly increased pH in the third section with no difference in the 25-43.5 mm section as compared to the unfertilized control soil (Table 3.2). For all P treatments, the increase in soil pH at the point of application was likely due to the chemisorption of dissolved P onto Fe and Al oxides with the resulting release of hydroxide ions, increasing soil pH (Stoop, 1983). The soil pH decreased with increasing distance from the center section, and all P treatments had relatively the same pH as the unfertilized control in the last (25-43.5 mm) section. Nelson The effect on soil pH diminished with distance from the point of application as P diffused outward and concentration decreased.

In contrast, for the Andisol, which had a pH of 5.6 initially (Table 3.2), both the APP (pH 6.0) and APP + FEP (pH 6.1) treatments had significantly higher pH than the control while all granular P treatments except MAP had significantly lower pH than the unfertilized control treatment (pH 5.6) in the 0-7.5 mm section (Table 3.2). There are two processes that could influence the soil pH; one would be an increase in pH from the release of hydroxide groups via chemisorption and the second would be a decrease in pH from the nitrification of ammonium-N that remained or moved into another section. The increased pH after incubation near the point of application for APP and APP+FEP suggests the diffusion of dissolved ammonium-N and nitrification occurred further from the point of application as compared to the granular P treatments. For the granular P fertilizers, the pH was significantly lower than the control except for MAP in the 0-7.5 mm section. The decrease in pH for the granular treatments is the result of the combination of the precipitation of P as well as nitrification of ammonium-N within the 0-7.5 mm section. The greater net acidification suggests much less ammonium diffusion away the point of application as compared to the APP treatments. We expect less diffusion of ammonium-N and P away from the granule compared to the liquid treatments because of the hygroscopic nature of the granules and the amount of water that moves toward the point of application as a result (Hettiarachchi et al., 2006; Lombi et al., 2005). For the P fluid fertilizer treatments, the significant drop in pH in the 7.5-13.75 mm and 13.75-25 mm sections as compared to the unfertilized control treatment suggests acidification from nitrification but to a lesser extent as compared to the granular treatments.

For the Ultisol, which had an initial pH of 4.5, the 0-7.5 mm pH values for all P treatments were significantly higher as compared to the unfertilized control treatment (Table 3.2). The increased pH near the point of application suggests chemisorption of P was greater

resulting in the increase in soil pH. (Sanchez et al., 1980; Stoop, 1983). All P treatments decreased in pH with increasing distance from the 0-7.5 mm section and only APP was significant higher in pH compared to all other treatments in the 25-43.5 mm section.

Percent P Added

In the Oxisol, much of the PPA was found within the 0-7.5 mm and 7.5-13.75 mm sections for all treatments (Figure 3.8; Figure 3.1). For the four granular P treatments, much of the PPA was restricted to the 0-7.5 mm section. The FEP treatment did not influence the diffusion of fertilizer P for DAP but was significant for MAP and APP treatments. Overall, there was significantly lower PPA in the 0-7.5 mm section for both fluid P applications compared to MAP, DAP and MAP + FEP treatments. In a similar experiment, Montalvo et al. (2014) found that after 35 days incubation in an Oxisol the percentage of added P remaining in the center dish sections were greater for granular P treatments than for the fluid P treatments. For the Oxisol, the PPA values suggest the greatest diffusion of added P into the 7.5-13.75 mm section was for the two P fluid treatments with the APP + FEP treatment having significantly greater PPA than DAP and MAP + FEP. Further, the APP treatment had significantly greater PPA in the 13.75-25 mm section as compared to all other P treatments. However, PPA in the 7.5-13.75 mm section for the APP treatment was not significantly higher than the APP + FEP treatment, this is most likely due to further movement of P into the 13.75 mm section. In general, for the granular P treatments, movement of soil water toward the hygroscopic P granule may have contributed to reduce diffusion of P from the granules compared to the P fluid applications where there was less restriction of mobility. Overall, the clay soil texture of the Oxisol likely contributed to reduced P movement from the center section. Lastly, sorption reactions with Fe and Al oxides contributed

overall to reduced P mobility beyond the 0-7.5 mm and 7.5-13.75 mm sections for all P fertilizer treatments due to sorption reactions.

For the Andisol, greater PPA values suggest greater P mobility into the 7.5-13.75 mm and 13.75-25 mm sections compared to the other two soils (Figure 3.9). Significantly greater PPA values were found in the 0-7.5 mm section for the four granular treatments and APP than with APP + FEP. All treatments except DAP + FEP had significantly greater PPA in the 7.5-13.75 mm section. The PPA values were low in the third section for all granular P treatments except DAP + FEP. Both liquid P treatments had significantly greater values in the 13.75-25 mm section. The diffusion of the P fertilizers is the result of several factors. For APP and APP + FEP higher pH compared to the granular treatments would decrease P adsorption. This combined with less hygroscopic flow of water toward the liquid P fertilizer would promote P movement away from the 0-7.5 mm and 7.5-13.75 mm sections as compared to the granular fertilizer. The soil pH was more acidic for all P granular treatments as compared to the fluid P treatments which would act to reduce P solubility in the 0-7.5 mm section. As described previously, the change in pH is the net effect of several processes but the net effect would still affect P solubility. Despite that, there was still much P diffusion into the 7.5-13.75 mm section for all granular treatments. The sandy loam texture possibly allowed for a general increase in P mobility in the Andisol as compared to the two other soils in the study.

Movement of fertilizer P in the Ultisol in general followed the order $DAP < MAP < APP$. Approximately 89-91% of the added P remained in the center section of the DAP treated soils, while 79-80.5% of added P remained in the center section of the MAP treated soils (Fig. 3.10). The APP + FEP and APP treatments had significantly lower PPA at 0-7.5 mm than did the other P treatments at 0-7.5 mm although the two liquid P treatments were not significantly different

from each other. Although phosphorus diffused into the 7.5-13.75 mm section for all P treatments, the PPA was significantly greater for both fluid P treatments with APP + FEP being significantly greater than APP. Overall, diffusion of P into the 13.75-25 mm section was minimal for most treatments except APP with a PPA of 3.8%, which was significantly greater than all other treatments. There could be combinations of factors reducing P mobility for the granular treatments in the Ultisol. Similar to the Oxisol in this study, the clay soil texture of the Ultisol helped to decrease P diffusion. The TOC of the Ultisol is 6%, which is roughly two times that of the Oxisol at 2.6% or Andisol at 3.1% (Table 3.1). The increased TOC likely absorbed more water, greatly decreasing hygroscopic mass flow and P diffusion from the application point as compared to the other soils. Similar to the Oxisol, the Ultisol contained high Fe and Al oxides that likely decreased P mobility through sorption reactions. Both soil mineralogy and texture play an important role determining overall P diffusion in addition to the P source effect, granular versus liquid.

Analysis of incubated P Fertilizer Granules

The percentages of mass of Fe, Al, and Ca remaining in the residue of incubated MAP and DAP granules for both the Andisol and Ultisol were similar and were 90% or greater (Table 3.3). These may have been insoluble Fe, Al, and Ca impurities from the original granules that did not dissolve upon incubation, and it is unlikely Fe and Al migrated inward from the soil during the dissolution of the P fertilizer granules since the solubility and mobility of Fe and Al in soil is quite low in general. However, it is possible that Ca is present as an initial impurity and that some Ca migrated into the P granule from the soil and formed a new solid with P. The summed molar amount of Fe and Al (0.025-0.030 mmol) remaining in the MAP and DAP granules from the Andisol and Ultisol correspond with the molar quantity of P (0.025-0.030 mmol) remaining

in the incubated granules. The P may be associated with insoluble Fe and Al compounds present in the fertilizer prior to incubation. Gilkes and Mangano (1983) found between 4.9-9.9% of the total P content of MAP and DAP fertilizers were present as compounds that were not water soluble and were complexed with Ca, Mg, Al and Fe. Similarly, Montalvo et al. (2014) summed up the molar amounts of Fe and Al remaining within various incubated P fertilizer granules, including MAP and DAP, in several acid soils and found the total molarity approximately matched the molarity of P remaining in the incubated granules. This indicates that the percentage P remaining in both P fertilizer sources incubated in the Andisol or Ultisol are likely associated with Fe and Al forms and are insoluble. Evidence of movement of Ca toward incubated granules was also found.

Energy dispersive X-ray spectroscopy dot maps of cross-sectioned view of DAP granules and a MAP granule incubated five weeks in the Andisol reveal the distribution of Al, P, Fe and Al inside the granule as per the different colors in the EDS formation (Fig. 3.11). Phosphorus appears to be evenly distributed throughout each incubated granule. The evidence lends additional support to the lack of P diffusion from the point of application for P granular fertilizer. Secondary electron micrographs of cross-sectioned view of nonincubated and incubated MAP and DAP granules in the Oxisol shows possible reaction products formed within the partially dissolved incubated P granule. For the Oxisol, inward movement of Ca was minimal, the precipitated P most likely with Fe and/or Al-P (Fig. 3.12).

Percent Resin P

In general, for all three acidic soils the greatest values of PRP were found within the 0-7.5 mm and 7.5-13.75 mm sections and decreased sharply in the 13.75-25 mm and 25-43.5 mm sections and all PRP values were quite similar and all treatments receiving P were significantly

greater than the unfertilized control treatment (Figs. 3.13, 3.14 and 3.15). For the Oxisol, there were no significant differences in PRP for all treatments in the 0-7.5 mm section (Fig. 3.13). The PPA values suggest greater movement of P out of the 0-7.5 mm section for the APP and APP + FEP treatments; however, the P remaining in the 0-7.5 mm section had increased PRP. There were significantly greater PRP values in the 7.5-13.75 mm section for APP, MAP + FEP, and APP + FEP compared to all other P treatments. Overall, based on PPA and PRP, all treatments receiving P behaved in a similar fashion which is most likely related to the minimal pH differences between treatments within dish sections.

In contrast to the Oxisol, there was significantly greater PRP in the Andisol for both the APP and APP + FEP treatments in comparison to the four P granular treatments in the 0-7.5 mm section (Fig. 3.14). Greater pH in the 0-7.5 mm section for both P fluid treatments are the result of chemisorption reactions, which enhanced P diffusion from this section with the portion of the remaining P being more extractable due to increased P solubility in comparison to the granular treatments (Table 3.2). There were no significant differences between P treatments in the 7.5-13.75 mm section; however, the two P fluid treatments were significantly greater in PRP in the 13.75-25 mm section compared to all other treatments. Decreased lability of the P fertilizers at 0-7.5 mm and limited diffusion into the 7.5-13.75 mm sections in the Andisol as compared to the Oxisol could be due to surface sorption and precipitation reactions with Fe and Al reducing the overall PRP for all P treatments (Sample et al., 1980).

As observed for the Oxisol and Andisol, the greatest resin P for the Ultisol was observed in the 0-7.5 mm section (Fig. 3.15). The MAP + FEP treatment had significantly greater PRP in the 0-7.5 mm section as compared to DAP, APP and APP + FEP. The decrease in PRP for the P fluid fertilizer treatments in the 0-7.5 mm section was related to increased adsorption reactions

due to increased opportunities for soil contact with both P fluid fertilizer treatments (Montalvo et al., 2014). It is also possible that the rate of hydrolysis for pyrophosphate was slow due to high exchangeable Al and a high clay content (Dick and Tabatabai, 1987); however, whether polyphosphate would remain after 5-weeks incubation is unknown. All PRP values were considerably less in the 7.5-13.75 mm section for the P treatments as compared to the values in the 0-7.5 mm section. The Ultisol had the greatest Fe_{CBD} , Al_{CBD} and higher Fe_{OX} and Al_{OX} of the three acidic soils in the study, and the sorptive capacity of this soil may have greatly decreased PRP through adsorption reactions given the limited amount of P that moved into the 7.5-13.75 mm section. This soil also had a TOC of 6.0% (Table 3.1). Ternary complexes with Fe and Al can act as a bridge between the negatively charged functional groups of the organic matter and the phosphate ions, greatly decreasing P lability (Hesterberg, 2010).

In the end, the lower PRP values for the three acidic soils are consistent with other studies that demonstrate P applied to these soils containing varying amounts of Fe and Al oxide materials may be subject to sorption reactions that significantly reduce the overall P extractability (Parfitt et al., 1989; Hedley and McLaughlin, 2005; Kizewski et al., 2011; Sanchez and Uehara, 1980; Sample et al., 1980). In addition, the acidic pH of the soils may have contributed to the solubilization of Fe and possibly Al, contributing to the precipitation with fertilizer P (Hesterberg, 2010; Hedley and McLaughlin, 2005). Lastly, a combination of increased Fe_{CBD} , Al_{CBD} and higher Fe_{OX} and Al_{OX} , along with ternary complex formation of organic matter and P may have contributed to P fertilizer retention resulting in decreased lability.

X-Ray Absorption Near-Edge Structure Analysis

Overall, for treatments receiving P fertilizer, P speciation was dominated by numerous Fe- and Al- P solids and P adsorbed to Fe and Al oxides. All P K-edge bulk XANES spectra and

linear combination fitting results for the three soils used in the study are shown in Fig. 3.16, 3.17, 3.18, and Table 3.4).

The unfertilized control treatment for the Oxisol contained P as Fe- and Al-adsorbed (Fig. 3.16, Table 3.4). The addition of the P fertilizer resulted in the relative increase in Fe-adsorbed P and Fe-P and a relative decrease in the amount of Al-adsorbed P as compared to the unfertilized control treatment. Both P granular treatments contained more Al-adsorbed or Fe-adsorbed as compared to the APP treatment, which had less Al-adsorbed P but a greater percentage of Fe-P. The increased adsorbed species for both granular treatments may be the reason for increased P added in the center dish section as compared to APP. There was some P movement to the second dish section regardless of P source. However, the increase in Fe-P for the APP treatment most likely is the reason for the lack of greater resin extractability from both the center section and second section.

For all three P sources, the inclusion of FEP resulted in greater percentages of Fe-P and less Fe- and Al-adsorbed P compared to the non-FEP treatments. The high percentage of Fe-P for the FEP treatments suggests the FEP itself may have enhanced the solubility of solid-phase Fe to a small degree via a ligand promoted dissolution and Fe then precipitated with P. This was not reflected in the PRP or PPA results; however, a significant ($P = 0.07$) negative correlation was found between Fe-P versus PRP, suggesting that P present as Fe-P was a form of P with low solubility and extractability. For acidic Oxisols, Fe and Al have a strong capacity to adsorb P and therefore the prevalence of the adsorbed species is not unexpected. In addition, depending on soil pH, there may be increased Fe and Al in solution which can precipitate with P.

The nonfertilized control treatment in the Andisol contained P as Al-adsorbed P and a small percentage of Fe-adsorbed P (Fig. 3.17, Table 3.4). The P fertilizer treatments resulted in a

decrease in Al-adsorbed P and an across the board increase in Fe-associated P. It is clear that both granular treatments had more Al-adsorbed and Al-P compared to the APP. Increased Al-P in the center section of the granular P treatment may be the reason for less P movement into the second section. In addition less PRP observed for the two granular treatments compared to the APP treatment suggests that the Al-P association is negatively correlated to resin extractability.

In comparing the non-FEP fertilizer treatments to the FEP-added treatments, the DAP + FEP treatment contained a greater percentage Fe-adsorbed P compared to DAP but both contained similar percentages of Fe-P. The MAP treatment contained less Al-adsorbed P and a greater percentage Al-P than the MAP + FEP treatment, which had a greater percentage of Fe-adsorbed P. The PRP was greater for MAP + FEP compared to MAP and may have been due to MAP containing more Al-P and less Fe-adsorbed P than MAP + FEP. In comparing the APP to the APP + FEP treatment, both were very similar and contained > 50% Fe-adsorbed P. Overall, there was a positive trend between Fe-adsorbed P and PRP. Both P fluid treatments contained the greatest percentage Fe-adsorbed P as compared to all other P treatments and this was consistent with both P fluid treatments having significantly higher PRP as compared to the other treatments. In addition, across all treatments, there was a significant negative correlation between Al-adsorbed P and PRP ($r^2 = 0.81$ $P \leq 0.05$) in the 0-7.5 mm section; as the percentage of Al-adsorbed P increased in the 0-7.5 mm section PRP significantly decreased.

The role of Al in immobilizing P in soils has been well established. Lindsay and Stephenson (1959) suggested that the formation of variscite as a P reaction product formed in the short term in acid soils with the application of P fertilizer was much more soluble than actual variscite; however, over time this may actually transform into variscite and coexist with other stable solid phases. Wada and Gunjigake (1979) found that it was Al-bound to humus followed

by Fe-bound to humus which controlled P adsorption in Andisolic soils. Molina et al. (1991) found in 10 Andisols from Costa Rica and Panama that P retention increased significantly with the increase of extractable forms of Al rather than Fe oxides.

Of the three soils, the Andisol had the lowest content of free Fe and Al oxides and amorphous Fe and Al indicating less weathering and likely a greater content of alumino-silicates favoring adsorption of P over precipitation as reaction products. In addition, the soil organic matter in the Andisol was higher compared to the Oxisol (TOC of 3.1% versus 2.6%). Soil organic matter can affect inorganic P sorption through ternary complexes formed with Al or Fe acting as a bridge between organic functional groups and the phosphate anion (Bloom, 1981; Gerke, 1993). Aluminum associated with organic matter influences P sorption capacity as well. Overall, P sorption capacity is largely influenced by these soil properties (Wada, 1985).

Phosphorus was generally present more as precipitated than adsorbed species across the treatments in the Ultisol (Fig. 3.18, Table 3.4). The unfertilized control treatment contained more or less equal amounts of Fe and Al-associated P. The introduction of P fertilizer resulted in the relative disappearance of Al-adsorbed P across all treatments. The MAP treatment contained more Al-P and Fe-adsorbed P and less Fe-P than DAP although there was no difference in the amount of P that diffusion from the center section (Fig. 3.10). The APP treatment had less Al and but increased Fe-adsorbed P or Fe-P which likely resulted in greater fertilizer P movement into the second dish section. Comparing the non-FEP fertilizer treatments to the FEP-added treatments, the three FEP-added treatments contained more Fe-adsorbed P and generally less Al-P and Fe-P solid phases than the non-FEP added treatments. The PPA in the center and second dish sections for the non-FEP treatments were similar to the FEP-added treatments. However, PRP for the non-FEP-added granular treatments were lower in the center dish section than for the

FEP treatments although they were not necessarily significantly different; PRP for the APP treatment was significantly lower than for APP + FEP in the center dish section. The overall prevalence of Al-P and Fe-P species in the non-FEP treatments may be related to proton induced dissolution of Fe and Al minerals from low soil pH (Table 3.2). The three non-FEP treatments resulted in a higher amount of the sum of the precipitated Fe-P and Al-P species and less Fe-adsorbed P species as compared to the three FEP treatments. The Ultisol in this study contained high concentrations of Fe_{CBD} and Al_{CBD} and moderate Fe_{OX} and Al_{OX} concentrations. Parfitt (1989) found that in very acidic soils, the types of fast and slow reactions of P sorption involved on various iron oxyhydroxide minerals varied considerably with time. There was less P sorbed onto crystalline minerals but the reaction was fast compared to other minerals in the study with little to no slow reaction with P.

Conclusions

Phosphorus mobility, expressed as PPA, was limited, and P generally did not move beyond the 7.5-13.75 mm section for the granular P treatments in all three soils. However, for the P liquid treatments, there was evidence of greater movement of P as compared to the granular treatments. In general, there was evidence the two liquid P treatments moved into the 13.75-25 mm section for the three soils, and no such evidence for the granular treatments. Overall, there was little indication the FEP increased mobility across the three soils.

The effect of P source on lability, as expressed as PRP, is dependent on the soil. In general for the Oxisol, there is little effect of P source on lability. In the Andisol, the liquid P treatments increased lability in the 0-7.5 mm section compared to the granular sources. In contrast, the opposite was true for the Ultisol, because in the Ultisol there was increased lability with the granular P sources than the P fluid treatments. There was no direct FEP effect for the Oxisol or Andisol. However, in the Ultisol, PRP was significantly higher in the 0-7.5 mm section when comparing all fertilizers with FEP to their equivalent without FEP. It is unknown whether this potential benefit of FEP would be valuable under field conditions.

Speciation results indicate the effect of P source and FEP added to the three soils were highly variable. The unfertilized control treatment for the Oxisol and Andisol did not contain any precipitated Al-P or Fe-P whereas the Ultisol contained both. For all three soils, the addition of P resulted in the presence of Fe-P, Al-P, or both. The only apparent effect of FEP was an increase Fe-P in the Oxisol and an increase Fe-adsorbed P for the Ultisol, for all P sources. There is evidence that speciation may have influenced PPA and PRP for the two P fluid treatments in the Andisol. Nevertheless, the results suggest any benefit of using a fluid P source over a granular source was more dependent on soil type. Overall, P source or FEP effects on mobility, lability

and speciation would need to be evaluated for production agriculture using well-designed field studies for the soil orders described here. The results of this current study help to provide a better understanding of the processes involved in the diffusion and lability, and a mechanistic understanding of P fixation with the addition of three P fertilizers with and without FEP to three different acidic soils.

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Table and Figures

Table 3.1 Selected initial properties of Soils

Properties	Oxisol†	Ultisol	Andisol
pH	3.9	4.4	5.9
Mehlich-3 P (mg kg ⁻¹)	3.6	21.2	4.0
CEC (cmol _c kg ⁻¹)	12.4	24.5	16.5
TOC (%)	2.7	6.0	3.1
Total P (mg kg ⁻¹)	220	960	430
Mineralogy	Kaolinite	Kaolinite	Quartz, Andesine, Kaolinite, weak Hematite
Amorphous Fe _{OX} (mg kg ⁻¹)	2610	5890	2130
Ammonium-oxalate extractable Al Al _{OX} (mg kg ⁻¹)	3620	6450	1140
Fe _{CBD} (mg kg ⁻¹)	46060	45100	6040
Al _{CBD} (mg kg ⁻¹)	5420	15100	4050
Textural class	C	C	SL

†Soil order: United States soil taxonomy classification Fe_{OX}, Al_{OX}: Amorphous Fe concentration, ammonium-oxalate extractable Al concentration; Fe_{CBD}, Al_{CBD}: Citrate-bicarbonate-dithionite extractable Fe or Al concentration; pH: (1:10) in water; TOC: Total organic carbon; Soil texture: C=clay, SL=sandy loam.

Table 3.2 Soil pH after 35-days incubation of three phosphorus fertilizer sources applied to the A horizon of three different soils, an Oxisol, Andisol and Ultisol, alone or in combination with a fertilizer enhancement product at four distances from the point of fertilizer placement.

Treatment †	Distance from the point of fertilizer placement (mm)			
	0-7.5	7.5-13.75	13.75-25	25-43.5
Oxisol				
Control	3.90b‡	3.88c	3.88b	3.88a
MAP	6.10a	5.27ab	4.15ab	3.86a
DAP	6.32a	5.34ab	4.11ab	3.80a
APP	5.96d	5.43ab	4.17a	3.87a
MAP + FEP	5.90a	5.03b	4.04ab	3.82a
DAP + FEP	6.40a	5.39ab	4.16a	3.89a
APP + FEP	6.34a	5.49a	4.04ab	3.87a
Andisol				
Control	5.63c	5.64a	5.62a	5.62a
MAP	5.56cd	4.88c	4.80c	5.12b
DAP	5.41e	4.90c	4.96b	5.12b
APP	5.95b	5.73a	4.97b	4.79c
MAP + FEP	5.45de	4.86c	4.96b	5.14b
DAP + FEP	5.49de	4.82c	5.02b	5.33b
APP + FEP	6.06a	5.52b	5.00b	5.20b
Ultisol				
Control	4.36e	4.35d	4.61cd	4.35bc
MAP	4.44d	4.37cd	4.31de	4.36bc
DAP	4.40cd	4.41bc	4.31de	4.36bc

APP	4.73a	4.61a	4.44a	4.49a
MAP + FEP	4.48c	4.39cd	4.36bc	4.36bc
DAP + FEP	4.53c	4.38cd	4.29e	4.35c
APP + FEP	4.57b	4.47b	4.38b	4.39b

†MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; MAP + FEP = monoammonium phosphate + FEP; DAP + FEP = diammonium phosphate + FEP; APP + FEP = ammonium polyphosphate + FEP.

‡Means within a soil and dish section with the same letter are not significantly different at P = 0.05 using Tukey's honest significance test. Means within a column with the same letter are not significantly different at P=0.05.

Table 3.3 Elemental analysis of MAP and DAP granules incubated in the Andisol and Ultisol. Weight and chemical composition of fertilizer granules after 35 days incubation.

Element	-----MAP†-----			-----DAP-----		
	Nonincubated	Incubated	Fraction remaining‡	Non-incubated	Incubated	Fraction remaining
	-----g kg ⁻¹ -----		%	-----g kg ⁻¹ -----		%
Andisol						
Al	10.3	131.4	92.0	8.5	127.8	92.5
P	200.6	278.2	8.8	195.0	254.7	7.8
Ca	2.3	106.2	329.3	2.5	116.0	290.0
Fe	8.2	116.1	101.6	7.0	103.8	90.5
Ultisol						
Al	10.3	70.6	103.8	8.5	74.3	98.6
P	200.6	135.2	10.2	194.1	129.4	7.5
Ca	2.3	19.8	127.7	2.5	25.1	114.5
Fe	8.2	53.9	98.9	7.0	56.2	89.8

†MAP = monoammonium phosphate; DAP = diammonium phosphate; ‡Based on the mass present in nonincubated granule and mass remaining in the granule residue after incubation.

Table 3.4 Oxisol, Andisol and Ultisol P K-edge XANES on 0-7.5 mm sections after 5-week incubation. The table presents relative proportions of adsorbed and precipitated P solid phase minerals expressed as a percentage. Energy range: 2.14 to 2.18 keV.

Treatment†	Al-P	Al-Adsorbed P	Fe-P	Fe-Adsorbed P	RCS‡
------%-----					
Oxisol					
Control	- §	91.4	-	8.6	0.13
MAP	-	-	27.9	72.1	0.32
DAP	-	47.3	52.7	-	0.04
APP	-	43.6	56.4	-	0.02
MAP + FEP	-	-	75.9	24.1	0.02
DAP + FEP	-	33.7	66.3	-	0.01
APP + FEP	-	21.4	78.6	-	0.01
Andisol					
Control	-	97.5	-	2.5	0.06
MAP	17.1	41.2	-	41.7	0.05
DAP	-	60.0	26.6	13.4	0.04
APP	26.4	-	-	73.6	0.03
MAP + FEP	-	84.1	-	15.9	0.03
DAP + FEP	-	37.6	26.9	35.6	0.02
APP + FEP	49.2	-	-	50.8	0.02
Ultisol					
Control	52.9	-	21.5	25.6	0.007
MAP	38.3	-	28.0	33.7	0.02

DAP	11.6	-	59.0	29.5	0.02
APP	20.8	-	48.3	31.0	0.02
MAP + FEP	-	-	47.2	52.8	0.03
DAP + FEP	28.7	-		71.3	0.02
APP + FEP	17.7	-	27.4	54.9	0.01

†MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product. ‡RCS is reduced chi-square and statistically represents the goodness of fit of the data with the P standards. The RCS is low when the fit is good.

§ Not a relevant solid phase in LCF

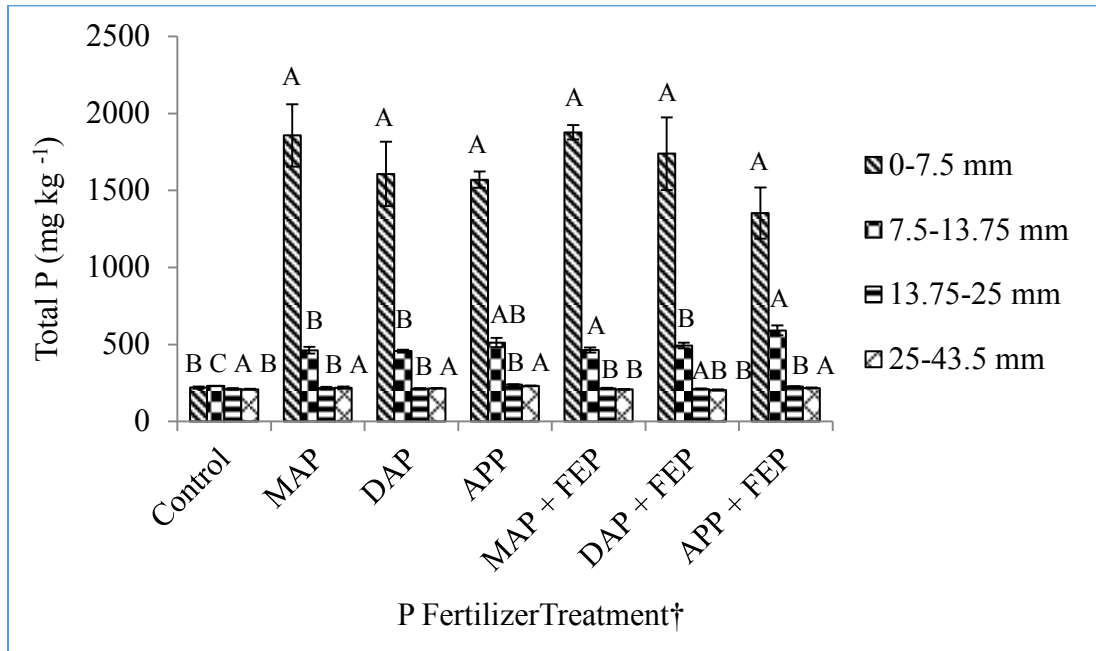


Figure 3.1 Total P concentration in soil sections of Oxisol collected at different distances from the point of fertilizer application. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

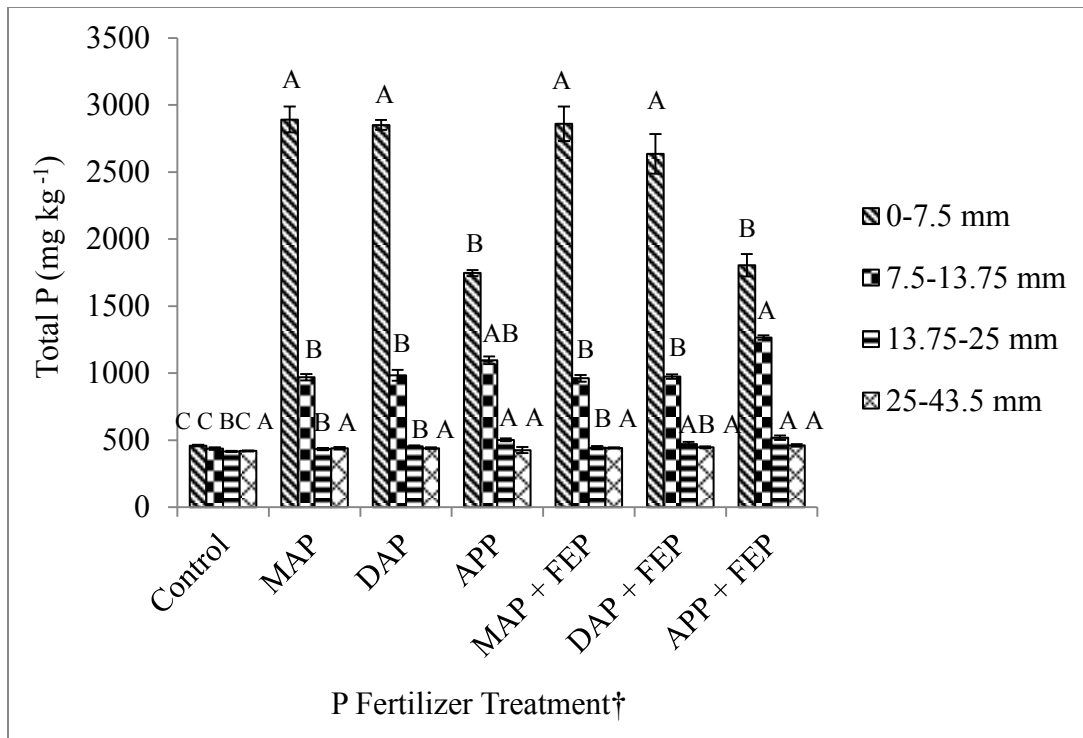


Figure 3.2 Total P concentration in soil sections of Andisol collected at different distances from the point of fertilizer application. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

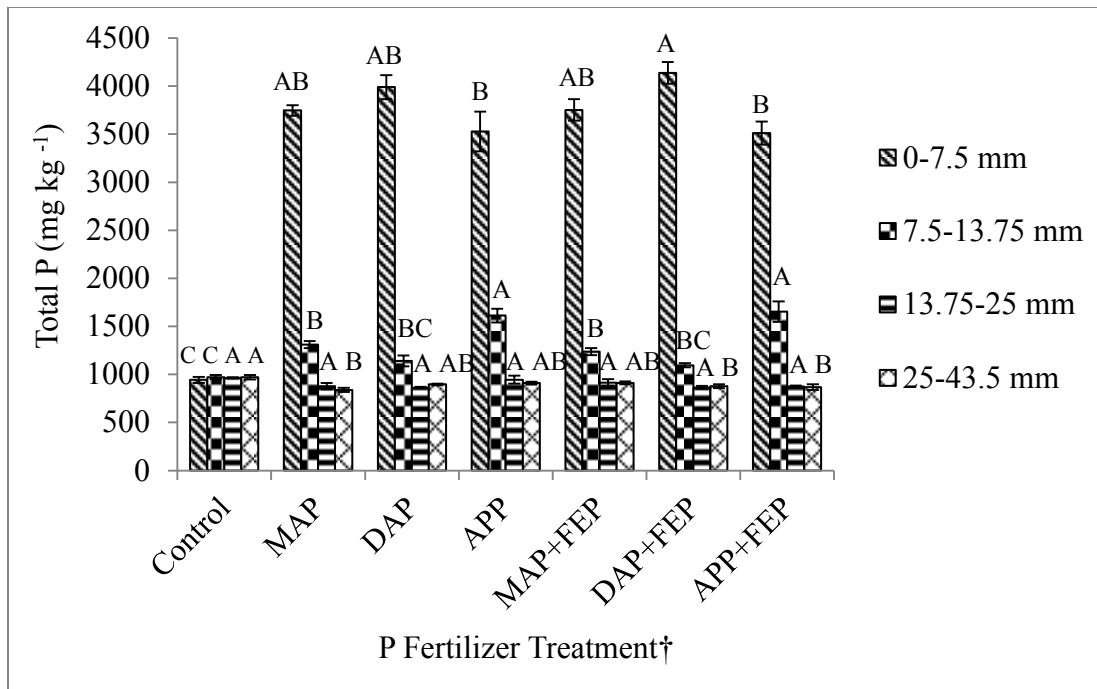


Figure 3.3 Total P concentration in soil sections of Ultisol collected at different distances from the point of fertilizer application. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

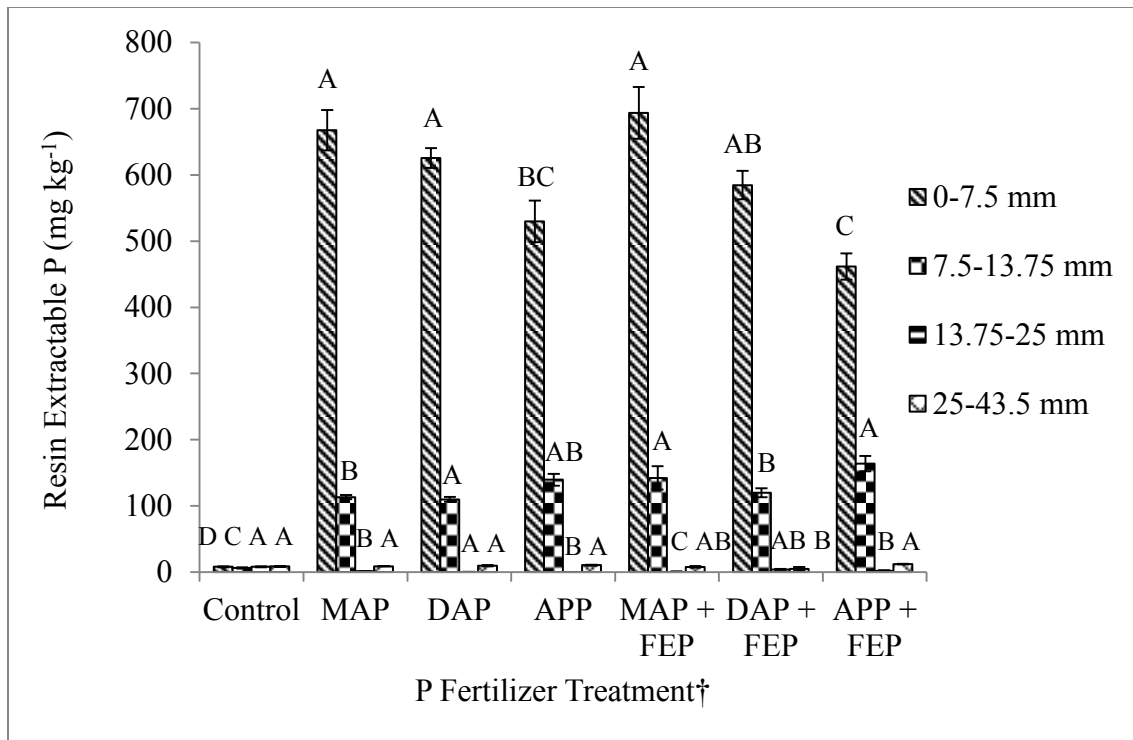


Figure 3.4 Resin extractable P concentration in soil sections of Oxisol collected at different distances from the point of fertilizer application. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

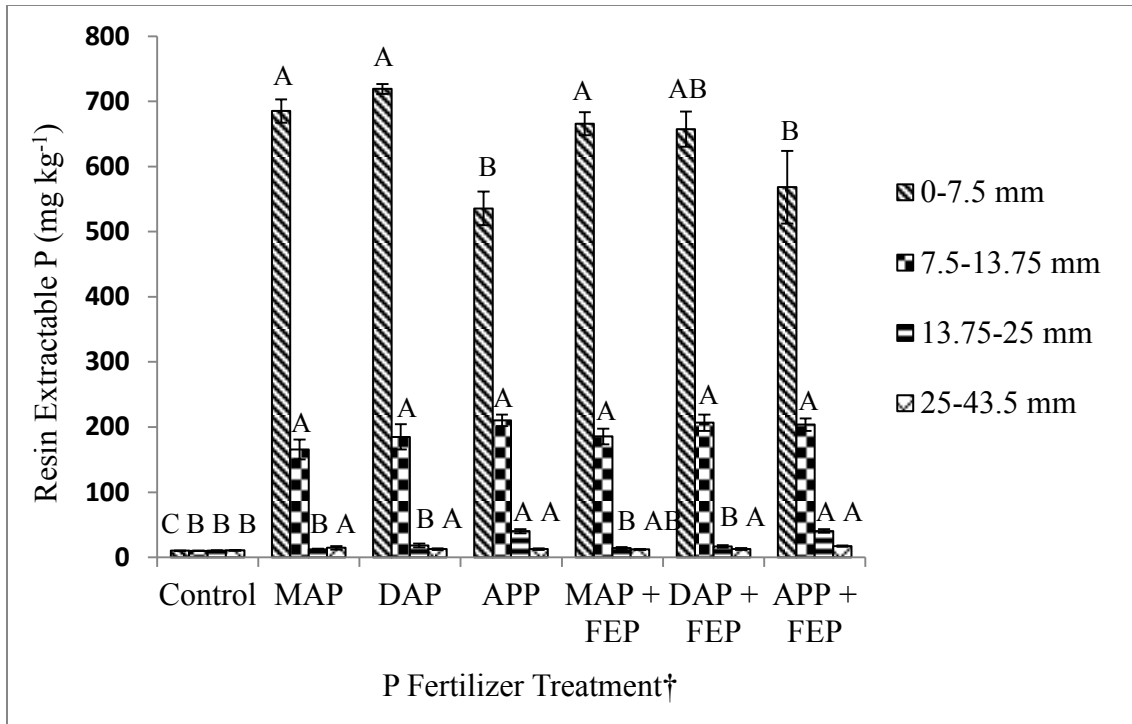


Figure 3.5 Resin extractable P concentration in soil sections of Andisol collected at different distances from the point of fertilizer application. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

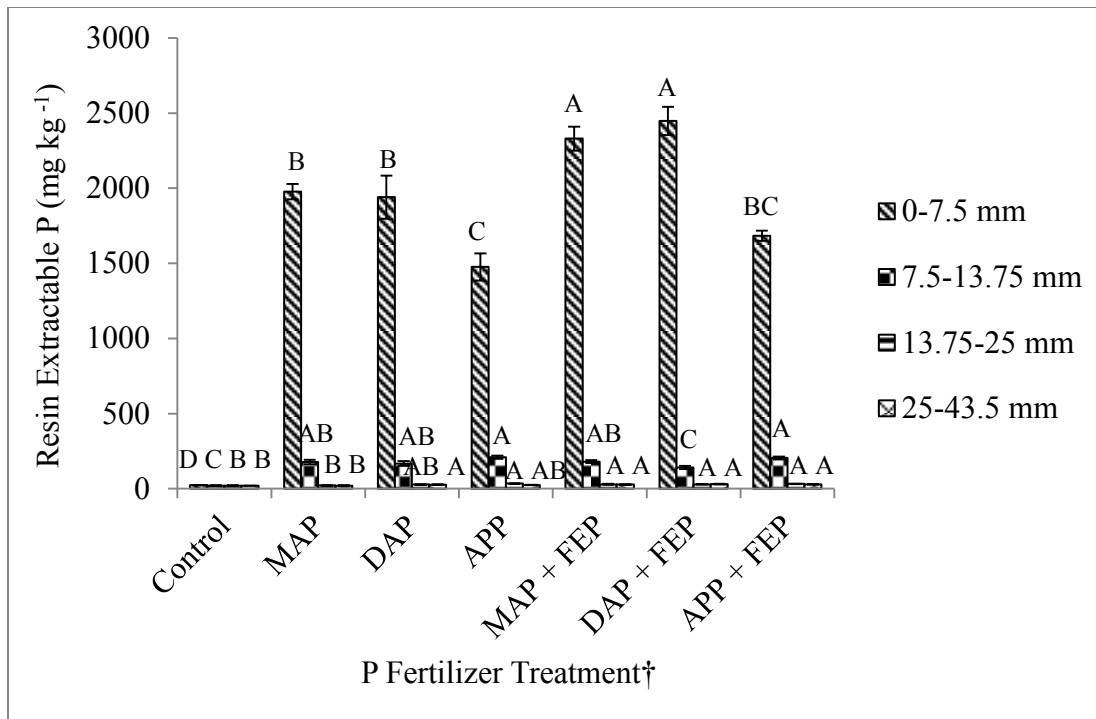


Figure 3.6 Resin extractable P concentration in soil sections of Ultisol collected at different distances from the point of fertilizer application. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

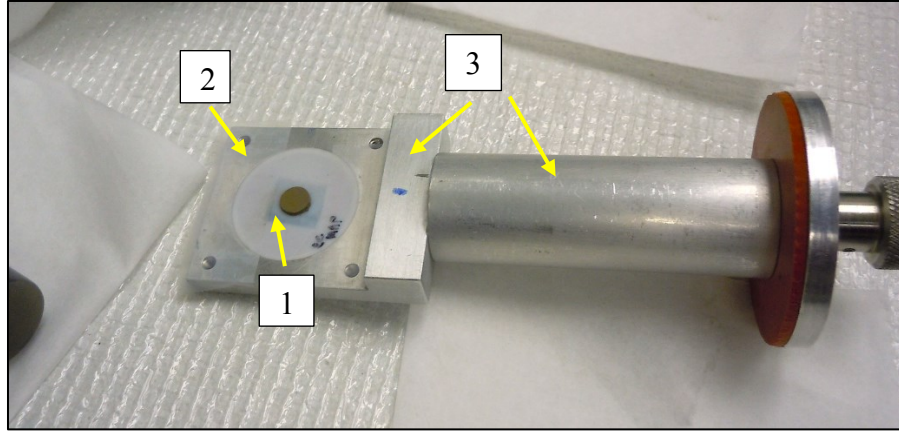


Figure 3.7 Sample preparation for P K-edge XANES data collection.

(1) 4-mm size soil pellet, (2) Teflon disk, (3) aluminum sample holder.

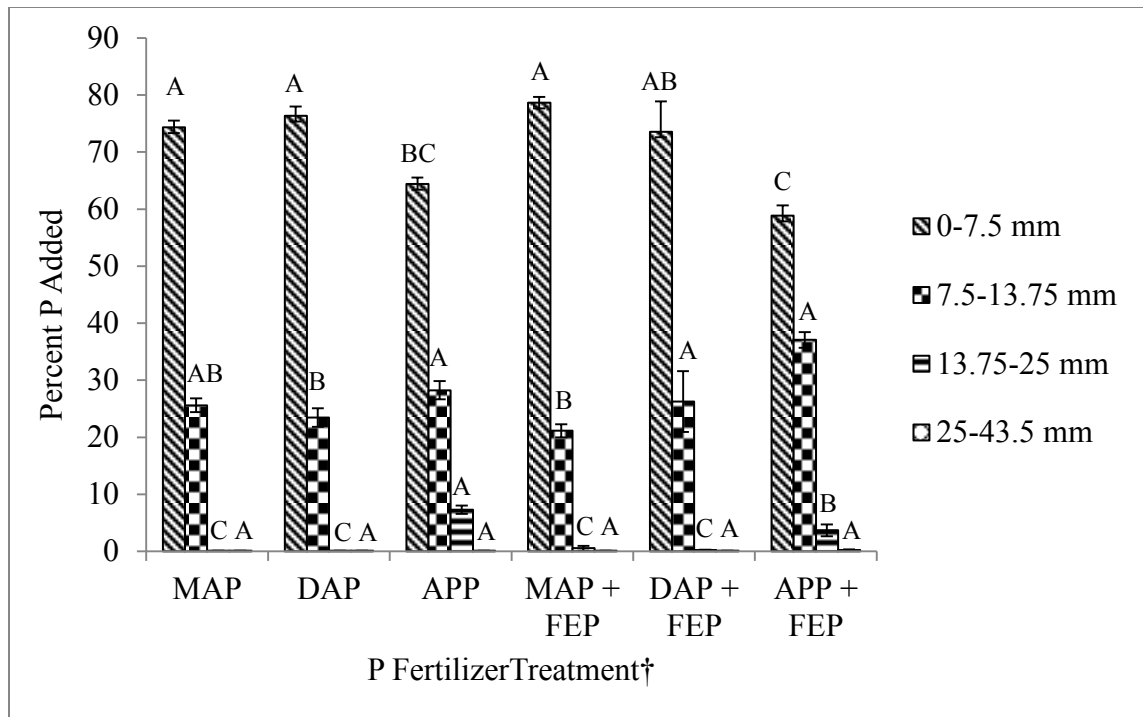


Figure 3.8 Percent P Added (PPA) in the soil sections of Oxisol collected at different distances from the point of fertilizer application. Percent P Added calculated for each dish section for all treatments, where PPA is defined as follows: $PPA = \left[\frac{(P_f)S_i \times M_i}{\sum_{i=1-4} [(P_f) S_i \times M_i]} \right] \times 100$. Where $i =$ dish section (1-4), $(P_f)S_i =$ the concentration of P fertilizer in each dish section, and $M_i =$ the mass of soil in each dish section. $[(P_f)S_i$ is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer

enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

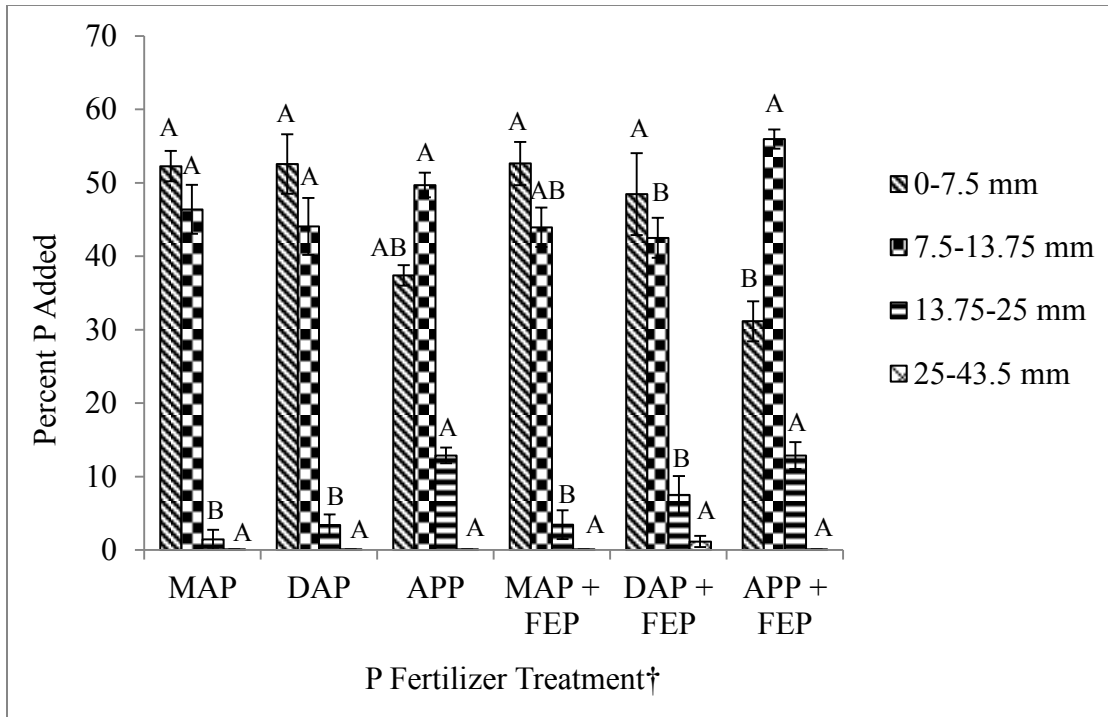


Figure 3.9 Percent P Added (PPA) in the soil sections of Andisol collected at different distances from the point of fertilizer application. Percent P Added calculated for each dish section for all treatments, where PPA is defined as follows: $PPA = \frac{[(P_f)S_i \times M_i]}{\sum_{i=1-4} [(P_f) S_i \times M_i]} \times 100$. Where $i =$ dish section (1-4), $(P_f)S_i =$ the concentration of P fertilizer in each dish section, and $M_i =$ the mass of soil in each dish section. $(P_f)S_i$ is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer

**enhancement product; APP + FEP = ammonium polyphosphate + fertilizer
enhancement product.**

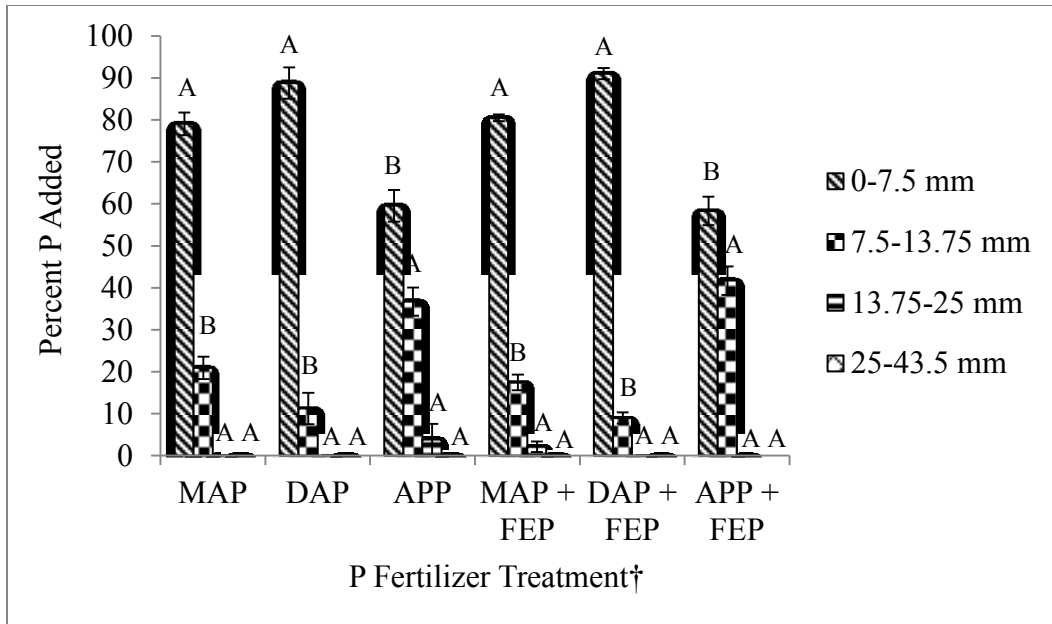


Figure 3.10 Percent P Added (PPA) in the soil sections of Ultisol collected at different distances from the point of fertilizer application. Percent P Added calculated for each dish section for all treatments, where PPA is defined as follows:

$$PPA = \left[\frac{(P_f)S_i \times M_i}{\sum_{i=1-4} [(P_f) S_i \times M_i]} \right] \times 100.$$

Where i = dish section (1-4), (P_f)S_i = the concentration of P fertilizer in each dish section, and M_i = the mass of soil each dish section. (P_f)S_i is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at P = 0.05 using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

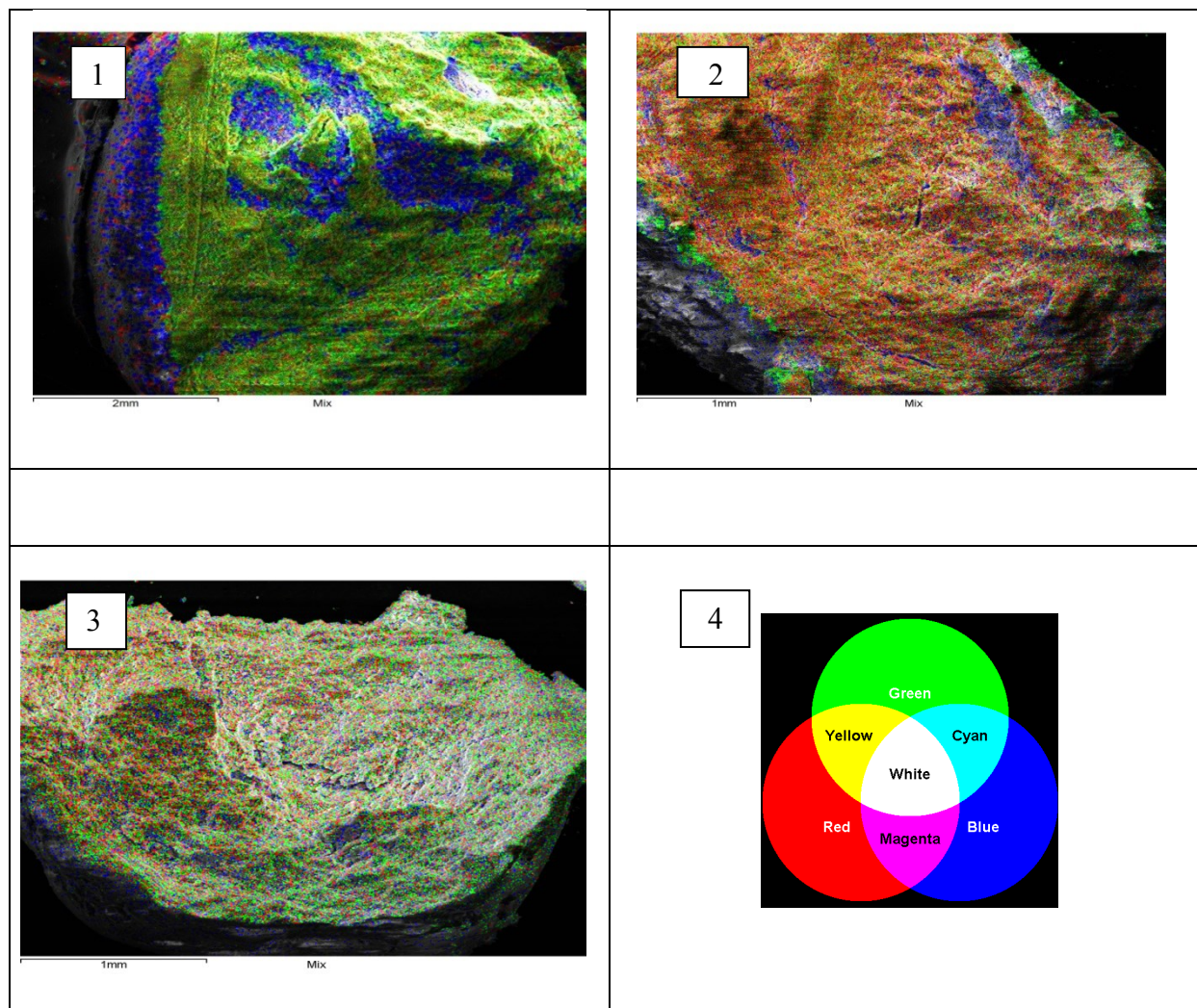


Figure 3.11 Energy dispersive x-ray spectroscopy microscopy dot-maps of diammonium phosphate (DAP) and monoammonium phosphate (MAP) granules incubated for 35 days in Andisol. Color differences in incubated granules are due to differences in granule orientation. (1) Cross section of DAP granule (2) Cross section of MAP granule (3) Cross section of DAP granule (4) The colors represent the distribution of Ca, P and Fe within the granule. Green=P; Red=Ca; Blue=Fe.

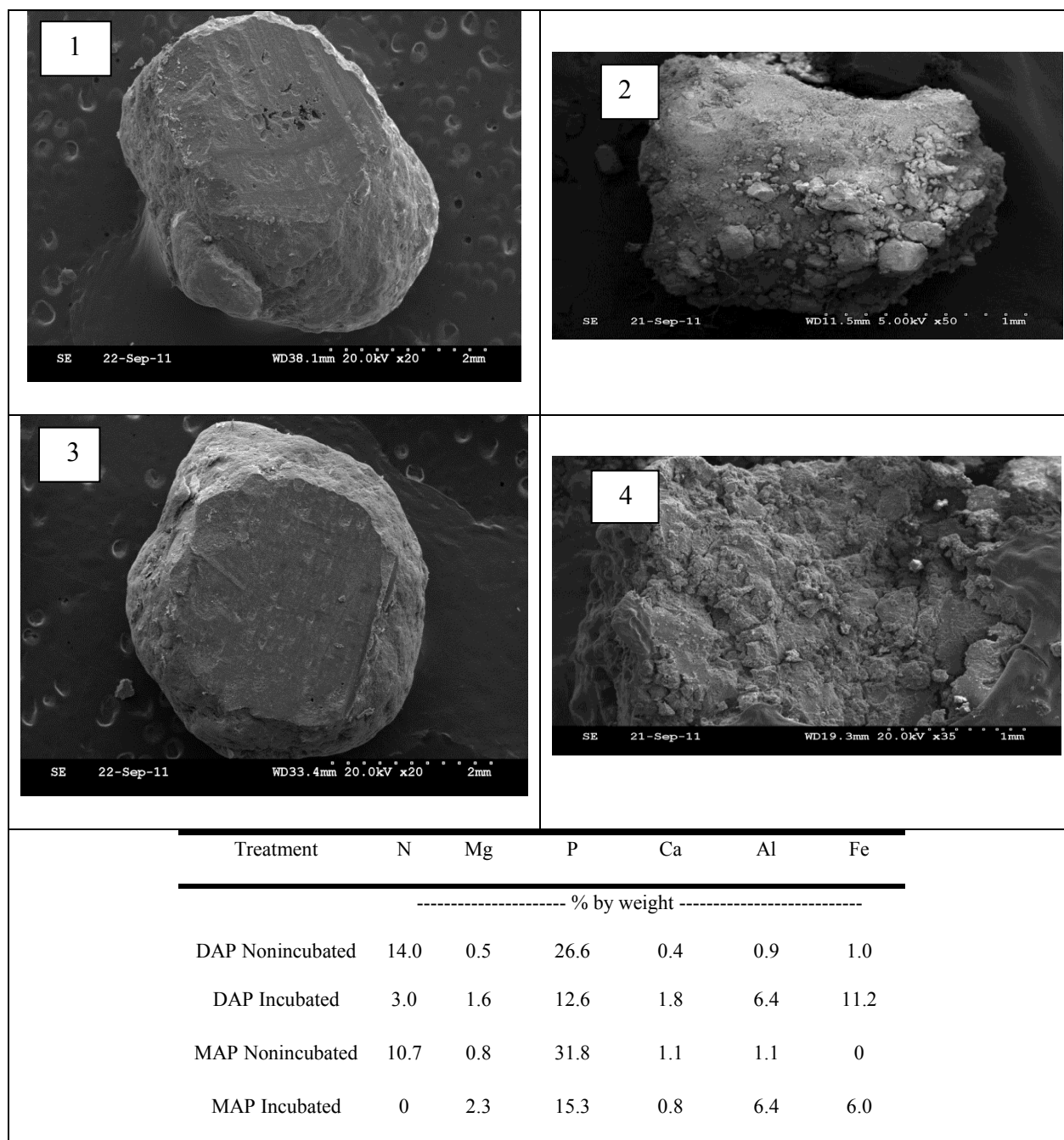


Figure 3.12 Secondary electron micrographs of monoammonium phosphate (MAP) and diammonium phosphate (DAP) granules, nonincubated and incubated for 35 days in the Oxisol, and elemental analysis. (1) Nonincubated MAP granule (2) Incubated MAP granule (3) Nonincubated DAP granule (4) Incubated DAP granule.

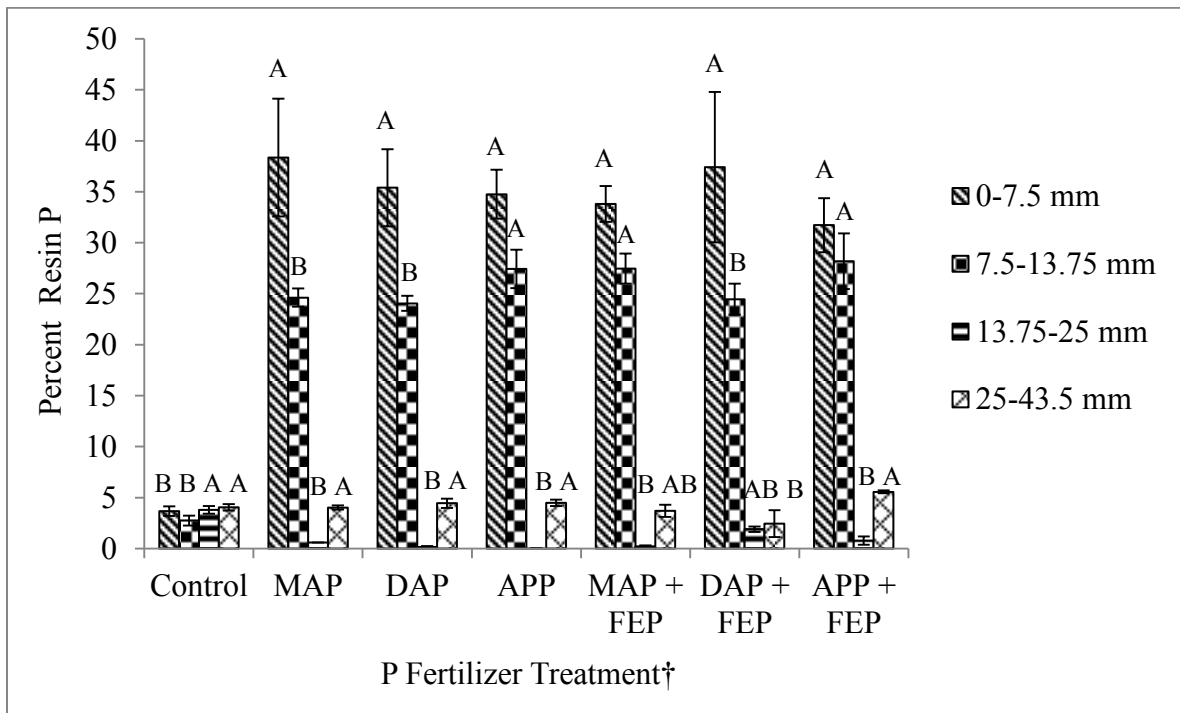


Figure 3.13 Oxisol Percent Resin P (PRP) calculated for each dish section for all treatments where PRP is defined as follows: $PRP_i = (\text{resin extractable } P_i / \text{total } P_i) \times 100$. Where $i = \text{dish section (1-4)}$, resin extractable $P_i = \text{the resin P concentration}$, and total $P_i = \text{total P concentration}$. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product

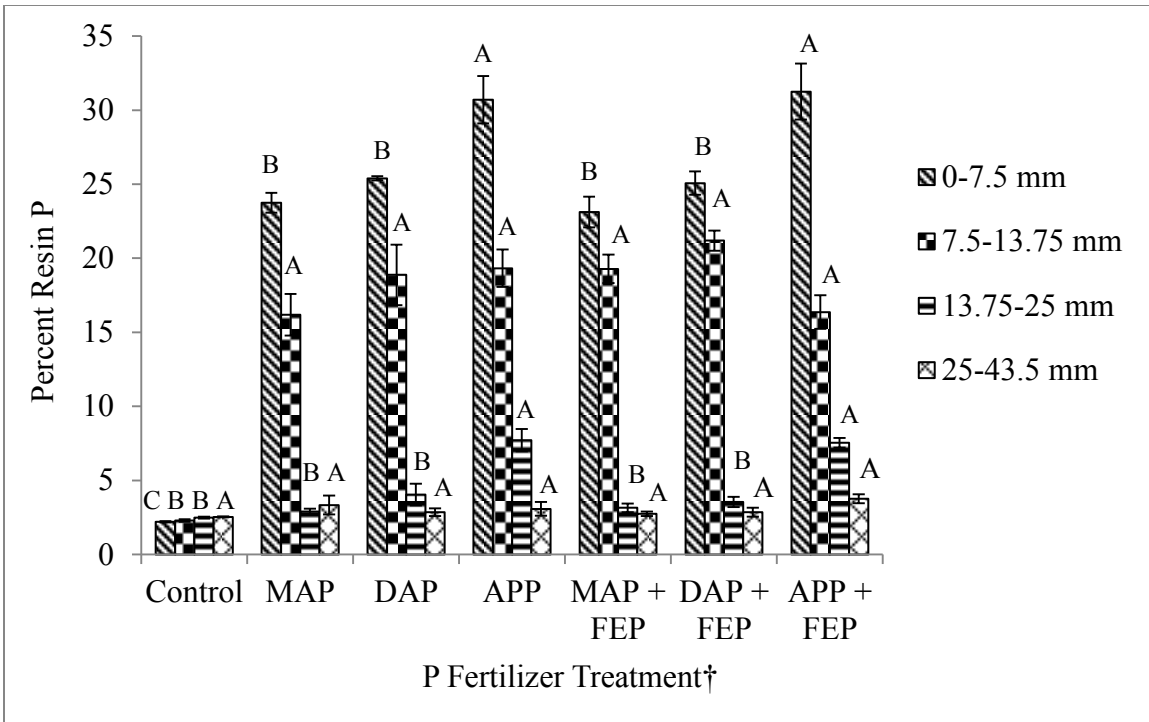


Figure 3.14 Andisol Percent Resin P (PRP) calculated for each dish section for all treatments where PRP is defined as follows: $PRP_i = (\text{resin extractable } P_i / \text{total } P_i) \times 100$. Where $i =$ dish section (1-4), resin extractable $P_i =$ the resin P concentration, and total $P_i =$ total P concentration. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

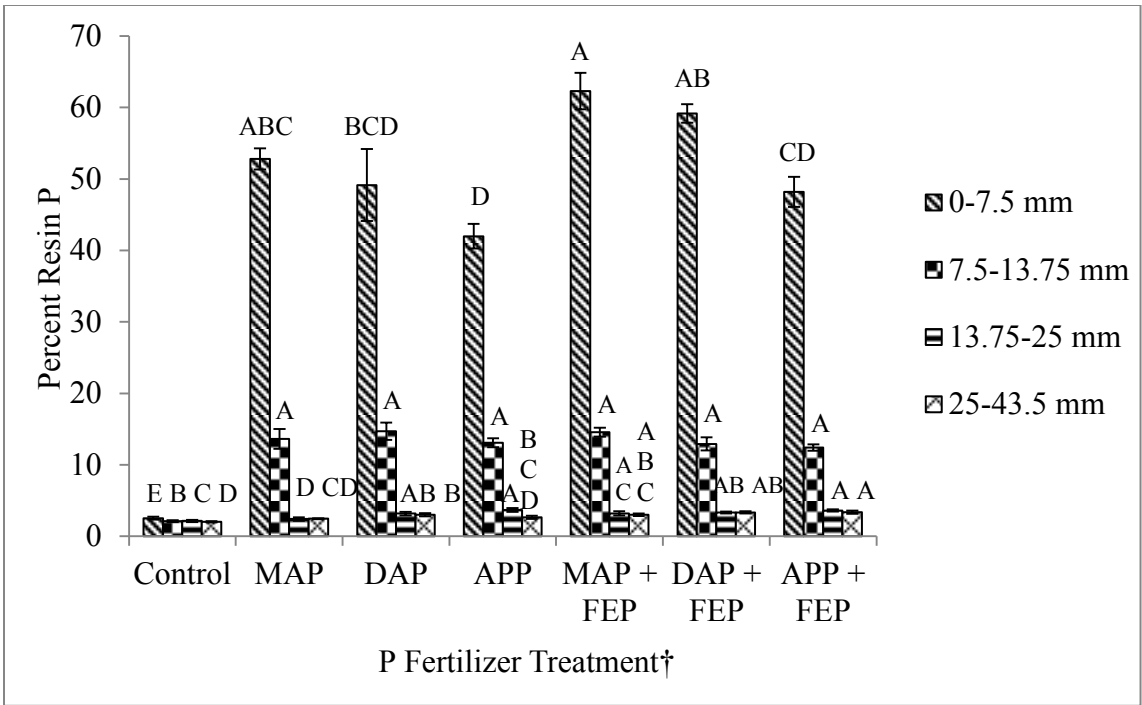


Figure 3.15 Ultisol Percent Resin P (PRP) calculated for each dish section for all treatments where PRP is defined as follows: $PRP_i = (\text{resin extractable } P_i / \text{total } P_i) \times 100$. Where $i =$ dish section (1-4), resin extractable $P_i =$ the resin P concentration, and total $P_i =$ total P concentration. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test.

†MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

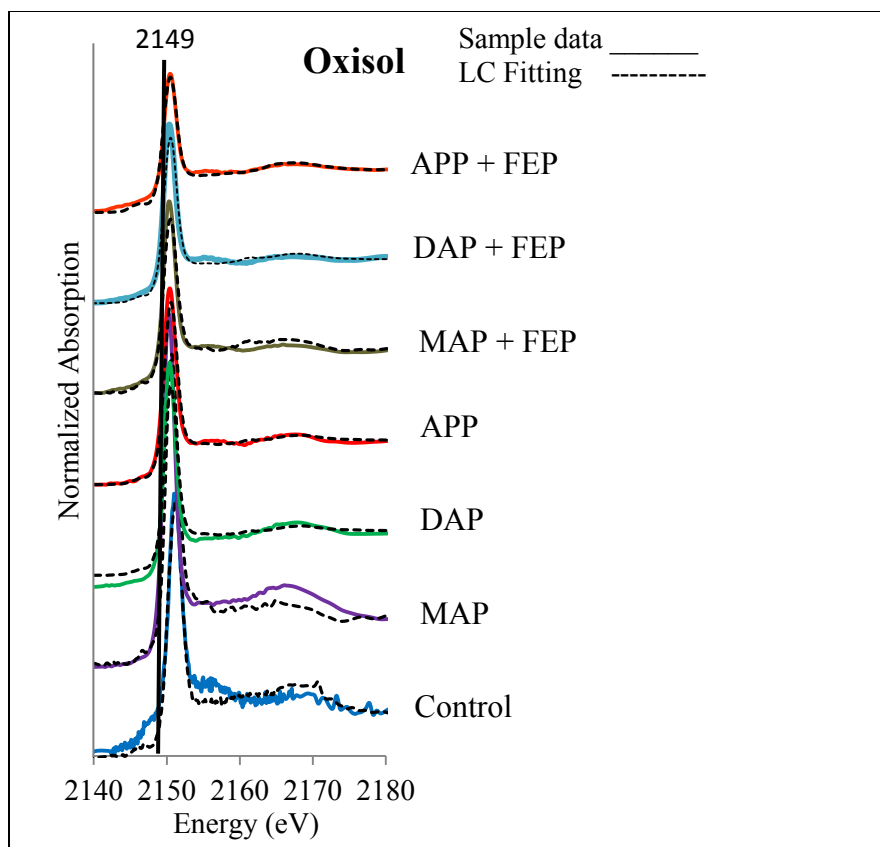


Figure 3.16 Normalized P K- edge XANES spectra for the Oxisol for the 0-7.5 mm section at 5-weeks incubation. The dotted lines are the linear combination fit line for each treatment and represents the linear combination fit with the P standards. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

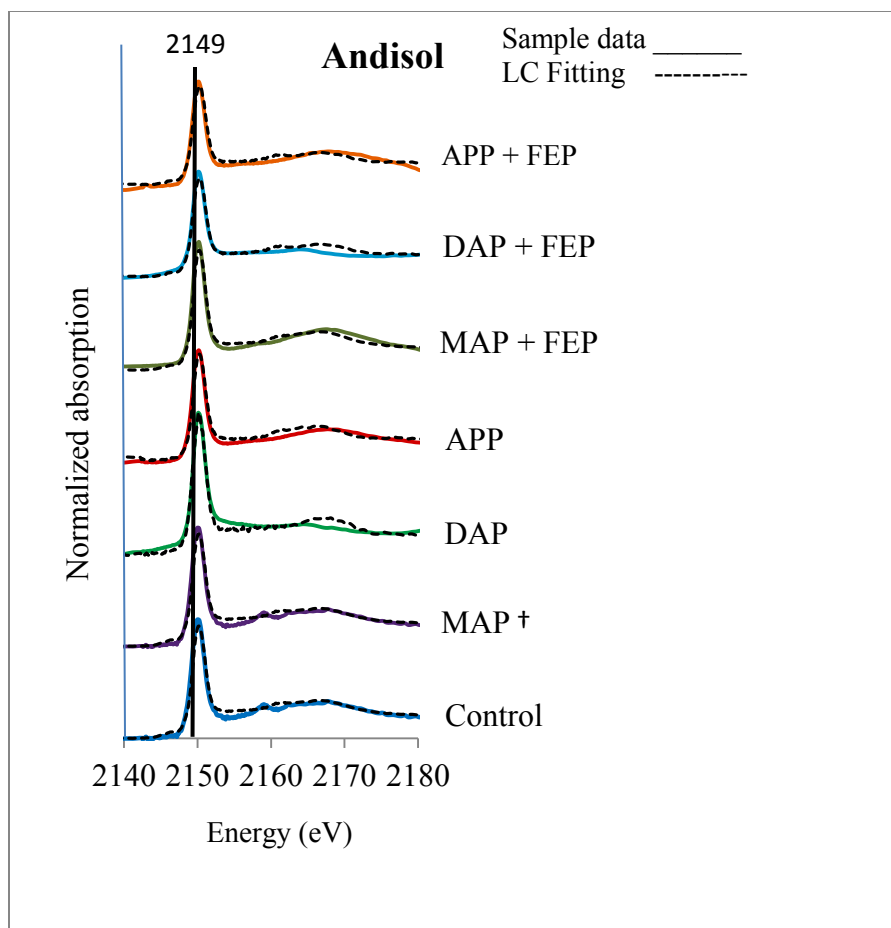


Figure 3.17 Normalized P K- edge XANES spectra for the Andisol for the 0-7.5 mm section at 5-weeks incubation. The dotted line at each treatment spectra is the linear combination fit line and represents the linear combination fit with the P standards. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

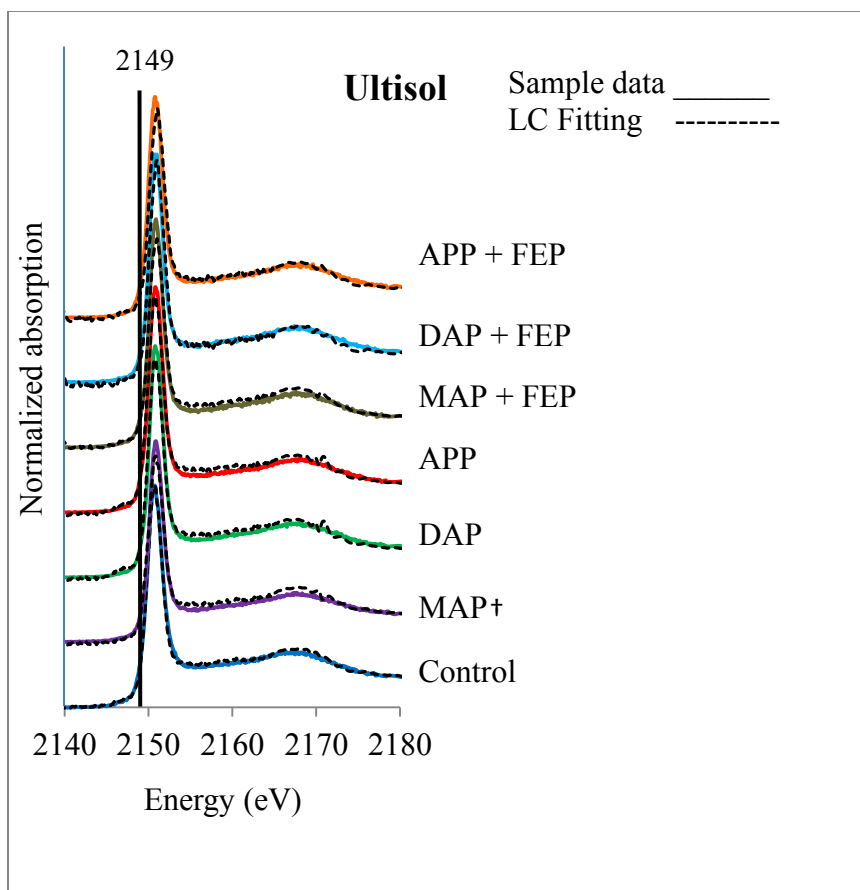


Figure 3.18 Normalized P K- edge XANES spectra for the Ultisol for the 0-7.5 mm section at 5-weeks incubation. The dotted line at each treatment spectra is the linear combination fit line and represents the linear combination fit with the P standards. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

Chapter 4 - Mobility, lability and reaction products of phosphorus fertilizer in three calcareous soils

Abstract

Limited phosphorus (P) availability in agriculture soils is a major factor that limits crop production for many areas of the world. In calcareous soils, fluid P fertilizers have been shown to be more effective over granular P fertilizer in increasing crop yields. We implemented a laboratory incubation study to pursue a more mechanistic understanding of responses associated with various P fertilizer sources in calcareous soils. The objectives of this study were to investigate and understand the interactions of various P sources on P mobility, availability and reaction products formed within and immediately around the P fertilizer application point. We used three fertilizers: monoammonium phosphate (MAP), diammonium phosphate (DAP), and ammonium polyphosphate (APP). In particular, we compared the effects of the fertilizers alone and enhanced with a fertilizer enhancement product (FEP) in three soils, an Entisol, a Mollisol, and an Inceptisol, to examine the associations among P fertilizer reaction products, P mobility, and potentially labile P. Mobility results showed that P fertilizer diffused to the 7.5-13.75 mm section for all P treatments. For the most part there was greater P diffusion from the liquid over P granular fertilizer treatments. Phosphorus fertilizer lability was increased for the liquid over granular P treatments in both the Entisol and Mollisol. Indirect evidence from the use of scanning electron microscopy plus energy dispersive X-ray spectroscopy suggests the formation of reaction products and complexation of P with calcium (Ca), aluminum (Al) and iron (Fe) within incubated P granules. The digestion of the incubated P granules indicated P reaction products complexed with Ca, greatly limiting P dissolution into the surrounding soil. Bulk XANES spectroscopy results directly confirmed that various Ca-P solid phases were the major

mechanism responsible for decreased P diffusion and availability in the calcareous soils. For the two P fluid treatments in each of the three soils, there were less Ca-P like precipitated forms and more Fe- and/or Al- adsorbed P forms than with the P granular treatments. There was few consistent enhanced P diffusion or lability effects with the addition of the FEP to the three soils used in this study.

Introduction

The reduction of plant available P in soils over a growing season may cause decreased crop yields (Stoop, 1983). Phosphorus is considered one of the most limiting soil macronutrient and is essential in numerous physiologic processes for optimal crop growth and development (Smil, 1999; Vance, 2001). Low P availability in agricultural soil limits plant growth by reducing the efficiency of use for other essential plant nutrients. When P fertilizer is incorporated into soils, fertilizer P undergoes a series of transformations over the growing season that limits its continued availability for plant uptake and use. So, heavy or frequent P fertilizer applications may be necessary to maintain adequate crop growth and yield (Pierzynski, 1991). This increases the risk of eutrophication of surface waters from soil runoff or erosion, or by subsoil leaching (Mengel et al., 2001; Pierzynski, 1991; Sharply et al., 2013; Andersson et al., 2015; Yan et al., 2016). A gradual decrease in P availability over time is an issue in agricultural soils regardless of the soil type, location, or the crop grown; however, it becomes significantly more of a problem in agricultural areas where crops are grown on very acidic or calcareous soils. Phosphorus fertilizer added on these soils is rapidly transformed to insoluble P forms in a process called P fixation. The distribution of high P fixing soils is widespread. Calcareous soils, which are the focus of this study, are in agriculture production all over the world; estimated at around 800 million hectares (FAO, 2004). Mechanisms behind increased P fixation in soils are complex and dependent on soil pH, climate, soil mineralogy, as well as the form of P fertilizer added to the soil (Sanchez and Uehara, 1980; Lombi et al., 2004; Sample et al., 1980).

Soils containing free calcium carbonate (CaCO_3) frequently have high P fixing capacity and present a unique challenge in terms of agriculture production. Generally, these soils are located in more arid environments and have a high pH as a result of slower weathering

processes; it is a challenge in agriculture in terms of producing crops with increased yield because with the addition of P fertilizers there can be the formation of insoluble Ca-P reaction products within and near the fertilizer application point that reduces plant availability of P (Sample et al., 1980; Hettiarachchi et al., 2006). Freeman et al. (1981) found that fertilizer P led to the formation of CaCO_3 , and precipitated species such as dicalcium phosphate dihydrate and octacalcium phosphate in addition to adsorbed species, suggesting there was greatly decreased potential P plant availability in the soil environment. In addition to Ca solid phases, iron (Fe) oxides are important in sequestering P in calcareous soils. Holford and Mattingly (1975) utilizing bonding energies using a two-surface Langmuir equation, found that high-energy P adsorption capacities were related more to hydrous oxides, such as citrate-dithionite soluble Fe, providing principle sites for adsorption in calcareous soils. Lower energy adsorption capacities were more correlated with the total surface areas of CaCO_3 . Low energy adsorption capacity was not related to pH, dithionite-soluble Fe or percent CaCO_3 . Similarly, Ryan et al. (1985) found that citrate-bicarbonate-dithionite (CBD) extractable Fe was associated with a longer term decrease in extractable P along with Ca-P solid phases in calcareous Mediterranean soils. Iron oxides were responsible for sorption reactions on amorphous surfaces with a gradual crystallization of various forms of Fe-P over time.

Increasing P use efficiency in P limiting soils is a continued topic of interest. The benefits of P supplied in fluid forms over conventional granular products as a method of increased P use efficiency in Australian soils have been demonstrated through x-ray, spectroscopic and laboratory-based chemical analyses (Lombi et al., 2004; 2006). Laboratory-based incubation results suggest the use of liquid P fertilizer on calcareous soils allowed for greater movement of P away from the point of application thereby reducing the formation of Ca-

P precipitates. Their X-ray diffraction (XRD) and microanalysis energy dispersive X-ray examination of MAP granules incubated in highly calcareous soil suggested an increased percentage of fertilizer P remained within the granule at the end of the incubation period.

Hettiarachchi et al. (2006) found that the form of P fertilizer added to calcareous soil greatly influenced diffusion and lability away from the point of application. X-ray computed microtomography was employed to look at differences in density over time with either a MAP granular or liquid technical-grade MAP (TG-MAP) added as a band incubated in a calcareous soil. For MAP granules, the hygroscopic water movement toward the granule decreased P diffusion resulting in increased density due to P precipitates immediately surrounding the granule. There was not decreased or restricted P diffusion with the fluid P source added to the soil. The reduced movement of dissolved fertilizer P away from the granule into the surrounding soil implies decreased potential plant availability of applied fertilizer P.

There are several management strategies that can be used to minimize P fixation in soils. Deep-banding verses broadcasting fertilizer has been investigated as a method to increase P efficiency in soils (Khaliwada et al., 2012; McBeath et al., 2005; Bordoli and Mallarino, 1998; Moody et al., 1995). Holloway et al. (2001) compared wheat yields in soils with deep-banded granular P fertilizers using MAP, DAP and triple super phosphate (TSP) verses liquid P fertilizer (TG-MAP) in field plot experiments on a highly calcareous Inceptisol in South Australia. The first year, field results showed that TG-MAP produced significantly more grain than the granular P sources. The liquid outperformed the granular P fertilizer with increased overall biomass when repeated the following year, as well.

Another approach to potentially increase P availability by minimizing soil P fixation has been the use of P fertilizer enhancement products (FEP). There are fertilizer enhancement

products that activate microbial growth using enzymes, which increase fertilizer nutrient uptake by crops. Some of the popular products include humic or fulvic acid-based compounds. There are products manufactured containing exclusive technologies, such as from Huma Gro® that use carbon-rich molecules as nutrient carriers which enable efficient nutrient absorption, including P, for an increased plant growth. Finally, there are numerous products that provide enhanced nutrient availability resulting in exceptional growth and development.

One widely used FEP is a long chain carbon compound composed of maleic-itaconic acid copolymer $(C_9H_6XO_8)_n$ (X= cation) marketed under the product name of Avail®. Due to its molecular structure, the product has a high cation exchange capacity (CEC) and complexes multivalent cations (Al, Ca, Fe) in direct proximity to the point of application that normally would precipitate or adsorb P anions thereby decreasing P plant availability (Karamanos and Puurveen, 2011). A number of field studies on different soils using P fertilizer at planting that contained Avail® have been summarized by Tindall (2007), although few have been done on calcareous soils. Independent field studies reportedly show increased yield and P use efficiency using Avail®. For example, in a field trial on calcareous soil in Idaho, Hopkins (2013) broadcast applied and incorporated MAP fertilizer at planting with or without Avail® for Russet Burbank potatoes. The MAP + Avail® addition resulted in greater petiole P concentrations and total yield increase in all but one site. Stark and Hopkins (2015) evaluated potato yields on calcareous soils with low to moderate soil test P, using spring verses fall applications of MAP and APP, with and without Avail®, over a four year time period. Some fertilizer treatment combinations that included Avail® increased yield. Although there was no differences in yield due to season of P application, there were yield increases with the fertilizer treatments that included Avail®. Positive effects with Avail were more likely in soils with high lime concentration. In contrast,

Cahill et al. (2014) conducted two field experiments using several DAP fertilizer rates with and without Avail® in order to evaluate the product's enhancement capabilities on corn growth and yield at several locations in North Carolina. The soil pH at the sites ranged from 5.5 to 7.2 and soil test P as measured with Mehlich-3 varied from low to very high. A variety of soil series and soil textures were included in this study. There were no significant differences with grain yields across the fertilizer treatments. There were no significant growth or yield increases comparing the control to the DAP+FEP treatment.

Degryse et al. (2013) developed a laboratory-based incubation experiment to test P fertilizer efficiency utilizing a group of cation-complexing compounds including enhancement products including one coated on MAP granules as part of a diffusion experiment on an Oxisol, Alfisol, Vertisol, and Inceptisol. There were no significant differences in terms of diffusion between the polymer coated and uncoated MAP treatments. A second experiment was developed to test whether chelates, citrate and Avail® added to P fertilizer at a higher rate may increase P efficiency. Phosphorus fertilizer granules were coated and incubated. After 28 days they found increased solution concentrations of P in one of the soils, however, there was no significant effect on P lability. Lastly, a greenhouse experiment was implemented on various soils, including a highly calcareous soil, to test the effect of coatings and the two chelates applied on low P rates, on wheat dry matter and production and shoot P. There were no significant effects of chelate treatments on either the shoot P or dry matter production on any of the soils.

Incorporating a specific form of absorption spectroscopy, X-ray absorption near edge structure (XANES), into the P fertilizer research is a tool that has been rather lacking in previous research on high P-fixing soils. The synchrotron-based spectroscopy method can be extremely useful in determining P solid-phase formation with an elevated degree of spatial resolution

(Beauchemin et al., 2003; Liu et al., 2014). Studies have employed other forms of spectroscopy such as XRD, scanning electron microscopy (SEM) and energy dispersive X-ray microanalyses (EDXMA) to look at P reaction products formed within and surrounding the P granule after incubation; or, X-ray computed microtomography (X-ray CT) in looking at changes in density surrounding P fertilizer granules versus P fertilizer banding (Lombi et al., 2004; Bell and Black, 1970; Freeman and Rowell, 1981; Hettiarachchi et al., 2006). A study that investigated P availability in granular and fluid P fertilizer in a highly calcareous soil incorporated nano X-ray absorption near-edge structure, nano X-ray fluorescence and P K-edge XANES to gain a mechanistic understanding of P reaction products in incubated P fertilizer granules (Lombi et al., 2006). However, the Lombi et al. (2006) study focused on a single, highly calcareous soil and used a granular P fertilizer and its fluid equivalent in their research. Phosphorus fertilizer research incorporating other moderately calcareous P-fixing soils from additional soil orders with other granular and liquid forms of P fertilizers and employing P K-edge XANES for direct speciation of P reaction products in close proximity to P fertilizer additions would be beneficial as we can learn more about reaction processes in a range of calcareous soils.

The objectives of this research were to (1) understand the mobility and availability of various P fertilizer sources in three different calcareous soils considered to have high P fixation capacity using a well-controlled, laboratory-based incubation experiment, (2) identify possible P reaction products formed within and around fertilizer granules or droplets that may contribute to decreased P efficiency, and (3) examine possible soil chemical changes related to P diffusion, lability and speciation with both granular and liquid P fertilizers in the presence of an FEP capable of interacting with cations that may influence fertilizer P reaction products.

Materials & Methods

Three soils were sampled for the study; an Entisol, Masuma soil series (coarse, loamy, mixed (calcareous) mesic Typic Torriorthent) from Elmore County, Idaho (N 43° 8' 13"; W 115° 41' 40"), a Mollisol, Ulysses soil series, (fine-silty, mixed, superactive, mesic Aridic Haplustoll) from Finney County, Kansas (N 38° 01' 10.22"; W 100° 49' 38.79"), and an Inceptisol equivalent to the Calcixerollic Xerochrept in the USDA system, (Soil Survey Staff, 1999) from the upper Western side of the Eyre Peninsula, South Australia (approximately S 32° 47' 50"; E 134° 12' 40"). None of the soils had a history of being fertilized. All soils were collected to a 15 cm depth, air dried and sieved to < 2 mm before being shipped from their place of origin.

The pH of the soil samples in the study were measured in a 1:10 soil:water extract (Watson and Brown, 1998); Mehlich-3 P as described in Frank et al. (1998); Cation exchange capacity (CEC) determined by displacement method (Soil Survey Staff, 2011); Carbonates determined by Allison and Moodie, 1965; Total organic carbon (TOC) determined using the dry combustion method of Nelson et al. (1996) using soil ground and sieved to $\leq 150 \mu\text{m}$ and treated with 1N phosphoric acid to remove inorganic C. Direct combustion of soils was completed using a Carlo Erba C/N analyzer (Carlo Erba instruments, Milan, Italy). Total P, Al, Fe and Ca determined according to Zarcinas et al. (1996), modified to use a digestion block instead of microwave. Total Fe oxide (Fe_{CBD}) and citrate-bicarbonate-dithionite extractable Al (Al_{CBD}) were determined using the citrate-bicarbonate-dithionite (CBD) method of Loeppert and Inskeep (1996).

Particle size analysis was determined using a combination of a modification of the pipette method by Kilmer and Alexander (1949) and method 3A-1 from the Soil survey

Laboratory Methods Manual (Soil Survey Staff, 2004). Clay mineralogy was completed using K-25 and Mg-25 combinations (Harris and White, 2008). The XRD analyses were performed on a PANalytical Empyrean Multi-Purpose X-Ray Diffractometer (Spectris Company, Surrey, United Kingdom) using a copper anode material and generator settings of 35 eV and 20 mA.

The maximum water holding capacity (MWHC) determination was made using the protocol from Jenkinson and Powlson (1976). Each soil was pre-moistened with 10% of the total distilled water needed for 60% MWHC. Soil was packed into petri dishes (87x11 mm) at a bulk density of 1.31 g cm⁻³ for the Entisol, 1.2 g cm⁻³ for the Mollisol, and 1.04 g cm⁻³ for the Inceptisol. Preliminary work indicated that these bulk densities were ideal for completely filling the volume of the petri dish without unnecessary compression. There were seven treatments with five replications. Two additional sets of petri dishes were prepared for scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS) analysis. The remaining distilled water was added to bring the soil to 60% MWHC by carefully dripping the water on the soil packed in each plate. The plates were sealed using Parafilm M® (Bemis Flexible Packaging, Neenah, WI), wrapped in aluminum foil and left to equilibrate over night at room temperature. The plates were unwrapped and the treatments introduced by placing the fertilizer just below the soil surface in the center of the dish and covering with soil as follows:

- (1) Unfertilized control soil sample
- (2) Monoammonium phosphate (MAP) granular (11-52-0; 11% N - 52% P₂O₅ - 0% K₂O by weight). A 43± 0.05 mg, (~9.76 mg P) standard grade MAP granule
- (3) Diammonium phosphate (DAP) granular (18-46-0; 18% N - 46% P₂O₅ - 0% K₂O by weight). A 49 ± 0.05 mg, standard grade DAP granule
- (4) Ammonium polyphosphate (APP) liquid (11-37-0; 11% N - 37% P₂O₅ - 0% K₂O by weight).

A 60.5 mg (49 μ L), standard grade APP liquid was placed using an Eppendorf Reference 100 μ L pipette (Eppendorf, Malaysia)

(5) MAP+FEP granular (11-52-0): A 43 ± 0.05 mg, standard grade MAP+FEP granule

(6) DAP+FEP granular (18-46-0): A 49 ± 0.05 mg standard grade DAP+FEP granule

(7) APP+FEP liquid (11-37-0): A 60.5 mg (49 μ L), standard grade APP liquid was placed using an Eppendorf Reference 100 μ L pipette (Eppendorf, Malaysia)

The FEP used in this research was described in Chapter 3. All fertilizer treatments received equivalent amounts of P, approximately 9.8 mg P per petri plate. Nitrogen was balanced against DAP using finely ground commercial grade urea (46-0-0): MAP and MAP + FEP treatments, APP and APP + FEP treatments.

After all treatment preparations were complete, Petri dishes were sealed with Parafilm M® and wrapped in aluminum foil. Soils were incubated (Precision Low Temp Incubator, Waltham, MA) in the dark at 25° C for 5 weeks. This particular incubation time was chosen because at five weeks root development would be limited and this is considered one of the critical P uptake periods for cereal crops (Hettiarachchi et al., 2010; Williams, 1948).

At the end of the five-week incubation period, the plates were opened, and the soil was collected in four concentric rings: 0-7.5 mm, 7.5-13.75 mm, 13.75-25 mm, 25-43.5 mm, using metal cylinders that were pushed gently into the soil to the bottom of the plate, starting with the smallest cylinder in the center and carefully collecting all soil from the circular section (Lombi et al., 2004). Each concentric soil sample was placed in a separate plastic specimen container (Fisher Scientific, Waltham, MA) with the soil weight recorded. The sample was then fast dried immediately at 40° C (Fisher Scientific drying oven, Waltham, MA). After drying, the weight of the soil was recorded, and a lid was placed and closed on the specimen cup to reduce the chance

of contamination. Two of the seven replicates from the granular treatments were utilized for scanning electron microscopy coupled with energy dispersive x-ray spectroscopy analysis. For these, the fertilizer granules were carefully removed using a small metal spatula and tweezers, placed in separate labeled containers, dried at 40° C. The samples were sealed and stored in the refrigerator until SEM-EDS analysis.

Aqua regia (1:3 volume to volume ratio of HNO₃:HCl) soil digestion was performed for total P analysis using a 0.25 g soil subsample from all 0-7.5 mm treatments and a 0.5 g subsample from the remaining three sections for all treatments. Each soil sample was placed in a separate 75 ml glass digestion tube containing 5 mL of aqua regia mixture and placed on a Foss Digester Unit (Fisher Scientific, Waltham, MA), slowly increasing the temperature from 75° C to 140° C until the acid mixture had evaporated to approximately 1.0 ml. The digested samples were cooled, diluted with 25 mL of 0.1% HNO₃ and filtered through a #42 Whatman filter paper. Each filtered sample was analyzed for total P using inductively coupled plasma emission spectrometry (ICP-OES, Varian 720-ES, Santa Clara, CA). Since slight variations exist in the amount of P applied to each petri dish due to the variability in fertilizer granule composition, total P data were normalized by calculating percent P added (PPA) for each dish section for all treatments (Hettiarachchi et al., 2010) is defined as follows:

$$PPA = \left[\frac{(P_f)S_i \times M_{i=1-4}}{\sum [(P_f) S_i \times M_i]} \right] \times 100$$

Where i = dish section (1-4), (P_f)S_i = the concentration of P fertilizer in each dish section, and M_i = the mass of soil in each dish section. (P_f)S_i is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section. Total P concentrations are shown in Fig. 4.1, 4.2, and 4.3.

A 0.5 g soil subsample from all 0-7.5 mm treatments and 1.0 g subsample from the remaining three sections for all treatments were extracted for potential plant available P (labile P) using one 50 x 25 mm resin exchange strip (VWR International Ltd., Radnor, PA) for the 0-7.5mm section and two strips for each one g sub-sample as described by Myers et al. (2005). The resulting filtered extract was analyzed according to the Murphy & Riley Method (Murphy and Riley, 1962) using a UV spectrophotometer (Beckman-Coulter, DU-800, Brea, CA). Since variations exist in the amount of P in each fertilizer granule and in an effort to minimize the effects due to granule variability that could be expected and difficult to control between P treatments, resin extractable P data were normalized by calculating percent resin P (PRP) for each dish section for all treatments as follows:

$$\text{PRP}_i = (\text{resin extractable } P_i / \text{total } P_i) \times 100$$

Where i = dish section (1-4), resin extractable P_i = the resin P concentration, and total P_i = total P concentration. Resin extractable P concentrations are shown in Fig. 4.4, 4.5, and 4.6.

To look for morphological changes within the granule, SEM analysis was performed on the original and P fertilizer granules incubated five weeks in the soils. After carefully removing residual P granule from the petri dish, the attached soil particles were carefully removed using a soft paint brush and tweezers. All fertilizer granules used for SEM-EDS were carefully fractured using a stainless steel blade, and mounted on an Al pin stub. The incubated granules from the Entisol sample was performed on a Hitachi S-3500N SEM equipped with a model *S-6542* absorbed electron detector (Hitachi Science Systems, Ibaraki Pref, Japan) with an accelerating potential of 5 kV using an X-ray Detector-Link Pentafet 7021 (Oxford Instruments Microanalysis Limited, Bucks, England). To minimize static electrical surface charging the

granules were sputter-coated with a 40% palladium (Pd) conductive coating four nm in thickness and allowed to dry before imaging. Due to unavailability of the previous SEM-EDS instrument, analysis of incubated fertilizer granules for SEM-EDS from the Mollisol and Inceptisol samples were examined using a FEI Nova Nano-SEM 430 with a helix detector (Hillsboro, Oregon) with high resolution and low-vacuum capability to enhance SEM image quality at low kV. A Schottky emitter allowed for stable emission and high current modes, with the immersion lens optimizing the collection of secondary electrons. We discounted the element used for sputter coating and therefore, results from both SEM are comparable.

The remaining P, Al, Fe and Ca in the incubated MAP and DAP granules were determined using another set of parallel incubation studies for the three soils. The granules were digested in aqua regia and analyzed for total P, Al, Fe and Ca to access the mass of each element remaining in the granule after incubation.

X-ray absorption near edge structure (XANES) analysis was performed on a composite sample of the 0-7.5 mm sections of the five replicates used for chemical analysis. The P K-edge XANES data were collected at two different synchrotron facilities. Entisol data collection was performed at Sector 9-BM-B, Advanced Photon Source (APS), Argonne National Laboratory, IL. The storage ring at the facility operates at 7 GeV with a maximum current of 100 mA. The energy range at Sector 9 is 2.1 to 23 keV. The beam line provides a focused x-ray beam for XANES as well as x-ray absorption fine structure (XAFS) experiments in an area of 500 μ m x 500 μ m. All spectra including the P pentoxide (P₂O₅) standard used for daily monochromator alignment were collected in fluorescence mode using a solid-state drift detector. The soils were ground to < 150 μ m using an agate mortar and pestle, and then a 4-mm size soil pellet was prepared from the soil using the KBr Quick Press Kit (International Crystal Laboratories,

Garfield, NJ) and carefully glued onto a 33-mm Teflon disk using double-sided carbon tape (SPI Supplies, West Chester, PA) before placing on an Al sample holder for analysis (Fig. 4.7).

Six scans were collected per sample at a range 2.1 to 2.4 keV and 12 scans were collected for each nonfertilized control soil using a three-step scan procedure. Due to beamline upgrades, sector 9 BMB at APS was not available, so the Mollisol and Inceptisol XANES data collection was performed at Canadian Light Source Inc., Soft X-ray Microcharacterization Beamline (SXRMB) 06B1-1, Saskatoon, Saskatchewan, Canada. The third generation storage ring at CLS operates at 2.9 GeV. The energy range at Sector 06B1-1 is 1.7 to 10 keV and the beam line provides a focused x-ray beam for XANES to 300 μ m x 300 μ m. All spectra including the phosphorus standard used for the InSb (III) monochromatic alignment were collected in fluorescence mode. The composite replications from the 0-7.5 mm section for each treatment were used in the spectral collection. All soils were ground to < 150 μ m using an agate mortar and pestle and evenly sprinkled onto double-sided carbon tape placed on a stainless steel sample holder in a vacuum chamber for analysis. Multiple scans were collected per sample at a range 2.1 to 2.2 keV, and 6 scans were collected for the control treatment soil. All data collected were analyzed using IFEFFIT-Athena software (Ravel and Newville, 2005). The scans of collected spectra were averaged for each treatment. The edge energy was calibrated, the fitting the pre-edge was subtracted by a linear function, the spectrum was normalized to a second-order polynomial.

All P standards were either prepared in the laboratory or purchased, and the pureness of each prepared standard was confirmed by using XRD. The following P standards were synthesized and collected by Khatiwada et al. (2012): variscite ($\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$), brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), strengite-1d, strengite-3d ($\text{FePO}_4 \cdot 2\text{H}_2\text{O}$), PO_4 -goethite, PO_4 -alumina, and PO_4 -

gibbsite. Apatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and vivianite $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$, University of Adelaide. Aluminum phosphate and Fe III-P spectra were obtained from K. Scheckel, EPA, Cincinnati, Ohio. The PO_4 -adsorbed to Al-saturated montmorillonite spectra were obtained from J. Prietzel, University of Technology, Munchen, Germany. The PO_4 -adsorbed kaolinite spectrum was obtained from D. Hesterberg, North Carolina State University. Phosphorus spectra were collected at Synchrotron Light Research Institute, Nakhon Ratchasima, Thailand, beam line scientist Wantana Klysuburn.

Spectra for the aligned standards were used to reconstruct the spectra of the unknown samples using a linear combination fitting (LCF) procedure that allowed for the determination of the relative proportion of the P solids in our samples. The Manceau Combo Method (Manceau et al., 2012) was also used to validate our data fitting procedure. The results closely matched the LCF results for the three acidic soils, providing an added confidence on the P solid phases identified by LCF.

Statistical Analysis

Soils data were statistically analyzed using the PROC MIXED Procedure in SAS (SAS 9.3, 2011). The experimental design was a complete randomized design (CRD). Data were analyzed using ANOVA with P fertilizer treatment as the main treatment, and dish sections as subplot treatments. The Tukey Pairwise Method was used for a comparison of all treatments at a 0.05 level of significance.

Results and Discussion

Physical and chemical properties of soils

Properties and chemical characteristics of the soils are provided in Table 4.1. Initial soil pH ranged from 8.5 in the Entisol to 8.7 in the Mollisol and Inceptisol. The reactive CaCO_3 was

variable between the three soils (Allison and Moodie, 1965). The Entisol contained 10.9% CaCO_3 , the Inceptisol was extremely high at 83% CaCO_3 while the Mollisol was lowest at 7.7% CaCO_3 . Soil texture varied between the soils with the Entisol and Inceptisol being sandy loam (SL) and the Mollisol being a silt loam texture (SiL).

Soil pH

The influence of the P treatments on soil pH was similar for the three calcareous soils. Regardless of P source, the greatest decrease in pH was in the 0-7.5 mm section with the soil pH increasing with increasing distance from the center section to a point relatively similar in pH to the control treatment by the 25-43.5 mm section (Table 4.2). In all three soils, APP and APP + FEP treatments had significantly lower pH as compared to the control and to most all other P treatments in the 0-7.5 mm section. Both P fluid treatments had the lowest pH in the 7.5-13.75 mm section as well. These were followed by MAP and MAP + FEP having the next lowest pH, in general, and DAP and DAP + FEP had the highest soil pH.

As stated previously, all P fertilizer treatments received equivalent amounts of P per plate. Since an equal mass of P was added to each plate, both APP and MAP required urea to equalize the amount of N in the DAP treatment, with MAP receiving 1.9 times more urea than the APP. The addition of urea to the soil consumed two moles of protons for each mole of urea being hydrolyzed, which initially resulted in an increase in the soil pH (Khaliwada et al., 2012; Isensee and Walsh, 1971). The solution pH of MAP and MAP + FEP is approximately pH 4.2, and for APP and APP + FEP approximately pH 6.0, in water. The greater amount of urea added with MAP likely produced a higher soil pH initially and this difference may have persisted after subsequent acidification due to nitrification. Over the five-week incubation period, decreases in pH for all P sources as compared to the control were also likely a result of acidification due to

nitrification of ammonium-N contained within the P fertilizers (Westfall and Hanson, 1985). However, this reaction was limited by the overall buffering capacity of the soil carbonates (Hettiarachchi, 2010; Lombi et al., 2004).

Percent P added

For all three calcareous soils, the greatest PPA values were found in the 0-7.5 mm and 7.5-13.75 mm sections with very low values beyond the 13.75 mm section (Fig. 4.8, 4.9, 4.10).

In the Entisol, added P was restricted to the 0-7.5 mm and 7.5-13.75 mm sections of the dish for both P granular and fluid treatments with very similar PPA in the center section for all P sources (Fig.4.8). The treatments appearing to have greater diffusion from the 0-7.5 mm section occurred with MAP, APP, DAP+FEP and APP+FEP with no significant differences in PPA among them. Although there was 10.9% CaCO₃ in this soil, other properties such as the low TOC and CEC, as well as its SL texture, suggests there was little to impede P movement for all treatments from the 0-7.5 mm to the 7.5-13.75 mm section. This may have been the reason for lack of treatment response on PPA in Entisol.

For the Mollisol, regardless of P source, almost all of the added P remained within the 0-7.5 mm section for all treatments (average of 89% of PPA) with significantly less PPA for the APP treatment in the center section (Fig. 4.9). Percent P added values in the 13.75-25 mm section for all treatment were low, however, both fluid P treatments had significantly higher PPA in the 7.5-13.75 mm section as compared to the granular treatments, indicating enhanced P movement. The overall reduction in diffusivity observed with all P treatments in the Mollisol may be the result of a combination of the impediment of P fertilizer movement outward by the silt loam texture and the hygroscopic movement of soil water toward the P granules in the center section. Furthermore, although the percentage CaCO₃ (7.7%) was less than the Entisol (10.9%),

the overall lack of fertilizer diffusion for the Mollisol P treatments suggested Ca-P precipitates formed either immediately around or within the P granule or at the injection point. This shows the importance of other soil parameters on fertilizer P diffusion and P reaction pathways.

In contrast to the Entisol with same soil texture, for the Inceptisol, significantly higher PPA was found in the 0-7.5 mm section for the granular treatments than for either liquid P treatment, indicating limited diffusion of P in granular fertilizers (Fig. 4.10) in the highly calcareous Inceptisol. Diffusion from the point of application was greatest with the APP + FEP treatment as indicated by the lowest percent P added in the center section, which was not significantly lower than the APP, followed by DAP + FEP and DAP. The APP + FEP had significantly higher PPA in the 7.5-13.75 mm section than all other P treatments except for the APP. Lombi et al. (2004) had similar findings in that the concentration of P in the granular MAP treatment remained significantly greater within the 0-7.5 mm section than its liquid equivalent. The general lack of P diffusion from the 0-7.5 mm section for all granular treatments in the Inceptisol may be due in part to the hygroscopic nature of the P granules which pulled soil water toward the granule via mass flow thereby decreasing P diffusion to other dish sections (Lawton and Vomocil, 1954; Hedley and McLaughlin, 2005; Hettiarachchi et al., 2006). Both P fluid treatments were not as impeded by this process and diffused further. The lack of fertilizer P diffusion with the P granular treatments suggests immobilization of fertilizer P possibly as Ca-P precipitation either immediately around or within the P granule which inhibited diffusion from the 0-7.5 mm section.

Although the Mollisol and Entisol contained a similar CaCO₃ content, the two soils had a different texture which greatly affected P fertilizer diffusion from the point of application. The Entisol sample had a sandy loam texture and the pore spaces between granules were large with a

low overall specific surface area, allowing both P granular and fluid fertilizers to diffuse from the point of application to the 7.5-13.75 mm dish section with little resistance (Brady and Weil, 2007). The Mollisol was a silt loam which allowed the soil to retain more water and elements such as P due to its increased surface area and cohesiveness properties compared to the Entisol sample. The movement of P from the fertilizers was physically inhibited allowing increased sorption of the P in the fertilizer by the silt and clay sized particles. This was evidenced for the Mollisol, there was reduced diffusion of both P granular and fluid fertilizers away from its application point.

Moreover, both the Mollisol and Entisol had similar CaCO_3 contents whereas the Inceptisol contained much more CaCO_3 . The differences had a large impact on P fertilizer lability and mobility in the soils. Although the Entisol and Inceptisol had the same soil texture, P diffusion was more inhibited in the Inceptisol compared to the Entisol by the high CaCO_3 content of the Inceptisol. Increasing CaCO_3 content in soils is related to greater P adsorption/precipitation capacity (Bertrand et al., 2003).

Analysis of incubated P Fertilizer Granules

The percentage of mass for Ca, Fe and Al remaining in the residue of incubated MAP or DAP granules was very high. The increase in Ca mass within the residues after incubation suggested the cation migrated inward from the soil (Table 4.3). The Fe and Al were likely insoluble impurities from the original granules that did not dissolve upon incubation since the average mass for both cations after incubation was close to the before-incubation mass. However, there was a great increase in percent of mass of Ca after incubation compared to before for both the MAP and DAP residues in the two calcareous soils. The increase in Ca mass within the residues as compared to the nonincubated granules suggests the cation migrated

inward from the soil. For P, the decrease in P mass with incubation indicates the dissolution and diffusion of the soluble forms of P. However, the similar P concentrations for incubated and nonincubated granules suggests the P exists as a solid phase while that remaining after incubation likely exists as an insoluble component present in the original granule in combination with Ca, Fe, and or Al. The summed molar amount of Ca, Fe and Al (0.034-0.040 mmol) remaining in the MAP and DAP granules in each of the calcareous soils corresponds with the molar quantity of P (0.03-0.04 mmol) remaining in the incubated granules. Lombi et al. (2004) had similar findings in that they found a significant percentage (12.3%) of P remained within MAP granules in a five-week incubation study in a highly calcareous soil from Eyre Peninsula, South Australia. Iron and Al were found within the residues as well. Montalvo et al. (2014) summed up the molar amounts of Ca, Fe and Al remaining within various incubated P fertilizer granules, including MAP and DAP, from a calcareous Inceptisol and found the total molarity approximately matched the molarity of P remaining in the incubated granules. Increased Ca within the residual granules most likely migrated in from the calcareous soil. Regardless of the varying CaCO_3 content in the calcareous soils, we noted a similar behavior in the moderately calcareous soils as well.

Energy dispersive X-ray spectroscopy dot maps of cross-section of DAP granule and a MAP granule incubated five weeks in the Inceptisol reveal the distribution of Ca, P, Fe and Al inside the granule as per the different colors in the EDS formation (Fig. 4.11). The P appears to be evenly distributed throughout each incubated granule. The appearance of Ca-P reaction products is indicated by the yellow-colored areas along the inside edge of the granule. The evidence lends additional support to the lack of P diffusion from the point of application for P granular fertilizer and migration of soil Ca into the granule in the calcareous soil. Scanning

electron microscopy micrographs of cross-sectioned view of nonincubated and incubated MAP and DAP granules in the Entisol shows possible reaction products formed within the partially dissolved incubated P granule (Fig. 4.12).

Percent Resin P

In general, due to a lower pH from the added P fertilizer and a subsequently higher P solubility in the center two rings, PRP values were greater in the 0-7.5 mm and 7.5-13.75 mm sections, with PRP values decreasing in the 13.75-25 mm and 25-43.5 mm sections.

The PRP results for the Entisol indicate that APP and APP + FEP had significantly greater PRP in the 0-7.5 mm section than all other treatments (Fig. 4.13). Increased PRP in the 0-7.5 mm section for both fluid treatments may partly be due to the lower solution pH of APP in the 0-7.5 mm section, allowing for greater P solubility. Additionally, APP is composed of ortho-, pyro- and polyphosphates forms. It has been found that pyrophosphate hydrolysis reactions decreased for APP in highly calcareous soils due to decreases in pyrophosphatase enzyme action, there is a negative correlation of the measured CaCO_3 equivalent in the soil (Stott et al. 1985; Tabatabai and Dick, 1979). For the P fluid treatments, this may have allowed for added diffusion and a delay in P fixation. The APP and APP + FEP treatments showed significantly greater P solubility in the 7.5-13.75 mm sections compared to all other treatments.

The lack of mass flow of water toward the point of application with the fluid treatments allowed for greater ammonium-N and P diffusion out to other section(s) as compared to the granular treatments in the Entisol. There were no significant differences in PRP for APP and APP + FEP in the 0-7.5 mm section, the PRP for the APP + FEP treatment was greater than the APP treatment. However, APP showed significantly more PRP in the 7.5-13.75 mm section as compared to APP + FEP. Both the DAP and DAP + FEP treatments contained significantly less

PRP in the 0-7.5 mm section as compared to all other P treatments. Although there was enhanced PPA in the center section for both DAP and DAP + FEP treatments, the significant decrease in PRP may be the combination of processes such as increased solution pH of dissolving DAP, which decreased P solubility within the granule, coupled with Ca migrating via mass flow toward the hygroscopic granule resulting in an incomplete dissolution of the P fertilizer granule and rapid P precipitation with the Ca and adsorption on the insoluble Fe and Al compounds within the granule.

Although the APP treatment had significantly greater PRP than all other treatments in the 7.5-13.75 mm section, overall PRP values were much lower than those in the 0-7.5 mm section regardless of P source. The reduction in PRP in the 7.5-13.75 mm section and beyond for all P sources may be the result of decreased fertilizer P diffusion with distance as a result of both precipitation and adsorption reactions. As granular P fertilizer diffused away from the application point, the change in fertilizer solution pH resulted in precipitation reactions with Ca, Fe cations in the soil solution. The decrease in P concentration as the fertilizer moved into the 7.5-13.75 mm section resulted in more adsorption reactions. With fluid P fertilizers, the quick diffusion into the soil reduced the chance of precipitation and adsorption reactions in the 0-7.5 mm and 7.5-13.75 mm sections thereby increasing P fertilizer availability (Hedley and McLaughlin, 2005).

The results for the Mollisol were very similar to the Entisol in that PRP was significantly greater for both P fluid treatments over the granular treatments in the 0-7.5 mm sections (Fig. 4.14). Both APP and APP + FEP treatments had significantly more available P in the 0-7.5 mm section than the other P treatments, and neither were significantly greater than the other. Similarly to the Entisol, the increased PRP in the center section for both fluid treatments most

likely due to same reasons discussed before. Very similar to the Entisol, both DAP and DAP + FEP treatments produced significantly less PRP in the center dish section as compared to all other P treatments for reasons discussed previously.

However, there are a couple key differences between the PRP in the Entisol and Mollisol. The P treatments in the 7.5-13.75 mm section of the Entisol in this study contained a greater overall PRP as compared to the PRP for the Mollisol. The Mollisol had a SiL texture, the finer texture likely slowed the P diffusion, and there may have been opportunities for increased P adsorption onto mineral surfaces and CaCO_3 , as the wetting front moved slowly through this soil, resulting in decreased PRP.

Percent resin P results for the Inceptisol were different than the results for the other two calcareous soils; although influenced by added P, PRP was low for all P sources in all dish sections; there were no PRP values above 17% in the 0-7.5 mm section (Fig. 4.15). In fact, PRP in all P treatments in the 0-7.5 mm and 7.5-13.75 mm sections of the Inceptisol approximately equaled the low PRP values observed for the DAP and DAP + FEP treatments in Mollisol and Entisol. The results indicated that the high CaCO_3 concentration in the Inceptisol exacerbated the reduction in P availability/extractability. There were no significant differences in PRP between treatments receiving P fertilizer in the 0-7.5 mm section of the Inceptisol. Though the effect of P fertilizer treatment on soil pH in all four dish sections was comparable to the pH values in the other two calcareous soils used in the study, the differences may be due the high reactive CaCO_3 content (83.3%) in this soil compared to the other soils. As a result, the high CaCO_3 concentration might have created an environment highly conducive to P sorption for all P sources, with and without FEP, near the point of application. Although the differences were small (~6%), in the 7.5-13.75 mm section APP, MAP + FEP, DAP + FEP and APP + FEP had

significantly greater PRP than the MAP and DAP alone, suggesting some positive influence of FEP for MAP and DAP in this soil. Both MAP and DAP treatments had significantly less PRP in the 7.5-13.75 mm section as compared to all other P treatments. This probably was due to limited P movement into the 7.5-13.75 mm section. Although the differences were small between P treatments in the 13.75-25 mm section, APP had significantly more PRP than any other treatment. In the 25-43.5 mm section all P treatments were similar in PRP as compared to the control. Though Lombi et al. (2004) used an isotope dilution technique for gathering labile P data from a highly calcareous soil from South Australia, containing approximately 67% effective CaCO_3 , the values reported were somewhat similar, but slightly higher than those found in this study.

X-ray Absorption Near-Edge Structure Analysis

The purpose of the bulk XANES data collection was to directly identify solid phases formed from the addition of the different P sources. All P K-edge bulk XANES spectra and linear combination fitting results for the three soils used in the study are shown in Fig. 4.16, 4.17, 4.18, and Table 4.4. Overall, for treatments receiving P fertilizer, P speciation was dominated by Ca-P followed by Al- and/or Fe-adsorbed P.

The nonfertilized control treatment in the Entisol contained P primarily as Ca-P (68.6%) with the remaining being Fe-associated P, either Fe adsorbed P or Fe-P (Fig. 4.16, Table 4.4). Calcium carbonates are an important factor controlling P solubility of fertilizer added to calcareous soils (Sample et al., 1980). Furthermore, P fertilizer adsorbed to Fe oxide forms are not uncommon in calcareous soils (Bertrand et al., 2003; Ryan et al., 1985). It was clear that MAP and DAP treatments contained more Ca-P and less Fe-associated P as compared to APP. The increased Ca-P for the two granular treatments compared to APP was not reflected in P

movement for the three P sources. However, significantly less PRP was observed for MAP and DAP treatments compared to the APP treatment and increased Ca-P most likely was the reason for decreased PRP as compared to the APP treatment. The increased Fe-adsorbed and Fe-P for the APP treatment did not seem to have a negative influence in terms of P extractability in the center dish section. In comparing the non-FEP fertilizer treatments to the FEP-added treatments, both MAP + FEP and DAP + FEP treatments contained a smaller percentage Ca-P and greater percentage of Fe-associated P compared to MAP and DAP, although all granular treatments had very similar P movement and P extractability. Both DAP and DAP + FEP treatments had the highest percentages Ca-P compared to all P treatments and lowest PRP in the first dish section (Fig 4.13). A saturated solution of DAP would have a much higher solution pH than a saturated solution of MAP. This increased pH may have led to a greater percentage of precipitated Ca-P species subsequently resulting the decreased PRP compared to the other P treatments. In comparing the APP and APP + FEP treatments, both contained the lowest percentages Ca-P compared to all P treatments, with APP containing less Fe-adsorbed P but a greater percentage Fe-P compared to APP + FEP. Both fluid P treatments had similar P movement from the center section and significantly greater PRP compared to the P granular treatments in the first dish section. The increased Fe-associated P for the APP + FEP treatment did not appear to have a negative impact in terms of P extractability in the first dish section compared to APP. Although the P K-edge XANES data showed differences in P speciation when comparing non-FEP to FEP-added treatments, this was not reflected as significant increases in P movement or extractability between the treatments. Overall, less PRP observed for the P granular treatments compared to both P fluid treatments suggests Ca-P is negatively associated with P extractability and the likely reason for decreased PRP compared to the P fluid treatments.

The nonfertilized control treatment in the Mollisol contained P as Ca-P and Al-adsorbed P (Fig. 4.17, Table 4.4). The P fertilizer treatments resulted in changes in the percentage of Ca-P and Al-adsorbed P and an increase in Fe-P for MAP and APP treatments (Fig. 4.17, Table 4.4). Both MAP and DAP treatments contained more Ca-P compared to APP; in addition, MAP contained almost half of its total P percentage as Fe-P, DAP contained Al-adsorbed P, while APP contained both Al- and Fe-associated P. The Ca-P coupled with the various percentages of Fe-P and Al-adsorbed P may be partial reason for decreased P movement into the second dish section for the three P treatments. However, decreased PRP observed in the center section for the two P granular treatments in comparison to the APP treatment suggests the greater Ca-P percentage may be negatively associated with resin extractability. In comparing the non-FEP added fertilizer treatments to the FEP-added treatments, both the MAP + FEP and DAP + FEP treatments contained a greater percentage Ca-P and DAP + FEP had a similar percentage of Al-adsorbed P compared to DAP. All granular treatments had very similar P movement, MAP had greater P extractability compared to MAP + FEP. This may have been because MAP + FEP contained more Ca-P over MAP. Both DAP and DAP + FEP treatments had the highest percentages Ca-P compared to all P treatments and both had the lowest PRP in the first dish section (Fig 4.14). The reason for this was stated previously in the Entisol P K-edge XANES section. Comparing the APP and APP + FEP treatments, both contained lower percentages Ca-P compared to the four granular treatments. Both fluid P treatments had similar P movement from the first dish section and significantly greater PRP in the first dish section compared to the P granular treatments. For APP, the increased Al- and Fe-associated P did not seem to have a negative impact in terms of P extractability. Although the P K-edge XANES data showed some differences in P speciation and percentages when comparing non-FEP to FEP-treated soils, this

was not reflected as significant increases in P movement or extractability for the treatments with the exception of MAP. For all three P sources in the Mollisol, the FEP-added treatments had very comparable percentages of Ca-P species with the non-FEP added treatments and did not appear to have an advantage compared to the FEP-added treatments. Overall, less PRP observed for the P granular treatments compared to both P fluid treatments suggests Ca-P is negatively associated with P extractability and the likely reason for decreased PRP compared to the P fluid treatments.

The nonfertilized control treatment in the Inceptisol contained high percentage Ca-P and a limited amount of Al-adsorbed P (Fig. 4.18, Table 4.4). The P fertilizer treatments resulted in the addition of Al-adsorbed P and a small percentage of Fe-adsorbed P solid-phase crandallite ($\text{CaAl}_3(\text{PO}_{3.5}(\text{OH})_{0.5})_2$), in addition to the Ca-P and Al-adsorbed P. Similar to the Entisol and Mollisol, both granular P treatments contained higher amounts of Ca-P as compared to the APP treatment. More so than the percentages Al-adsorbed P and crandallite, the increased percentage Ca-P resulted in less P movement from the center section for both P granular treatments compared to APP. A comparison of the non-FEP fertilizer treatments and the FEP-added treatments, the FEP-added treatments overall contained a higher percentage Ca-P and generally more Al-adsorbed P than the non-FEP added treatments. However, MAP and APP contained small percentages of crandallite and Fe-adsorbed P, respectively. Similar PPA and PRP for MAP and MAP + FEP, DAP and DAP + FEP, are the results of high percentage of Ca-P limited P movement and lability. Comparing APP to APP + FEP, both contained a small amount less of Ca-P and more Al-adsorbed P which may have been the reason for the increased P movement from the center dish section. However, it did not increased P lability for either P fluid treatment.

The non-FEP added fertilizer treatments contained relative Ca-P percentages very comparable to the FEP-added fertilizer treatments in this highly calcareous Inceptisol, and appeared to be no advantage to using the FEP-added fertilizer over the non-FEP added fertilizer.

The overall relationship between Ca-P and PRP on the soils was negative (Fig. 4.19). The results of the linear regression clearly illustrated that the fraction of added P remaining potentially plant available greatly decreased as the percentage of Ca-P-like species increased. This relationship in the three very different calcareous soils suggests that as added P from the non-FEP fertilizer or FEP-added fertilizer is converted to Ca-P like species, it may no longer be extractable or available for plant uptake.

Conclusions

Phosphorus diffusion in these calcareous soils, as measured using PPA, was limited and generally did not move beyond the 7.5-13.75 mm section for the granular P treatments. However, for the P liquid treatments, there was evidence of greater movement of P into the 13.75-25 mm section. Overall, there was no evidence the FEP-added treatments consistently increased PPA over the non-FEP added treatments for the three soils tested.

The effect of P source on lability was dependent on the soil type. In general for both the Entisol and Mollisol, the fluid P treatments had increased PRP compared to the granular P sources. Percent resin P for the Inceptisol was limited and values were comparable for both P granular and P fluid treatments. There was no consistent enhanced P diffusion and lability from the FEP-added fertilizer additions on the calcareous soils.

Phosphorus speciation in the three soils was variable. The addition of P fertilizer resulted in the presence of Ca-P, Fe- and/or Al-P, and Al- and/or Fe-adsorbed P. There was evidence that percentage Ca-P influenced PRP and the fraction of P as Ca-P was significantly and negatively

correlated with PRP. The results suggest there was a benefit of using a fluid P source over a granular source both in highly and moderately calcareous soils. The formation of the Ca-P species was the dominant mechanism for P fixation. The Fe- and Al-adsorbed P and Fe- and Al-P species likely contributed to the lack of mobility and lability to a much lesser extent.

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Tables and Figures

Table 4.1 Selected initial properties of soils

Properties	Entisol†	Mollisol	Inceptisol
pH	8.5	8.7	8.7
Mehlich-3 P (mg kg ⁻¹)	32	16	39
CEC (cmol _c kg ⁻¹)	7.0	18.4	5.9
TOC (%)	0.42	0.94	6.0
Total P (mg kg ⁻¹)	468	662	691
Mineralogy	Quartz, clay mica, kaolinite, vermiculite	Quartz, clay mica, kaolinite, smectite, calcite	Calcite
CaCO ₃ (%)	10.9	7.7	83.3
Fe _{CBD} (mg kg ⁻¹)	1411	2960	4080
Al _{CBD} (mg kg ⁻¹)	146	187	724
Textural class	SL	SiL	SL

†Soil order: United States soil taxonomy classification; CaCO₃: Calcium Carbonate; Fe_{CBD}, Al_{CBD}: Citrate-bicarbonate-dithionite extractable Fe or Al concentration; pH: (1:10) in water; TOC: Total organic carbon; Soil texture: SL=sandy loam, SiL=Silt loam.

Table 4.2 Soil pH after 35-days incubation of three phosphorus fertilizer sources applied to the A horizon of three different soils, an Entisol, Mollisol and Inceptisol, alone or in combination with a fertilizer enhancement product at four distances from the point of fertilizer placement.

Treatment †	Distance from the point of fertilizer placement (mm)			
	0-7.5	7.5-13.75	13.75-25	25-43.5
Entisol				
Control	8.53a‡	8.71a	8.72a	8.65a
MAP†	7.58cd	8.24bc	8.55b	8.57a
DAP	8.07b	8.46ab	8.56b	8.58a
APP	7.38d	8.00c	8.41b	8.56a
MAP + FEP	7.64c	8.45ab	8.52b	8.53a
DAP + FEP	7.99b	8.47ab	8.56ab	8.58a
APP + FEP	7.38d	8.05c	8.51b	8.59a
Mollisol				
Control	8.68a	8.77a	8.70a	8.77a
MAP	8.04bc	8.49b	8.56ab	8.64b
DAP	8.28b	8.54ab	8.53ab	8.62b
APP	7.58d	8.13c	8.46b	8.66b
MAP + FEP	7.95bc	8.56b	8.63ab	8.67ab
DAP + FEP	8.30b	8.48b	8.62ab	8.65b
APP + FEP	7.78cd	8.49b	8.47b	8.68b
Inceptisol				
Control	8.70a	8.70a	8.72a	8.75ab
MAP	8.23cd	8.54c	8.62bc	8.65bc

DAP	8.14d	8.55c	8.67ab	8.67abc
APP	7.72f	8.35d	8.56c	8.56c
MAP + FEP	8.33bc	8.62ab	8.71a	8.77a
DAP + FEP	8.40b	8.63ab	8.72a	8.77a
APP + FEP	7.96e	8.43cd	8.62c	8.60c

†MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product. ‡Means within a soil and dish section with the same letter are not significantly different at P = 0.05 using Tukey's honest significance test. Means within a column with the same letter are not significantly different at P=0.05.

Table 4.3 Elemental analysis of MAP and DAP granules incubated in the Entisol and Mollisol. Weight and chemical composition of fertilizer granules after 35 days incubation.

Element	-----MAP†-----			-----DAP-----		
	Non- incubated	Incubated	Fraction remaining ‡	Non- incubated	Incubated	Fraction remaini ng†
%	-----g kg ⁻¹ -----		%	-----g kg ⁻¹ -----		
Entisol						
Al	10.3	48.7	97.9	8.5	49.3	98.7
P	251.0	128.2	13.2	195.0	105.7	9.2
Ca	2.4	87.9	774.3	2.5	82.3	565.6
Fe	8.2	37.9	95.1	7.0	38.4	92.7
Mollisol						
Al	10.3	56.7	98.6	8.5	58.2	89.8
P	200.6	130.7	11.7	194.1	125.3	8.4
Ca	2.3	80.1	610.4	2.5	91.3	484.1
Fe	8.2	40.4	87.7	7.0	45.3	84.2

†MAP = monoammonium phosphate; DAP = diammonium phosphate; ‡Based on the mass present in nonincubated granule and mass remaining in the granule residue after incubation.

Table 4.4 Entisol, Mollisol and Inceptisol P K-edge XANES on 0-7.5 mm sections after 5-week incubation. The table presents relative proportions of adsorbed and precipitated P solid phase minerals expressed as a percentage. Energy range: 2.14 to 2.18 keV.

Treatment†	Ca-P	Al-P	Al-Adsorbed P	Fe-P	Fe-Adsorbed P	Crandallite	RCS‡
------%-----							
Entisol							
Control	68.6	- §	-	-	31.4	-	0.06
MAP	59.2	-	-	9.8	31.0	-	0.02
DAP	64.1	-	-	-	35.9	-	0.04
APP	27.8	-	-	24.0	48.2	-	0.04
MAP + FEP	43.2	-	-	20.2	36.6	-	0.06
DAP + FEP	57.1	-	-	-	42.9	-	0.06
APP + FEP	32.0	7.1	-	-	61.0	-	0.16
Mollisol							
Control	72.1	-	27.9	-	-	-	0.02
MAP	54.5	-	-	45.5	-	-	0.006
DAP	79.2	-	20.8	-	-	-	0.03
APP	47.5	-	22.1	30.4	-	-	0.01
MAP + FEP	74.7	-	25.3	-	-	-	0.007
DAP + FEP	78.1	-	21.9	-	-	-	0.03
APP + FEP	64.5	-	35.6	-	-	-	0.02
Inceptisol							
Control	74.1	-	25.9	-	-	-	0.004
MAP	67.0	-	17.8	-	-	15.2	0.004
DAP	70.2	-	29.8	-	-	-	0.002

APP	55.6	-	33.7	-	10.7	-	0.005
MAP + FEP	75.8	-	24.2	-	-	-	0.002
DAP + FEP	73.5	-	26.5	-	-	-	0.002
APP + FEP	62.5	-	37.6	-	-	-	0.005

†MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product. ‡RCS is reduced chi-square and statistically represents the goodness of fit of the data with the P standards. The RCS is low when the fit is good. § Not a relevant solid phase in LCF.

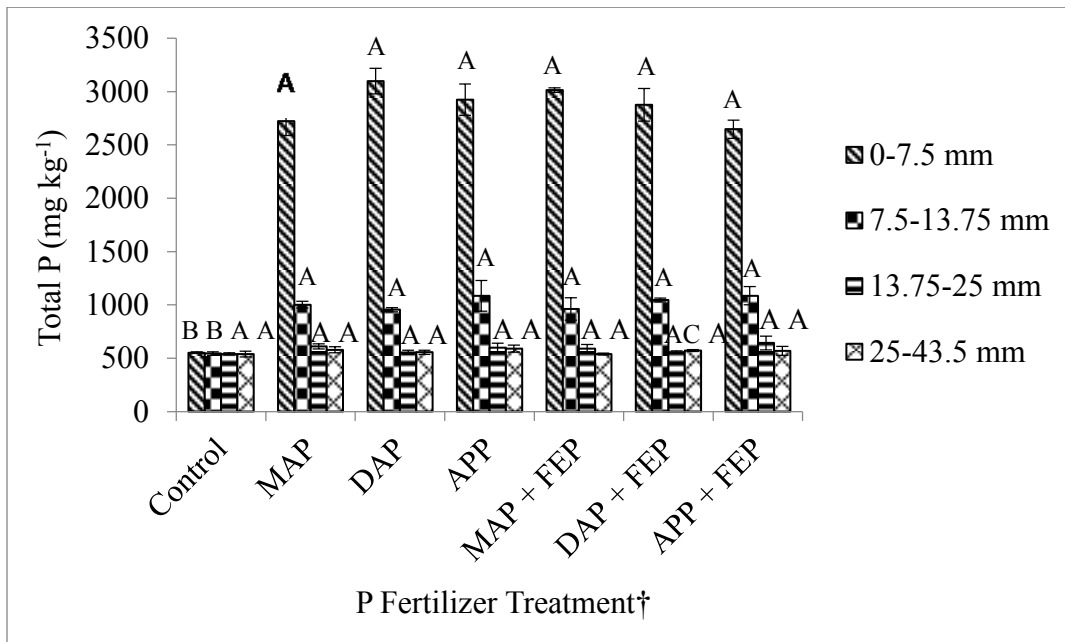


Figure 4.1 Entisol total P concentrations in each dish section for all treatments. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

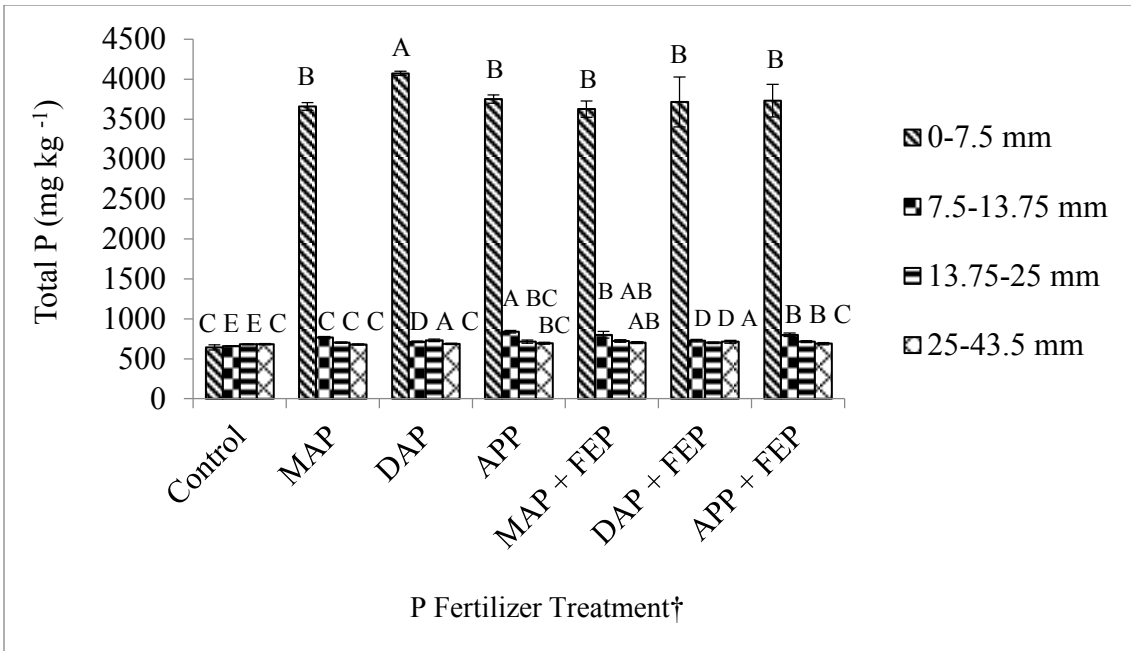


Figure 4.2 Mollisol total P concentration in each dish section for all treatments. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + fertilizer enhancement product = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

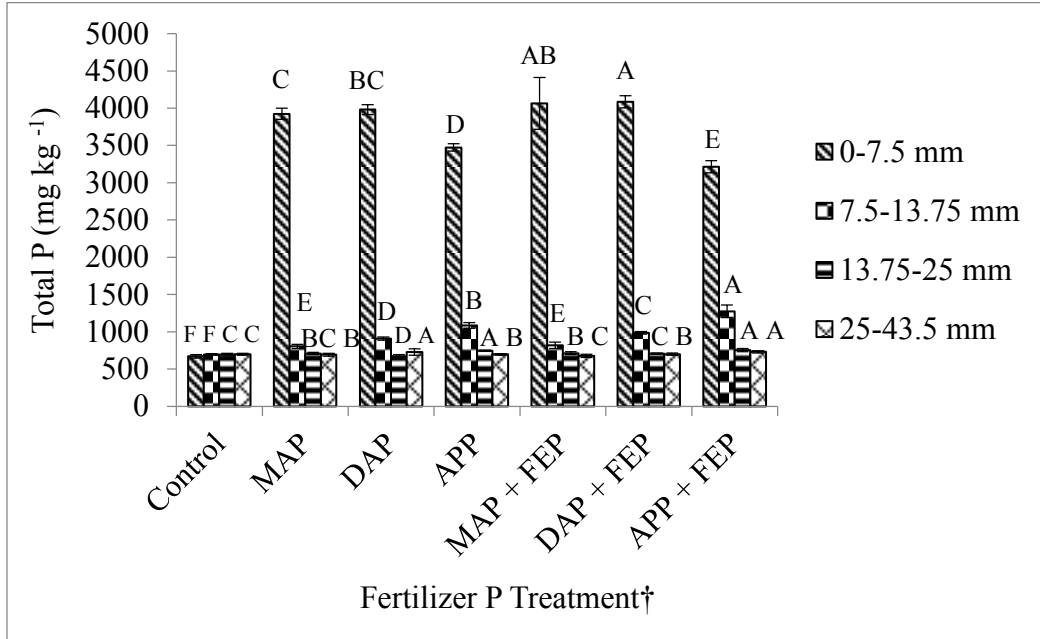


Figure 4.3 Inceptisol total P concentration in each dish section for all treatments. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

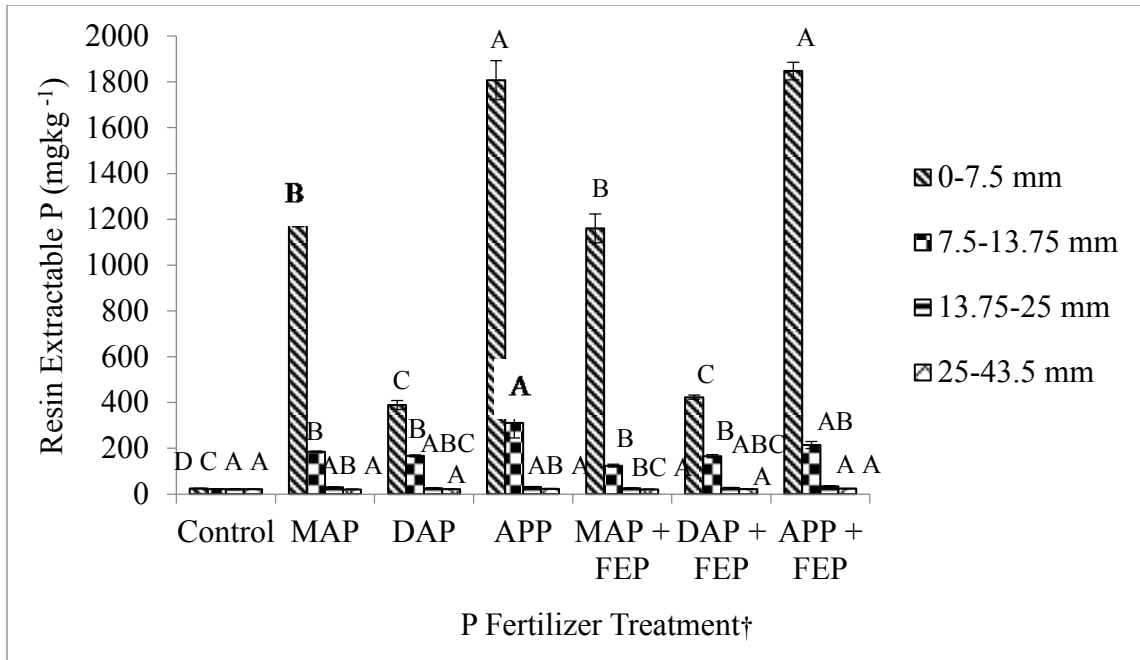


Figure 4.4 Entisol resin extractable P concentration in each dish section for all treatments. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

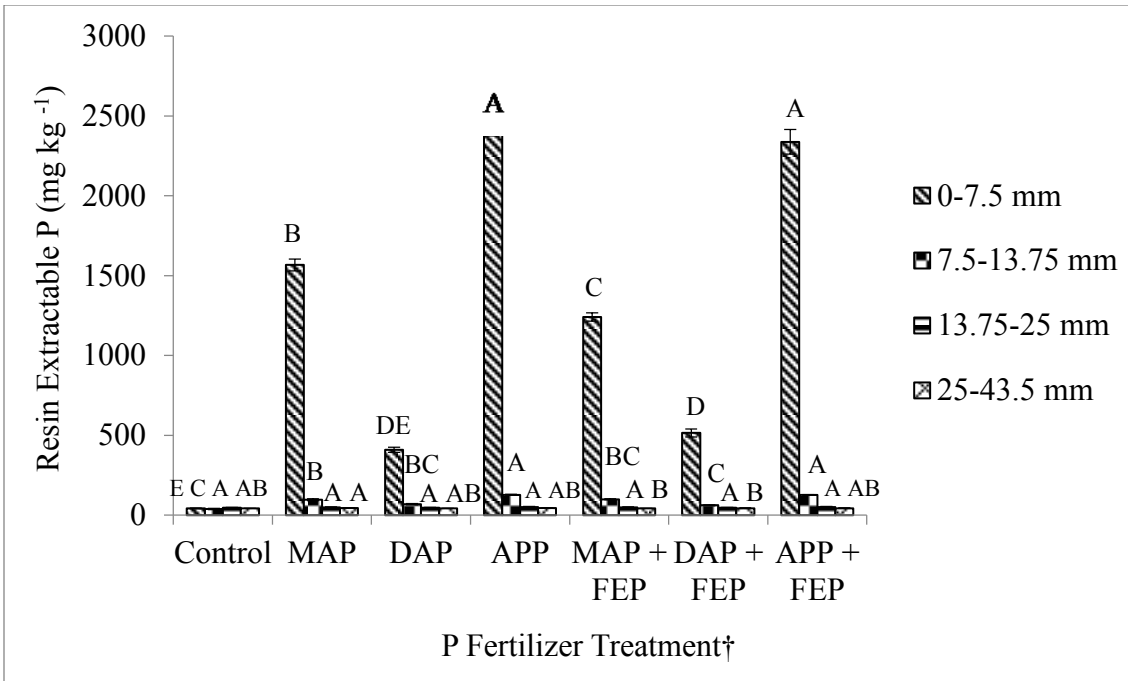


Figure 4.5 Mollisol resin extractable P concentration in each dish section for all treatments. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

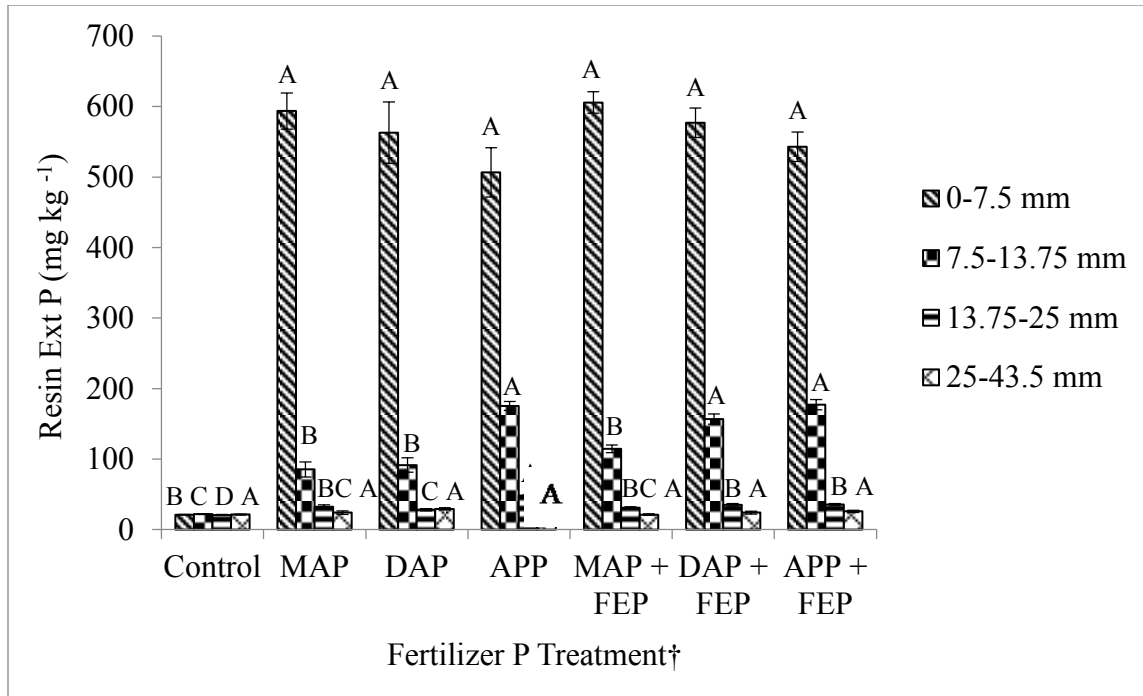


Figure 4.6 Inceptisol resin extractable P concentration in each dish section for all treatments. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

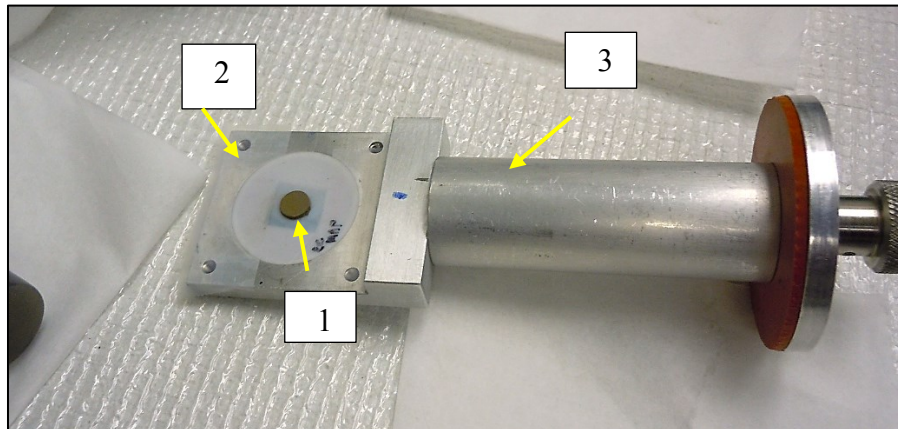


Figure 4.7 Sample preparation for P K-edge XANES data collection.

(1) 4-mm size soil pellet, (2) Teflon disk, (3) aluminum sample holder.

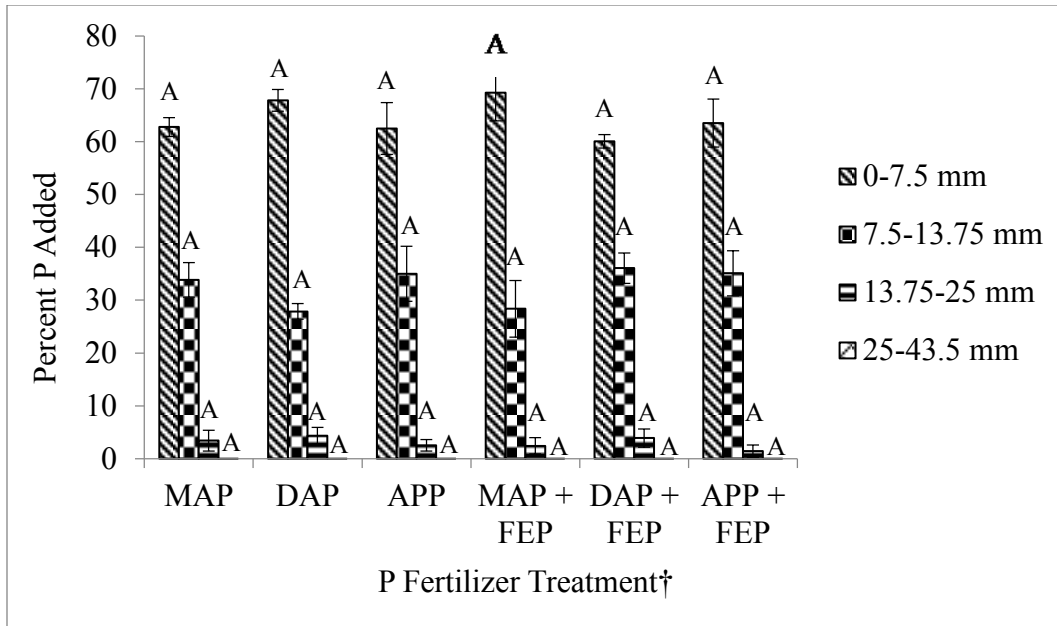


Figure 4.8 Entisol Percent P Added (PPPA) calculated for each dish section for all treatments, where PPA is defined as follows: $PPA = \left[\frac{(P_f)S_i \times M_i}{\sum_{i=1-4} [(P_f) S_i \times M_i]} \right] \times 100$ Where i = dish section (1-4), $(P_f)S_i$ = the concentration of P fertilizer in each dish section, and M_i = the mass of soil in each dish section. $(P_f)S_i$ is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

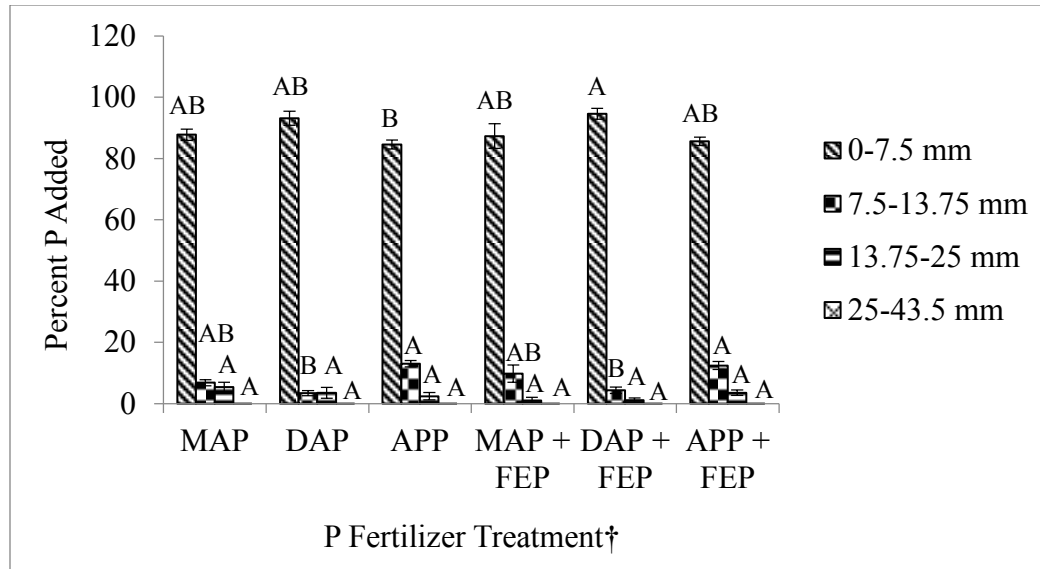


Figure 4.9 Mollisol Percent P Added (PPA) calculated for each dish section for all treatments, where PPA is defined as follows: $PPA = \left[\frac{[(P_f)S_i \times M_i}{\sum_{i=1-4} [(P_f) S_i \times M_i]} \right] \times 100$ Where $i =$ dish section (1-4), $(P_f)S_i =$ the concentration of P fertilizer in each dish section, and $M_i =$ the mass of soil in each dish section. $(P_f)S_i$ is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

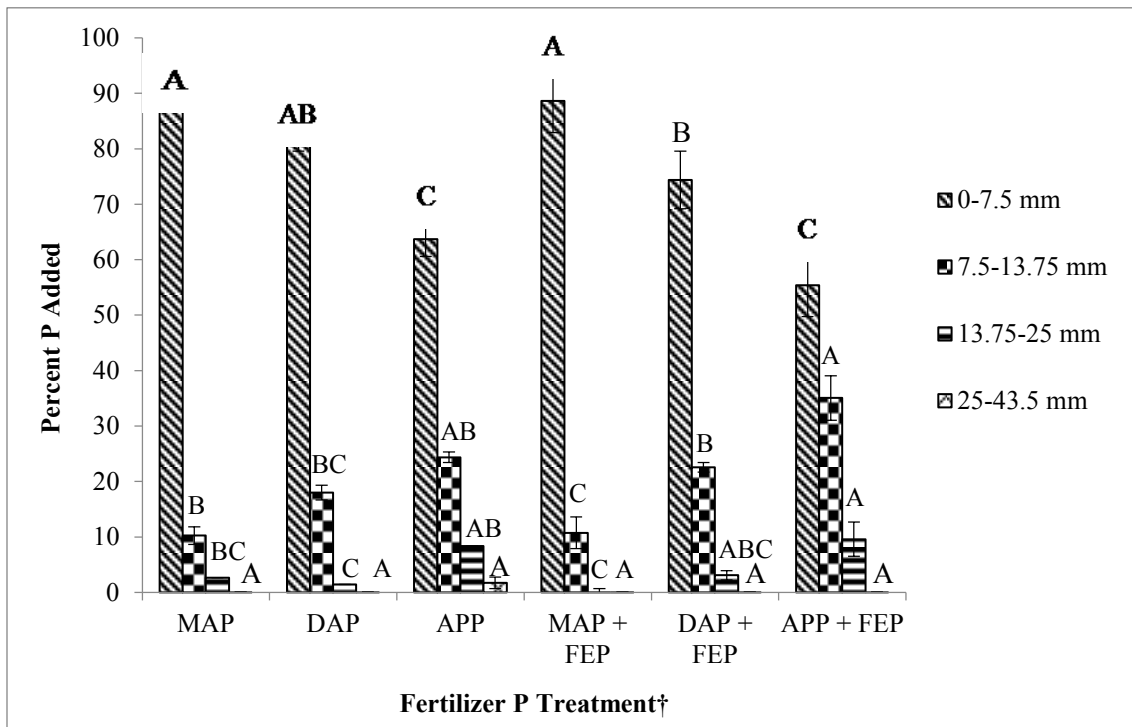


Figure 4.10 Inceptisol Percent P Added (PPA) calculated for each dish section for all treatments, where PPA is defined as follows: $PPA = \left[\frac{[(P_f)S_i \times M_i]}{\sum_{i=1-4} [(P_f) S_i \times M_i]} \right] \times 100$. Where i = dish section (1-4), $(P_f)S_i$ = the concentration of P fertilizer in each dish section, and M_i = the mass of soil in each dish section. $(P_f)S_i$ is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP +

FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

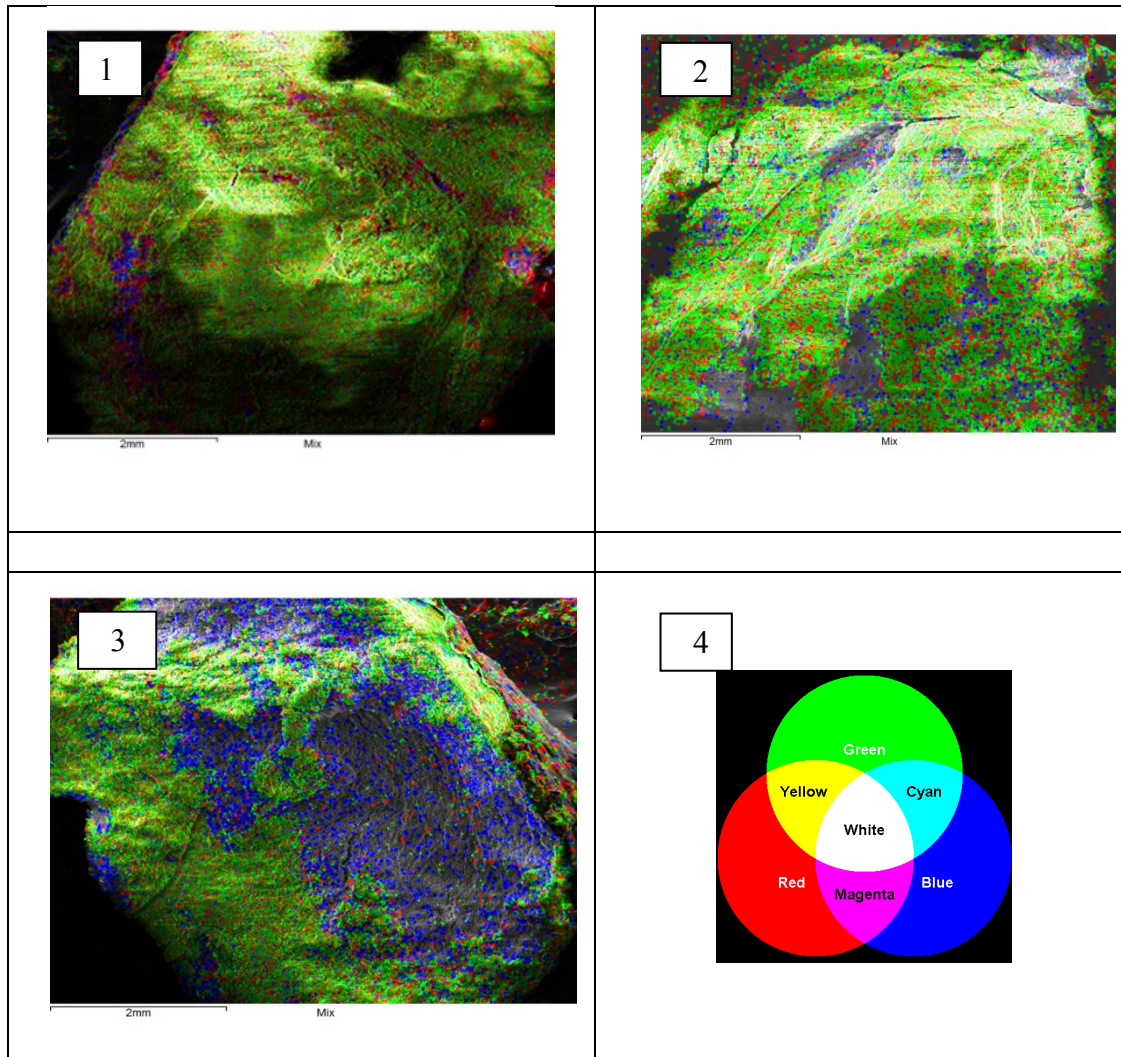


Figure 4.11 Energy dispersive microscopy dot-maps of diammonium phosphate (DAP) and monoammonium phosphate (MAP) granules incubated for 35 days in Inceptisol. (1) Cross section of DAP granule (2) Cross section of MAP granule (3) Cross section of DAP granule (4) The colors represent the distribution of Ca, P and Fe within the granule. Green=P; Red=Ca; Blue=Fe.

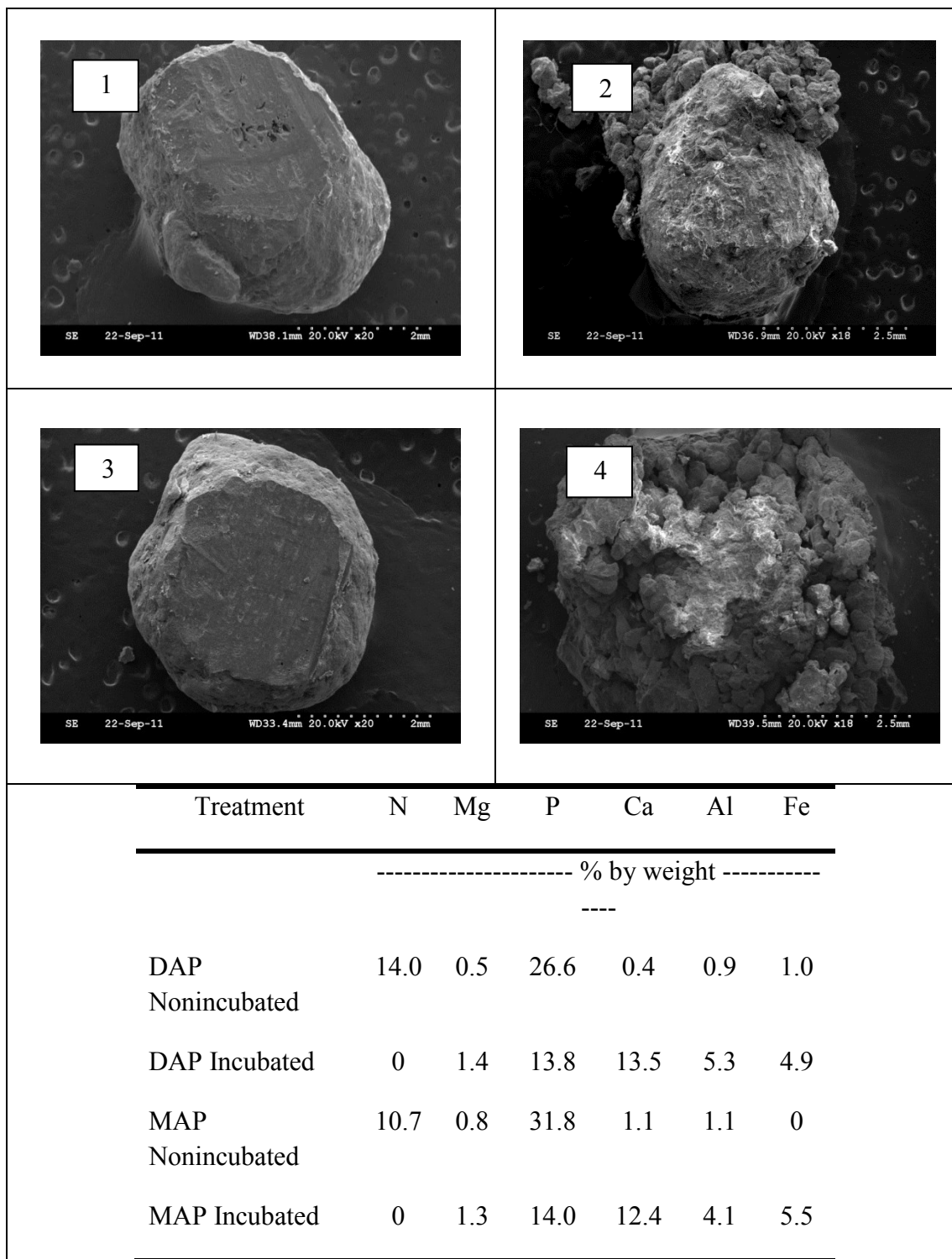


Figure 4.12 Secondary electron micrographs of monoammonium phosphate (MAP) and diammonium phosphate (DAP) granules, nonincubated and incubated for 35 days in the

Oxisol, and elemental analysis. (1) Nonincubated MAP granule (2) Incubated MAP granule (3) Nonincubated DAP granule (4) Incubated DAP granule.

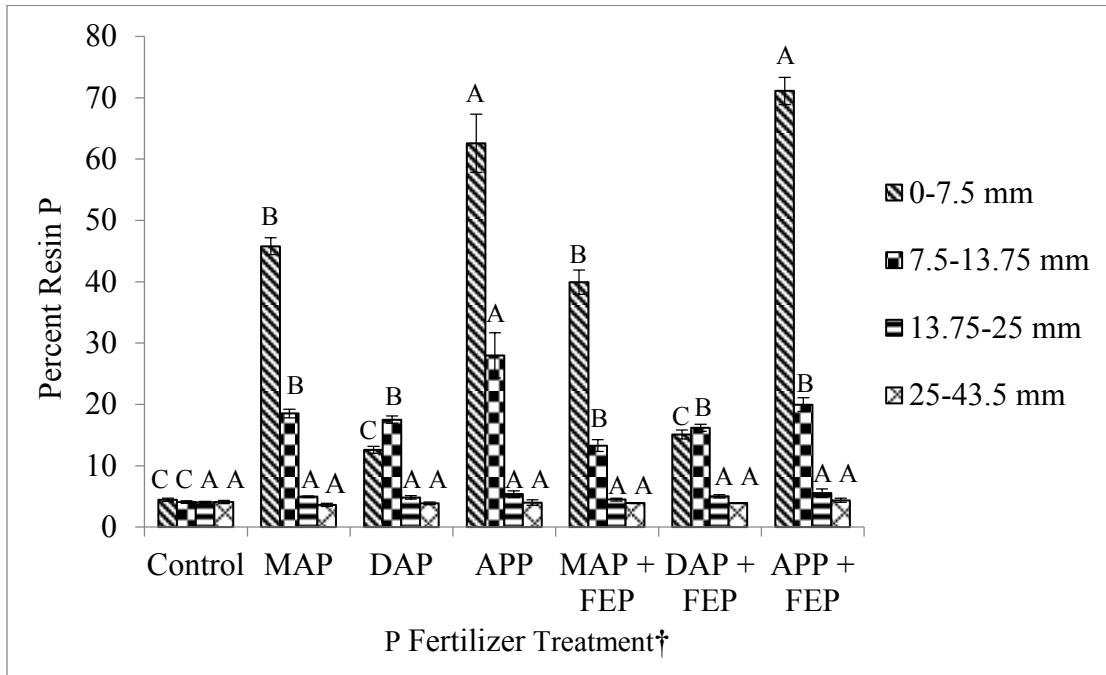


Figure 4.13 Entisol Percent Resin P (PRP) calculated for each dish section for all treatments where PRP is defined as follows: $PRP_i = (\text{resin extractable } P_i / \text{total } P_i) \times 100$. Where $i =$ dish section (1-4), resin extractable $P_i =$ the resin P concentration, and total $P_i =$ total P concentration. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test.

†MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

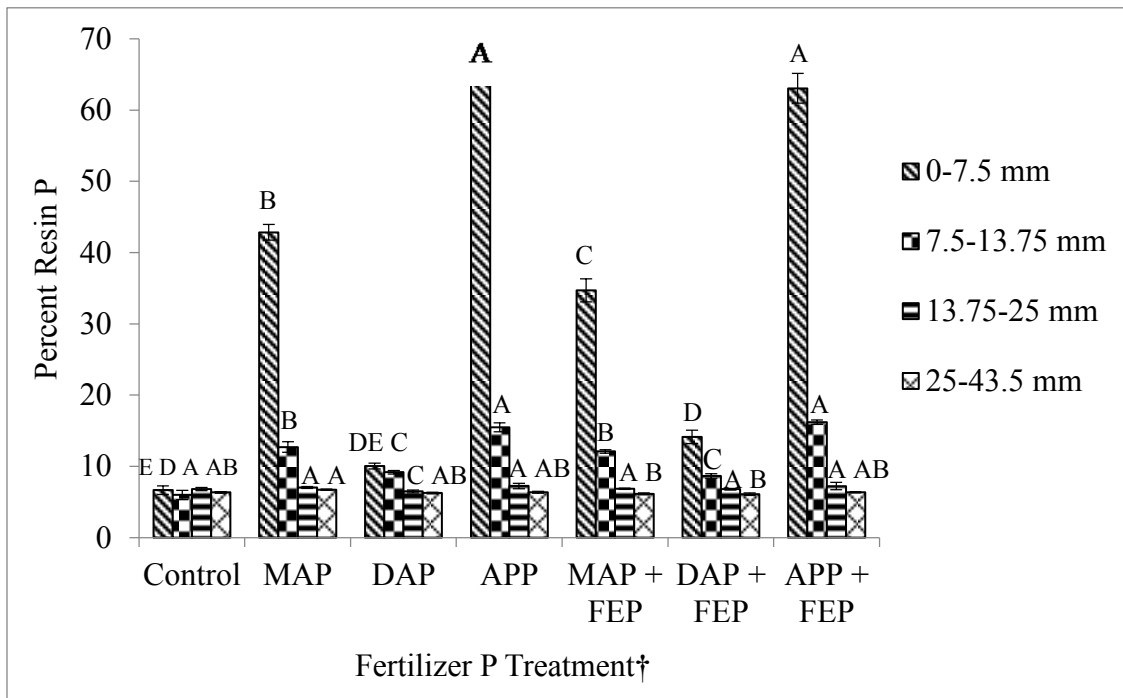


Figure 4.14 Mollisol Percent Resin P (PRP) calculated for each dish section for all treatments where PRP is defined as follows: $PRP_i = (\text{resin extractable } P_i / \text{total } P_i) \times 100$. Where $i = \text{dish section (1-4)}$, resin extractable $P_i = \text{the resin P concentration}$, and total $P_i = \text{total P concentration}$. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

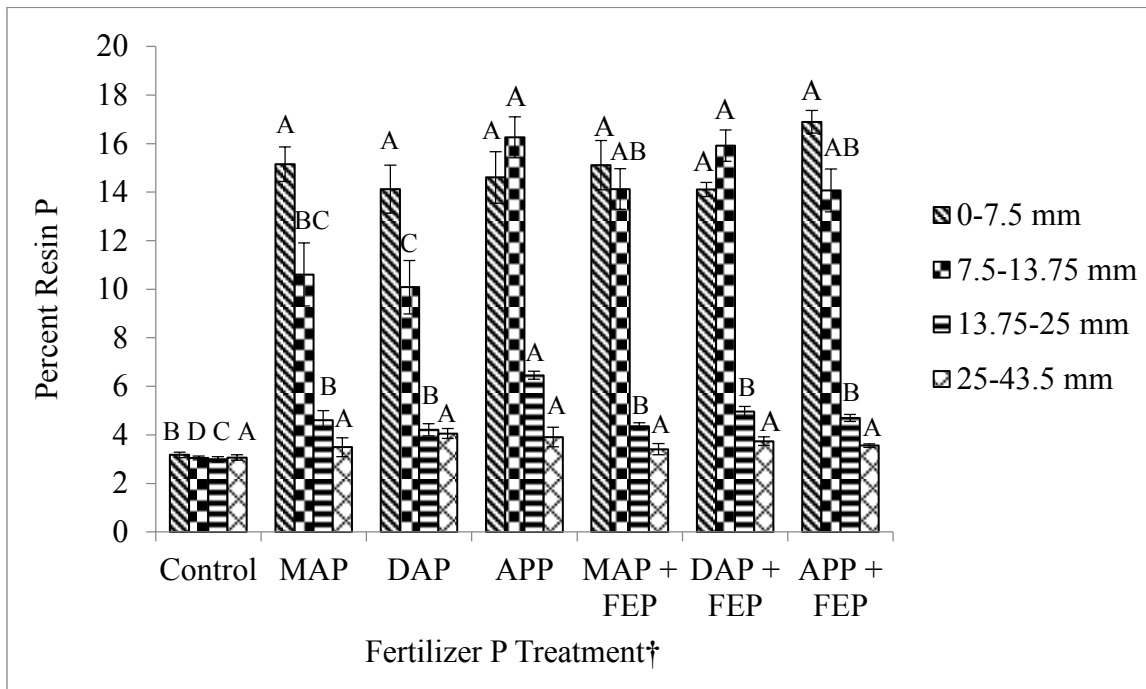


Figure 4.15 Inceptisol Percent Resin P (PRP) calculated for each dish section for all treatments where PRP is defined as follows: $PRP_i = (\text{resin extractable } P_i / \text{total } P_i) \times 100$. Where i = dish section (1-4), resin extractable P_i = the resin P concentration, and total P_i = total P concentration. Standard error bars were averaged from the five replications for each dish section. Means within a soil section for each treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance test. †MAP = monoammonium phosphate DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

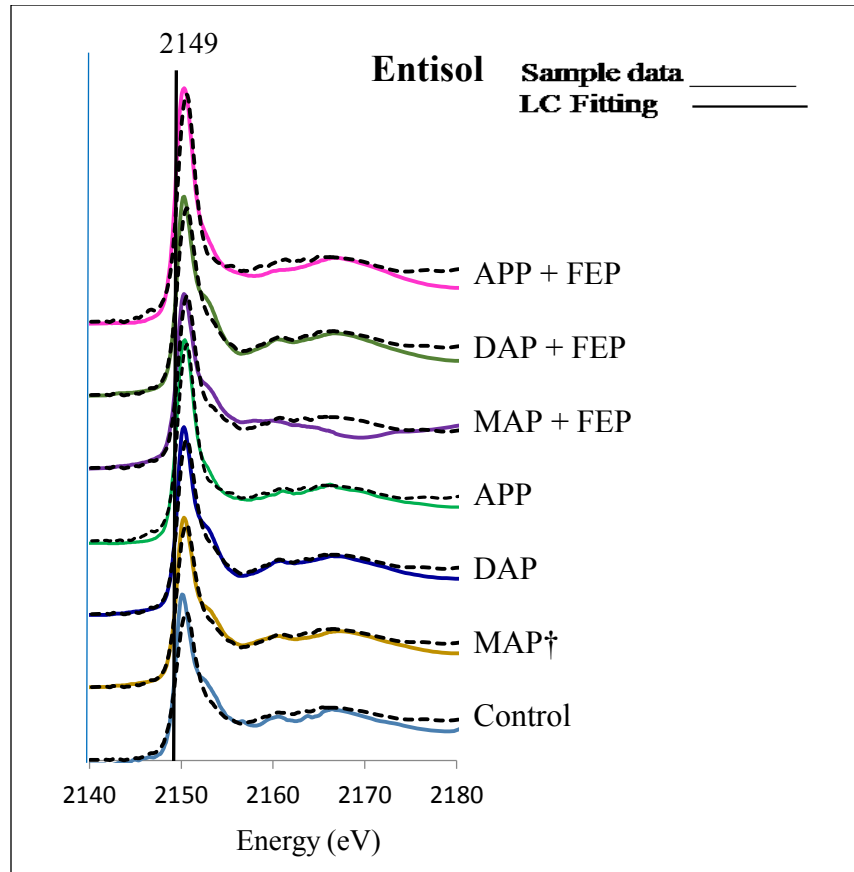


Figure 4.16 Normalized P K- edge XANES spectra for the Entisol for the 0-7.5 mm section at 5-weeks incubation. The dotted lines are the linear combination fit line for each treatment and represents the linear combination fit with the P standards. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

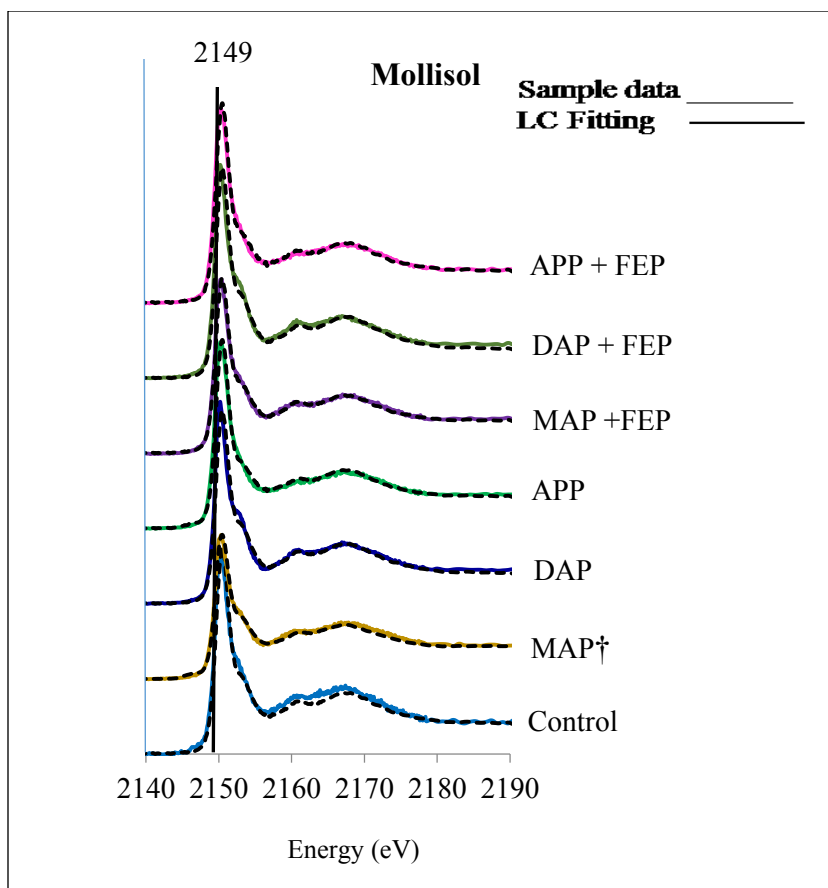


Figure 4.17 Normalized P K- edge XANES spectra for the Mollisol for the 0-7.5 mm section at 5-weeks incubation. The dotted line at each treatment spectra is the linear combination fit line and represents the linear combination fit with the P standards. †MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

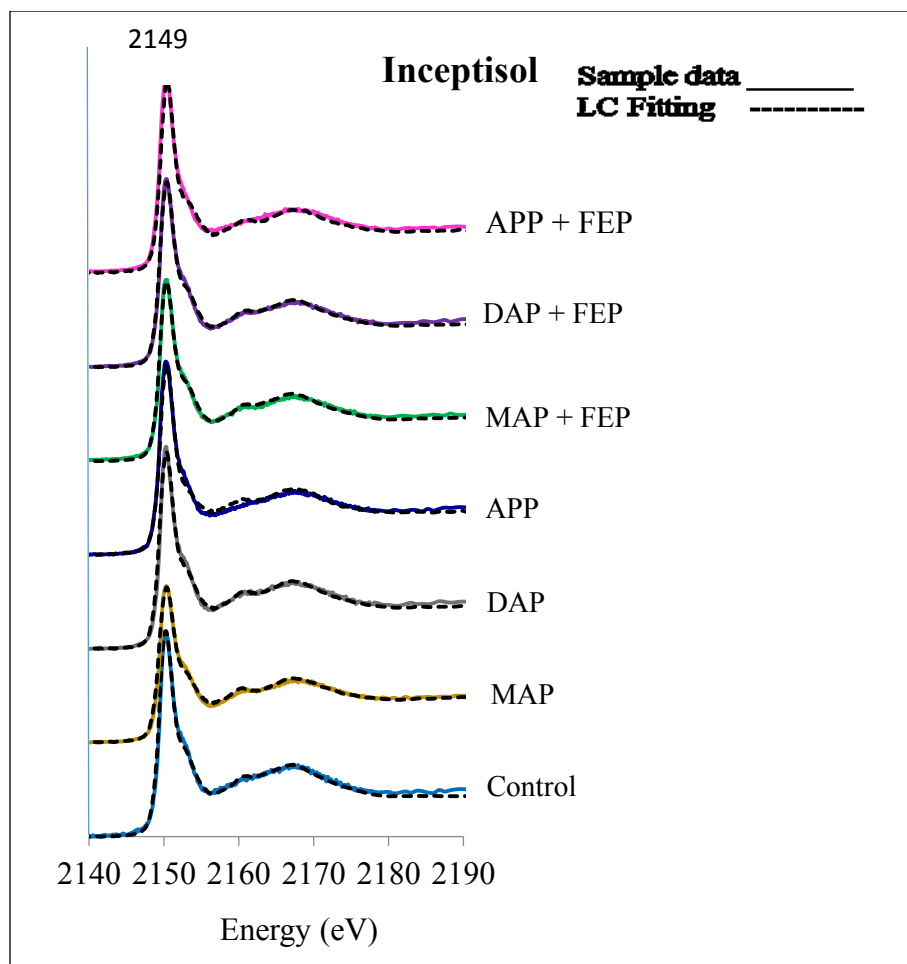


Figure 4.18 Normalized P K- edge XANES spectra for the Inceptisol for the 0-7.5 mm section at 5-weeks incubation. The dotted line at each treatment spectra is the linear combination fit line and represents the linear combination fit with the P standards.

†MAP = monoammonium phosphate; DAP = diammonium phosphate; APP = ammonium polyphosphate; FEP = fertilizer enhancement product; MAP + FEP = monoammonium phosphate + fertilizer enhancement product; DAP + FEP = diammonium phosphate + fertilizer enhancement product; APP + FEP = ammonium polyphosphate + fertilizer enhancement product.

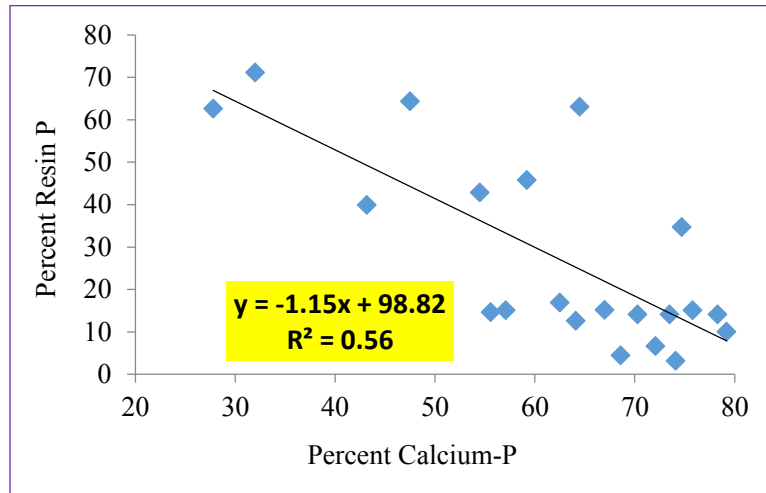


Figure 4.19 Percent resin P results verses Ca-P speciation for the Entisol, Mollisol and Inceptisol.

Chapter 5 - Soil Phosphorus Cycling in a Non-Agriculture Grassland Ecosystem: The Influence of Fertilization

Abstract

The tallgrass prairie is an ecosystem inherently low in plant available nutrients including phosphorus (P). The main objectives of this study were to observe how the long-term addition of P, with or without N, influenced the inorganic and organic P pools in this grassland soil system. In 2002, a P and N fertilization experiment was initiated on a well-drained, upland Mollisol on the Konza Prairie Biological Station, Manhattan, KS. The randomized 4x2 factorial design included four levels of superphosphate fertilizer (0, 2.5, 5, 10 g P/m²) and two levels of ammonium nitrate additions (0, 10 g N/ m²) with six replications for a total of 48 plots in factorial arrangement. Sequential fractionation of soil P after 14 years of annual fertilizer additions revealed the effects of P were dependent on N. There were significant interactions with the addition of P plus N fertilizer for most fractions. For the residual P fraction, there was a significant P main effect and residual P was significantly less when N was added, suggesting that either the addition of N enhanced plant growth and P was removed from this pool, or the addition of N limited the amount of P that moved into this pool. Phosphorus K-edge X-ray absorption near-edge structure (XANES) spectroscopy revealed that the addition of P with or without N resulted in the formation of calcium phosphorus (Ca-P) species. The P plus N fertilizer treatments showed reduced phytic acid species, suggesting a shift or possible mineralization of organic P (P_o) to a more labile P pool. In addition, the utilization in plant productivity was consistent with the decreased residual P fraction. The results of ³¹P-NMR suggested the addition of P fertilizer without N did not change the concentration of monoester forms of P, consistent

with the phytic acid results by XANES. However, P plus N increased the concentration of monoester forms of P as determined by ^{31}P -NMR. The monoester includes phytic plus other P_o entities and the increase may have reflected an overall greater biological activity from the addition of N. The overall results suggest that P and N fertilization and associated changes in plant productivity resulted in significantly different P distributions.

Introduction

Native grasslands occur around the world and have been estimated to have covered upwards of 25% of the land surface at one time (Grasslands Conservation Council of British Columbia, 2016). The major differences among the grassland ecosystems are the annual variable rainfall and temperature which influences the species present, productivity and soil formation. The grasslands of the Great Plains region cover approximately 1.5 million km² (Knapp and Seastedt, 1998). These grassland ecosystems perform useful ecosystem services which include grazing for livestock and habitat for indigenous grazing species, both important to the function and structure of the grasslands.

While the native vegetation has greatly influenced soil formation on the grasslands, the inherent chemical as well as physical properties of the soils have also helped support the vegetation and the subsequent microbial community of this ecosystem (Ransom et al., 1998). Grasslands with native vegetation are generally not fertilized, although there is interest due to the possibility of increasing productivity and to understand the impacts of nutrient enrichment through atmospheric deposition. Precipitation and temperature help to regulate biological activity and weathering which both influence P transformations in grassland soils (Chadwick et al., 2007).

Native grassland soil can be inherently low in plant available P as there are limited P additions such as recycling of P in biomass and ash following burning, dust additions, and weathering of primary minerals in the system. Plants in this environment use numerous strategies to obtain P needed for growth. An important source of orthophosphate for plant uptake is the solubilization of immobile P by arbuscular mycorrhizae (AM) fungi. These fungi form symbiotic relationships with plant hosts and assist plants with obtaining P (Wilson and Harnett,

1997; Cox et al., 1995). Arbuscular mycorrhizae have some of the largest biomass in the grassland soils they colonize and as a result are a large part of the soil microbial nutrient pool (Olsson et al., 1999). The AM invade and live within the roots of vascular plants and extend external hyphae into the soil and are easily able to colonize a very diverse plant community. This results in an effective increase in the root surface area with which to absorb water and nutrients. In exchange for photosynthate, the fungus enhances P and N availability to plants by releasing organic acids and phosphatase enzymes (Hamel, 2004; Bolan, 1991). The P and N are translocated from the soil inorganic and organic sources to the plant hosts. In general, an increase in carbon (C) to the AM by the host will increase nutrient uptake and transfer by the fungus to the host (Bücking and Shachar-Hill, 2005; Smith et al., 2003). Other methods for obtaining P by the plant include acidification with root exudates within the rhizosphere zone influencing the pH and microbial activity that promotes the solubilization of nutrients. In addition, root exudates can facilitate the chelation of metal ions allowing for the uptake and transfer of P by AM and plant roots to the plant. Lastly, the imbalance of P chemical equilibria within the soil as a result of plant uptake of P will promote desorption of P and the transfer of P into the soil solution. The uptake and redistribution of P by fungi are important parts of the organic P cycle (Stewart and Tiessen, 1987). The complex relationship influences the species interactions as well as belowground ecosystem processes (Hartnett and Wilson, 1999).

There are a number of methods for studying soil P in grassland-type systems. Using P sequential fractionation, Ippolito et al. (2010) looked at P forms and transformations in four different grassland ecosystems varying in precipitation within the Midwest; shortgrass steppe, two mixed grass prairies, and the tallgrass prairie. They found at all four sites just a small fraction of the total P was in soluble or labile P form within the A horizon. With the increase in

precipitation across the four sites there was a decrease in Ca-bound P and an increase in occluded P, and for non-calcareous sites within the four, varying amounts of Al- and Fe-bound P were found. Sharpley and Smith (1985) used P fractionation in looking at changes in P pools of fertilized cultivated and unfertilized noncultivated soils. The inorganic P fractions in the unfertilized soil increased in the order of labile, nonoccluded, occluded and Ca-bound, with no change in P pool size with cultivation. In contrast, Liu et al. (2014) used a sequential fractionation method to look at P transformations in agricultural fields receiving P on a long-term basis versus those without P fertilization for 15 years after long-term fertilization. The accumulation of plant-available P was in the Al-bound fraction. Unfertilized soils had a higher organic P content as compared to the fertilized equivalent suggesting redistribution of organic P to inorganic P pools due to P and N fertilization and/or cropping.

Sequential fractionation schemes cannot determine the distinct soil P species, however. Two methods, thus far, commonly used for direct P speciation are P K-Edge X-ray absorption near-edge structure (XANES) and liquid state ^{31}P - nuclear magnetic resonance (^{31}P -NMR). There has been valuable research employing the use of XANES P fertilization research in agriculture (Khatiwada et al., 2012; Liu et al., 2014). However, no recent studies have been done using P K-edge XANES on native grassland soils. Taranto et al. (2000) used these techniques to look at P transformations of native and added P and plant growth change in P pools in woodland and adjacent grassland soils. The presence or absence of species-specific pre- or post-edge signals allows identification of main P species with reasonable certainty. Due to similarities between some P XANES spectra, care should be taken during data analysis to reduce uncertainty associated with speciation (Ingall et al., 2011; Kizewski et al., 2011; Hesterberg et al., 1999). Combining the use of XANES and ^{31}P -NMR is a valuable approach, useful in determining P

solid-phase formation and valuable soil P composition information in soils (Abdi et al., 2014; Liu et al., 2014; Turner et al., 2003; Liu et al., 2014). The technique of ^{31}P NMR alone is not suited for overall P speciation in soils due to inherent weaknesses associated with identifying inorganic P species. It is much better suited for organic P speciation in soils (Cade-Menun, 2005; Cade-Menun and Liu, 2013). Hence, combining these techniques will allow better understanding of changes in P pools. To our knowledge, there are no studies that have combined the use of P K-Edge XANES and liquid state ^{31}P -NMR with P sequential fractionation to closely examine soil P on native, virgin or unplowed grassland soils.

Phosphorus soil test results on soil from the Konza, using the Bray-1 extraction method, showed available P at 6 mg kg^{-1} and KCl extractable $\text{NO}_3\text{-N}$ at 15 mg kg^{-1} (Wilson and Harnett, 1997). Kansas State University Research & Extension Soil Test Recommendations indicated the Bray-1 extractable P levels or extractable $\text{NO}_3\text{-N}$ of this magnitude would be considered very low for any crop (Leikam et al. 2003).

Nutrient enrichment of grasslands could impact both vegetation and consequently, soil P. Avolio et al. (2014) explored the effects of fertilizer additions on the plant community composition and aboveground net primary productivity (ANPP) in a long-term N and P fertilizer study at the Konza Prairie Biological Station, Manhattan, KS. Among their findings, there was little change in the ANPP, the percent grass, and forb biomass over a ten year period with annual applications of superphosphate alone as compared to the control. There was significant increases in all three parameters during the same time period with the addition of N compared to the control treatment. Further, resin bags containing anion and cation resin beads, installed at a 10 cm depth during the growing season, and were used as an index of labile P and available N in the

soil. There was a significant increase in resin extractable P as the amount of P added increased. There was no significant increase in resin exchangeable P with the addition of N.

The initial objectives of Avolio et al. (2014) were to examine ANPP and to quantify plant community structure and composition effects as a result of annual P and N fertilizer additions to a tallgrass prairie system. Detailed analyses of the soil were not conducted. Our study extended the experiment described in Avolio et al. (2014) to gain a better understanding of the fate of P and N fertilizers in prairie soils to contribute to the overall knowledge and understanding of the soil P cycle. The objectives of this research were to determine how P and N fertilizer additions influenced the forms of P in a grassland soil system utilizing a variety of approaches including soil P sequential fractionation, XANES and ^{31}P -NMR. We hypothesized that the addition of P and/or N fertilizers would influence both inorganic and organic pools of P through a combined effect of soil chemical processes and changes to plant community structure and productivity. Specifically, P addition alone would increase stable forms of mineral P in the soil and N addition alone would deplete stable mineral forms of P while increasing organic P. The addition of P and N together would increase both stable mineral forms of P and organic P.

Materials and Methods

In 2002, a P and N fertilization experiment was initiated on a biennially burned watershed (2C) of the Konza Prairie Biological Station, Manhattan, KS. The soil series is Florence, which classifies as a Mollisol (clayey-skeletal, smectitic, mesic Udic Argiustoll) (Soil Survey Staff, 2004). The complete randomized multi-level P addition, with and without N addition, experiment is on a 30 x 40 m area divided into 5 x 5 m plots. The plots consist of four levels of triple superphosphate fertilizer (0, 2.5, 5.0, 10.0 g P/m²) along with two levels of ammonium nitrate fertilizer additions (0, 10 g N/m²) on six replications, for a total of 48 plots in

factorial arrangement. In spring 2014, soil cores were collected to a 15 cm depth, with three cores per plot. Each soil core was divided into 3 sections: 0-5 cm, 5-10 cm and 10-15 cm. All soils were air dried at room temperature and sieved to < 2mm before analysis.

The soil pH in the study was measured in 1:10 soil:dionized water (Watson and Brown, 1998); Mehlich-3 P determined as described in Frank et al. (1998); cation exchange capacity determined using a summation method of Chapman, (1965); total P was determined according to Zarcinas et al. (1996), modified to use a digestion block instead of microwave. Total organic carbon (TOC) determined using dry combustion method of Nelson et al. (1996) using soil ground and sieved to $\leq 150 \mu\text{m}$ and treated with 1N phosphoric acid to remove inorganic C. Direct combustion of soils was completed using a Carlo Erba C/N analyzer (Carlo Erba instruments, Milan, Italy).

Particle size analysis was determined using a combination of a modification of the pipette method by Kilmer and Alexander (1949) and method 3A-1 from the Soil Survey Laboratory Methods Manual (Soil Survey Laboratory Staff, 2004). Clay mineralogy was completed using K-25 and Mg-25 combinations (Harris and White, 2008). The X-ray Diffraction (XRD) analyses were performed on a Panalytical Empyrean diffractometer using a copper anode material and generator settings of 35 eV and 20 mA.

Phosphorus sequential fractionation was performed using a modified Hedley soil fractionation scheme that estimated operationally defined fractions of soil inorganic P and organic P as per Tiessen et al. (1984). The fractions included the bioavailable P (BA-P) using anion exchange resin, the loosely bound sorbed inorganic and organic P extracted with 30 mL of 0.5M sodium bicarbonate, Fe- and Al-bound inorganic and organic P extracted with 30 mL of 0.1M sodium hydroxide, for removing P_i and P_o held within the internal soil aggregate surfaces a

2 minute ultra-sonification of the soil residue using 20 mL of 0.1M sodium hydroxide followed by filling to a final volume of 30 mL, a 30 mL of a 1M hydrochloric extract was used to remove Ca-bound inorganic P. The residual inorganic P (P_i) was digested using aqua regia for dissolution of insoluble P_i and P_o forms. The P_o for the BA and HCl fractions were not included as P_o recovery was negligible.

To identify organic P species directly, four samples were chosen for ^{31}P - NMR, which represented the treatments most likely to show P treatment differences. The soils were extracted using a NaOH-EDTA extraction protocol, and the lyophilized soil was treated with deuterium oxide and sodium sulfide to remove paramagnetic ions (Vestergran et al., 2012), and transferred to 5 mm diameter NMR tube in preparation for liquid ^{31}P -NMR. The samples were run on a Varian 400MHz Mercury system (Agilent Technologies, Santa Clara, California) using a switchable probe tuned for ^{31}P at 161.90MHz at the Department of Chemistry, Kansas State University, KS. Acquisition time was 0.38 seconds and delay time 2 seconds. The pulse used was a 45° and the sweep width was 130 ppm. Number of scans was set at 12k. The external reference was an 85% H_3PO_4 sample referenced at 0 ppm. The processing was done with a 16k multiplication and line broadening of 5Hz on all samples.

The phosphorus K-edge XANES data was performed at Canadian Light Source Inc., Soft X-ray Microcharacterization Beamline (SXRMB) 06B1-1, Saskatoon, Saskatchewan, Canada. The third generation storage ring at CLS operates at 2.9 GeV.

The energy range at Sector 06B1-1 is 1.7 to 10 keV and the beam line provides a focused x-ray beam for XANES to $300\mu\text{m} \times 300\mu\text{m}$. All spectra including the phosphorus standard used for the InSb (III) monochromatic alignment were collected in florescence mode. The composite replications from the 0-5 cm soil depth for each treatment were used in the spectral collection.

All soils were ground to ≤ 150 μm using an agate mortar and pestle and evenly sprinkled onto double-sided carbon tape placed on a stainless steel sample holder in a vacuum chamber for analysis. Multiple scans were collected per sample at a range 2.1 to 2.2 keV, and six scans were collected for the unfertilized control treatment soil. All data collected were analyzed using IFEFFIT-Athena software (Ravel and Newville, 2005). The scans of collected spectra were averaged for each treatment. The edge energy was calibrated, the fitting the pre-edge was subtracted by a linear function, the spectrum was normalized to a second-order polynomial.

All P standards were either prepared in the laboratory or purchased, and the pureness of each prepared standard was confirmed by using XRD. The P standards were synthesized by Khatiwada and used for data fitting included variscite ($\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$), brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), strengite-1d, strengite-3d ($\text{FePO}_4 \cdot 2\text{H}_2\text{O}$), PO_4 -goethite, PO_4 -alumina, PO_4 -gibbsite, hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$). Details in standard preparation can be found in Khatiwada et al. (2012). Apatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and vivianite $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ standards were collected at Sector 2, APS, Chicago, IL. Aluminum phosphate and FeIII-P standard obtained from K. Scheckel, EPA. The PO_4 -adsorbed to Al-saturated montmorillonite standard was obtained from J. Prietzel, University of Technology, Munchen, Germany. The PO_4 -adsorbed kaolinite standard was obtained from D. Hesterberg, North Carolina State University. Spectra from both of these P standards were collected at the Synchrotron Light Research Institute, Nakhon Ratchasima, Thailand. All XANES data collected at CLS were using IFEFFIT-Athena software (Ravel and Newville, 2005). The scans of collected spectra were averaged for each treatment. The edge energy was calibrated, the fitting

the pre-edge was subtracted by a linear function, the spectrum was normalized to a second-order polynomial.

The spectra of the standards were used to reconstruct the spectra of the unknown samples using XANES linear combination fitting (LCF) fitting procedure. The weighting factors were forced to sum to one and the combination of solids with the lowest reduced Chi-Square was selected as the best fit. The Manceau Combo Method (Manceau et al., 2012) was also used to validate our data LCF procedure. The Combo method results closely matched the LCF results for the Konza samples, providing an added confidence on the P solid phases identified by the LCF.

Statistical Analysis

The experiment was a 4 x 2 factorial arranged with P and N rates in a completely randomized design with six replications, different P and N levels in a two-factor factorial arrangement. Data were analyzed using the PROC MIXED Procedure in SAS software (SAS 9.3, 2011). The Tukey Pairwise Method was used for a comparison of all treatments at $P=0.05$.

Results and Discussion

Select initial properties of the soil used in this research are provided in Table 5.1. The pH of the unfertilized control soil slightly decreased with depth: pH 6.8 at 0-5 cm, 6.5 at 5-10 cm and 6.4 for the 10-15 cm soil depth. The trend was similar for total P concentrations for the three depths; the decreased total P with depth is most likely from P stratification due to surface P litter inputs and deposition of P in ash from biennial controlled burning (Messiga et al., 2013; Cade-Menun et al., 2010). Cation exchange capacity changed very little with depth. Ammonium-N (NH_4^+ -N) concentration of 11 mg kg^{-1} at the surface decreased slightly with depth; in contrast, the nitrate-N (NO_3^- -N) concentration decreased dramatically from 3 mg kg^{-1} at 0-5 cm to 0.9 mg kg^{-1} at the 10-15 cm soil depth. The influence of P and N fertilizer treatments on surface soil pH were

different for each of the two fertilizers used in the experiment (Table 5.2). Regardless of the amount of P added there was little influence on soil pH at 0-5 cm soil depth as compared to the unfertilized control soil. The addition of the 10 g N/m² impacted the soil pH in each level of added P decreasing by 0.3 to 0.5 a pH unit likely the result of nitrification. For all treatments, the greatest total P concentration was found in the 0-5 cm soil depth and decreased to the 10-15 cm soil depth (Table 5.1). Much of the P cycling in this grassland occurred within surface soil layer with minimal mixing, resulting in P stratification.

Fractionation of phosphorus in soils

The average P recovery, calculated as the sum of the fractions for the sequential fractionation scheme divided by the independently determined total P concentration (by aqua-regia digestion) for the soil, on the eight treatments was 92% and ranged from 79.1 to 102.5% (Table 5.3). Liu et al. (2014), had comparable P sequential total P recoveries as part of characterizing soil P study in agriculture soils prone to runoff collected from five different Ultisols in China. The BA-P concentration for the Konza control treatment (0 g P/0 g N) was 16.9 mg P kg⁻¹ and comprised 5.6% of the total P concentration; the 0 g P/10 g N treatment was 11.9 mg P kg⁻¹ and 5% of the total P concentration. The low values may be related to the rapid plant uptake of available P by microorganisms and grasses (Ippolito et al., 2010; Hedley et al., 1982).

The BA-inorganic P (P_i) concentration increased with increasing increments of added P as did the proportion of BA-P_i as a percentage of the total P concentration, and BA-P_i concentrations with 5 g P/0 g N and 10 g P/0 g N treatments were higher as compared to the 0 g P/0 g N or 2.5 g P/0 g N treatment (Fig.5.2; Table 5.3). Schmidt et al. (1996) found resin extractable soil P increased with annual P applications in excess of crop removal as part of an

experiment looking at soil P changes in a continuous cropping system. The BA-P_i concentration for the 0 g P/10 g N treatment was 11.9 mg P kg⁻¹ and comprised 4.2% of the total P. Very similar to the treatments without added N, the BA fraction increased in concentration and as a proportion of the total P as the amount of P added increased. The 10 g P/10 g N treatment contained the greatest total P concentration but was not significantly different from the 10 g P/0 g N treatment. The P main effect and the P*N interaction were significant while the N main effect was not significant.

The moderately to more strongly sorbed P_i fractions were estimated as P_i- sodium bicarbonate (P_i-NaHCO₃), which extracts P_i adsorbed onto soil surfaces; P_i-sodium hydroxide (P_i NaOH) extraction, which releases P held more strongly to Fe and Al components on soil surfaces; and sonified P_i-NaOH extraction which releases P held within the aggregates (Hedley et al., 1982; Zhang and MacKanzie, 1997).

For the P_i- NaHCO₃, there was a significant main effect for P but not for N and there was a significant P*N interaction (Fig. 5.3). The 0 g P/0 g N, 2.5 g P/0 g N, 0 g P/10 g N, 2.5 g P/10 g N, and 5 g P/10 g N treatments were not significantly different than the control. The 10 g P/10 g N treatment had significantly more P in the P_i-NaHCO₃ fraction, which suggests a significant amount of added P was transformed into this fraction. In addition, it suggests the plants in the 10 g P/10 g N treatment plots had more than adequate levels of available P for plant growth.

For the organic P-sodium bicarbonate (P_o-NaHCO₃), by difference, there was a significant main effect for P but not for N, and there was a significant P*N interaction (Fig. 5.4). There was no significant difference between the 5 g P/0 g N - 5 g P/10 g N and 10 g P/0 g N - 10 g P/10 g N treatments; and, the 5 g P/0 g N - 5 g P/10 g N treatment was significantly greater than both the 0 g P/0 g N - 0 g P/10 g N and 2.5 g P/0 g N - 2.5 g P/10 g N treatments. These

results suggest the addition of P_i resulted in the accumulation of P_o -NaHCO₃ extractable P in these particular treatments. The P_o -NaHCO₃ fraction is considered to be the fraction containing predominantly labile or more soluble P_o compounds in soils (Negassa and Leinweber, 2009; Turner and Leytem, 2004).

Similarly the P_i -NaOH extraction (supposedly more strongly sorbed P) indicated a significant main effect for P but not N, and a significant P*N interaction (Fig. 5.5). The 0 g P/0 g N, 0 g P/10 g N, and 2.5 g P/10 g N fractions were not significantly different from one another. For the P treatments alone, the P_i -NaOH fraction increased in concentration and as a proportion of total P as the amount of P added increased (Fig. 5.5). With the P and N treatments, this fraction also generally increased with the added increments of P. The 10 g P/10 g N treatment contained significantly more P_i -NaOH than all other treatments.

The P_i -hydrochloric acid (P_i -HCl) extraction is believed to extract non-labile P precipitated as secondary Ca-P minerals. There was a significant P main effect, non-significant N main effect, and a significant P*N interaction (Fig. 5.6). With the additional increments of added P for the 2.5 g P/0 g N, 5 g P/0 g N and 10 g P/0 g N treatments, the P_i -HCl concentration increased in a similar step-wise fashion as seen with the previous fractions. The P_i -HCl ranged from 6.0% in 0 g P/0 g N to 16.3% in 10 g P/0 g N of the total P. The 0 g P/10 g N treatment contained 13 mg P/kg and comprised 5.5% of the total P concentration. With each additional increment of added P at 10 g N/m², the P_i -HCl concentration generally increased in a step-wise fashion, but to a lesser extent than the treatments that did not receive N. Similar to what was seen previously, the P_i -HCl 10 g P/10 g N treatment was significantly greater compared to all other treatments.

The residual P fraction estimates the most stable, non-labile forms of P_i and P_o remaining in the soil (Hedley et al., 1982). Statistics on the eight treatments in this fraction revealed that the P main effect was not significant while the N main effect was significant and the P*N interaction was not significant (Fig. 5.7). It was clear there was significantly less residual P, averaged across the four N treatments, with the addition of N. This indicated that for the plots receiving the 10 g N/m², in addition to accessing P from the other fractions, microorganisms and the plant species are mobilizing P from the residual fraction or the addition of N limited the amount of P that moved into this pool. The residual P fraction had the highest concentration of P as compared to all other P pools and comprised 26 to 56% of the total P in the fertilized plots.

X-ray absorption near edge structure

The purpose of the bulk XANES collection of data on the eight treatments on the grassland soil was to directly identify solid phases formed from the addition of the different combinations of P and N. Normalized spectra for bulk XANES can be found in Fig. 5.8. All P K-edge bulk XANES spectra LC fitting results for soils used in the study are shown in Table 5.4.

The unfertilized control soil treatment contained primarily phytic acid (64%) with the remaining P species being Fe-P and a small amount of Al-adsorbed P. Phytic acid is a common form of organic P in soils, in grassland soils in particular (Cade-Menun et al, 2010; Anderson, 1980). Phytic acid, a form of inositol phosphate, is part of a group of phosphate esters. Of all the inositol forms it is the one found in the largest amount in the soil, much of it the result of plant debris (Turner et al., 2003; Abdi et al., 2014; Cade-Menun et al., 2010). All eight treatments contained a large amount of phytic acid. Here phytic acid represents the organic matter associated P (Prietzl et al., 2013). This is not surprising as undisturbed ecosystem soils generally have higher concentrations of soil organic matter (Cade-Menun, 2005).

In general, the addition of P to the grassland system changed the proportions of P solid phases. Overall, with the addition of superphosphate fertilizer there was an increasing presence of Ca-P in all P treated soils likely resulting from the combination of Ca present in the soil and added P. Also, Al-adsorbed P was prevalent in the treatments that did not receive 10 g N. With the addition of the 10 g N, there was the increase of Fe-adsorbed P, the disappearance of Al-adsorbed P solid phases and appearance of Al-P as compared to the P-alone treatments. The addition of 10 g P increased the percentage of phytic acid for the P-alone treatments as compared to all 10 g N added treatments. The percentage of phytic acid-like P (organic P) was lower in the N added soils compared to the unfertilized control soil and the P-alone treatment. Phytic acid, representing a majority of organic P in soils, could be one of the P forms that are reflected in the residual P fractions and the reduction would suggest that enhanced plant productivity from the N additions was capable of transforming organic P pools to inorganic P to meet plant P needs. This is opposite of the results by Liu et al. (2014) who reported higher phytic acid proportions in a fertilized soil as compared to its unfertilized equivalent which was utilizing legacy P in an aridic haploboroll located at the Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Saskatchewan, Canada..

³¹P Nuclear Magnetic Resonance

This analysis provides information about organic P cycling in the system. Four treatments were chosen that would likely represent the greatest differences among the eight treatments. Although solution ³¹P-NMR spectroscopy using extractions from the NaOH-EDTA protocol can identify many P_o and P_i compounds found in soils it only identifies those extracted in this procedure. This is useful for our purposes in looking at relative treatment differences. Species detected by ³¹P-NMR were P_i forms of orthophosphate and pyrophosphate, and within P_o

general monoester and diester groups (Figs. 5.9 and 5.10). The sum of the areas of the peaks of these four groups was assumed to represent 100% of the P in the extract since no other significant peaks were present in the spectra. Hence, the proportion of each of the four forms of P could be calculated. In addition, the NaOH-EDTA extractable P was expressed as a concentration of P in the original soil sample and multiplying by the proportion of each P species found with ^{31}P -NMR allowed us to express the concentration of each of the species in the soils (Table 5.5).

Pyrophosphate occurred in all four treatments at similar proportions. The pyrophosphate in soils are representative of microbial turnover and may represent a type of soil C stabilization (Turner et al., 2003). In addition, pyrophosphate in the grassland soils are linked to microbial activity and through adsorption in the soil may remain stable for long periods of time (Blanchar and Hossner, 1969).

The monoester P group was the dominant P fraction in all four soil treatments. Turner et al. (2003) had similar finding as part of the characterization of fractionated pasture soils using ^{31}P -NMR. The monoester group includes all forms of inositol, choline phosphate, glucose-1-phosphate, beta-glycerophosphate and phosphatidic acid, although some of these may be produced by the hydrolysis of diesters during extraction (Turner et al., 2003; Li et al., 2013). The ^{31}P -NMR results for the 0 g P/0 g N treatment showed 80% of the P in the extract was in the monoester P group, equivalent to 135 mg P kg^{-1} , which was the highest organic P proportion of the four treatments (Fig. 5.9; Table 5.5). Abdi et al. (2014) identified monoester groups in the 0-5 cm soil depth for the no-till treatments using ^{31}P -NMR, including myo-, neo-, scyllo- forms of phytic acid, while studying the effects soil conservation tillage practices on soil organic P. The no-till treatment resulted in an accumulation of monoesters especially with increasing depth.

There was a total of 20% P_i as orthophosphate and pyrophosphate which is typical in native grassland soils relative to agriculture soils (Harrison, 1987). The equivalent soil concentration of orthophosphate determined with ^{31}P -NMR would be 28 mg kg^{-1} which is similar to the amounts extracted with the BA-P and $P_i\text{-NaHCO}_3$ extractions. The Mehlich-3 P for the 0-5 cm soil depth was extremely low, as well. Turner et al (2003) reported that the largest percentage of total extracted P was P_o , including the orthophosphate monoester group, for permanent pasture soils in England and Wales.

In the 10 g P/10 g N treatment, there was an increase in the proportion P_i orthophosphate and a large drop in the proportion of the monoester P group as compared to 0 g P/0 g N treatment (Fig. 5.10; Fig. 5.9). When expressed as concentration the P_i orthophosphate increased from 28 to 298 mg kg^{-1} while the monoester P group remained unchanged (Table 5.5). The increase in P_i orthophosphate was most likely from the fertilizer addition and adding P did not change the concentration of the monoester P group. Little change in the monoester P group concentration is consistent with the lack of increase in ANPP upon P fertilization (Avelio et al., 2014). The addition of P alone did not stimulate plant growth.

In the 0 g P/10 g N treatment, there was little change in the orthophosphate and pyrophosphate groups as compared to 0 g P/0 g N treatment while the concentration of monoester P increased, reflecting an increase in plant growth from the addition of N (Fig. 5.9; Table 5.5). The proportion of monoester P in the 0 g P/10 g N treatment remained similar to the 0 g P/0 g N treatment. This concentration increase reflects the increased plant growth due to N. For the 10 g P/10 g N treatment, there was a relative increase in P_i orthophosphate and pyrophosphate groups, as would be expected with the addition of phosphate fertilizer. However, there was a drop in the proportion of the monoester P group, but an increase in the monoester P

concentration as compared to all other treatments. This increase may be a reflection of the enhanced plant growth from P and N additions (Avelio et al., 2014). The appearance of the orthophosphate diester P group suggests an enhanced P microbial biomass (Turner et al., 2003).

Conclusions

Our sequential fractionation results indicated the effects of P addition were dependent on the addition of N. There were significant P*N interactions for most of the fractions. In general, the P concentrations in all fractions except the residual P fraction increased with increasing amounts of P added. The residual P fraction was not affected by P-alone additions; however, residual P was affected by N addition and the residual P fraction was significantly less when N was added suggesting addition of N resulted in slower transformation of other P pools towards the residual P pool. The XANES analysis showed that in general the addition of P fertilizer favored the formation of Ca-P species. The addition of P and N fertilizers together or alone favored formation of more Fe-adsorbed P species as compared to Al-P. In addition, there was a reduction of phytic acid forms for all P plus N treatments compared to P alone, suggesting an increase or enhancement in plant productivity that utilized phytic acid to supply plant available P. This was consistent with the results for our residual P fraction results. The ^{31}P -NMR results suggested the addition of P fertilizer greatly increased the concentration of P_i orthophosphate but did not change the concentration of the monoester group and was consistent with the phytic acid results obtained with XANES. The addition of 10 g P/10 g N increased the P_i orthophosphate concentration compared to the 0 g P/0 g N treatment; however, the concentration of the P_o monoester group greatly increased as compared to all other treatments. This increase in concentration reflects the enhanced plant growth from P and N addition.

This work was able to track the fate of P as applied to these grassland soils low in native P. Phosphorus sequential fractionation, which was used to operationally separate P_i and P_o soil P fractions and provided valuable information on P lability in this soil. The ^{31}P -NMR technique identified P_o species, however, it had limitations in terms of the identification of specific P_i species. Phosphorus K-edge XANES analysis was able to characterize inorganic solid-phase Ca-P, Fe- and Al-associated species and identify phytic acid. The results from the three approaches were in general agreement in that each reflected changes in P_i and P_o soil P pools upon P and N additions to the soil and in some cases were in close agreement regarding the P species present. These results may be useful in trying to better understand the impact of nutrient enrichment in grassland soils.

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Tables and Figures

Table 5.1 Selected initial soil chemical and mineralogical characteristics.

Properties	0-5	5-10	10-15
pH	6.8	6.5	6.4
Mehlich-3 P (mg kg ⁻¹)	3.6	2.5	2.1
CEC (cmol _c kg ⁻¹)	27.4	25.4	27.7
NH ₄ -N (mg kg ⁻¹)	10.9	9.0	1.0
NO ₃ -N (mg kg ⁻¹)	3.0	1.0	1.0
Total P (mg kg ⁻¹)	358	276	251
TOC (%)	4.0	2.6	2.5
TON (%)	0.3	0.2	0.2
Textural class	SiCL	SiCL	SiCL
	Mica,		
Minerology†	Montmorillonite,	--‡	--
	Kaolinite		

† National Soil Characterization Data Base. pH; (1:10) in water. CEC: cation exchange capacity.

TOC: Total organic carbon. TON: Total organic nitrogen. ‡ Mineralogy not available. Soil

texture: SiCL=silty clay loam.

Table 5.2 Soil pH as influenced by P and N fertilizer additions, 0-5 cm soil depth, illustrating the P * N interaction.

P Additions (g/m ²)	----- N Addition (g /m ²) -----	
	0	10
0	6.8	6.3
2.5	6.8	6.4
5.0	7.0	6.5
10.0	7.0	6.7

Table 5.3 Soil P sequential fractionation of 0-5 cm soil depth.

P Fraction	Treatment (g/m ²)							
	0 g P/ 0 g N	2.5 g P/ 0 g N	5 g P/ 0 g N	10 g P/ 0 g N	10 g N/ 0 g P	10 g N/ 2.5 g P	10 g P/ 5 g P	10 g N/ 10 g N
-----mg/kg-----								
P _i Bioavailable	16.9 (5.6)†	46.6 (11.1)	93.2 (16.3)	101.0 (16.7)	11.9 (5.0)	29.8 (8.4)	68.7 (14.7)	134.2 (18.7)
P _i NaHCO ₃	6.8 (2.3)	7.9 (1.9)	31.0 (5.4)	29.1 (4.8)	6.7 (2.8)	16.8 (4.8)	14.5 (3.1)	48.3 (6.7)
P _o NaHCO ₃	12.1 (4.0)	12.3 (2.9)	41.1 (7.2)	45.0 (7.5)	33.4 (14.1)	23.8 (6.7)	47.9 (10.3)	36.4 (5.1)
P _i NaOH	19.3 (6.4)	52.5 (12.5)	63.2 (11.1)	71.3 (11.8)	33.5 (14.1)	27.9 (7.9)	58.4 (12.5)	115.2 (16.1)
P _o NaOH	18.9 (6.3)	56.6 (13.5)	25.1 (4.4)	51.7 (8.6)	8.7 (3.7)	56.1 (15.9)	72.3 (15.5)	53.0 (7.4)
P _i NaOH sonicate	2.3 (0.8)	8.8 (2.1)	9.1 (1.6)	16.6 (2.8)	6.2 (2.6)	3.0 (0.8)	9.7 (2.1)	18.0 (2.5)
P _o NaOH sonicate	38.9 (12.9)	13.1 (3.1)	55.8 (9.8)	31.5 (5.2)	9.0 (3.8)	30.8 (8.7)	19.9 (4.3)	31.9 (4.4)
HCl Extract	18.2 (6.0)	40.1 (9.6)	64.3 (11.3)	98.7 (16.3)	13.0 (5.5)	33.9 (9.6)	46.1 (9.9)	113.4 (15.8)
Residue digest	168.3 (55.6)	181.8 (43.3)	188.6 (33.0)	159.2 (26.4)	115.5 (48.5)	131.4 (37.2)	129.6 (27.7)	167.2 (23.3)
Sum of Fraction	301.6	419.8	571.2	604.0	237.9	353.5	467.1	717.5
Separate Total P	357.4	440.9	585.9	601.8	300.7	404.3	513.2	699.9
P Recovery	84.4	95.2	97.5	100.4	79.1	87.4	91.0	102.5

†Values in parentheses represent the percentage of total P for a given fraction. P_i = inorganic P.

P_o = organic P. NaHCO₃ = sodium bicarbonate. NaOH = sodium hydroxide. HCl = hydrochloric acid.

Table 5.4 Phosphorus solid phase speciation as determined by P K-edge XANES on the 0-5 cm soil depth. The table presents relative proportions of adsorbed and precipitated P solid phase minerals expressed as a percentage. Energy range: 2.14 to 2.19 keV.

Treatment	Ca-P	Al-P	Al-Adsorbed P	Fe-P	Fe-Adsorbed P	Phytic Acid	Red. Chi.†
g/m ²	-----%						
0 g P/0 g N	- ‡	-	15.1	20.9	-	64.0	0.020
2.5 g P/0 g N	11.0	-	-	10.6	-	78.4	0.002
5 g P/0 g N	15.1	-	6.6	15.0	-	63.3	0.005
10 g P/0 g N	15.3	-	20.7	-	-	64.0	0.006
0 g P/10 g N	6.6	-	-	-	50.3	43.0	0.009
2.5 g P/10 g N	14.7	19.2	-	-	6.0	60.0	0.006
5 g P/10 g N	6.5	7.1	-	28.4	5.2	59.9	0.008
10 g P/10 g N	14.7	-	-	28.6	-	56.7	0.006

Red. Chi.† = reduced chi-square and statistically represents the goodness of fit of the data with the P standards. The RCS is low when the fit is good. ‡ Not a relevant solid phase in LCF.

Ca-P; calcium phosphate. Al-P; aluminum phosphate. Al-adsorbed P; aluminum adsorbed phosphate. Fe-P; iron phosphate.

Table 5.5 Total soil P and NaOH-EDTA extractable P concentrations and the proportion and concentration of P species as determined by ³¹P-NMR.

Treatment	Total P	NaOH-EDTA Extractable P	P _i -Orthophosphate	P _r -Pyro-phosphate	P _o Monoester	P _o Diester
g/m ²	---- mg kg ⁻¹ -----		----- % -----			
0 g P/0 g N	357.5	169.4	17 (28)†	3.00	80 (135)	--‡
10 g P/0 g N	601.8	450.2	66 (298)	4.20	30 (133)	--
0 g P/10 g N	300.7	224.1	19 (42)	5.80	76 (170)	--
10 g P/10 g N	669.9	497.4	51 (251)	4.30	44 (219)	1.1

† The equivalent soil concentration (mg kg⁻¹). ‡ Not a relevant organic P species.

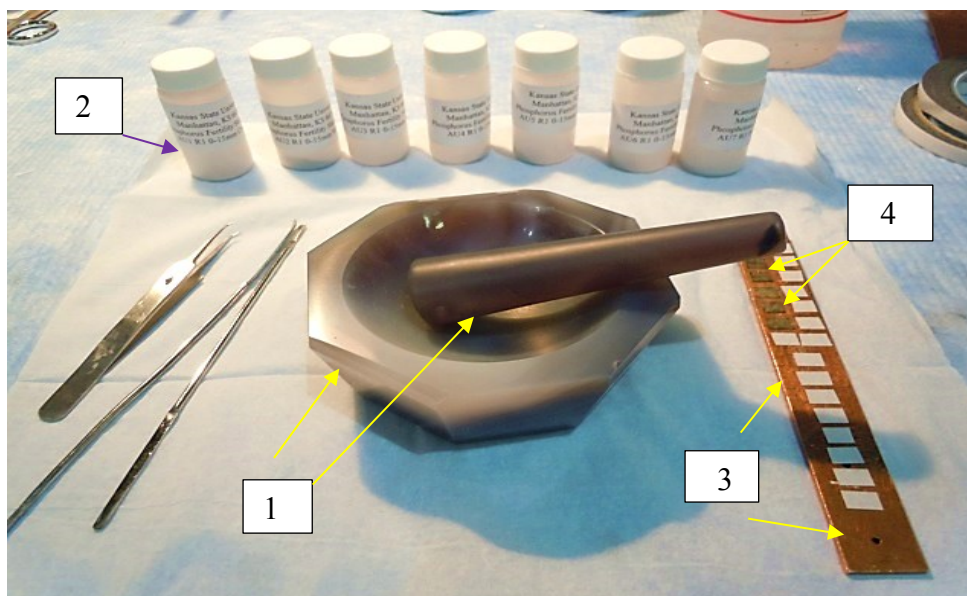
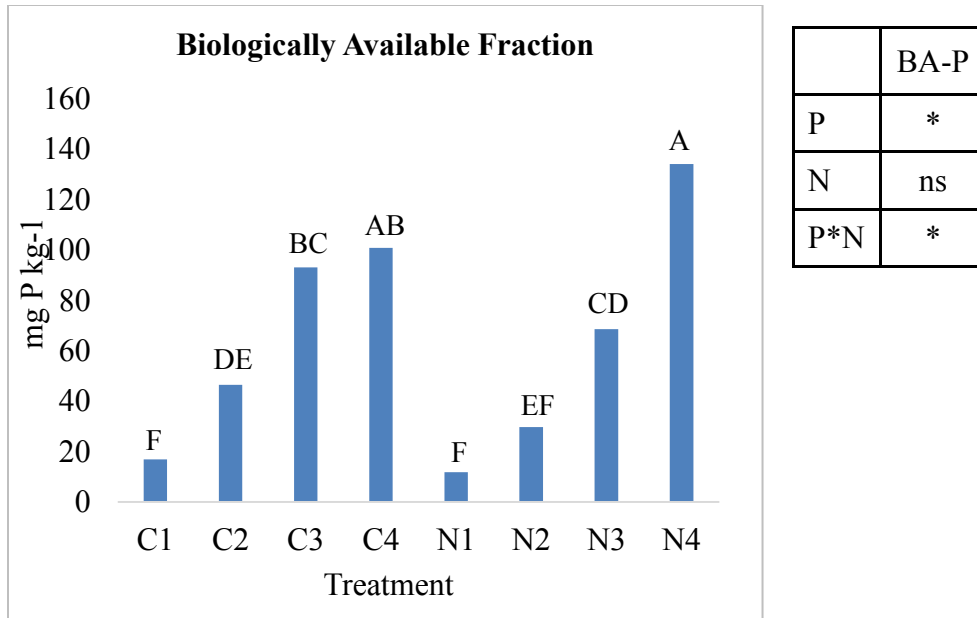


Figure 5.1 Sample preparation for P K-edge XANES data collection. (1) Agate Mortar and pestle (2) Soil samples (3) copper sample holder (4) double-sided carbon tape holding soil sample.



	BA-P
P	*
N	ns
P*N	*

Figure 5.2 The influence of P and N treatments on the biologically available P fractionation (BA-P). Bars with the same letter are not significantly different at P=0.05. C1= 0 P/0 N, C2= 2.5 P/0 N, C3=5.0 P/0 N, C4= 10 P/0 N, N1= 0 P/10 N, N2= 2.5 P/10 N, N3=5.0 P/10 N, N4= 10 P/10 N. Units= g/m²

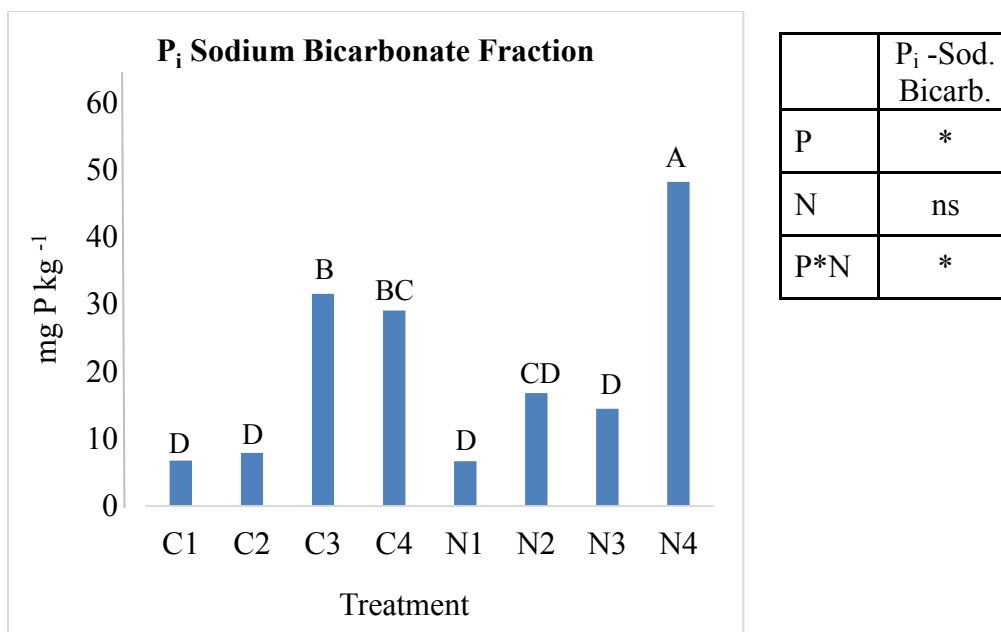
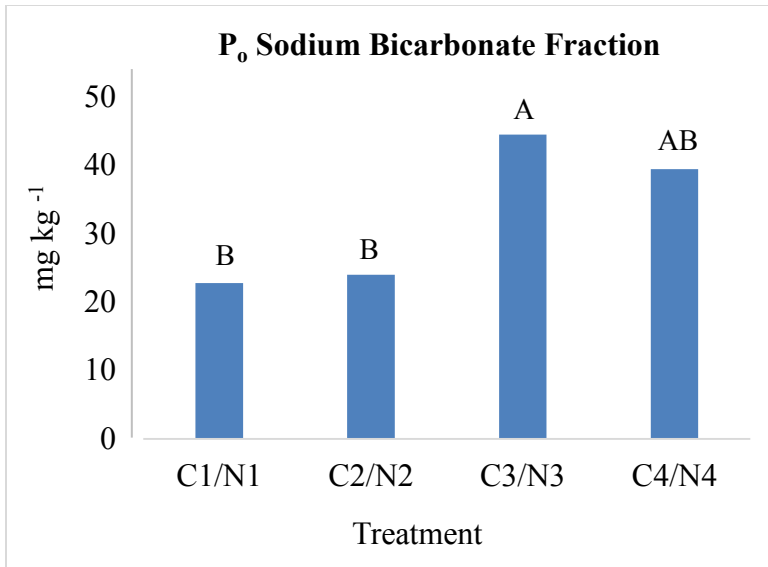


Figure 5.3 The influence of P and N treatments on sodium-bicarbonate extractable inorganic P (P_i). Bars with the same letter are not significantly different at P=0.05.

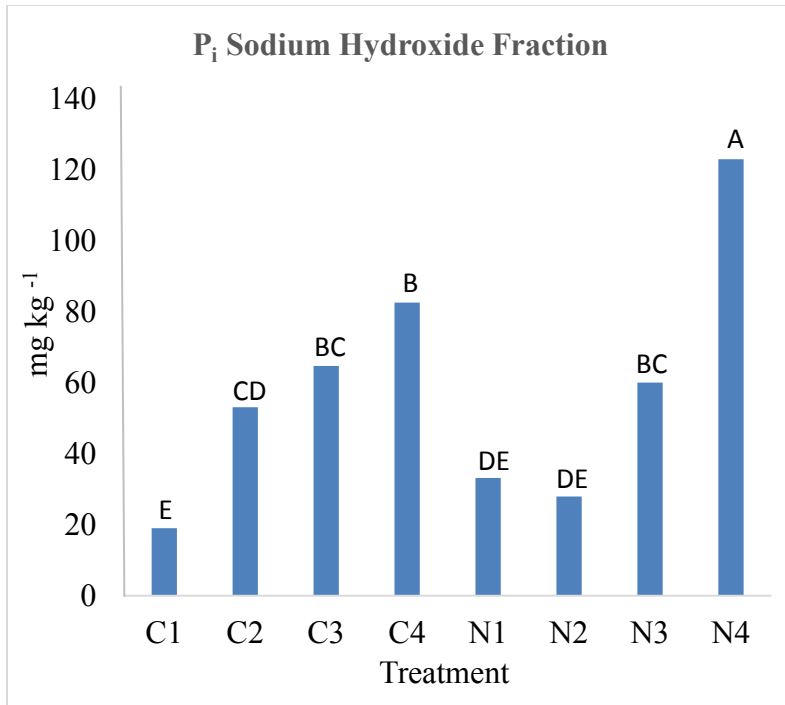
C1= 0 P/0 N, C2= 2.5 P/0 N, C3=5.0 P/0 N, C4= 10 P/0 N, N1= 0 P/10 N, N2= 2.5 P/10 N, N3=5.0 P/10 N, N4= 10 P/10 N. Units= g/m².



	P ₀ Sod. Bicarb.
P	*
N	ns
P*N	ns

Figure 5.4 The P main effect on the bicarbonate extractable organic P (P₀).

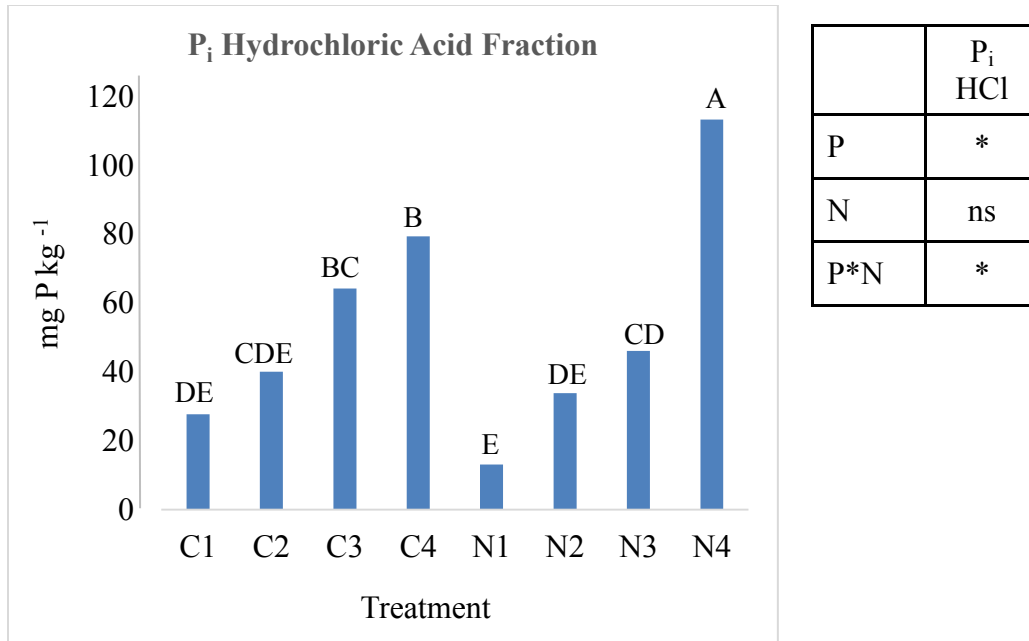
Each bar is the average of each P rate with and without N. Bars with the same letter are not significantly different at P=0.05. C1= 0 P/0 N, N1= 0 P/10 N. C2= 2.5 P/0 N, N2= 2.5 P/10 N. C3=5.0 P/0 N, N3=5.0 P/10 N. C4= 10 P/0 N, N4= 10 P/10 N. Units= g/m².



	P _i Sod. Hydrox.
P	*
N	ns
P*N	*

Figure 5.5 The influence of P and N treatments on Sodium Hydroxide inorganic P (P_i).

Bars with the same letter are not significantly different at P=0.05. C1= 0 P/0 N, C2= 2.5 P/0 N, C3=5.0 P/0 N, C4= 10 P/0 N, N1= 0 P/10 N, N2= 2.5 P/10 N, N3=5.0 P/10 N, N4= 10 P/10 N. Units= g/m².



	P _i HCl
P	*
N	ns
P*N	*

Figure 5.6 The influence of P and N treatments on hydrochloric acid extractable inorganic P (Pi). Bars with the same letter are not significantly different at P=0.05.

C1= 0 P/0 N, C2= 2.5 P/0 N, C3=5.0 P/0 N, C4= 10 P/0 N, N1= 0 P/10 N, N2= 2.5 P/10 N, N3=5.0 P/10 N, N4= 10 P/10 N. Units= g/m².

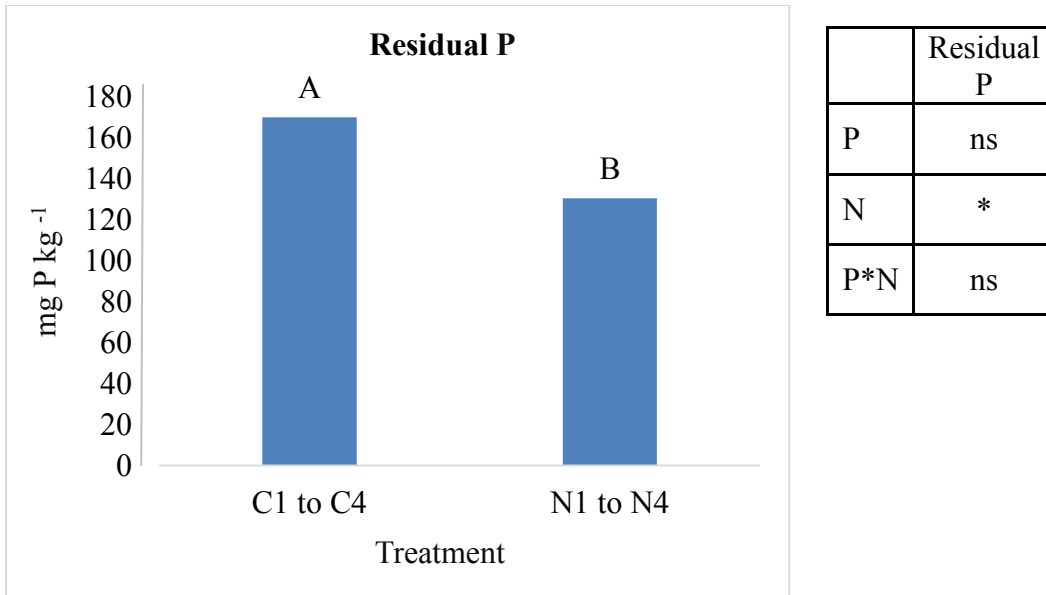


Figure 5.7 The N main effect on the residual soil P fraction. Each bar is the average of all P rates with or without N. Bars with the same letter are not significantly different at P=0.05. C1= 0 P/0 N, C2= 2.5 P/0 N, C3=5.0 P/0 N, C4= 10 P/0 N, N1= 0 P/10 N, N2= 2.5 P/10 N, N3=5.0 P/10 N, N4= 10 P/10 N. Units= g/m².

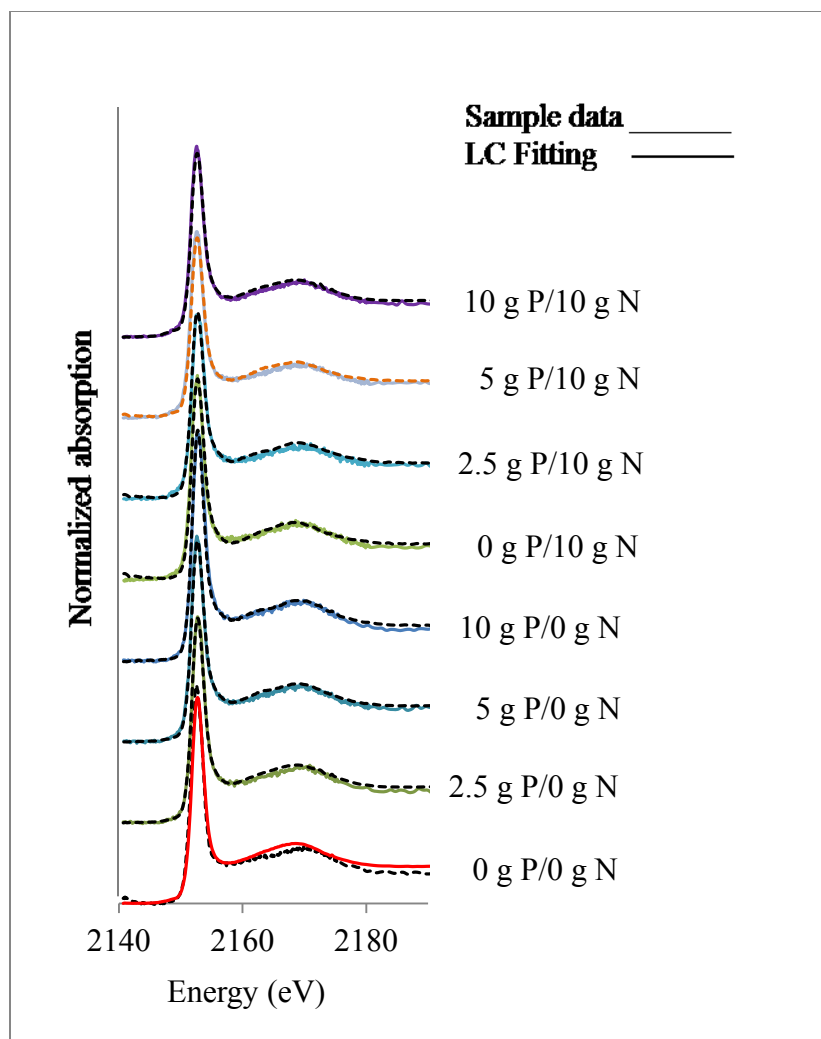


Figure 5.8 Normalized P K- edge XANES spectra on 0-5 cm soil depth for all treatments. The dotted lines are the linear combination fit line for each treatment and represents the linear combination fit with the P standards.

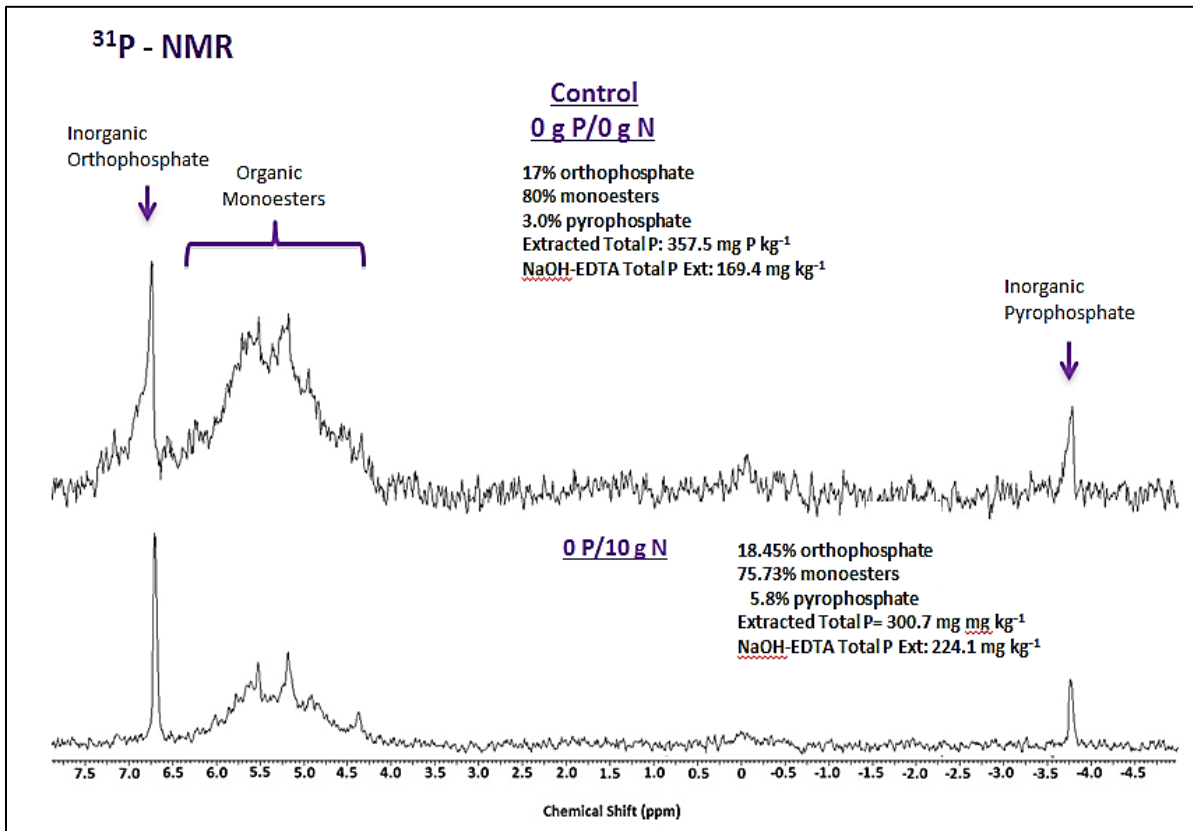


Figure 5.9 ³¹P-NMR spectra for unfertilized control treatment. 0 g P/0 g N, and 0 g P/10 g N fertilizer treatment.

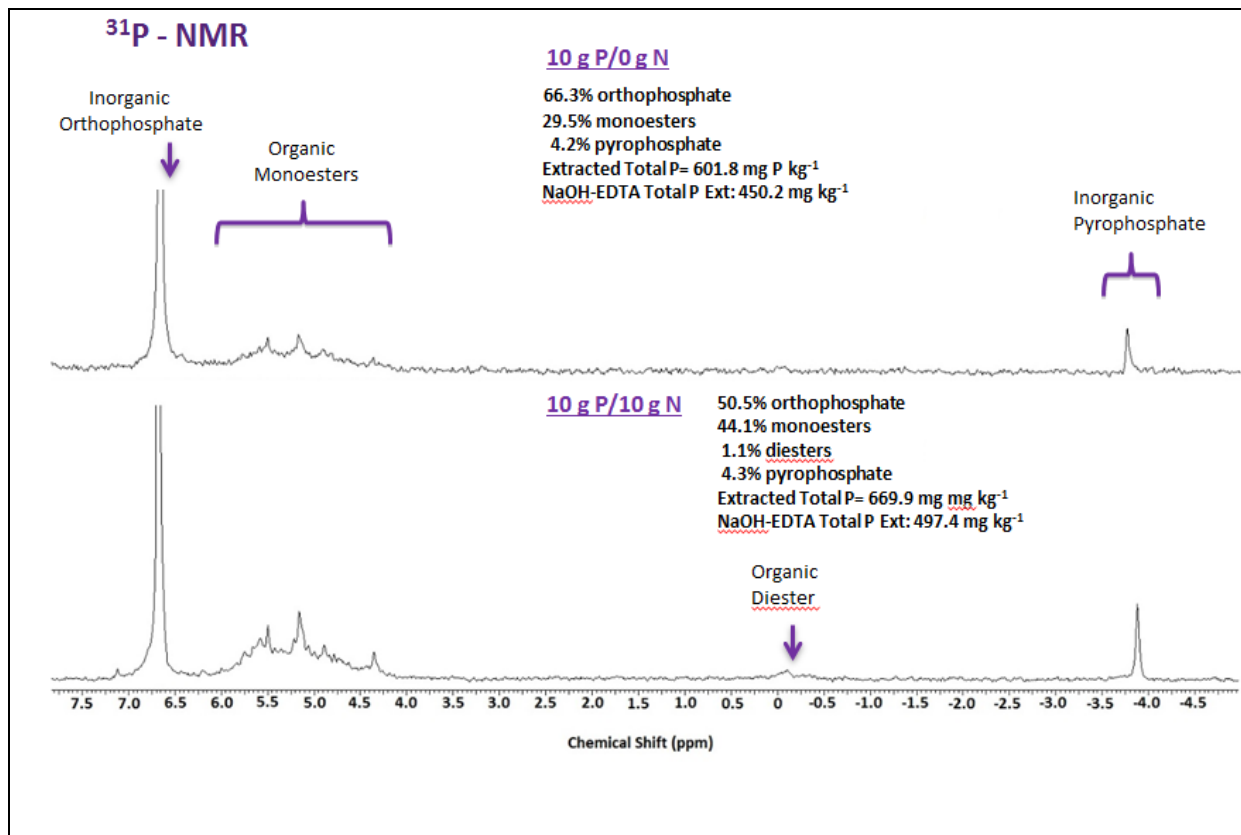


Figure 5.10 ³¹P-NMR spectra for 10 g P/0 g N and 10 g P/10 g N fertilizer treatments.

Chapter 6 - Summary and Conclusions

This research was designed to study phosphorus (P) fertilizer reaction products in a number of different soil systems. In each of the systems, P fertilizer added was expected to be “fixed” or transformed into fairly insoluble P by being either strongly adsorbed onto mineral or clay surfaces or precipitated as secondary solid phases, because of the high P fixation capacity of the soil, or because of low native soil P. The P reaction products formed in the different systems were expected to reduce P mobility and potentially lability in each of the soils.

In the laboratory-based incubation studies implemented on three acidic or three calcareous soils (Chapter 3 and 4) using different P fertilizer sources, MAP, DAP and APP, with and without FEP, results varied. On the acidic soils, P diffusion with the granular fertilizers was limited in all three soils; however, diffusion was enhanced in two of the soils with the liquid P treatments as compared to the granular P sources. The Oxisol showed that P source had minimal effect on lability, but in the Andisol, lability was significantly higher near the point of application with two liquid P treatments (with and without FEP) compared to the granular P treatments.

Phosphorus lability was estimated as the percentage of total P in the soil that was resin extractable. For the Ultisol only there was generally greater lability for the granular P treatments compared to the liquid P treatments in the center dish section. In addition, lability was higher near the point of application for APP + FEP when compared to APP alone. In general, the addition of the FEP did not consistently increase P mobility or lability in the acidic soils. The P K-edge XANES results indicated that P source had variable effects on P fertilizer reaction-product formation across the three soils in the study. The addition of the P increased or decreased

the percentages of Fe- and Al- precipitated and adsorbed phosphate-like forms compared to the unfertilized control treatment.

The only apparent effect of FEP was an increase in Fe-P-like forms in the Oxisol and an increase in Fe-adsorbed P-like forms in the Ultisol for all P sources as compared to the unfertilized control treatment. Speciation did influence P lability. For the Andisol, regardless of P source, or the presence or absence of FEP, the fraction of P present as Fe-adsorbed P-like form was positively correlated with P lability. Overall, this research suggests that there was not a consistent benefit of using a fluid over a granular P source in the acidic high P-fixing soils most likely due to a more or less irreversible sorption of diffused P by soil minerals. Similarly, adding P with FEP did not result in any increased mobility of lability of applied P.

Laboratory-based incubation studies on the three calcareous soils (Entisol, Mollisol, Inceptisol) (Chapter 4) showed that the added P fertilizer was transformed into Ca-P, Fe- and/or Al-P, and Al- and/or Fe-adsorbed P. The formation of the Ca-P species was the dominant mechanism for P fixation. The fraction of P species present as Ca-P was significantly and negatively correlated with P lability. Phosphorus lability was notably lower overall for the highly calcareous soil as compared to the two moderately calcareous soils. For all three calcareous soils, the results suggested a benefit of using fluid P fertilizer over a P granular in calcareous soils most likely due to a reduction in Ca-P precipitation due to enhanced mobility. This is in contrast to the acidic soils where there were no such benefits were found. The FEP did not consistently increase P diffusion or lability.

In gaining a better understanding of the fate of P and N fertilizers in soils of the native tallgrass prairie we wanted to determine how P and N fertilizer additions influenced the transformation of P in this soil system and did so by utilizing a number of approaches; soil P

sequential fractionation, P K-edge XANES and ^{31}P -NMR (Chapter 5). Soil P was influenced by the addition of N fertilizer as demonstrated with P sequential fractionation. With the addition of N fertilizer, there was increased P extractability from the labile and moderately labile fractions but there was decreased residual P. The ^{31}P -NMR results suggested inorganic and organic P changed with the addition of N and P fertilizers most especially with the highest P and N treatment rate. The increase in the monoester group represented increased contributions from plant growth and enhanced decomposition of plant residues, allowing for the organic P species represented in the monoester group to increase over time. The direct speciation of P using XANES spectroscopy revealed the addition of the P fertilizer alone promoted Ca-P formation. In addition, the XANES data showed that for the treatments receiving both P and N, there was a decrease in phytic acid and an increase in Fe-associated P. This supported the sequential fractionation results in that the increased above ground net primary productivity (ANPP) corresponding to a reduction in residual P fraction most likely due to more aggressive transformations of residual inorganic and organic P fractions. It is also possible that the increased demand for P due to the addition of N did not allow for the residual P fraction to increase.

There are several practical applications implied from this research. No strong evidence to select one P source over another, or to use this specific FEP were found for the acidic, high P fixing soil. Therefore, the existing knowledge promoting the use of banded P or high rates of P applications to manage high P sorption in acid soils is further supported. For the calcareous soils, the evidence suggests using liquid P fertilizer over P granular forms, particularly for moderately calcareous soils. The specific FEP did not seem to enhance diffusion or lability in a clear consistent manner in the calcareous soils. So no clear recommendation can be made with regard to FEP. While P and N fertilization is not common, or recommended, for native

grasslands, this research demonstrated that P added to the soils as fertilizer is partitioned to various P pools and this partitioning is significantly influenced by enhanced ANPP due to N fertilization.CC