

DISSERTATION

SOIL PHOSPHORUS AVAILABILITY AND TRANSFORMATIONS FOLLOWING BIOSOLIDS APPLICATIONS

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ABSTRACT

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Land application of biosolids has many benefits such as providing nutrients for plant growth as well as adding organic matter to soil. However, there is the potential for negative impacts due to the high concentration of phosphorus (P) relative to nitrogen (N). Much work has been done looking at the effects of over application of P and best management practices for the application of biosolids in the eastern United States. However, little work has been done in the western United States where the environment is very different due to lower rainfall and higher soil pH and calcium (Ca) concentrations.

This study was composed of four parts to help understand how P reacts in the western United States and to look at the implications for plant growth. These parts included: (1) fractionation of P in biosolids, (2) vertical distribution of P following long-term biosolids applications, (3) seasonal soil P cycling, and (4) plant uptake of P. The fractionation of P was designed to look at several biosolids and determine a method to be able to characterize the P that is present. This information would have the potential to aid in decision making about best managing the P from various sources. The vertical distribution of P following long-term application was designed to characterize the P in the soil profile. It is usually assumed that P is not mobile, but over long periods of time and with high application rates it can move. Seasonal changes of the different pools of P were studied to better understand how time of year affects the amount of P available in soil samples, and potentially to plants and the environment. The last study was designed to show how different sources of organic amendments supply different amount of P to a plant. The objective was to be able to better determine the amount of plant available P from an amendment.

The results of the different studies begin to show the similarities and differences in the behavior of P in the western United States and in the east. The fractionation study found that the assumptions that are used for manure cannot be applied to all fractions of P in biosolids and that a longer shaking time is needed. This study also found that there are differences among wastewater treatment plants that is mostly related to differences in treatment methods in an individual wastewater treatment plant. The vertical distribution of P study found that over time with repeated applications of P in dryland wheat (*Triticum aestivum L.*)-fallow rotations there is a significant effect on occluded P. Even with repeated applications most of the accumulations of P were limited to the plow layer and in a system where P chemistry is dominated by Ca, Fe still plays an important role. The seasonal change in the fractions of soil P study showed the changes in soil P were greatly affected by soil properties, water availability, climate, and application rates of biosolids. The high concentration of Ca in the soils favored the formation of Ca bound P, and saturated soils affected the forms of Fe bound P. The addition of organic matter and Fe with biosolids applications increased the concentration of soluble P, microbial biomass P, and Fe bound P. The plant uptake study found that there is a much larger effect of the soil on plant uptake than the source of the amendment. There is an inverse relationship between a soil's ability to adsorb P and the concentrations of plant tissue P when the plants are young. As the plants age and develop large root masses they can increase their tissue P concentrations.

This work shows that in the western United States there is more of a need to focus on the soils than the biosolids being applied to be able to make the best management decision. All the studies that looked at the P after it had been added to soil found that Ca affects the transformations and uptake of P by plants. In the western United States, there are areas that have large amount of Ca in the soil and areas that do not. As a result, interactions of Ca and P need to be focused on and further studied to ensure adequate plant nutrition as well as being a steward of the environment.

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CHAPTER 1: INTRODUCTION OF PHOSPHORUS AND BIOSOLIDS MANAGEMENT IN THE WESTERN UNITED STATES

Introduction:

Land application of municipal biosolids (sewage sludge) and manure as a method of beneficial use provides major nutrients, such as nitrogen (N), phosphorus (P), and micronutrients, needed for crop production (Barbarick et al., 2012). However, the application of organic material, such as manure and biosolids, can lead to accumulation of soil P (Elliott et al., 2002). As the concentration of P in the soil increases, so does the potential for movement to surface water and the resulting negative environmental impacts due to eutrophication (Carpenter et al., 1998). The risk assessment for environmental impacts of P is based mostly on the risk of offsite transport by runoff (Domagalski and Saleh, 2015). The movement of P from agricultural fields to waterways has been extensively studied (Sharpley et al., 2013), but mostly in the eastern portion of the United States. This work may not relate to what would be expected in the western United States, due to the lower rainfall and the difference in soil chemistry. In the eastern United States soil P chemistry is dominated by iron (Fe) and aluminum (Al), but western soil P is largely controlled by calcium (Ca) and to a smaller extent magnesium (Mg) and manganese (Mn) (Lindsay, 1979). The different chemical forms of P affect the availability of P to both plants and the environment (Elliott et al., 2002, Sharpley and Moyer, 2000).

To understand how P behaves in arid soils, several interactions of P, soil, and plants need to be studied further. These include how P portions between different soil P pools, what is the vertical distribution of P in the soil profile following applications of biosolids at varying rates, and the extent plants uptake different amounts of P depending on the source of P fertilizer.

In soils, there are three recognized pools of inorganic P (sorbed, secondary minerals, and primary minerals) and one pool of organic P that are in equilibrium with each other and the soil solution

P (Figure 1.1, Pierzynski, 2005). The soil solution P is the pool that plants use as their nutrient source and the form that is available for leaching. Primary minerals contribute P to the soil solution pool by dissolution of P through weathering. The weathering of primary minerals contributes P to the soil solution pool by the leaching of bases, loss of carbonates, and increased activity of Fe and Al (Walker and Syers, 1976). The secondary minerals are in equilibrium with the soil solution through dissolution and precipitation reactions (Pierzynski, 2005). Sorbed P is the most loosely held P and is connected to the soil solution P through sorption and desorption reactions to clay particles and metal oxides. Organic P is connected through microbial driven transformations of mineralization and immobilization.

Phosphorus is not highly mobile through the soil profile, but there have been cases shown where P will leach (Elliott et al., 2002). Phosphorus may also redistribute in the profile by translocation in plant roots and subsequent deposition of P by root biomass turnover. A better understanding of soil P vertical distribution is needed for understanding how soil P is accumulated through the application and incorporation of biosolids and to determine if there is deep accumulation of P taking place.

The amount of plant available P that is supplied by different amendments is of great importance to both crop producers and the producers of large amounts of P rich organic waste. The producers of both livestock manure from confined feeding operations or municipalities with the production of biosolids through the treatment of wastewater are interested in the proper management of P. As the political and environmental climates have changed over the last 20 years, there has been more importance placed on the management of P leading to changes in the regulations that control the application of biosolids. Currently the regulations assume that all the P that is applied is plant available; however, it would be expected that during the wastewater treatment process some of the P would be in forms that would be unavailable to plants in the soil. During the treatment of wastewater Fe is added to

reduce gas production during digestion and this Fe may bind to P creating less plant and environmentally available forms of P.

If the wastewater treatment process affects the amount of P that plants take up from biosolids, then a need exists to characterize the biosolids in terms for plant available P. Currently, available methods are techniques that have been used for manures, but due to the large differences in the inputs that create manure and biosolids, it is expected that the methods used for manure will need to be modified for use with biosolids.

Importance of Phosphorus Management:

Phosphorus is an essential plant macronutrient. In agronomic production, crops are usually fertilized to meet the N, P, and potassium (K) needs. Over the years the sources of the P have changed. Initially, P fertilizer was from organic sources, including manure and guano (Stewart et al., 2005). In the mid-1800s, rock phosphate (RP) deposits were found in the United States and around the world. The RP for fertilizer production is a limited resource, and it has been estimated (Stewart et al., 2005), that the current reserves will be depleted in the United States within 100 years and worldwide in approximately 340 years. Beginning in the late 1900s there was growing interest in recycling biosolids as a fertilizer source. The main benefit to using biosolids is the presence of both plant macro- and micronutrients at a much lower cost than traditional fertilizer. In recent years there has been a growing concern with the over-application of P leading to increased focus on P management (Sharpley et al., 2013). As the deposits of RP continue to decrease, it becomes more important to look at other sources of P and potential changes to management practices to increase the efficiency of P application.

The management of P is very important in maintaining the ability to produce large amounts of food to feed the growing world population. Many of the advances in agriculture that have led to increases in food production are a result of improved genetics and proper management of the

agroecosystem. In the developed world there is a tendency for the over-application of fertilizer, while there tends to be under-application of P in the developing world (Bindraban et al., 2015). The over-application of P has increased the risk of P loss through runoff (Elliott et al., 2005) or leaching (Elliott et al., 2002), and in some cases has elevated soil P to levels sufficient for short-term crop production without additional P fertilization (Withers et al., 2014). The need for improved understanding of how P interacts with soils becomes very important in matching the amount of P that the crop needs and the total amount of P that can be supplied by fertilizer and residual soil P. This becomes even more important with biosolids management where elements in the biosolids have the ability to sequester P and change the soil P dynamics.

Biosolids as P Fertilizer:

The uptake of P by plants concentrates P to a level that when humans and livestock consume the plants, large amounts of P are released in their waste. However, there is a growing disconnect in the P cycle because P from waste products of animals and humans is not being recycled back into production of feed stocks (Sharpley et al., 2015). The locations of large animal feeding operations and feed sources are often geographically separated resulting in excess nutrients from the feeding operations not being returned to crop production land. Similarly, human waste is concentrated in urban centers; typically, well removed from locations of possible agricultural use.

One of the limitations for the efficient reuse of biosolids as a fertilizer for agricultural production is the over-application of P that arises from the application of biosolids at the agronomic rate of N. The amount of P that plants need relative to N is lower and biosolids have a higher concentration of P relative to N. The future reduction in the amount of P that wastewater treatment plants will be permitted to discharge into surface water will result in increased amounts of P in the biosolids. This change will further increase the imbalance of the ratio of P and N in biosolids relative to the needs of

plants. There has been some transition to the use of mechanical and chemical methods to recover P from biosolids and manures, but many of these can be cost prohibitive (Sharpley et al., 2015).

A simple solution to the over-application of biosolids is to change the application rate from the agronomic rate of N to the agronomic rate of P (Chinault and O'Connor, 2008). This approach would reduce the excess P applied to the soil but may lead to unforeseen consequences such as the reduction of additional carbon (C) added to the systems to help replace the C removed with the harvest of biomass. There is a strong relationship between P availability and microbial activity, and the changes in C in the soil will affect microbial activity. The shift in the application of biosolids from an N to P basis would require a larger area of land to apply biosolids due to the lower application rates. One of the limitations to increasing the acreage available for land application is the public acceptance of biosolids. When biosolids were first applied in the 1970s, concerns were raised due to metals and the unknowns associated with the application of biosolids. Many of these objections have been overcome, but there is still the need to continue to improve public perception and acceptance of the land application of biosolids to aid in the sustainable management of P (Sharpley et al., 2015). Others have speculated that the change from the agronomic rate of N to P may result in the end of land application of biosolids due to increased cost of spreading and hauling (Chinault and O'Connor, 2008).

As more information is learned about nutrient management, best practices have been developed such as the “4R” approach of the right rate, right source, and the right time with the right placement. However, the application of biosolids is not able to always follow the “4R” approach. Larger wastewater treatment plants produce multiple semi-truck loads of biosolids every day and do not have the ability to store the biosolids until it is the best time to apply. Also, the application of biosolids is regulated, and there are limitations on who can apply biosolids making it harder to apply over the smaller application window when fertilizer application would be optimal. For nutrients that primarily

move through the soil by diffusion, like P, plants receive the most benefit by placing the nutrients close to the roots. Biosolids are generally applied by broadcasting a cake and then incorporating it into the soil. Biosolids are generally applied as evenly as possible across the field and this results in the nutrients being distributed across the field, not just near the plant roots. These challenges with the application of biosolids make it very difficult to match “the right time and the right placement.” If it is assumed that this half of the “4R” approach cannot be followed, then it would become more important to ensure that the other half of the approach is followed as closely as possible.

As a “right fertilizer source,” biosolids have been shown to supply both macro- and micronutrients to plants (Barbarick et al., 2012). Application of biosolids also provides C to the soil that improves the physical properties of the soil that help with water retention and flow (Ozores-Hampton et al., 2011). The challenge is to understand the imbalance of N:P and how to properly manage it.

The last part of the 4R approach is the “correct rate”, which has the most room for improvement with the management of P from biosolids. As mentioned earlier, the application of biosolids at the agronomic rate of N leads to the over-application of P. The best way to manage P so that it is not used in excess would be to develop application practices for biosolids that are only at the agronomic rate, but this is not currently feasible. The application of biosolids at the agronomic rate of P would not supply enough N and the addition of C and the micronutrients would be greatly reduced. This may reduce the willingness for producers to use biosolids because they have been using it as the N fertilizer and gaining the benefits of the additional nutrients. Much of the reluctance by the producers would be due to the large inconvenience of biosolids application by a separate party at a time that would not always be optimal and not meet all the plant fertility needs. The remaining nutrients would have to be applied to the soil by an additional fertilizer application at a higher cost. The problems with the changes in the application rate from an N to P basis would make it more difficult to find land for the

application of biosolids while also increasing the amount of land that is needed for application.

Therefore, a balance needs to be reached on the practical agronomic and environmental concerns.

To protect the environment and also supply the needed nutrients to plants, the use of risk assessments has been developed to reduce the risk of the over-application of P causing environmental damages (Sharpley et al., 2003). The use of these assessments allows for determining the risk of environmental impacts from the application of P fertilizer and then requires management practices to mitigate the risk. The use of these assessments is very important for biosolids management in that they allow the change from N to P application rates to be done on a field-by-field basis.

The data used for many of the decisions on P management is based on the use of research in the eastern United States. Most of this research on the management of P from biosolids and manure has been where there is a history of over-application of P resulting in soils with high P (Sharpley et al., 2013). In the western United States, the agronomic and management practices are different due to the large difference in climate and soil properties. The soils in the semi-arid west have a higher amount of Ca and calcium carbonate (CaCO_3) and lower concentration of Fe and Al. The difference in chemical properties of the soil has a large affect on the P dynamics. The assumption that the effects seen in the eastern United States will be the same in the west may lead to large errors in the management of P. Like in the eastern US, there is still the risk of P transport to waterways and the reduction in water quality (Chinault and O'Connor, 2008), but with lower rainfall in the west there is a higher potential loss of P from wind erosion which currently is not well understood (Sharpley et al., 2015).

Relationship Between Calcium Carbonate and Phosphorus:

The bioavailability of P in soils is controlled by organic matter in the short term and by geochemical transformation in the long term (von Wandruszka, 2006). The sorption of P to clays is dominant at low concentrations of soil P, but as the concentration of P increases, the sorption to CaCO_3

becomes more important (Ryan et al., 1985, Samadi and Gilkes, 1999, Zhou and Li, 2001). Some studies have shown that there is a negative relationship between P availability and CaCO₃ concentrations (Afif et al., 1993, Sharpley and Smith, 1985). Soils in the arid western United States have a high amount of CaCO₃ or calcite and also tend to have a high pH which affects the forms of P present (Lindsay, 1979). While it is generally viewed that carbonates reduce plant availability of P fertilization (Cole et al., 1953), studies have shown that calcite can act as a sink or a source of P depending on the conditions of the soil (So et al., 2011). In soils that have high calcite it becomes very important to determine the interactions between calcite and P to better understand the availability of P to both plants and the environment. Also, the ability of calcite to hold or release P under different conditions will have a large impact on the amount of P that would be extracted by different soil test methods and this could lead to mistakes in management decisions. A large amount of work has been completed (House and Donaldson, 1986, Madsen, 2001, Millero et al., 2001, So et al., 2011, Tomazic et al., 1989, vanderWeijden et al., 1997) on the relationship between calcite and phosphate in batch studies, and this work can be used to help theorize how calcite in soils will react with the addition of P.

The ability of calcite to bind with P depends on the ionic strength (So et al., 2011), organic matter content (von Wandruszka, 2006), surface area (von Wandruszka, 2006), pH (Cole et al., 1953), and the surface charge of calcite (Madsen, 2001). So et al. (2011) also found that the sorption of phosphate to calcite does not reach the theoretical amount of P that could be bound and that it was 10 to 15 times less than the sorption maximum. They suggested that there are physical limitations, repulsion forces between adsorbed phosphates, and competition for the binding sites between phosphate, carbonates, and bicarbonates. In soils, there are many anions present in the soil solution that may further reduce the amount of P that could be bound. The kinetics of phosphate binding to calcite in batch studies is rapid; initially a large amount of P is sorbed and after 2-3 hours the concentration in the liquid phase will become constant (So et al., 2011). This fast adsorption (3 hours of

reaction time between the calcite and phosphate) has been shown to be reversible if the solution is changed to one that does not contain P (So et al., 2011).

The degree to which CaCO_3 will bind with P and affect the amount of P that is available is most likely a function of the concentration of P in the soil solution. Zhou and Li (2001) show that the isotherms that represent the sorption of P are dependent on the concentration range. If the concentration of P is $0\text{--}1 \mu\text{g mL}^{-1}$ there is a linear relationship, from $1\text{--}400 \mu\text{g mL}^{-1}$ both Freundlich and Langmuir isotherms fit well, and between 400 and $600 \mu\text{g mL}^{-1}$ there was precipitation taking place.

Following the rapid initial binding of P to calcite, slower reactions occur resulting in the formation of precipitates (So et al., 2011). Even though the precipitation of phosphates does have an effect on the overall chemical activity, this does not have an effect on the kinetics of the reaction (vanderWeijden et al., 1997). The formation of precipitates is believed to initially start by the formation of intermediate amorphous and metastable crystalline phases before the formation of more stable crystalline forms of calcium phosphates (Tomazic et al., 1989). The formation of hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) tends to be the initial precipitate when the saturation index is between 6.43 and 8.04 and calcite is present (So et al., 2011). (Saturation index is the ratio between the ion activation product, which is the product of the actual activity of the species that form the solid, and the thermodynamic solubility product, K_{sp} .) The addition of P will lead to the formation of very insoluble carbonate apatite (McGeorge and Breazeale, 1931). In soils that have a high level of exchangeable cations, precipitation appears to be the dominate mechanism for the retention of P (Tunesi et al., 1999).

The effect of ionic strength on phosphate sorption to calcite appears to be related to the activity of aqueous phosphate (So et al., 2011). As ionic strength increases, aggregation will reduce the surface area, thus reducing the potential for adsorption. The desorption of P is not affected by the ionic strength of the solution because complete desorption has been seen in seawater (Millero et al., 2001).

As a note of practical importance, the extraction of phosphate that is sorbed to calcium carbonate surfaces is easily achieved with a solution of sodium bicarbonate (NaHCO_3) due to reduction in solution Ca and the competition of bindings sites between phosphate, bicarbonate, and carbonate ions (Cole et al., 1953). Sodium bicarbonate extracting solutions are used in the Olsen extraction method (Kuo, 1996), and it has been shown that when humic materials are added to calcareous soils there will be increases in extractable P (von Wandruszka, 2006). Humic materials interact with metals and the phosphates that were bound to the metals releasing them for plant absorption (Riggle and von Wandruszka, 2005).

In summary, the fast adsorption of P and its reversibility suggest that added P is quickly bound with calcite, but readily released so that it would still be very available to cause environmental impacts. However, if there is a long reaction time (greater than 3-8 days), then there is the potential for the formation of precipitates that would be more stable and less available. These time frames are for batch studies that would have more chemically optimal conditions than would be found in fields. It would then be expected that the reactions would be slower in field soils and dependent on water content and temperature.

Soil Organic P Dynamics:

Organic soil P is classified as any form of P that is associated with non-carbonate C. The mechanisms that are involved in the transformations of organic P and the exact role of organic P in plant and environmental availability of P is not completely understood (Condon et al., 2005). One of the limitations to better understanding organic P is that there is not a method for direct measurement of organic P, and indirect methods lead to potential inaccuracies. A large amount of total soil P can be organic P and must be transformed to inorganic P before being taken up by plants. Harrison (1987) showed that between 30 and 60% of the total P in a plant is from organic forms of soil P. This fraction of

P can have a high rate of turn over and in production fields 18 to 38% of the P taken up by plants is returned to the soil in the form of litter and roots each growing season (Hanway and Olson, 1980). The mineralization of organic P to inorganic P can occur at a daily rate that produces as much inorganic P as the equilibrium soil concentration of orthophosphate (Oehl et al., 2001).

The rates of reactions for both mineralization and immobilization control organic P turnover. There are two primary methods for immobilization in the soil, (1) the uptake of P into plants that is then returned in the form of litter, roots, or animal waste and (2) the uptake of P into microbial biomass with the decomposition of organic matter especially when the C:P ratio is >300:1 (Condrón et al., 2005). The transformation of organic P to inorganic P through mineralization is not well understood, but it has been shown that it is controlled by extracellular activity primarily through the enzyme activity of phosphatase (Frossard et al., 2000, Magid et al., 1992, Stewart and McKercher, 1982). Some studies that have shown that phosphatase has a role while other researchers were not able to find a relationship (Condrón et al., 2005). However, further investigation into the mechanism has found that what may be the most important factor in mineralization is the availability of suitable substrates rather than the activity of phosphate (Gressel et al., 1996). The rate of mineralization is influenced by physical properties that affect soil-water content, including particle size, aggregate stability, and the influence of wetting and drying (Chepkwony et al., 2001, Perrott et al., 1999, Rubæk et al., 1999). The large interaction of environmental factors and organic P factors would lead to seasonal fluctuations that have a large effect on the amount of P that is available for plant uptake.

Plant Uptake of Phosphorus:

Phosphorus is one of the three macronutrients that plants need to grow and produce large yields. Unlike N, P is not found in large concentrations in the soil solution, usually not exceeding 10 μM (Bieleski, 1973). Plants have a tissue concentration of 0.1-0.5% P (Havlin et al., 2005) showing that

plants need to take in large amounts of soil water to supply P to support their development and growth. Phosphorus is needed for almost every aspect of plant growth from metabolism to the transport of nutrients through the plant (Schachtman et al., 1998). Plants take up only inorganic P which is found in the soil as H_2PO_4^- and HPO_4^{2-} , but there is preferential uptake of H_2PO_4^- (Chen and Barber, 1990).

Plants interact with soil to extract P through both a passive and active process. Soil P will slowly diffuse through the soil or move with the soil solution and come into contact with the roots of the plant. Plant roots are usually closer to the soil surface where higher concentrations of nutrients are present, and as the roots grow they will bump into pockets of P, which has been termed root interception (Barber et al., 1963). Using the assumptions of Barber (1963) that the volume of soil that will come in contact with roots is equal to the volume of the roots, only 1% (the volume of roots for an annual crop (Barber, 1995)) of the soil in the top 20 cm will come in contact with the roots. To better extract P from the limited amount of soil the roots contact, the roots will exude organic acids in high quantities (up to 23% of net photosynthesis) to acidify the soil causing the mobilization of P and micronutrients by chelating metal ions around the roots (Marschner, 1995). Mass flow, the process by which the soil water moves as plants transpire and removes water from around the roots and additional water moves to that area (Kovar and Claassen, 2005), usually supplies about 5% of the P to the plant (Barber, 1995). In soils that have had a long history (greater than 10 years) of organic amendments such as manure or biosolids, the soil solution will have a higher concentration of P and mass flow will supply a higher percentage of P to the plants (Frossard et al., 2000).

Due to the involvement of water flow on the distribution of P in the soil profile, many soil physical properties will have an effect on how P is transported in the soil (Kovar and Claassen, 2005). When soils are under different management practices such as no-till versus conventional tillage, or have

different clay and organic matter content, then there also are potential changes in the amount of P that would reach the plant in addition to the reactivity of the soil that would affect the mobility of P.

As plants absorb P from the soil, a concentration gradient that forms which drives diffusion. Plant uptake of P creates non-equilibrium in the soil and will deplete P that is in contact with the root surface, but a soil test still may show an adequate amount of P in the soil. Different plants are able to take P out of the soil solution at different P concentrations, and for most production crops the concentration can be as low as 1 to 5 μM (Asher and Loneraga, 1967, Barber, 1995, Breeze et al., 1984). In response to the plant removal of solution P, soil P will move from solid phase to liquid phase forms. However, when large amounts of soluble P is added to the system from the addition of organic amendments such as manure and biosolids, the equilibrium will be shifted in the other direction and P will precipitate out of the soil solution (Kirk, 1999).

The soil pore-size distribution and total porosity affect water flow. To explain some of the differences in P uptake that are seen between different soil textures, soil physical properties need to be studied. As the soil water content changes, there will be different amounts of P that go into solution due to equilibrium of the solid and liquid phases, and this can lead to challenges with management of P in soils that experience rapid changes in water content. If the soil bulk density changes, there will be fluctuations in the amount of air space and water in the soil that will then affect the amount of P in the soil solution. As the plant roots grow they compress the soil around the roots which makes for a more tortuous path for the water flow and this reduces the amount of P that reaches the root by mass flow and concomitantly slows the rate of P diffusion (Bruand et al., 1996).

Due to different mechanisms, studies have shown both an increase and a decrease in solution P as the soil temperature changes. As water cools the diffusion of P will slow (Barber, 1995), but the amount of P that is adsorbed to soil solids will decrease and result in more P staying in solution (Kovar

and Claassen, 2005). As soils warm the concentration of organic labile P will increase, resulting in a 1-2% increase in solution P per degree increase in temperature (Kovar and Claassen, 2005).

Plant root kinetics:

Due to the higher concentration of P in the plant compared to the soil, the process of acquiring P by the roots is an energy-intensive process. There are specific binding sites and “carriers” (Marschner, 1995) where P will travel through the cell wall of plants, and recently there is evidence of specific genes that regulate this process (Smith et al., 2003). The transfer of P is also dependent on the concentration of Mg in the soil and P uptake can be reduced when a plant is deficient in Mg (Havlin et al., 2005). Many of the forms of soil P are not very mobile and are unavailable to plants. As plants have evolved, they have developed strategies and methods for changing the soil in the rhizosphere around the roots to become more favorable for P solubilization. The strategies can involve the release of organic acids that cause Fe, Al, and Ca bound P to go into solution or the plant may change the architecture of the roots to have more branches with increased root hairs that can find pockets of P in the soil. Plants have the ability to affect the soil chemically for only short distances from the root surface, ranging from <1 mm (Hubel and Beck, 1993) to approximately 4 mm (Gahoonia and Nielsen, 1992). Due to this small zone of influence that the roots can affect, the plant produces many additional roots and root hairs to increase the surface area of the roots thus increasing the ability of the plant to find more P. Taiz and Zeiger (2006) have shown that the majority of the surface area of roots is from the small root hairs.

Translocation and storage of P in plants:

The distribution of P between the roots and shoots of a plant, is dependent on the amount of P the plant is able to extract from the soil. The P is translocated through the xylem (where only inorganic forms of P are transported) and through the phloem where both organic and inorganic forms of P are transported (Schachtman et al., 1998). If the plant is experiencing optimal or surplus soil P the roots will

extract soil P and transport it through the xylem to the young leaves (Schachtman et al., 1998). When the plant becomes P deficient, the amount of P in the xylem will decrease from approximately 7 mM to 1 mM in barley (Mimura et al., 1996). As a result, P in the older leaves is metabolized and translocated into the growing roots and shoots (Schachtman et al., 1998). However, approximately half of the P that is translocated to the roots through the phloem is then translocated back to the growing shoots by the xylem (Jeschke et al., 1997).

Practical Implications:

With the unique differences in soils in the western United States, there are several areas where additional research is needed to address some of the challenges that have been presented. The first is; if the practices that have been used for many years are continued, what are the expected impacts? Before large changes are made to existing management practices, it is important to document a problem with the practice that needs to be addressed. If there is evidence of P buildup in sites that have received long term application of biosolids at the agronomic rate of N, then it would suggest that there may be a problem with over-application of P. However, for the P to be of concern for environmental impacts to surface water the P also would need to be near the soil surface and close enough to water bodies it would lead to negative environmental impacts.

The objective of this project is to gain a better understanding of the transformations and availability of P in Colorado soils that receive biosolids. Several research questions need to be answered to accomplish the objective:

1. To what extent is there downward movement of P in soil following the addition of biosolids?
2. Is there seasonal cycling between the different pools of soil P following the application of biosolids to agroecosystems?

3. What fraction of the total P in the biosolids applied is plant available, and does the source of P being applied make a difference?
4. Do the assumptions made for the fractionation of P from manure hold for the fractionation of biosolids P?

Downward Movement of P:

To evaluate the effects of long-term application of biosolids managing for N, several experiments were established to investigate the vertical distribution of P in the soil. One site was a long-term research field, with 20 years of biosolids management and a wheat (*Triticum aestivum L.*)-fallow rotation. The research plots allowed for monitoring under very controlled conditions with varying biosolids application rates. The second site has an eight-year application history that has been in continuous irrigated corn. The sites vary in soil properties helping to determine if any difference seen are site specific or if the results can be projected to other locations.

Seasonal Cycling of Soil Phosphorus:

With the large impacts of high levels of soil CaCO_3 on P availability, it is important to determine the P dynamics during a growing season that may affect P availability to both plants and the environment. Cycling of soil P can affect the long-term buildup of P in agroecosystems that are only managed for N. The equilibrium between the soil solution P and the pools of inorganic and organic P needs to be understood to determine how much P is available. There have been studies that investigate the dynamics of P moving between different soil P pools over multiple years and very short time frames but not over a single growing season. The P cycle involves transformations that are both chemically and biologically driven. During a single season, there may be times when transformation take place that affect P availability. This is important information to know because the loss of P is primarily due to runoff. It would be important to know these times of year so that possible management practices can

be put in place that would reduce the environmental risk. Also, the transformation between different forms will affect the soil P index value received from a soil test and it may be possible that certain times of the year do not provide an accurate accounting of the P that is in the soil. It is generally assumed that soil test P is a measure of the amount of inorganic P that is in the soil and plants only use inorganic P. If there is a large amount of organic P in the soil, accounting for this in making P recommendations would be necessary.

To investigate the seasonal changes of P, research plots are used in this work as well as production fields to determine how P changes seasonally. The research plots have a long history of application at various rates. The production fields were used to determine if the same effects are seen under production management practices but with different soil and different crops being produced. The samples were collected and P was fractionated into 12 different forms. The forms of P were limited to those described as functional forms based on the extraction method; however, this still allows for comparisons that affect P availability.

Plant Availability of Phosphorus:

The seasonal changes of P in the field can be useful for making management decisions, but there are still limitations in using this information. After knowing how P changes during the growing season it becomes important to know how much of the P applied is taken up by plants and if there is an effect of different sources of P amendments. To investigate the uptake of P by plants a greenhouse study was established to study the interaction between different amendment sources (both biosolids and manure) to different soil types at varying rates. This study used sorghum-sudan grass (*Sorghum bicolor* (L.) Moench ssp. *drummondii*) due to its relative high need for P (Ketterings et al., 2006) and the ability to harvest the same crop multiple times. Both the soil and the plant tissue were analyzed after each harvest and an accounting of the P in the system was conducted. The intent was to determine the

amount of P that was being used as a proportion of the total amount of P that was applied. It was hypothesized that the fertilizer source would be more an important variable than the soil type so there were five amendments and three soils used.

Fraction of Biosolids Phosphorus:

To use the information about how different amendments, affect plant uptake of P, a method to characterize the P forms in biosolids was needed. In many studies where the P in biosolids is characterized the method that is used is from work on manure. It was expected that manure and biosolids would have different properties due to the large difference in the input sources for the two materials. As a result, research tests were conducted to determine if the assumptions that were used for the fractionation of manure P could also be used for biosolids.

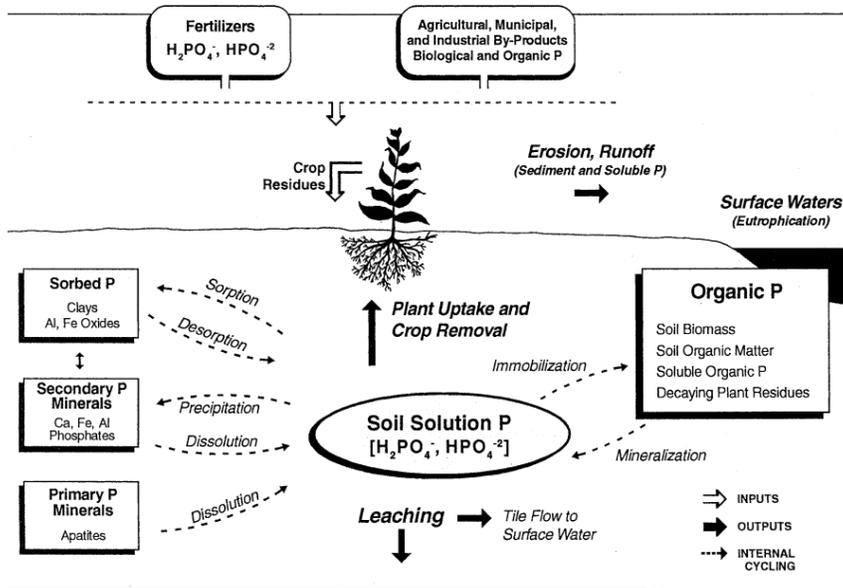


Figure 1.1. Phosphorus cycling diagram from Pierzynski (2005).

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CHAPTER 2: PHOSPHORUS FRACTIONATION FROM BIOSOLIDS

Introduction:

There have been many studies that determined methods to evaluate the fractions of phosphorus (P) in manure (Dou et al., 2000). However, there is a lack of information on the forms of P present in municipal biosolids. Most studies of P forms in organic soil amendments have focused on raw manure from a livestock producer. The method that is typically used for manure (Dou et al., 2000) was modified from the soil P fractionation procedure (Hedley et al., 1982). The modifications were based on data and assumptions that the P in manure would be in simple forms. Compared to soil P analysis methods (Kuo, 1996), this reduced the time for each step of the analysis. However, since biosolids contain many cations that can form complexes with P, the method required to fractionate the forms of P from biosolids may require a procedure that is more similar to the soil method. Biosolids are the product of extensive processing of a municipal waste stream that may result in a product that is not at chemical equilibrium at the time that the biosolids leave the plant. During the production of biosolids, there can be the addition of Fe or Al compounds to reduce the production of toxic gases during anaerobic digestion or to aid in the removal of P from the liquid effluent prior to discharge. This allows for the formation of more complex forms of inorganic P in the biosolids compared to manure.

Material and Methods:

Biosolids P Fractionation Method Development:

Biosolids samples were collected from four wastewater treatment plants along the Front Range of Colorado. Four replications of each sample were analyzed at Colorado State University (CSU) using a sequential extraction method to segment the inorganic P in each sample. The extraction solutions were deionized water, 0.5 M NaHCO₃, 0.1 M NaOH, and 1.0 M HCl. The extraction solutions were chosen to match the solutions being used for manure analysis (Dou et al., 2000). Each of the samples was shaken

at a ratio of 1:100 (0.3g of biosolids and 30.0 mL of extraction solution). Previous work has shown that drying biosolids samples will change the availability of metals and nutrients (Akinremi et al., 2003); therefore, wet samples were used. Three subsamples of each biosolids were dried to determine the water content. The subsamples were placed in a convection oven at 105°C for 16 hours. Then 0.3g of dry matter equivalent was placed in pre-labeled tubes for each of the analyses that would be conducted. The samples were then kept frozen until needed for analysis. Following the collection of the samples a subsample of each biosolids was also analyzed by the Soil, Water and Plant Testing Lab at Colorado State University for total metals and nutrients (Table 2.1).

To determine the amount of time that the samples should be shaken to extract the P, samples were shaken for 1, 2, 4, 8, 16, and 24 hours using a horizontal reciprocating shaker. While determining the amount of P extracted from each fraction the samples were centrifuged and then filtered through a 0.45 um filter. The sample was then analyzed with the modified molybdenum blue method (Rodriguez et al., 1994). Initially the thought was that we should replicate each of the extractions until no more P was released. However, as we continued to do the extractions it was noticed that there was a point where the amount of P extracted with each replication remained constant. Possibly P was being removed mechanically that was not actually made available by that extracting solution. Under these conditions, the complete chemical removal of P by an extractant is indicated by a constant slope of the cumulative P released (less than 5% change in total P extracted for a given fraction) and this criterion was used to judge when further extraction steps were unnecessary.

Results and Discussion:

Biosolids P Fractionation:

One of the guiding principles for the study was to develop a method that would be practical to use. Therefore, times were chosen that would allow for the work to be completed during a typical work

day. For each of the fractions, the largest quantity of P from each of the different fractions was extracted from the 24-hour shaking period. However, to compete the extraction with a 24-hour shaking periods would be very impracticable due to the repeated extractions that would be required for each of the fractions and the multiple extraction solutions. The entire procedure would take more than a month the complete. Therefore, we chose to look at a method (combination of extractant and shaking time) that would best show the different fractions but also be practical to complete. The cumulative P extracted from biosolids using the alternative extractants was averaged across the wastewater treatment plant sources to determine a method that would be widely useful.

Water Extractable:

For water extractable P, Table 2.2 contains the number of extractions that are required for each source at each of the shaking times. There was a different number of replications needed for the different treatment plants and this is mostly a function of the differences in the makeup of the wastewater that was entering the treatments plants and the treatment processes that were used by the treatment plants. Table 2.3 contains the cumulative amount of inorganic P that was extracted from the different shaking periods. Since there was not a difference between each of the extraction times for water extractable the 1 hour shaking time was chosen.

There was a significant ($P=0.010$) interaction between the length of the shaking period and the treatment plants suggesting that there are differences in the makeup of the biosolid samples. For three of the biosolids samples there was little difference between the different shaking times but for the other treatment plant there was a trend for a longer shaking time removing higher amounts of P. This treatment plant uses a process that is closer to biological reduction of P, and it is believed that this process results in higher amounts of water soluble P being removed with the longer shaking times.

Sodium Bicarbonate Extractable:

The NaHCO₃ extraction showed the largest amount of P was extracted from the 24-hour shaking time, but again it was decided that it would not be practical to use (Tables 2.5 and 2.6). The second largest amount of P extracted was statistically the same between the 16 and 2-hour extractions. The 2-hour shaking time was chosen due to the increase in efficiency from the shorter shaking period. For the four treatment plants that were used for this study it was determined that the NaHCO₃ extraction would need to be repeated 11 times for each sample to no longer extract an amount of P that would result in a significant effect on the total amount of extracted P.

Sodium Hydroxide Extractable:

A 16-hour extraction was needed to completely extract the P with NaOH (Tables 2.5 and 2.6). This fraction of P would represent Fe bound P (Kuo, 1996). The shorter shaking periods did not allow enough time for the chemical reactions to take place to release all the Fe bound P. The longer shaking time mostly allowed for a more complete extraction. This reduced the number of replications that were needed.

Hydrochloric Acid Extractable:

The amount of P extracted from the biosolids with HCl was statistically the same for the 2-hour through 24-hour shaking periods; therefore, the 2-hour shaking time was chosen (Tables 2.5 and 2.6). This fraction required the fewest replications and for most treatment plants, only one extraction replication was needed to remove the P from the samples, and unlike the water or NaHCO₃ fraction there was not a small amount of P that was extracted with each subsequent extraction.

Conclusion:

This study found similar results to manure studies that showed that a shaking time of 2 hours was sufficient to extract the various fractions of P. However, there was a difference for the NaOH

extraction, which represents the Fe bound fraction (Kuo, 1996). This was mostly due to the higher amounts of Fe in biosolids than manure.

The results of fractionation study showed that more P was extracted with a longer shaking time and that approximately 5-10 replications of each method were needed. This led to a problem with maintaining the goal that the method needs to be practical to use. If the method required a 24-hour shaking time for each repetition of each extraction, then it would take over a month to complete the analysis. The method determined from this study (DDI – 1 hour extractions, NaHCO_3 – 2 hour extractions, NaOH – 16 hour extraction, and HCl – 2 hour extractions with each extraction repeated until there is not a significant increase (less than 5% increase) in the total amount of P extracted from the step) does still take longer to complete than would be optimal for doing large amounts of analysis, but it does allow for the comparison of different treatment plants or between different treatment methods within a treatment plant (Table 2.6).

Overall this method would allow for the evaluation of how changes to treatment process would affect the amount of P that is found in each fraction. This information would help in making management decisions.

Table 2.1. Analysis of biosolids. The values below are obtained by the Soil, Water and Plant Testing Lab at Colorado State University.

Sample ID #	-----%-----		-----paste-----		% Organic		-----mg kg ⁻¹ -----		-----Total-----	
	Total Solids	Volatile Solids	pH	EC mmhos cm ⁻¹	N	NH ₄ -N	NO ₃ -N	K	P	
Metro	21.6	69.9	8.8	5.5	1.16	3107	1.9	0.5	0.69	
Littleton/Englewood	18.9	72.1	8.6	3.1	1.07	1389	0.4	0.3	0.53	
Boulder	23.0	73.4	8.7	2.5	1.33	2748	1.2	0.3	0.50	
Fort Collins	21.8	70.5	8.2	3.3	1.37	1895	0.7	0.5	0.81	
-----Totals-----										
Sample ID #	-----mg kg ⁻¹ -----									
	Al	Fe	Cu	Zn	Ni	Mo	Cd	Cr	Pb	
Metro	1170	5843	161	167	4.4	3.3	0.39	5.5	8.5	
Littleton/Englewood	935	5424	158	154	3.7	1.7	0.29	7.4	4.5	
Boulder	1117	6681	181	126	5.9	2.1	0.31	7.4	5.8	
Fort Collins	1186	3989	118	118	4.1	2.4	0.22	4.7	4.4	
-----Total-----										
Sample ID #	-----mg kg ⁻¹ -----									
	As	Se	Hg	Be	Ag	Mn	Ba			
Metro	<0.001	1.40	0.012	0.03	34.3	68.5	65.1			
Littleton/Englewood	<0.001	3.10	0.003	<0.01	32.3	81.3	74.4			
Boulder	<0.001	0.32	0.001	<0.01	46.9	192	80.5			
Fort Collins	0.930	2.00	0.001	<0.01	43.1	37.2	393			

Table 2.2. Number of extractions replications need to extract the water extractable P. The complete chemical removal of P by an extractant was indicated by a less than 5% change in total P extracted for a given fraction.

Source	Extraction Time				
	1 Hr	2 Hr	4 Hr	16 Hr	24 Hr
	Number of Replications				
Metro	8	7	7	5	6
Littleton/Englewood	8	6	7	7	5
Fort Collins	8	9	7	5	5
Boulder	7	10	7	8	8

Table 2.3. Total amount of inorganic P extracted with water at differing lengths of shaking. The samples were averaged over the four treatment plants to determine the shaking time that worked best for the most treatment plants.

Length of shaking period (hours)	Inorganic P (mg kg⁻¹)	Standard error
1	7662.73	1135.42
2	7875.73	1135.42
4	7707.53	1135.42
16	8174.04	1135.42
24	9436.27	1135.42

Table 2.4. ANOVA table for the comparison of extraction time and biosolids source. The extraction solutions used were deionized distilled water (DDI), 0.5 M NaHCO₃, 0.1 M NaOH, and 1 M HCl. Each of the fractions was analyzed separately.

	DDI	NaHCO₃	NaOH	HCl
Effect	P Value	P Value	P Value	P Value
Extraction Time	<0.001	<0.001	0.005	0.049
Treatment Plant	<0.001	<0.001	<0.001	<0.001
Time x Treatment Plant	0.010	0.108	0.097	0.689

Table 2.5. Amount of P extracted by length of extraction shaking period. Each of the extraction periods was analyzed individually and averaged over treatment plant. The extraction solutions used were deionized distilled water (DDI), 0.5 M NaHCO₃, 0.1 M NaOH, and 1 M HCl. Times with different letters within an extraction solution are statically different.

	DDI	NaHCO₃	NaOH	HCl
Extraction Shaking Period	P (mg kg ⁻¹)			
1 Hour	7662.73 a	5563.00 d	2546.09 c	1924.77 b
2 Hours	7875.73 a	7896.75 b	2745.55 bc	2079.42 a
4 Hours	7707.53 a	7414.14 c	2712.39 bc	2059.15 a
16 Hours	8174.04 a	7891.65 b	3035.78 a	2017.27 ab
24 Hours	9436.27 a	8354.20 a	2927.58 ab	2104.84 a

Table 2.6. Percent of P removed by each extraction by treatment plant. The extraction solutions used were deionized distilled water (DDI), 0.5 M NaHCO₃, 0.1 M NaOH, and 1 M HCl.

	Total (mg kg⁻¹)	DDI	NaHCO₃	NaOH	HCl
Boulder	15018.02	29%	46%	19%	5%
Fort Collins	26776.88	57%	27%	7%	9%
Littleton/Englewood	18825.25	27%	44%	18%	11%
Metro	21082.54	37%	35%	14%	14%

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CHAPTER 3: VERTICAL DISTRIBUTION OF P FOLLOWING BIOSOLIDS APPLICATION

Introduction:

Land application for the beneficial use of biosolids has long been shown to supply the needed nutrients for crop production (Barbarick et al., 2012). The application of biosolids is currently regulated by state and federal regulations at the agronomic rate of nitrogen (N), but this results in the over-application of phosphorous (P) (Wang et al., 2015). The over-application of P enhances the risk of environmental impacts due to transport of P to surface water by runoff (Sharpley et al., 2015) and erosion or in some cases downward movement (Elliott et al., 2002) to groundwater.

The plant and environmental availability of P is typically low in calcareous soils due to the ability of free calcium (Ca) in the soil to bind P (Zhang et al., 2014). Previous work has shown that there is a negative correlation between calcium carbonate (CaCO_3) and P availability (Afif et al., 1993, Sharpley and Smith, 1985). In acidic soils, a saturation index has been used to predict the risk for P movement based on the ratio aluminum (Al), iron (Fe), and P. In basic soils, Fe and Al are less important and the formation of Ca and magnesium (Mg) phosphates dominate the solid phase phosphorus minerals. The degree of P saturation in soils (the ratio between the amount of bound P in the soil and the amount of P binding sites) has been used as a method to predict the risk for P leaching (O'Connor et al., 2005). Ratios of Ca, Mg, and P can be used in basic soils to predict the ability of P to leach (Casson et al., 2006). So et al., (2011) also found that the amount of P that is bound to calcite does not reach the maximum expected based on isotherm data, indicating that caution would be needed if using a saturation index for basic soils to evaluate the risk of downward P movement.

In fields in the western United States that have a history of biosolids application at the agronomic rate of N, many of the potential environmental impacts that are seen in the eastern United

States have not been considered due to the ability of CaCO_3 to bind P and the greater distances to surface water. However, because of P losses from surface erosion, it is important to understand how long-term applications of biosolids affect the vertical distribution of P in the soil profile. The different forms of soil P in the soil profile have varying potential for environmental impacts due to differences in availability/mobility. Complicating the issue is the availability of P changes with time since application of the biosolids, with organic matter controlling the initial P availability and geochemical transformations becoming more important in the long-term (von Wandruszka, 2006).

Three objectives were addressed in this study: 1) to determine how the long-term application of biosolids at that agronomic rate of N affects distribution and forms of P in the soil profile, 2) to determine if the rate of biosolids application affects the forms and distribution of P in the soil, 3) to investigate how the distribution and forms of P are impacted by different agroecosystems.

Materials and Methods:

Field Locations:

Two field locations (referred to as research and production) were used for this study. The research location was established 20 years prior to sampling in a randomized complete block design with four replications. This site is located near Bennett, Colorado, and is a wheat (*Triticum aestivum*) - fallow system, where the soil is a Weld loam (fine, smectitic, mesic Aridic Argiustoll) (Soil Survey Staff, 2012). There was minimal tillage following biosolids application for incorporation. The production site is a field that had received biosolids applications for eight years and was producing irrigated maize (*Zea mays*), with conventional tillage. This production field is located near Wiggins, Colorado, and the soil is a Bijou sandy loam (mesic Ustic Haplargids) (Soil Survey Staff, 2012). Soil samples were collected on a transect line across the field sampling. Three samples were collected within the irrigation pivot (area

with biosolids application), and three samples were collected from the pivot corners. The pivot corners served as the control for the study. They had grasses established and were not irrigated or fertilized.

Soil Sample Collection:

Three biosolids application rates were sampled from the research plots; control (no fertilizer additions), biosolids applied to meet the N agronomic application rate, and biosolids application at 2.5 times the N agronomic application rate. For the production field, biosolids were applied at the N agronomic rate only. To sample the site, a transect line was made across the center pivot that would be in the same expected soil type. Three samples were collected inside of the application area and three samples were collected from the unfertilized grass in the pivot corners. Since the production field was not designed for research, the corners were considered representative of what the field samples would have been prior to biosolids application.

Soil samples were collected using a pickup mounted Giddings soil probe and zero contamination sampling tubes. Samples were collected down to 150 cm, but the sampling was stopped when the probe was no longer easily penetrating the soil to prevent the compaction of the sample. The tubes were brought back to the laboratory where the soil was cut into smaller sections. The top 50 cm of soil was separated into 5-cm segments, and the remaining 100 cm separated into 10-cm segments. The soil was allowed to air dry before it was ground with a mortar and pestle and passed through a 2-mm sieve. The samples were then stored at room temperature in sealed zip top bags until analysis. Composite samples were made with each of the soil cores to reduce the number of samples to be analyzed and to focus on the areas that were showing indications for differences. The composite samples were made on a mass basis and were composited based on the analysis of CaCO_3 and total or index P concentration.

Laboratory Analysis:

Total P:

To determine the extent of the downward movement of P in the soil from the research plots, the soil samples were analyzed to determine the total amount of soil P using the alkaline oxidation method (Dick and Tabatabai, 1977). The method was modified slightly by placing the samples in 50-mL digestion tubes instead of a boiling flask and then placing the digestion tubes in a digestion block and not a sand bath. After digesting the first several sets of samples in the boiling flask and sand bath, it was noticed that there was very uneven heating and that this was affecting the amount of P that was extracted from the samples. The digestion block provided a much more even and consistent heating. The concentration of CaCO_3 was also determined for all soils with the modified pressure transducer method (Sherrod et al., 2002).

Olsen P Index:

The Olsen P Index (Kuo, 1996) was used to characterize the vertical distribution of P in both the research and production location. Olsen extractable P is used in the Colorado P risk assessment and is a common P test value that can be easily compared to many other studies and soil test values.

Statistical Analysis:

To determine differences in the concentration of CaCO_3 , total P, and Olsen P Index in the soil due to application rate or depth, Proc Glimmix in SAS Version 9.4 (SAS Institute, 2012) was used with an alpha value of 0.05. The data was checked for homogeneity of variance and when necessary a log transformation was used.

Soil P Fractionation:

The composited samples were analyzed to determine the amount of P that was present in several different fractions of inorganic and organic P following the methods outlined by Kuo (1996). Due

to the high concentrations of CaCO_3 in the greater soil depths for the research plots, the calcareous soil method was used for the inorganic P fractionation. The inorganic P sequential extraction consisted of three steps that separated the inorganic P into (1) soluble, Al, and Fe bound, (2) occluded, and (3) Ca bound (Figure 3.1, Pierzynski, 2000). The composite samples from the production field were fractionated into five forms of inorganic P ((1) soluble, (2) Al, (3) Fe bound, (4) occluded, and (5) Ca bound) following the method outlined by Kuo (1996) since there were not elevated concentrations of CaCO_3 . For the non-calcareous method soluble, Al, and Fe can be separated into three fractions instead of one with the calcareous method. Modifications were not made to the organic fractionation method (Kuo, 1996) to account for the higher CaCO_3 since others have found that it is not necessary (Kuo, 1996). The organic P was fractionated into six labile pools, (1) labile organic, (2) microbial P, (3) moderately labile organic, (4) fulvic, (5) humic, and (6) non-labile organic (Figure 3.2, Pierzynski, 2000).

The data was analyzed with Proc Glimmix in SAS Ver. 9.4 (SAS Institute, 2012) using the repeated measures portions of the program. The repeated measures analysis includes an expectation for higher correlation in samples of similar depths to be taken into account. An alpha value of 0.05 was used for determining statistical differences. The data was checked for heterogeneity of variance and a log transformation was used when needed.

Results and Discussion:

Soil CaCO_3 :

Research Location:

Figure 3.3 shows the vertical distribution of soil CaCO_3 at the research location increases with depth down to approximately 40 cm before beginning to decline. The analysis of the CaCO_3 indicated that there was a treatment effect ($P=0.005$) and a depth effect ($P<0.001$) but not a treatment by depth interaction ($P=0.111$). An interesting finding is that, as the rate of biosolids application increased, the

concentration of soil CaCO_3 decreased (Table 3.1). This may be due to the increased plant respiration and release of carbon dioxide (CO_2) from the increased biomass production with higher application rates of biosolids. As the soil receives more nutrients, the plants produce more biomass, and therefore more CO_2 . The CO_2 then creates carbonic acid (H_2CO_3) that will dissolve the CaCO_3 . There was a statistical difference in the CaCO_3 concentration with depth (Table 3.2) that showed the highest concentration of CaCO_3 was near the middle of the depth sampled (30-60 cm). Even though there was not a statistical interaction of treatments and depth, there was a trend of a higher concentration of CaCO_3 in the control soil between 20 and 40 cm than the plots that received biosolids (Figure. 3.3). Another possible reason for the decrease in the CaCO_3 concentration in the soil with increasing biosolids application would be due to the addition of organic acids from the biosolids. These acids would dissolve the CaCO_3 as they moved through the soil profile.

Production Field:

The analysis of soil for CaCO_3 in the production field showed that there is no detectable CaCO_3 present in the top 100 cm. This finding is further supported by the soil survey stating that the parent material is from noncalcareous granite and other alluvial deposits (Soil Survey Staff, 2012).

Total Soil P:

Research Plots:

The analysis of the total soil P from the research location showed only a significant difference with depth ($P < 0.001$) and most of the differences were only at the soil surface (Figure 3.4). The effect was most likely due to the P being surface applied and only incorporated into the top 15 cm for the first eight applications (16 years of wheat-fallow). The last two applications of biosolids, were surface applied and only incorporated with hand-raking. The downward movement of P below the plow layer appears to be very small, and it is possible that there could have been downward movement that was

not detected in the analysis. In the control soil, the total amount of P was between 600 and 800 mg kg⁻¹ for the entire profile (Figure 3.4), and the analytical method used for analysis required the samples to be diluted to determine the sample concentration thus reducing the resolution for small changes. In a greenhouse study, Elliott et al (2002), showed that most biosolids applied to sandy soils, which would have the highest potential for leaching, had less than 1% leaching of the P applied.

The soil P profile shows an interesting increase between 30 and 60 cm. This zone has an elevated P concentration due to the parent material or management practices prior to the beginning of our study, since we also see the increase with the control soil. This area of increase also corresponds to the increase in soil calcium carbonate (Figure 3.3). The field contains an argillic horizon that varies with depth across the field but is typically found in the 30-60 cm depth range.

Olsen Extractable P:

Research Location:

Olsen extractable P (Figure 3.5) had a similar distribution to total P near the soil surface and, similar to total P, there was only a depth effect ($P < 0.001$). There was a trend for a depth by application rate interaction ($P = 0.399$) for increased Olsen extractable P near the soil surface and from 60-100 cm with the 2.5 times N agronomic application rate. This indicated that there might be some transformation to more available P at this deeper depth. The low application rates at this location and spatial variation, possibly lead to there not being a significant effect of application rate.

Production Location:

There was an increase in Olsen extractable P, compared to the control, between 0-20 and 60-90 cm (Figure 3.6) as was seen in the research location. The accumulation at the soil surface was expected due to the addition of biosolids incorporated into the top 20 cm. The increase below the surface indicated that there was potentially downward movement taking place. The increase that was observed

between approximately 40 and 80 cm with the biosolids applications is correlated with a change in clay content of the soil. The upper 38 cm of the Bijou series is a loamy sand, between 38 and 84 cm the soil is a sandy loam, and below that depth it becomes a loamy sand again (Soil Survey Staff, 2012). The increased concentration of Olsen Extractable P at the soil surface in the control samples is likely due to the turnover of organic matter from the decomposition of the grasses.

Elliott et al. (2002) have also found that P can leach under certain conditions (sandy soils). In soils in the eastern United States it is more of an environmental risk to have P movement in the soil profile below the root zone due to the higher water table. Even though there currently is not a high risk for the downward movement of P from this site to groundwater, it is important to better understand how P moves in this system to know how to manage P to ensure it does not become a risk for groundwater contamination.

Research Location:

Inorganic P Fractionation:

Inorganic Soluble, Fe, and Al Bound P:

There was only a depth effect ($P < 0.001$, Figure 3.7) on the composited soil samples for soluble, Fe, and Al bound P (due to the high CaCO_3 in the soil these three fractions were analyzed as one pool and could not be separated (Kuo, 1996)). The lack of a treatment effect (Figure 3.8) suggests that the soluble P from the biosolids was quickly changed to more stable forms. Soluble, Fe, and Al bound P was found only in the plow layer. The elevated soluble P pool near the surface may be the result of wheat residue left on the field and microbial communities mineralizing the organic P into a soluble pool. In a study of the distribution of P following the long-term application of biosolids at high rates Ippolito et al. (2007) found the Fe bound P would move into the soil profile below the plow layer.

Occluded P:

When biosolids are initially applied, the soluble P from the biosolids will likely react with the Fe and Al in the soil but also in biosolids to form more stable P forms including occluded P. At the research site, there was a depth by treatment effect for occluded P ($P < 0.001$, Figure 3.9). The differences were only seen in the top 10 cm of soil (Figure 3.9) where occluded P was the highest in the highest application rate and lowest in the lowest application rate. The only elevated concentrations below this depth were for the agronomic rate in the 5-10 cm depth. The dry conditions would favor the formation of occluded P forms because Fe will remain in the oxidized form and encapsulate P. There also was a trend for an increase in occluded P over the control below 40 cm for the highest application rate of biosolids. As this form of P moves through soils containing large amounts of Ca, the P is protected by the Fe and, therefore, not able to react with Ca. The occluded P did not show this increase for the agronomic rate of application. However, there was an increased accumulation of occluded P in the soil below a layer of soil with elevated Ca bound P (Figure 3.10) further supporting the non-reactivity of occluded P with Ca. The Fe additions to the soil from the application of biosolids may have led to increased Fe in the soil profile at the high application rates, and the Fe was reacting with P that was already present in the soil.

Ca Bound P:

Calcium bound P exhibited a significant depth effect ($P < 0.001$, Figure 3.10) but no treatment effect (Figure 3.11). As with the total soil P, it is possible that this increase in Ca bound P was too small, relative to the total amount of Ca bound P in the system to be able to see a significant difference. The increase with depth followed what was seen with CaCO_3 (Figure 3.4), supporting the theory that CaCO_3 would serve as an indicator to soil P in calcareous soils. The total soil inorganic P concentrations below the plow layer were composed of mostly Ca bound P. (The largest fraction of soil P was Ca bound,

making up approximately 30-50% of the total soil P.) Ippolito et al. (2007) also found that Ca bound P was the largest fraction of P from 20-60 cm in a similar soil that had received biosolids application.

Organic Soil P:

Total Organic P:

Total organic soil P (sum of the labile, moderately labile, microbial, fulvic acid, humic acid, and non-labile organic pools) had a significant treatment by depth interaction ($P=0.045$). Both rates of biosolids applications elevated the organic soil P in the upper 40 cm of soil (Figure 3.12).

Labile Organic P:

The labile organic P pool (log transformed data to correct for heterogeneity of variance) showed a significant depth effect ($P<0.001$). Concentrations of labile organic P increased in the top 20 cm, which is the depth of incorporation when the field was tilled, and below this depth there were no differences. These results suggest that most of the P in this fraction is from the decomposition of plant biomass and not from the biosolids since there was not a significant treatment effect ($P=0.948$, Figure 3.13). Over the last several years there has not been a significant effect seen in the yield or biomass produced with these application rates (data not shown).

Microbial Biomass P:

The microbial biomass pool showed a significant effect ($P=0.038$) from the addition of biosolids (log transformed data). There was a higher concentration of microbial biomass P for the agronomic application rate, and the other treatments were near zero, (Figure 3.14). This is a pool of organic P that can release P quickly (Bayley et al., 2008). At the time of sampling the soil was dry and without a growing crop. The low amount of P in the biomass is a result of the low moisture content reducing the microbial populations at the time of sampling.

Moderately Labile Organic P:

Moderately labile organic P pool data also required a log transformation to correct for heterogeneity of variance and only showed a depth effect ($P < 0.001$). Figure 3.15 shows that there was an increase in moderately labile organic P between 25-50 cm. This is an area in the soil profile that has an argillic horizon and is most likely restricting the downward movement of organics in the soil water solution and allowing the organic P to accumulate and be transformed into a more stable form. The time it takes for labile organic P to be transformed to moderately labile P has been shown to be at least one year (Audette et al., 2016), and given the long history of the test plots, it is likely there has been labile P transformed and there is not an application rate effect (Figure 3.16).

Fulvic and Humic Acid P:

Fulvic acid organic P (Figure 3.17) and humic acid organic P (Figure 3.18) both were significantly different with soil depth ($P = 0.004$ and $P < 0.001$, respectively). These forms are related to microbial activity that breaks down organic matter into more stable forms and from fulvic acid to more stable humic acid (Bayley et al., 2008). There was not an effect of the addition of biosolids (Figures 3.19-20), implying that these forms were more affected by the production and turnover of wheat biomass.

Nonlabile Organic P:

Nonlabile organic P showed a significant depth effect ($P < 0.001$, Figure 3.21) but no treatment effects (Figure 3.22). There was an increase with depth through the plow layer and then a trend for a decrease with depth (Figure 3.21). In previous work on several calcareous soils from Florida, Zhang et al. (2014) found that the amount of residual P in the soil (nonlabile) decreases with production intensification of production. If this form is mostly affected by the change in management of the system, then it would be expected that the similar management across these plots would have a larger effect than the addition of biosolids.

Production Location:

Inorganic Fractionation:

Soluble Inorganic P:

There was a significant ($P < 0.001$) effect of depth on soluble P, but there was not a significant treatment effect ($P = 0.202$, Figure 3.23). It would be expected to find a higher concentration of soluble P near the soil surface due to higher amounts of residue that are mixed into the soil and mineralized by microbes. The lack of a difference between the control and the treatments indicate that the application of biosolids is not affecting soluble P and that the amount of residue incorporated into the soil is having the same effect as the turnover of the grasses for the control samples. Soluble inorganic P is a form of P that is in equilibrium with the solid phases of P that are also present in the soil. At the end of the growing season this pool should be depleted because the plants take up large amounts of P during the growing season and a large portion of the P being contributed from the mineralization of organic P (Oehl et al., 2001).

Aluminum bound P:

Aluminum bound P had a significant treatment ($P = 0.004$) and depth ($P < 0.001$) effect, but there was not an interaction ($P = 0.256$, Figure 3.24). There was a higher concentration of Al-P in the top 20 cm that decreased with depth. Even though there was not a significant interaction, there was a trend for an increase in Al-P at the soil surface with the application of biosolids. The soils at this site are slightly acidic at the soil surface, which would favor the formation of Al-P. The treatment effect produced almost three times as much Al-P than the control. Free Al and Fe in soils and from the addition of biosolids will act as strong binding sites to sorb soluble P.

Iron bound P:

Iron-bound P exhibited a significant depth × treatment interaction ($P=0.016$, Figure 3.25). There was a large increase in Fe-P near the soil surface then decreased with depth. The addition of Fe in the biosolids created similar differences to the effects of Al. In addition, during the production of biosolids, Fe that is in the biosolids can react with P and the application may add Fe bound P directly. Below the plow layer was a trend for higher Fe bound P with the application of biosolids. In a dryland study there is an increase in Fe bound P with depth with high rates of biosolids application (Ippolito et al., 2007). Our experimental site was irrigated and was a sandy soil, so it would be expected that there would be a higher risk of downward movement of P.

Occluded P:

Occluded P only showed a depth effect ($P<0.001$, Figure 3.26) that was limited to higher concentrations in the plow layer. The high concentrations of Fe bound P in the same depth indicates that over time the P is being transformed from Fe-P to occluded P. Occluded P is a more stable form of P due to the Fe encapsulation. Occluded P will release P and is not stable if it is under reducing conditions. Therefore, it is possible that with the higher water content, and likelihood of creating reducing conditions in the soil due to irrigation, occluded P is only found in areas with higher amounts of air movement.

Calcium bound P:

For Ca-P, the treatment ($P=0.081$) and depth ($P=0.133$) effects and their interaction ($P=0.216$, Figure 3.27) were not significant. The soil in this field does not contain any CaCO_3 and has a low amount of total Ca in the soil. Also many soils in the western United States were formed from parent material that was high in calcium phosphates, and this soil was formed from river sediment deposits (Soil Survey

Staff, 2012). Due to the lower pH of the soil, the P chemistry will be more affected by Fe and Al chemistry than would be expected in many other Colorado fields.

Organic Fractionation:

Labile Organic P:

Labile organic P (Figure 3.28) exhibited both a significant depth ($P=0.011$) and treatment ($P=0.006$) effect. There was approximately three times higher concentration of labile organic P resulting from the addition of biosolids than in the control. However, caution must be taken in the interpretation of this result because of the difference in vegetation between the treatment (maize production) and control (perennial grasses). Labile organic P is the pool of organic P that is the most available and can be greatly influenced by the vegetation that is growing. With the production of a highly managed crop (such as maize), it would be expected that there would be a much larger amount of biomass produced and therefore the total amount of P returned to the soil at the end of the season would be higher for production field than the grass control.

Moderately Labile Organic P:

A significant depth \times treatment interaction affected the moderately labile organic P ($P=0.024$, Figure 3.29) where there was a higher concentration in the top 10 cm with biosolids, but then the control was higher down to 70 cm, and from 70-90 cm the biosolids application was higher. This form of P takes longer to form than labile P (Audette et al., 2016), and since the control is not being disturbed it would be more likely to accumulate moderately labile P throughout the root zone.

Humic Acid P:

Humic acid organic P (Figure 3.30) only exhibited a treatment effect ($P=0.031$) where biosolids had a concentration of 34.8 mg kg^{-1} and the control was 13.0 mg kg^{-1} . Similar to the labile organic P pool it would be expected that with the increased biomass production from maize over grass there would be

an increase in organic material in the soil. This fraction is associated with root biomass and this would explain why there was a trend for increased concentrations with biosolids down to 70 cm.

Microbial Biomass P, Fulvic Acid P, and Non-Labile P:

Three additional fractions (microbial, fulvic acid (Figure 3.31), and non-labile) of organic P were extracted and analyzed, but there were not significant effects at a probability level of 0.05.

Determination of microbial biomass associated P was limited by the available analytical methods and the spatial and temporal variability of microbial populations (Condrón et al., 2005). Nonlabile organic P (Figure 3.32) is the most stable form of organic P and thus, the time scale of the study may not have been long enough to observe differences. One of the limitations of this study was that the samples were collected once and this only supplies us with a snapshot of what is taking place and does not show how the pools are changing with time.

Discussion:

The only fractions that showed a significant biosolids effect with depth were occluded P (research location) and Fe bound P (production location). However, all the other fractions evaluated only had a significant depth effect. This suggests that in semiarid calcareous dryland agroecosystems, there is limited evidence of downward movement of P from biosolids application. Ippolito et al, (2007) showed that P will accumulate in calcareous soils in Colorado in the Fe bound P fraction. This study was following 20 years of biosolids application, but the method used for the fractionation of inorganic P was different in that the authors did not use the calcareous soil fractionation method for the surface soil, but it was used for the deeper samples. In both studies it is possible that the P applied was not moving but that Fe was moving in the profile and resulting in the formation of Fe bound P over Ca bound P.

The soils used in this study presented some challenges in determining the P transformation processes that were taking place in the soil. The high soil CaCO₃ interferes with the extraction of Al

bound P due to the precipitation of calcium fluoride (CaF_2) with the NH_4F extraction solution (Smillie and Syers, 1972). The calcareous inorganic P fractionation method was used for all samples in our study to allow for depth comparisons with the same extraction method. There was CaCO_3 found at all depths. With the calcareous method Fe, Al, and soluble P were all extracted as one phase, and this fraction was only present in the top 20 cm.

Not being able to separate soluble, Al-bound and Fe-bound P reduces our ability to potentially see the effects of adding an amendment that is high in Al and Fe. For soils in the western United States the P chemistry is expected to be dominated by Ca and Mg due to the higher soil pH (Lindsay, 1979). However, with the large additions of Fe and Al in biosolids, these elements could affect P dynamics, and the data shows that Fe is very important for the research (occluded P) and production sites (Fe-bound P). This may have large implications in the management of P because it shows that Fe dynamics that are often ignored in calcareous systems need to be considered.

Conclusions:

The long-term application of biosolids at the N agronomic rate does not appear to result in large accumulations of P, and the effects that were seen were limited to the plow layer. The forms of soil P found suggest that the P is not readily available for environmental impact due to the lower concentrations of soluble and labile forms. The data also suggest that even in a system that is dominated by Ca-bound P, there is still an important effect of Fe chemistry on P dynamics since the only fraction that showed downward movement was occluded (Fe encapsulated P). Further work is needed to determine if the Fe effect that was seen is a result of application of biosolids with P and Fe or if similar effects would be seen with just the addition of Fe.

Table 3.1. Soil calcium carbonate concentration comparison of treatments from the research location. The average concentrations of CaCO₃ in the soil for three application rates of biosolids (0, 5, and 11 Mg ha⁻¹) averaged over all depths are shown in the table below. Concentrations with different subscripts are statistically different at a probability level of 0.05.

Treatment (Application Rate)	% CaCO₃
Control (0 Mg ha ⁻¹)	7.83 % _a
Agronomic Rate (5 Mg ha ⁻¹)	7.08 % _b
2.5 Times Agronomic Rate (11 Mg ha ⁻¹)	6.48 % _b

Table 3.2. Soil calcium carbonate concentration with depth from the research location. The average soil CaCO₃ concentration is shown averaged over treatment at different depths in the soil profile following 20 years of biosolids applications. Concentrations with different subscripts are statistically different at a probability level of 0.05.

Depth (cm)	CaCO ₃
0-5	0.49 % _i
5-10	0.52 % _i
10-15	1.44 % _i
15-20	3.78 % _h
20-25	7.14 % _{ef}
25-30	9.46 % _{cd}
30-35	11.23 % _{ab}
35-40	11.57 % _a
40-45	11.80 % _a
45-50	10.42 % _{abc}
50-60	9.86 % _{bcd}
60-70	9.60 % _{cd}
70-80	8.72 % _{ef}
80-90	7.32 % _{ef}
90-100	7.38 % _{ef}
100-110	6.57 % _{fg}
110-120	5.88 % _{fg}
120-130	5.16 % _{gh}

Noncalcareous Soils

Calcareous Soils

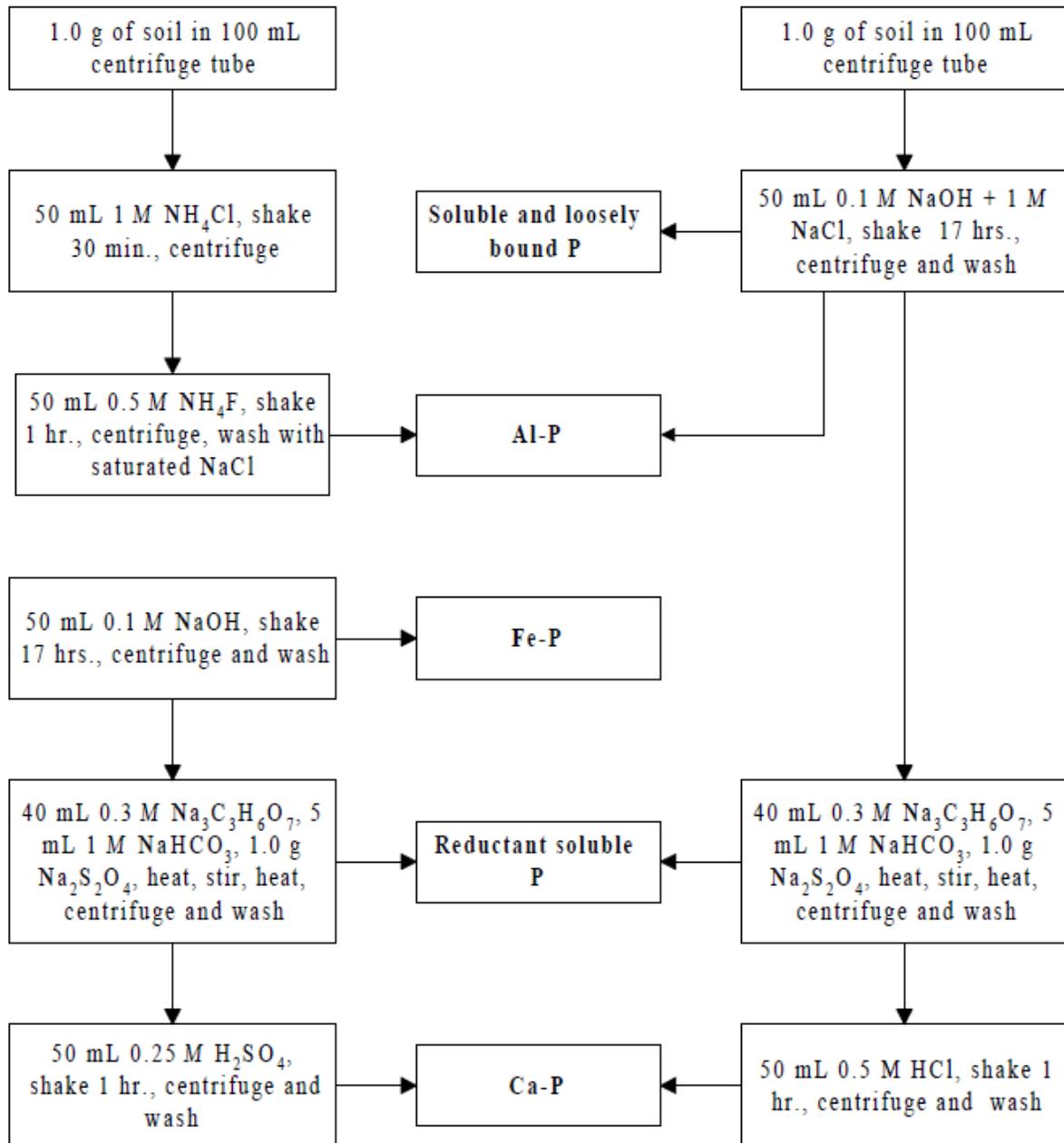


Figure 3.1. Inorganic fractionation method. (Pierzynski, 2000)

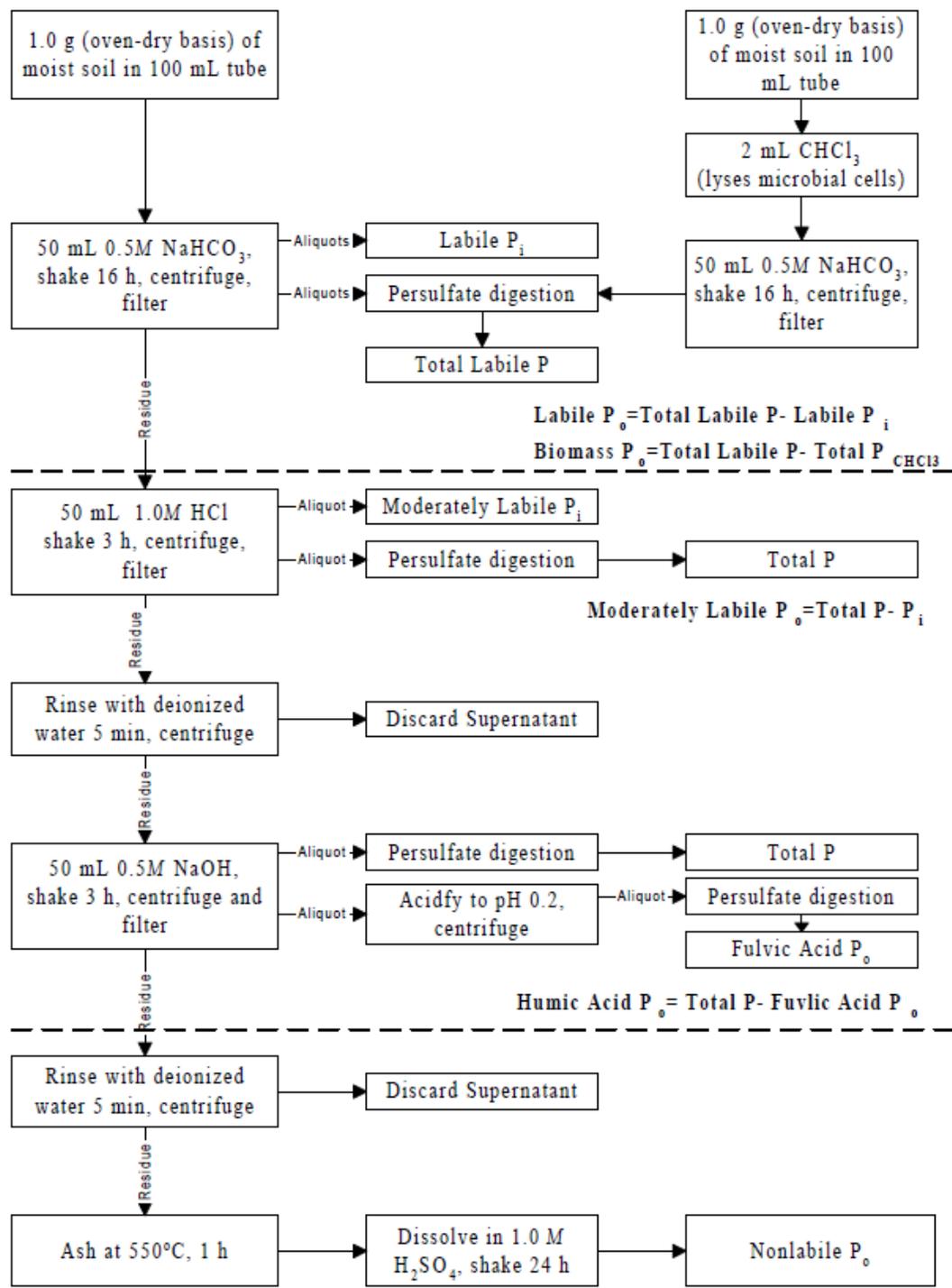


Figure 3.2. Organic fractionation method. (Pierzynski, 2000)

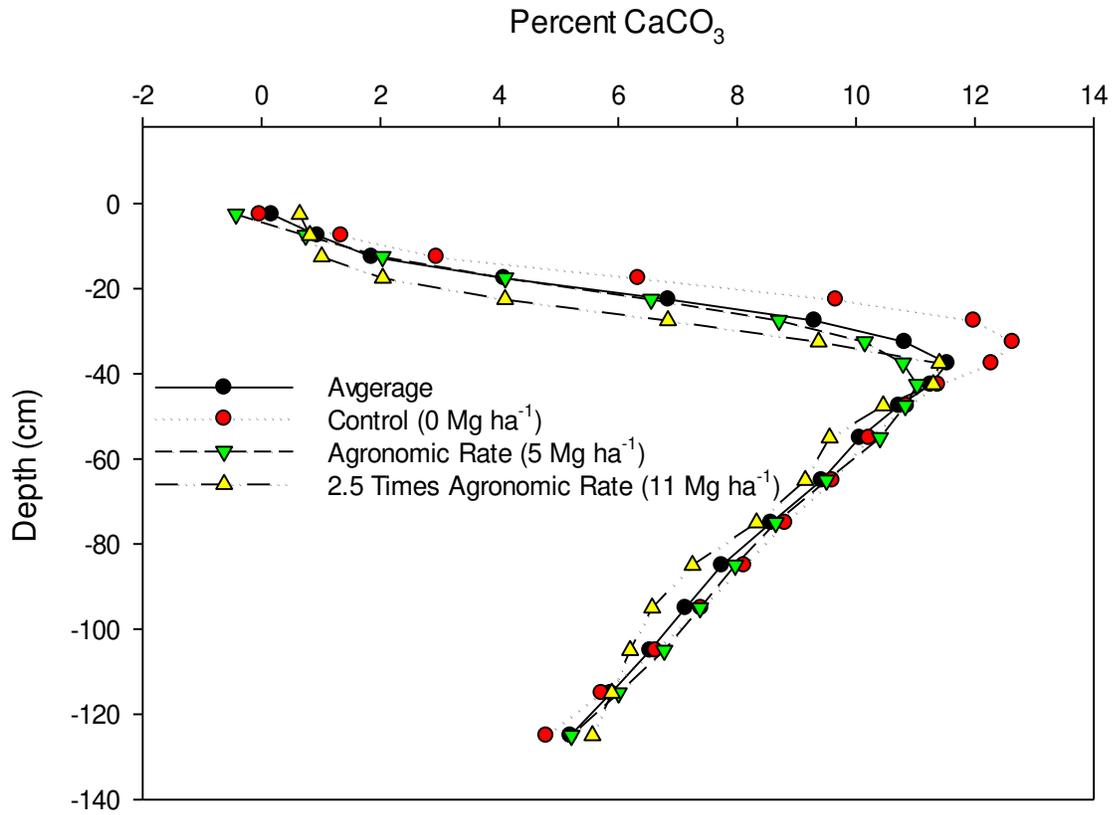


Figure 3.3. Concentration of CaCO₃ with depth following 20 years of biosolids application at the research location. The figure above shows the concentration of CaCO₃ in the soil for three different application rates of biosolids. Even though there was not a significant interaction ($P=0.111$) between depth and application rate, there was a trend for decreased CaCO₃ in the plow layer with the application of biosolids.

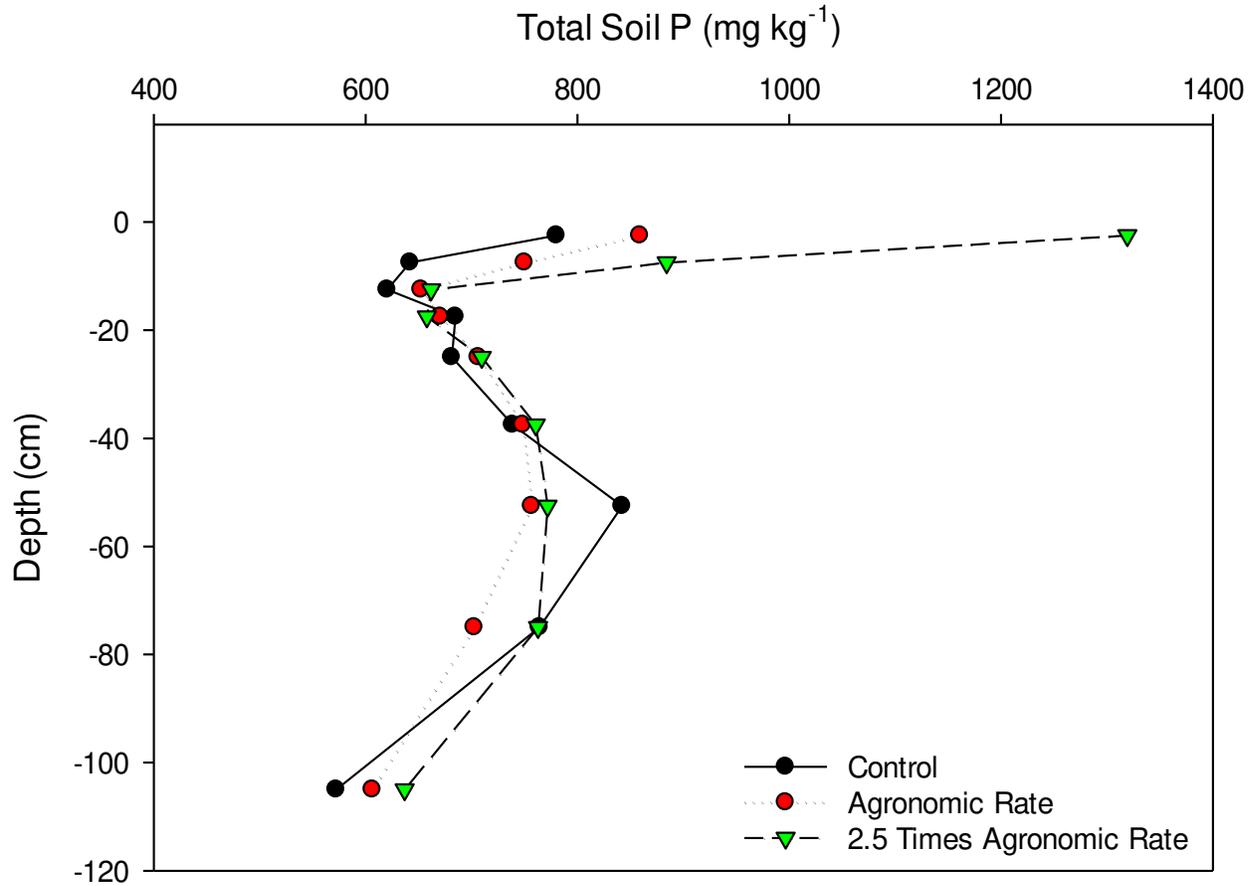


Figure 3.4. Concentration of total soil P with depth following 20 years of biosolids application at the research location. The figure above shows the concentration of P in the soil for three different application rates of biosolids. There was not a significant depth by application rate interaction, but there was a trend for increased concentration in total P at the soil surface.

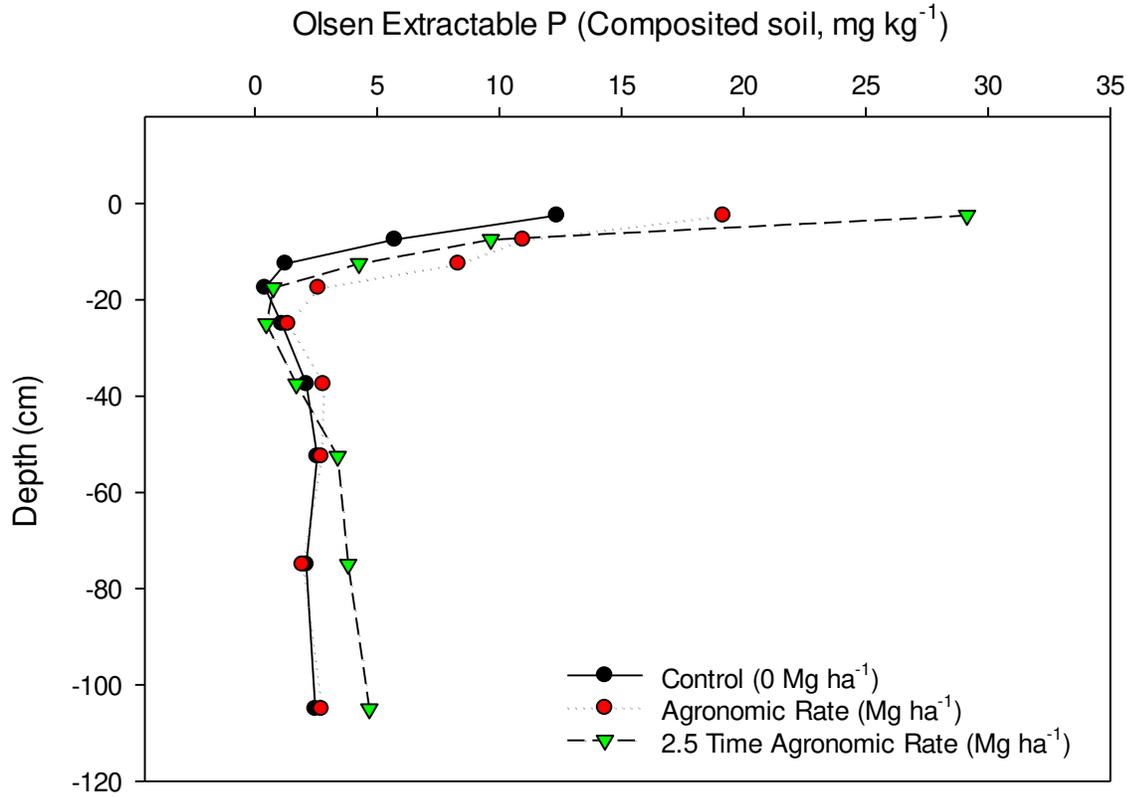


Figure 3.5. Olsen Extractable P concentrations from the research location. The concentrations of Olsen Extractable P from the soil samples collected from the research location shows that there was a trend for an increase with higher application rates at the soil surface and from 60 cm down, but this was not statistically significant ($P=0.399$). There was a significant depth effect ($P<0.001$).

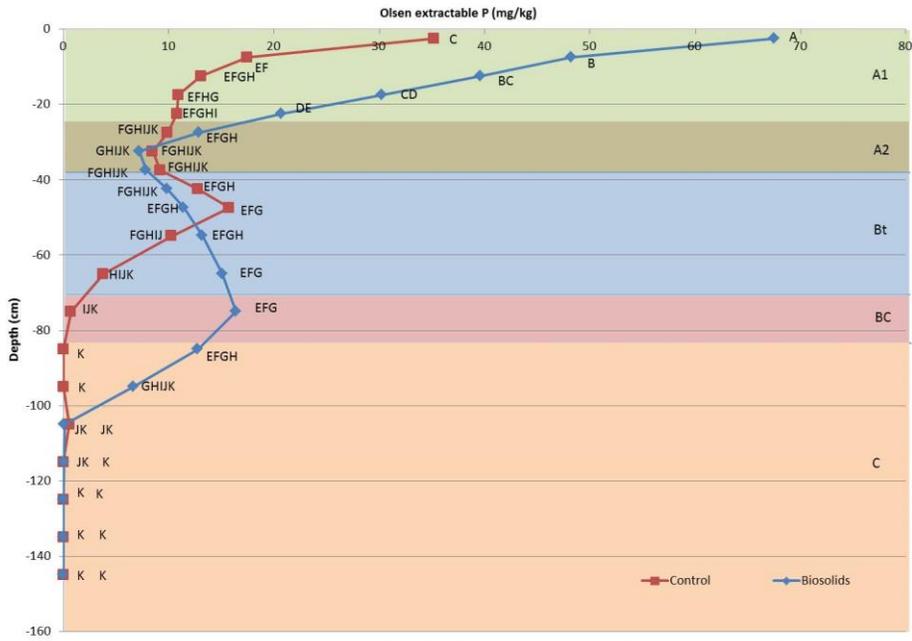


Figure 3.6. The figure above shows the distribution of Olsen Extractable inorganic soil P from the production location. The red line is the average of three control soil cores and the blue line is the average of three cores from across the field. Points with different subscripts are statically different from each other at a probability level of 0.5. The different background colors correspond to the soil horizons as described by the NRCS.

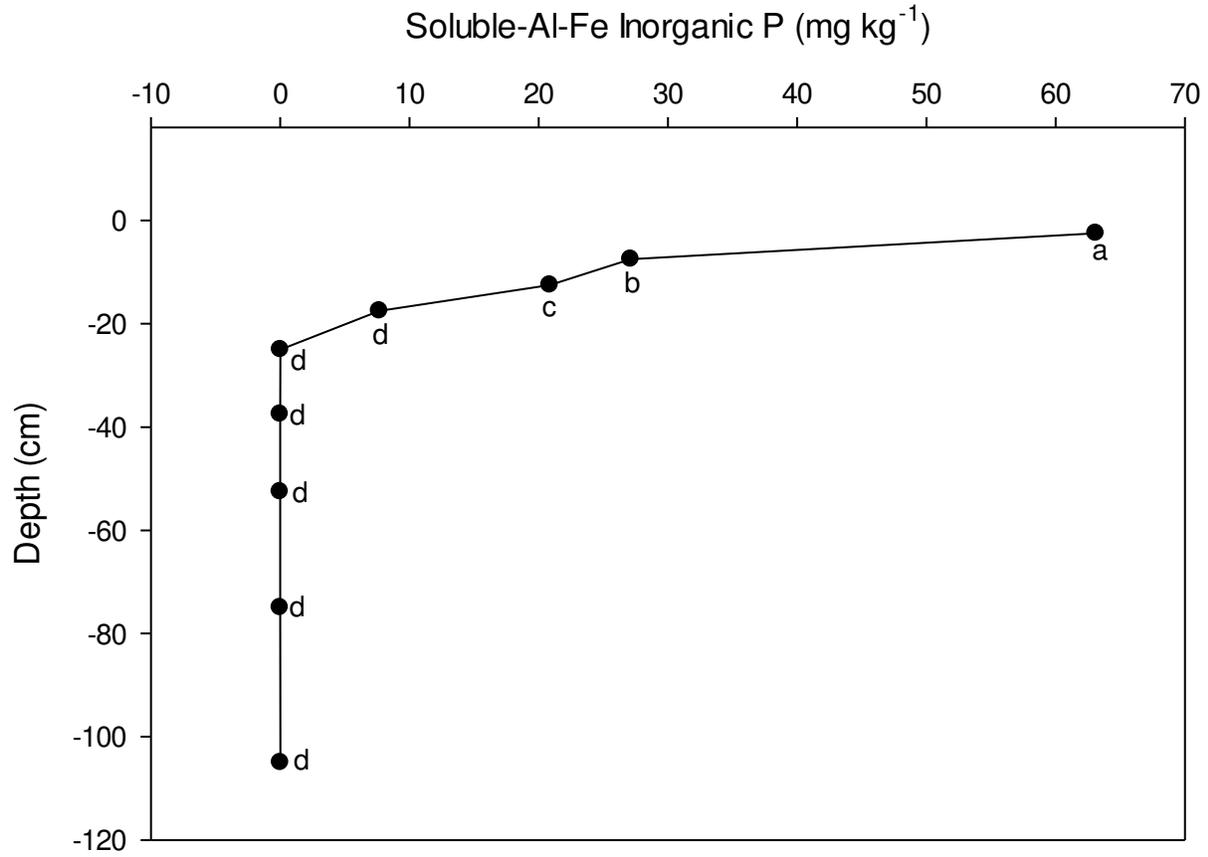


Figure 3.7. Distribution of soluble-Al-Fe inorganic P following 20 years of P application to the research location. There was a significant ($P < 0.001$) depth effect on the distribution of these forms for inorganic P. The data presented was averaged over application rate. There was an increase in the amount of P in the soil down to 20 cm, and below this depth it was statistically zero. Concentrations with different subscripts are statistically different at a probability level of 0.05.

Soluble-Al-Fe Bound Inorganic P

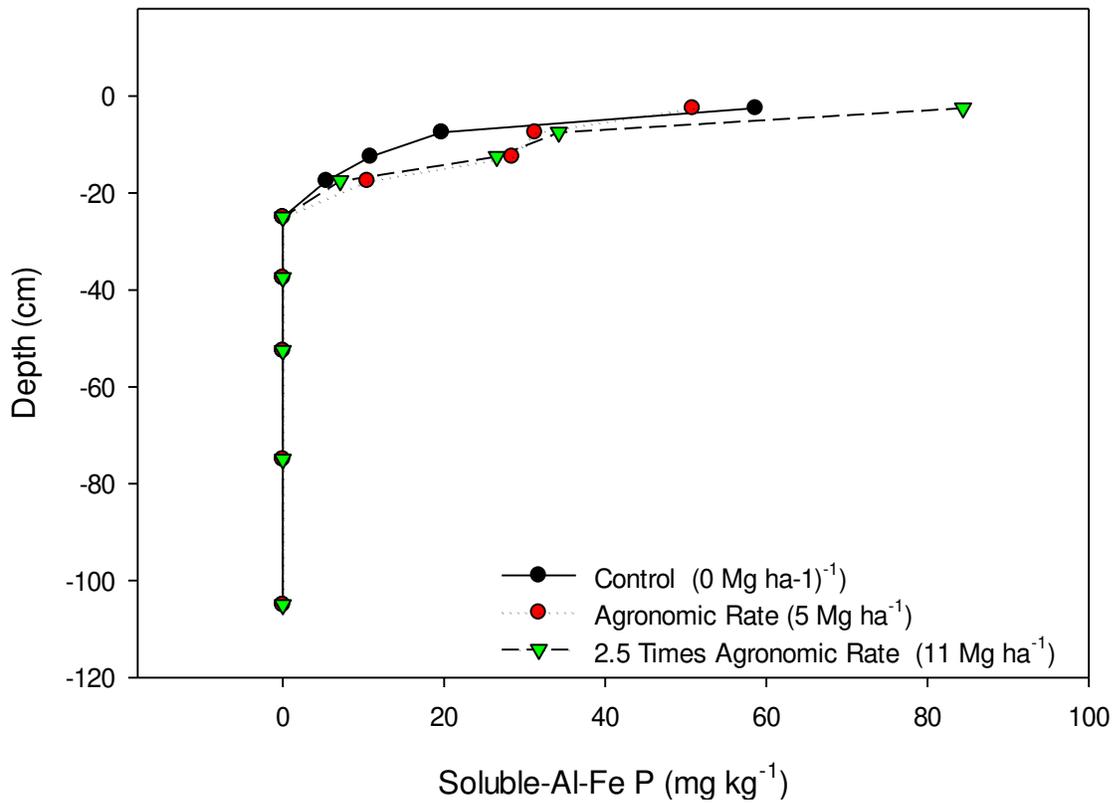


Figure 3.8. Distribution of soluble-Al-Fe inorganic P following 20 years of biosolids application at the research location. There was not a significant treatment effect, but there was a significant ($P < 0.001$) depth effect on the distribution of these forms for inorganic P.

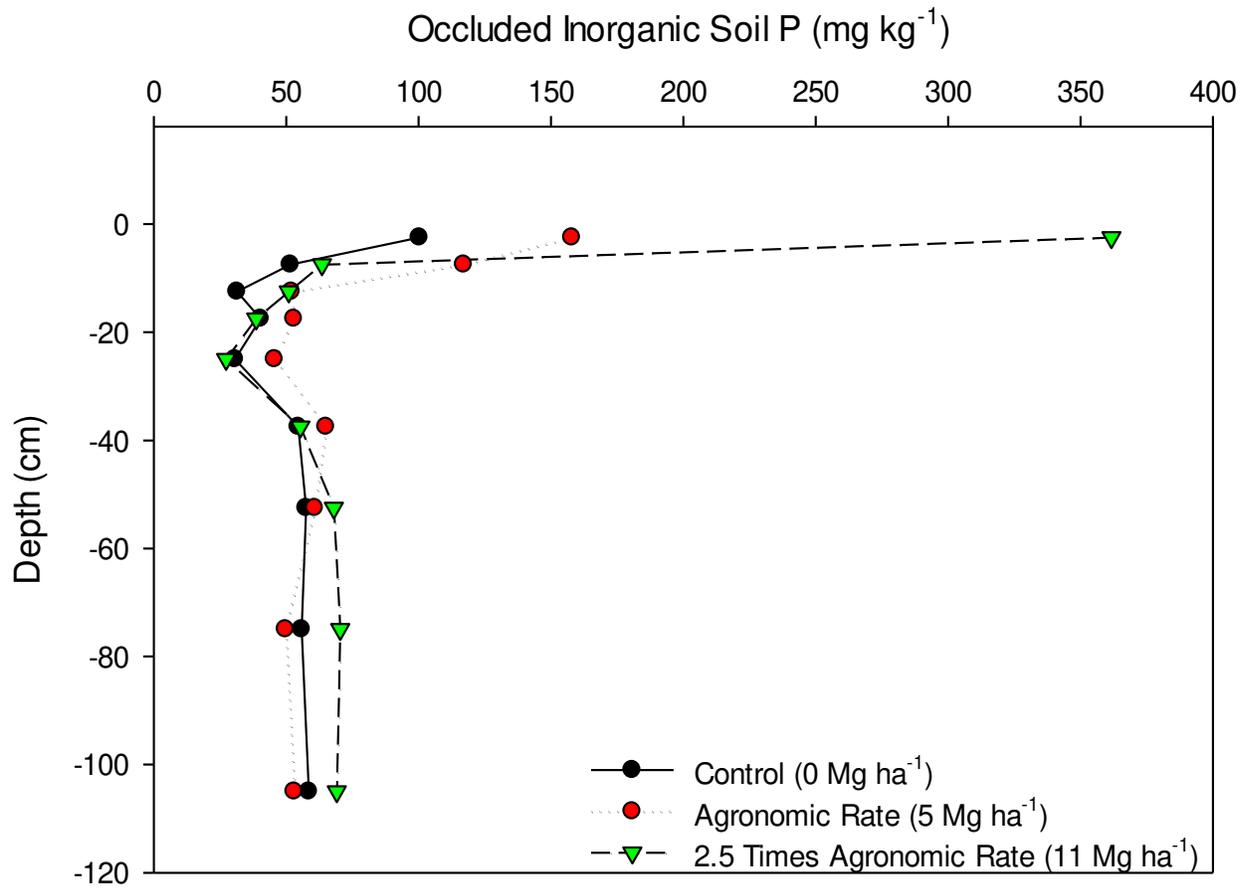


Figure 3.9. Distribution of occluded inorganic P following 20 years of biosolids application to a wheat-fallow agroecosystem at the research location. There was a significant interaction ($P < 0.001$) between the application rate and the concentration with depth.

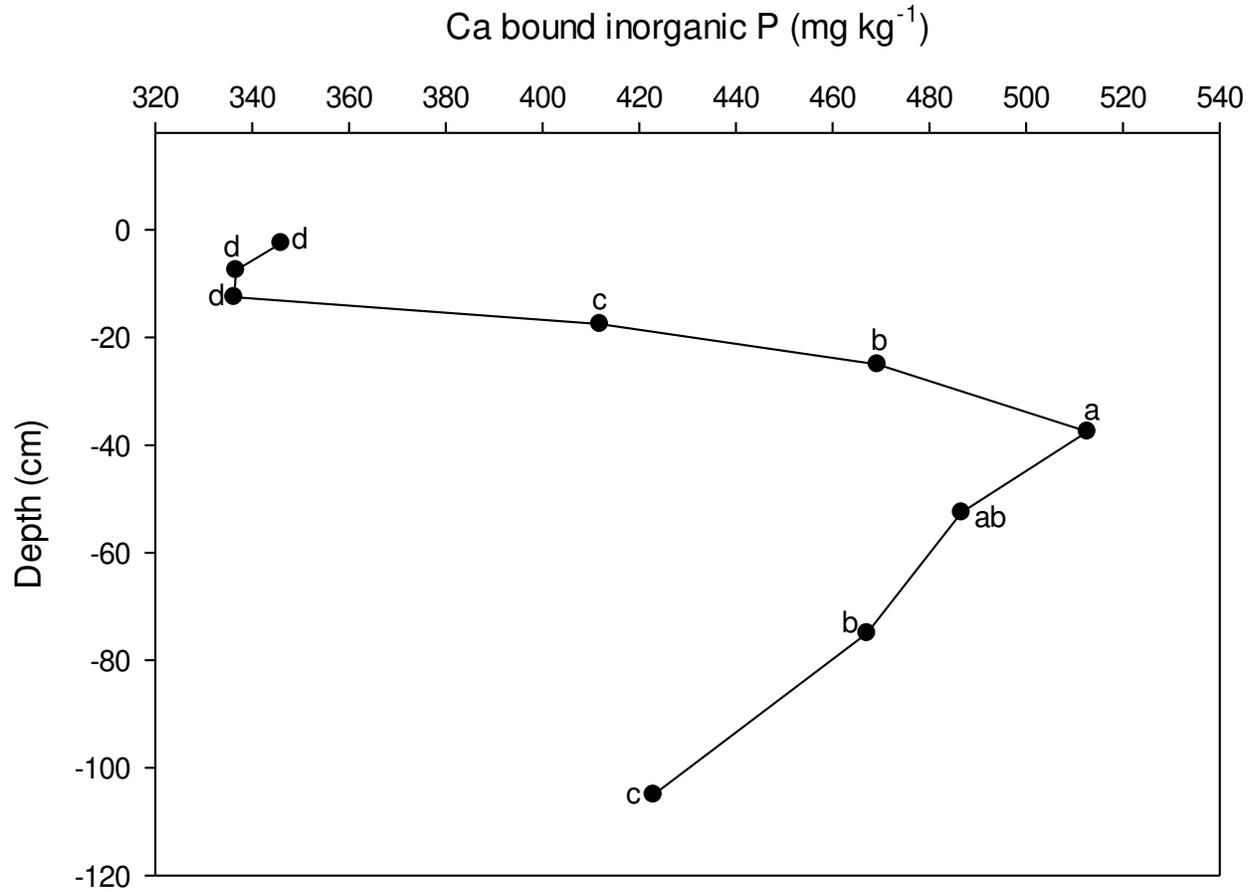


Figure 3.10. Distribution of Ca bound inorganic P following 20 years of biosolids application at the research location. There was only a significant ($P < 0.001$) depth effect and the distribution of Ca bound P was similar to the distribution of CaCO_3 (Fig. 2). The data presented was averaged over application rate at each depth point. Depths with different subscripts are statistically different at a probability level of 0.05.

Ca Bound Inorganic P

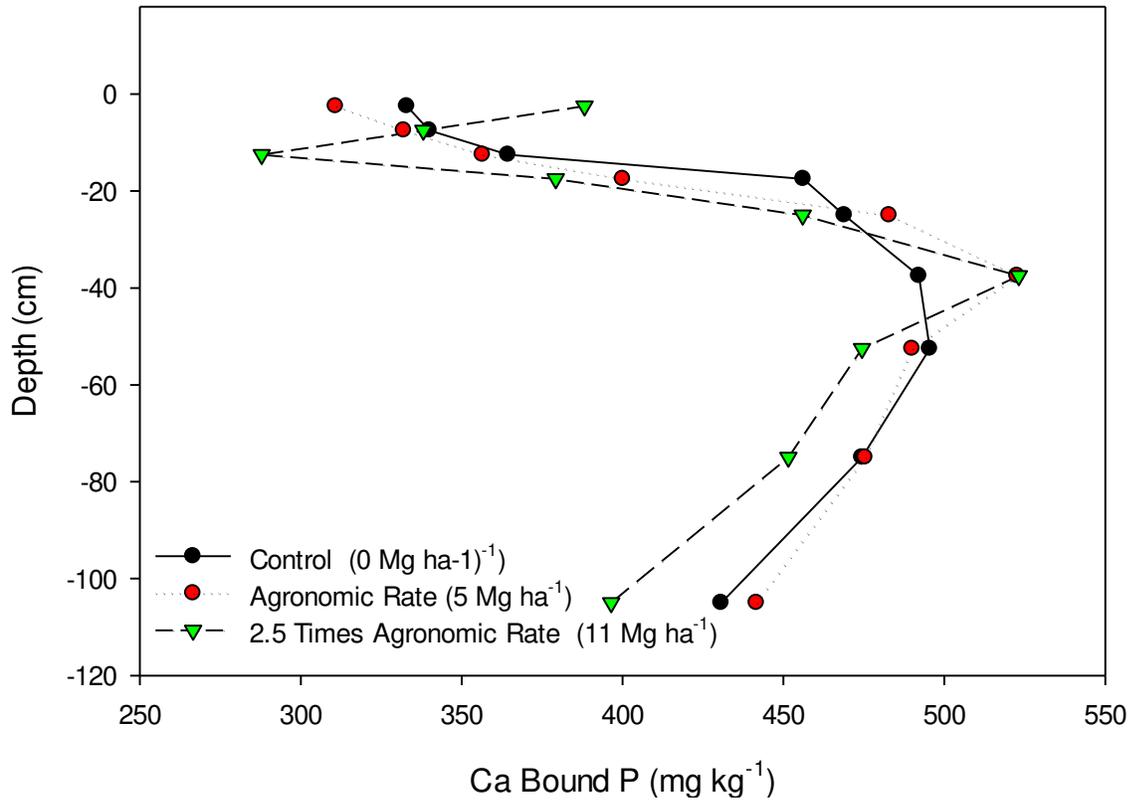


Figure 3.11. Interaction of biosolids application rate and depth on Ca bound inorganic P following 20 years of biosolids application at the research location. There was not a significant interaction seen between depth and application rate.

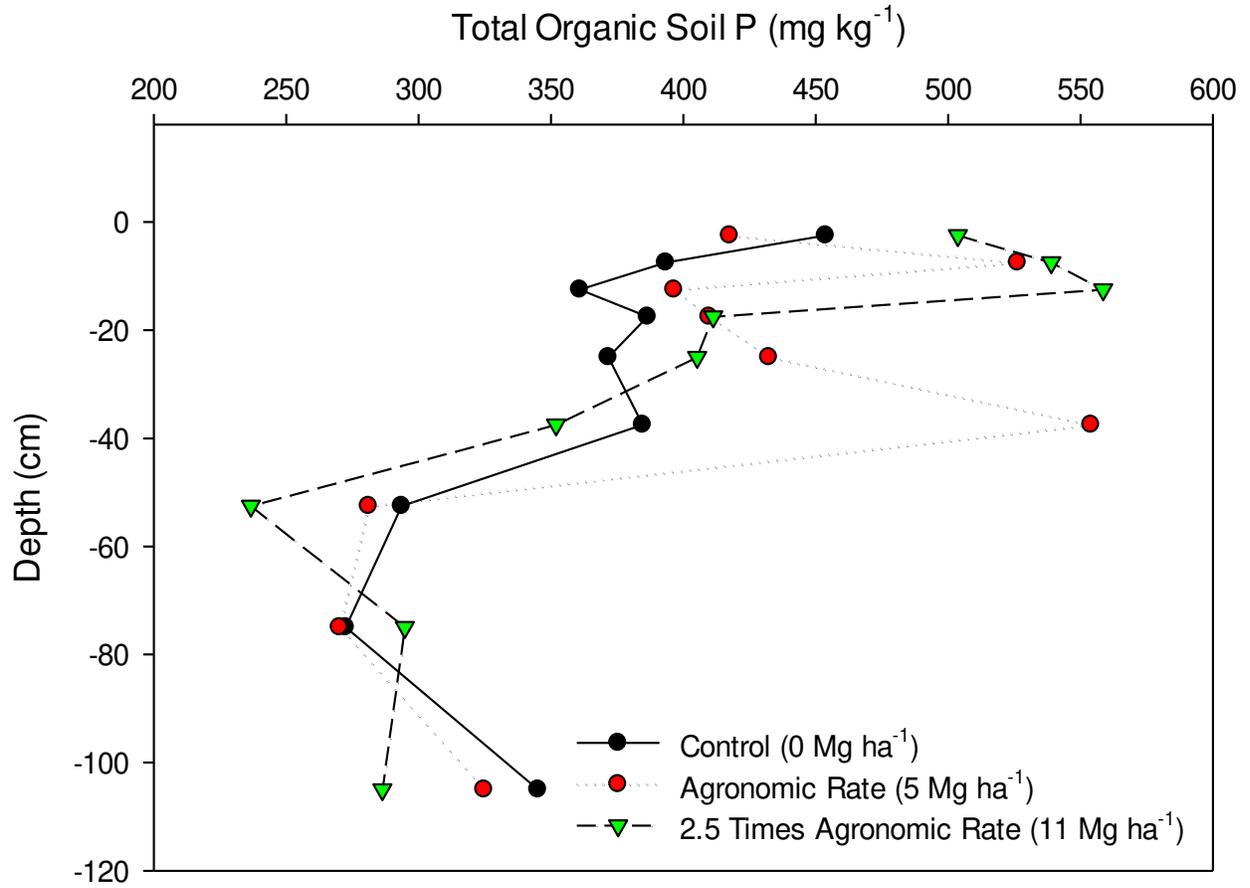


Figure 3.12. Distribution of total organic P following 20 years of biosolids application at the research location. There was a significant interaction ($P=0.045$) between application rate and depth, where a difference was seen in the top 20 cm.

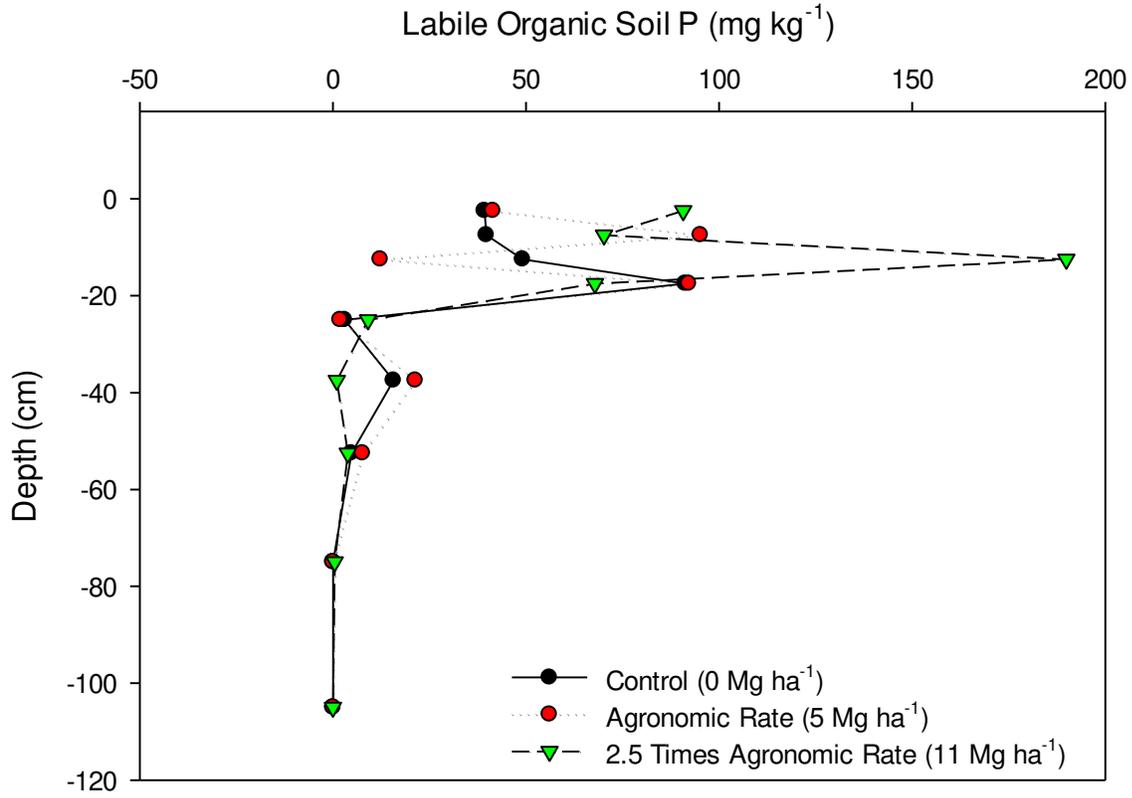


Figure 3.13. Interaction of biosolids application rate and depth on the distribution of labile organic P at the research location. There was not a significant interaction but there was a trend for increased concentrations at the soil surface with the higher application rates.

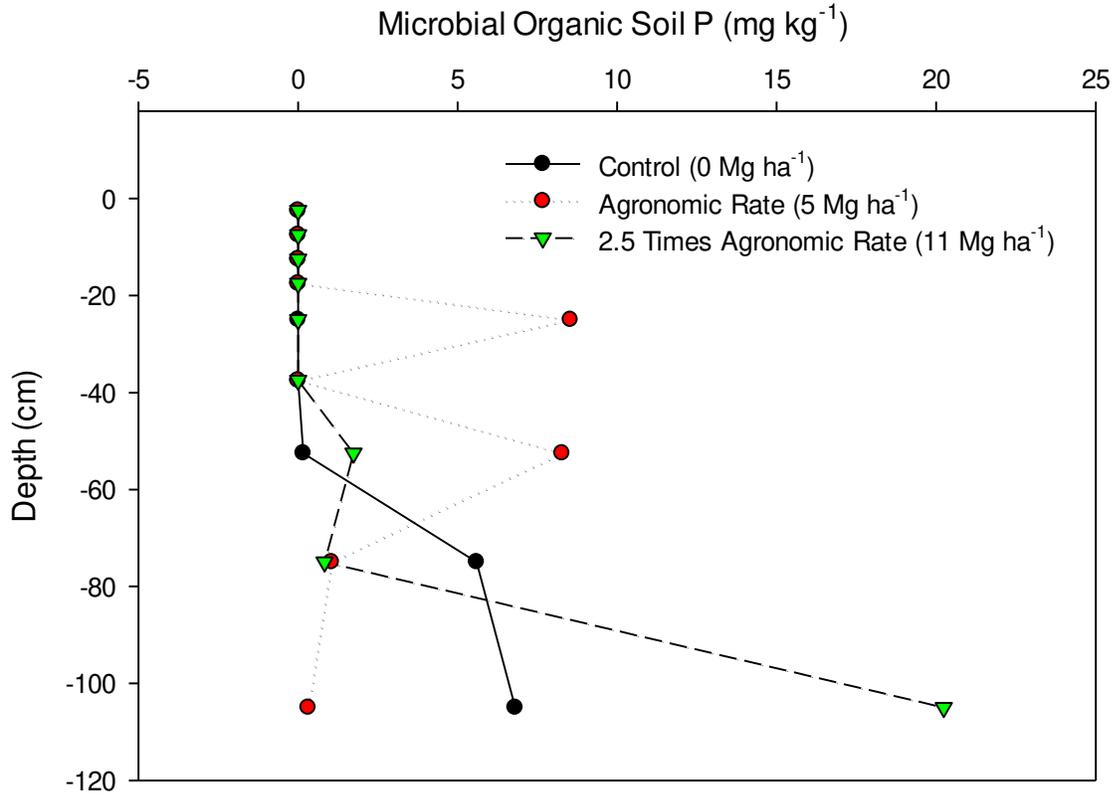


Figure 3.14. Interaction of biosolids application rate and depth on microbial biomass P at the research location. There was not a significant interaction or a clear trend to the data.

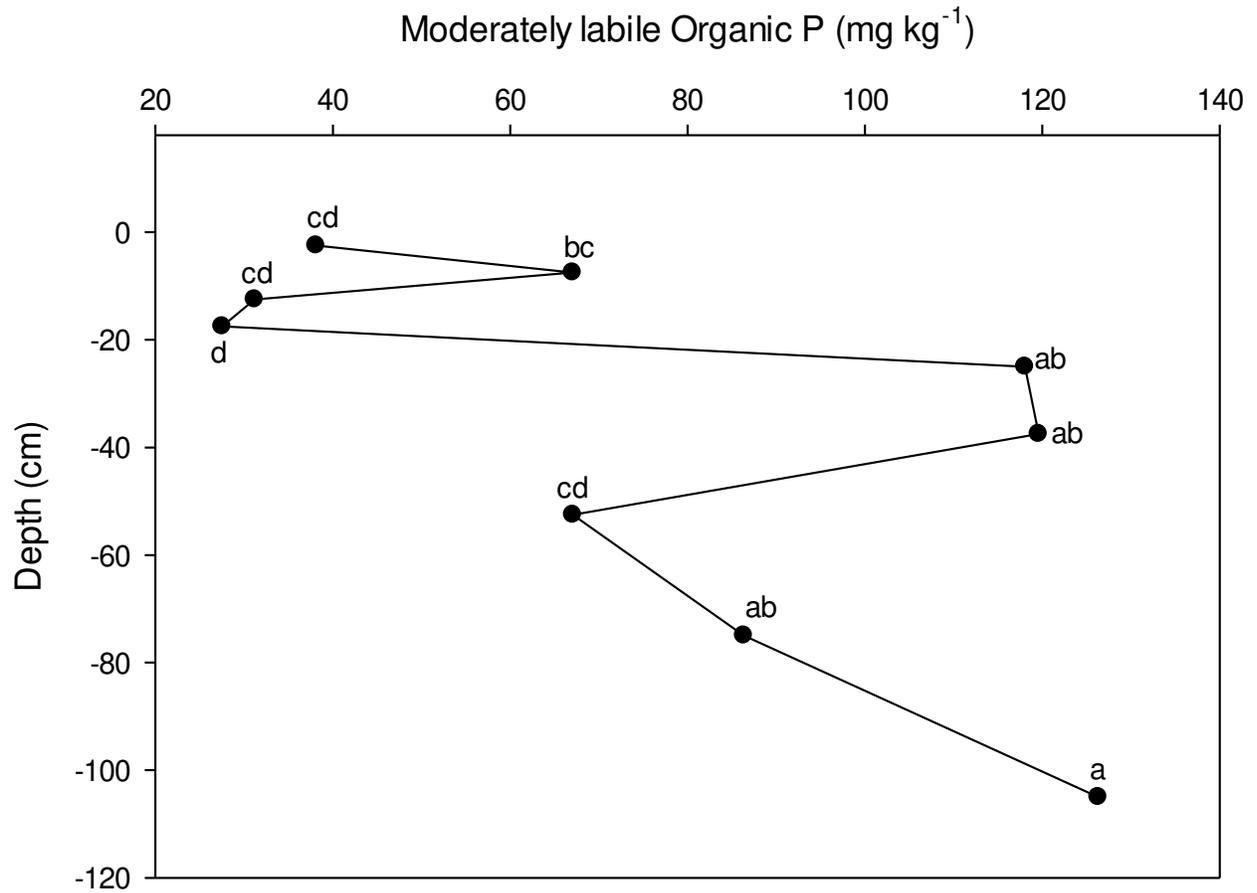


Figure 3.15. Distribution of moderately labile organic P following 20 years of biosolids application at the research location. There was only a significant ($P < 0.001$) depth effect and the distribution of moderately labile organic P. The data presented was averaged over application rate at each depth point. Depths with different subscripts are statistically different at a probability level of 0.05.

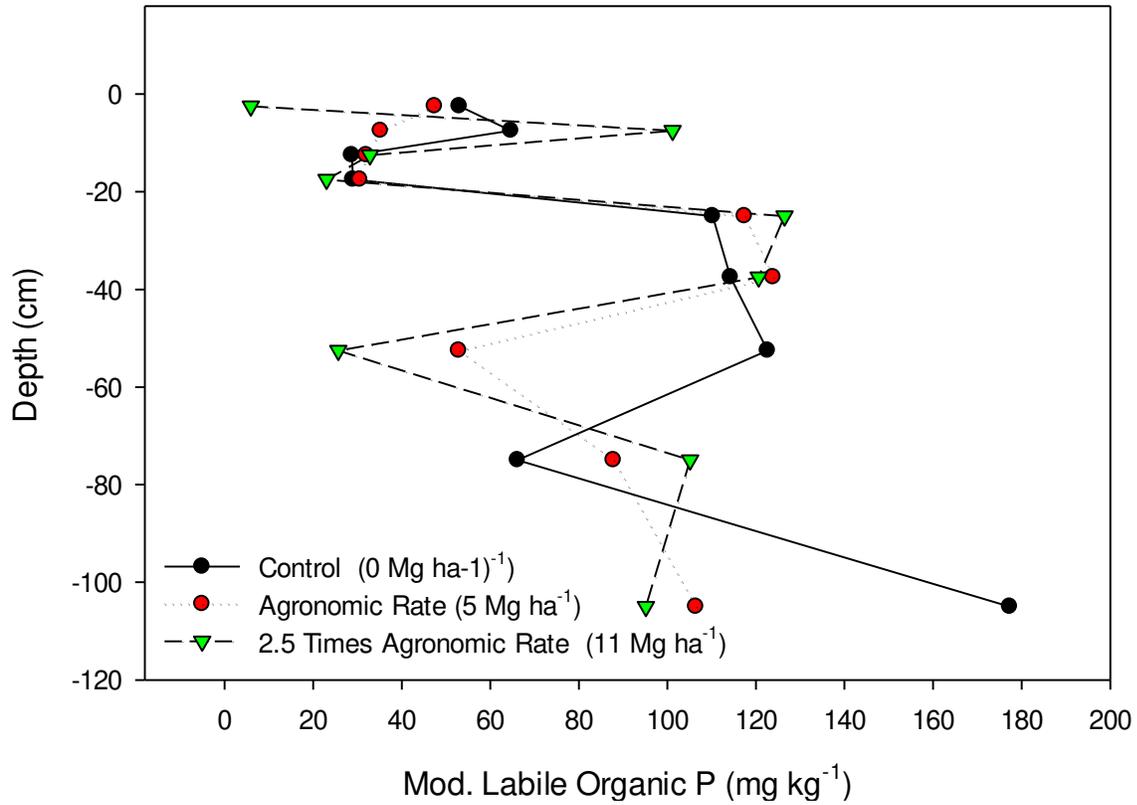


Figure 3.16. Interaction of biosolids application rate and depth on the concentration of moderately labile organic P at the research location. There was not a significant interaction indicating that there were other factors such as a change in texture that was controlling the vertical distribution of moderately labile organic P.

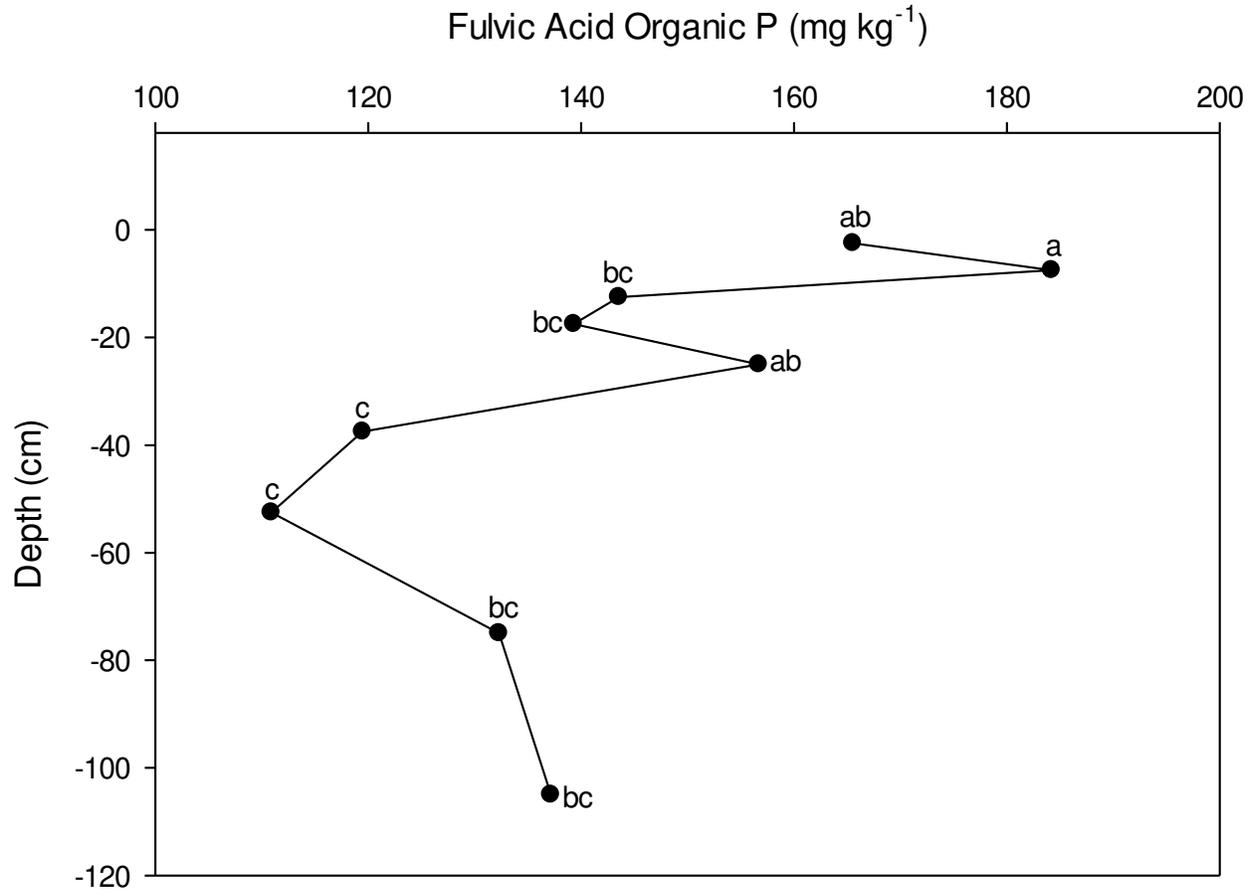


Figure 3.17. Distribution of fulvic acid organic P following 20 years of biosolids application at the research location. There was a significant depth effect ($P=0.004$). The data presented was averaged over application rate for each depth. Depths with different subscripts are statistically different at a probability level of 0.05.

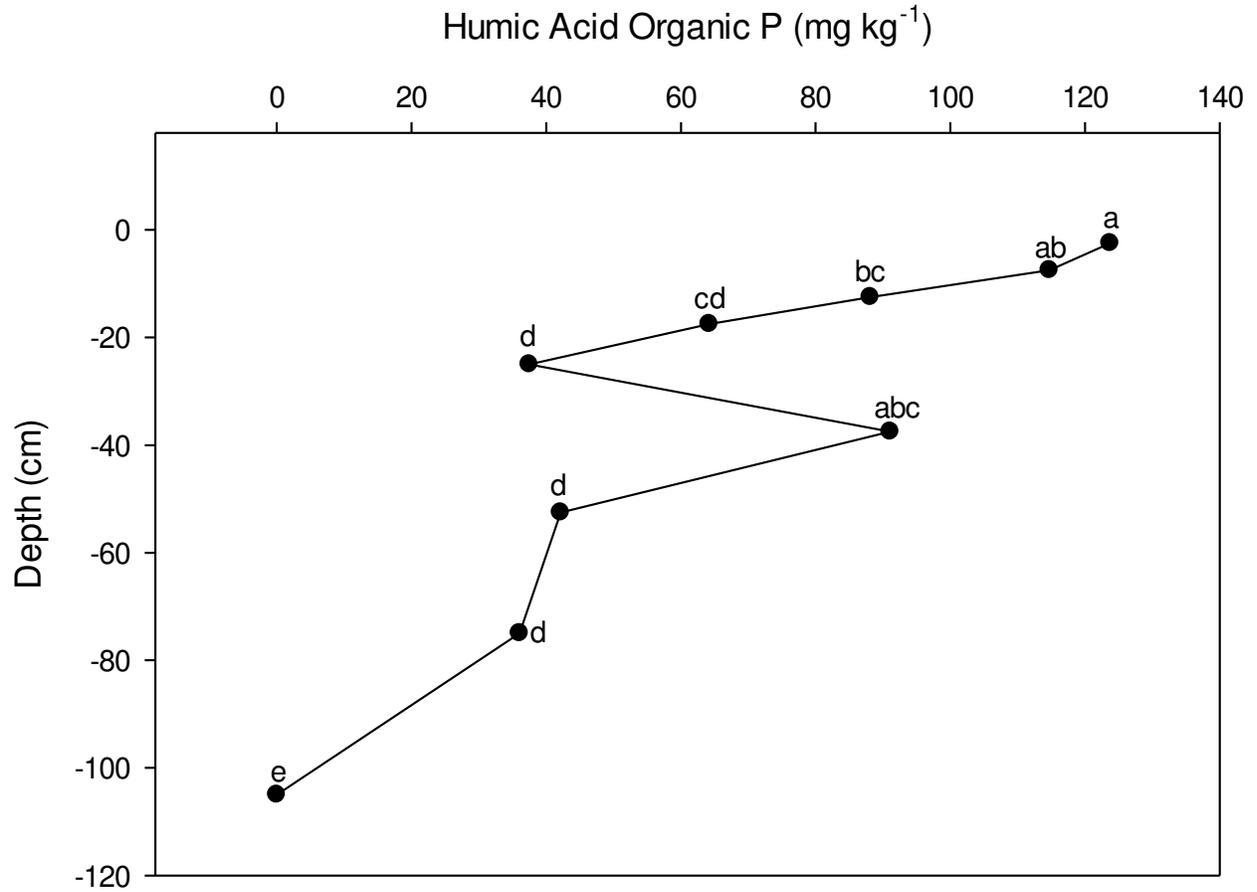


Figure 3.18. Distribution of humic acid organic P following 20 years of biosolids application at the research location. The data presented was averaged over application rate for each depth. Depths with different subscripts are statistically different at a probability level of 0.05.

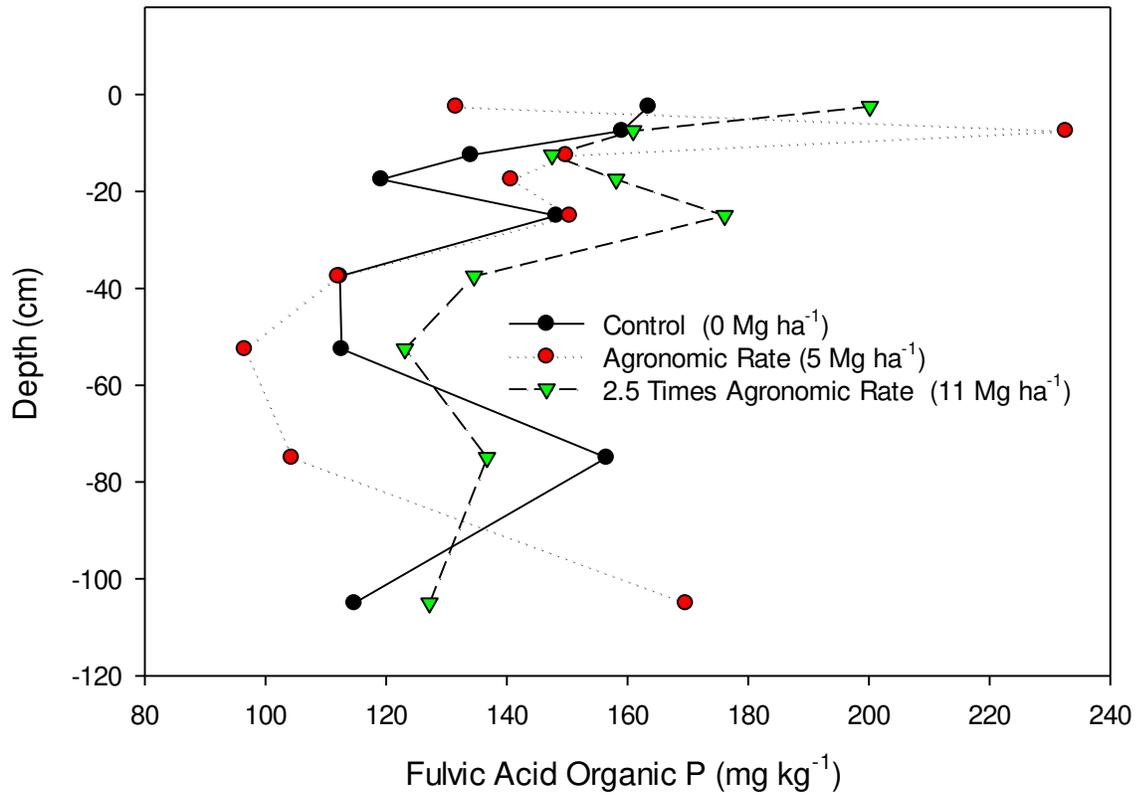


Figure 3.19. Interaction of fulvic acid organic P and depth following 20 years of biosolids applications at the research location. There was not a significant difference interaction indicating that factors other than application rate are controlling the distribution of fulvic acid organic P.

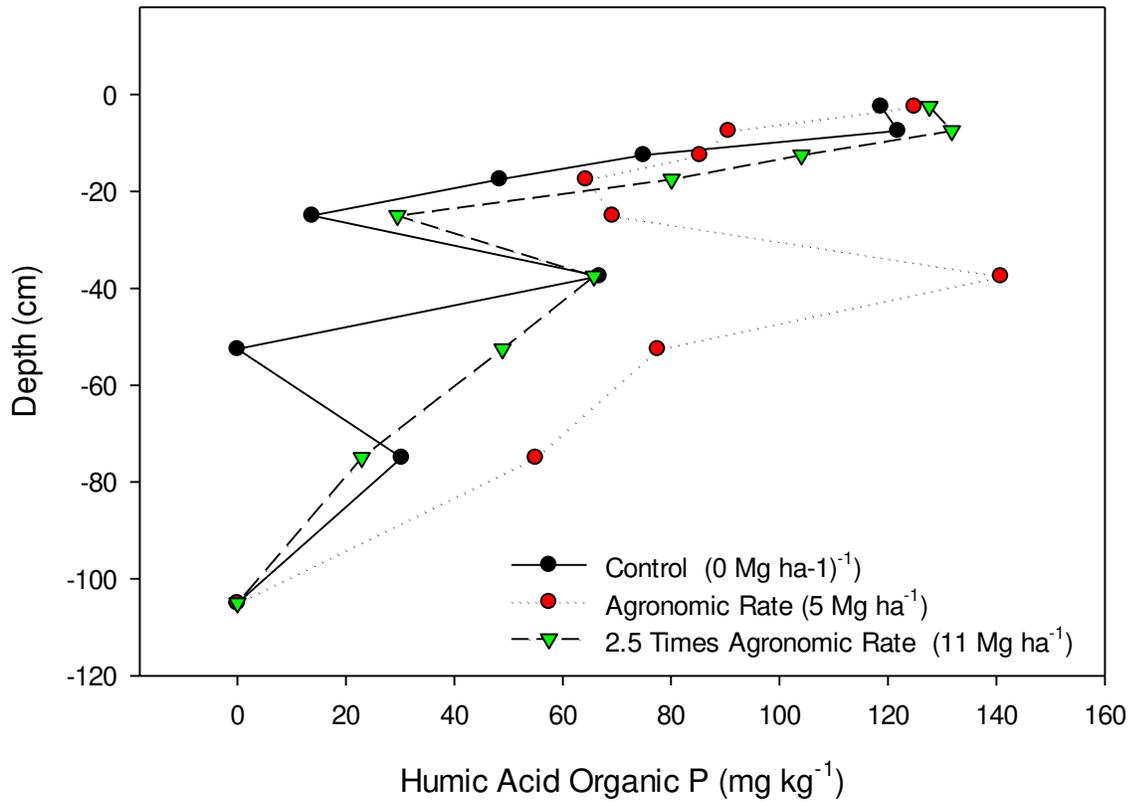


Figure 3.20. Interaction of humic acid organic P and depth following 20 years of biosolids applications at the research location. There was not a significant difference interaction indicating that factors other than application rate are controlling the distribution of humic acid organic P.

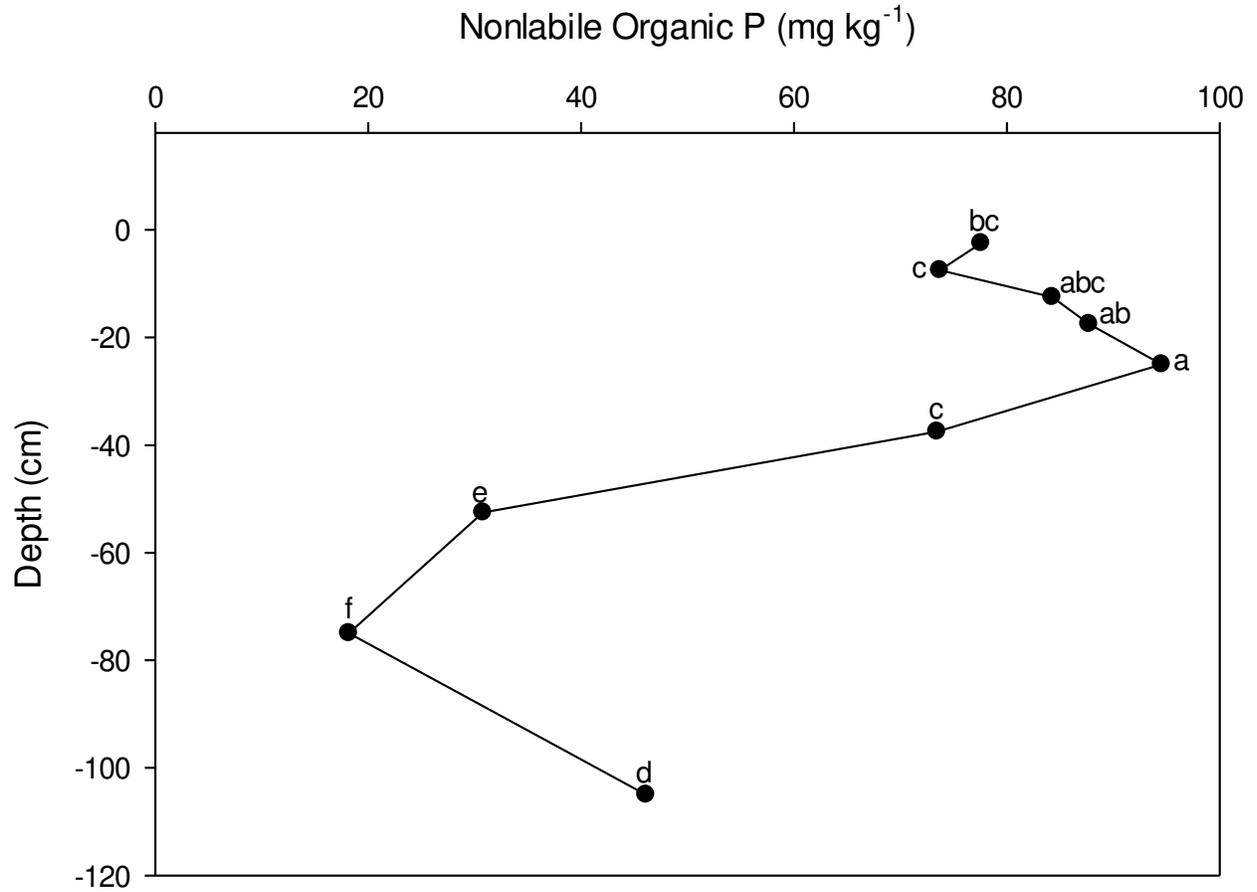


Figure 3.21. Distribution of nonlabile organic P following 20 years of biosolids application at the research location. The data presented was averaged over application rate for each depth. Depths with different subscripts are statistically different at a probability level of 0.05.

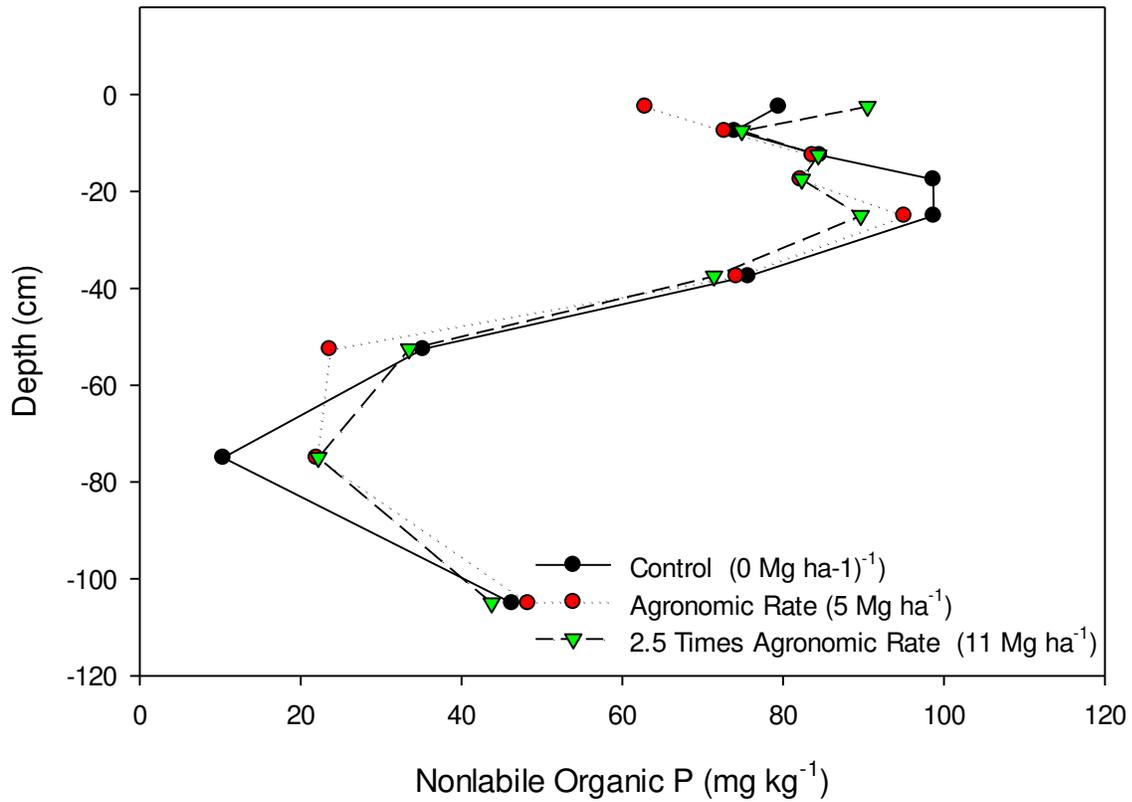


Figure 3.22. Interaction of nonlabile organic P and depth following 20 years of biosolids applications at the research location. There was not a significant difference interaction indicating that factors other than application rate are controlling the distribution of nonlabile organic P.

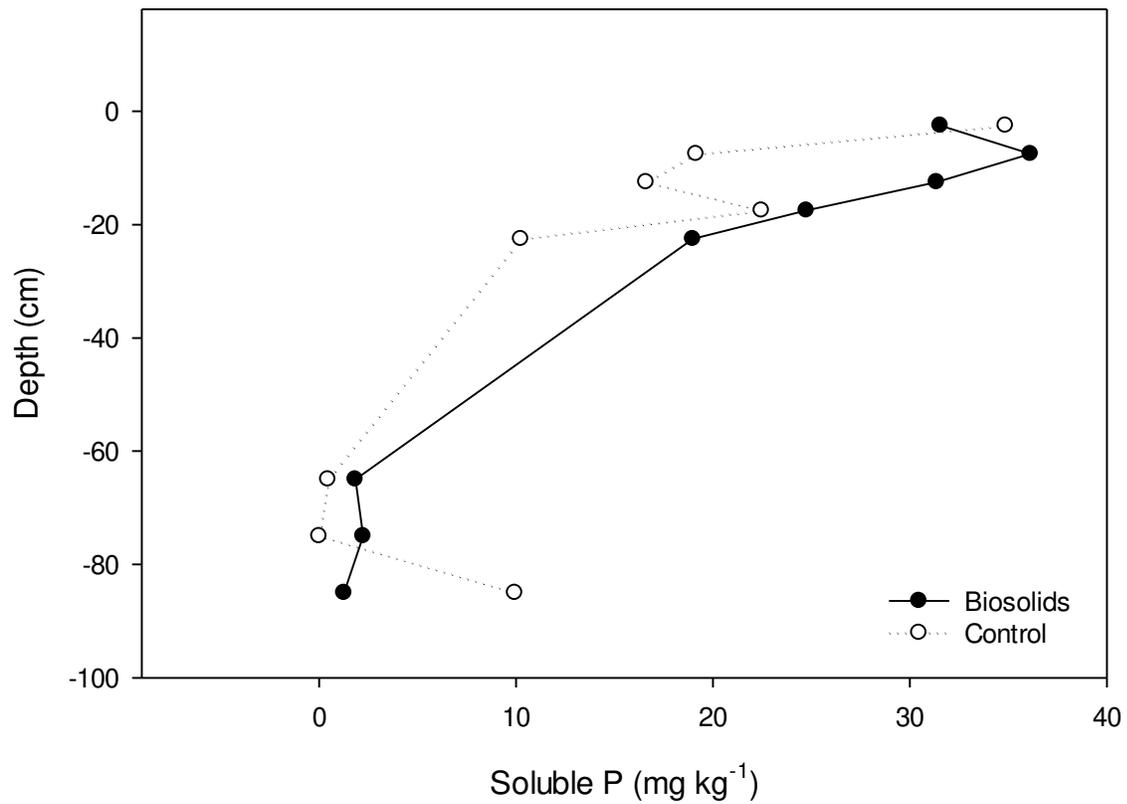


Figure 3.23. Distribution of soluble inorganic P in soil profile receiving biosolids applications at the production location. There was not a significant treatment effect or interaction of depth and treatment. There was a depth effect that showed an increase in soluble P near the soil surface then a rapid decrease.

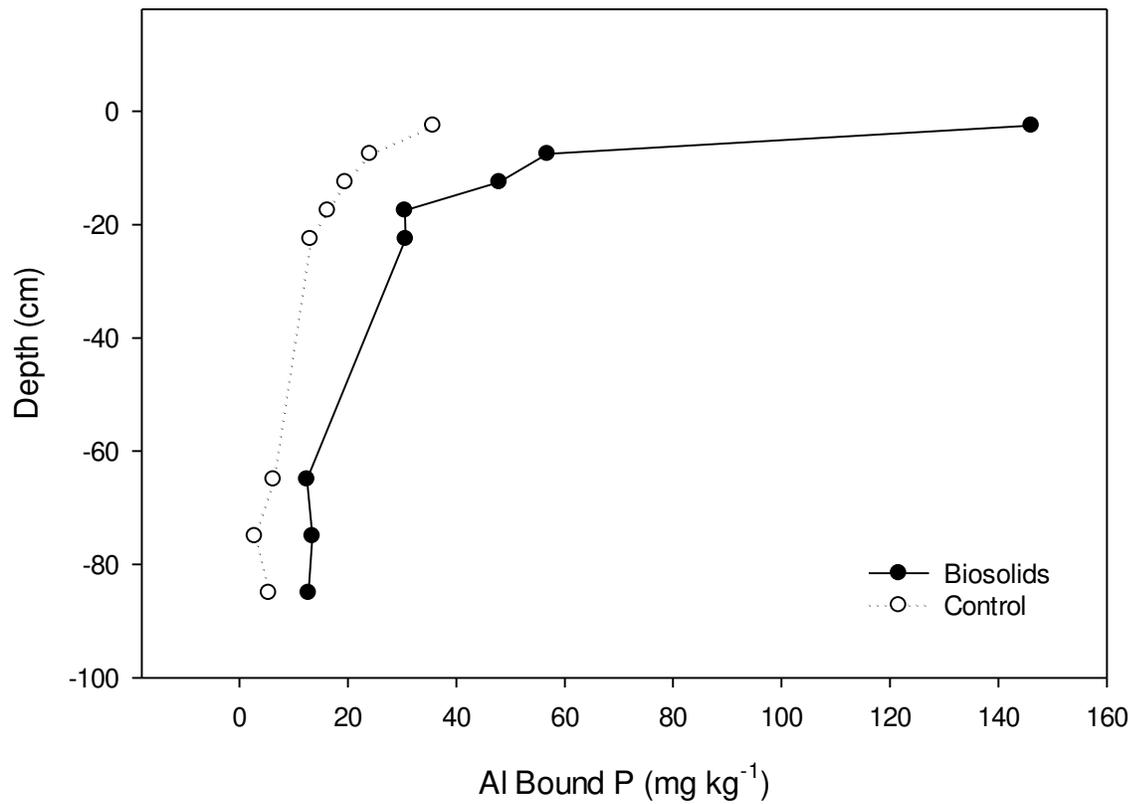


Figure 3.24. Interaction of biosolids application and depth on Al bound inorganic P at the production location. There was not a significant depth by treatment effect, but both of the main factors were significant at a probability level of 0.05.

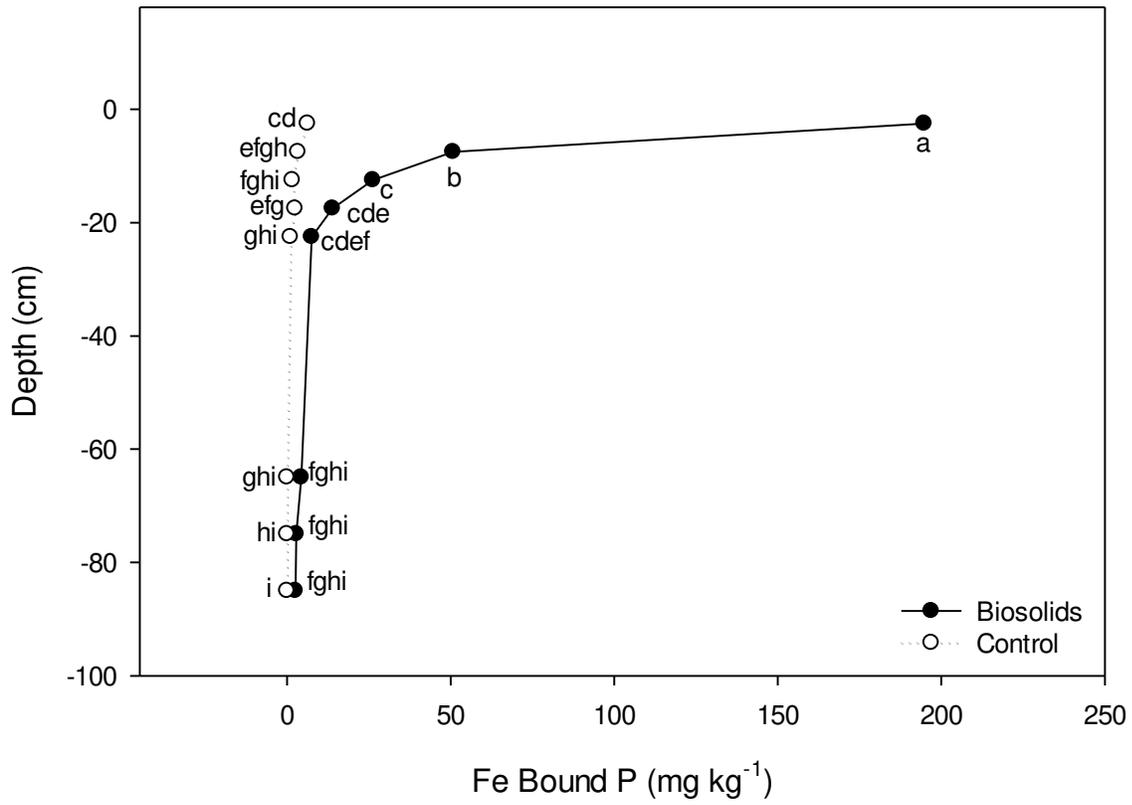


Figure 3.25. Interaction between biosolids application and depth at the production location. There was a significant interaction ($P=0.016$) that showed increased Fe bound P in the plow layer.

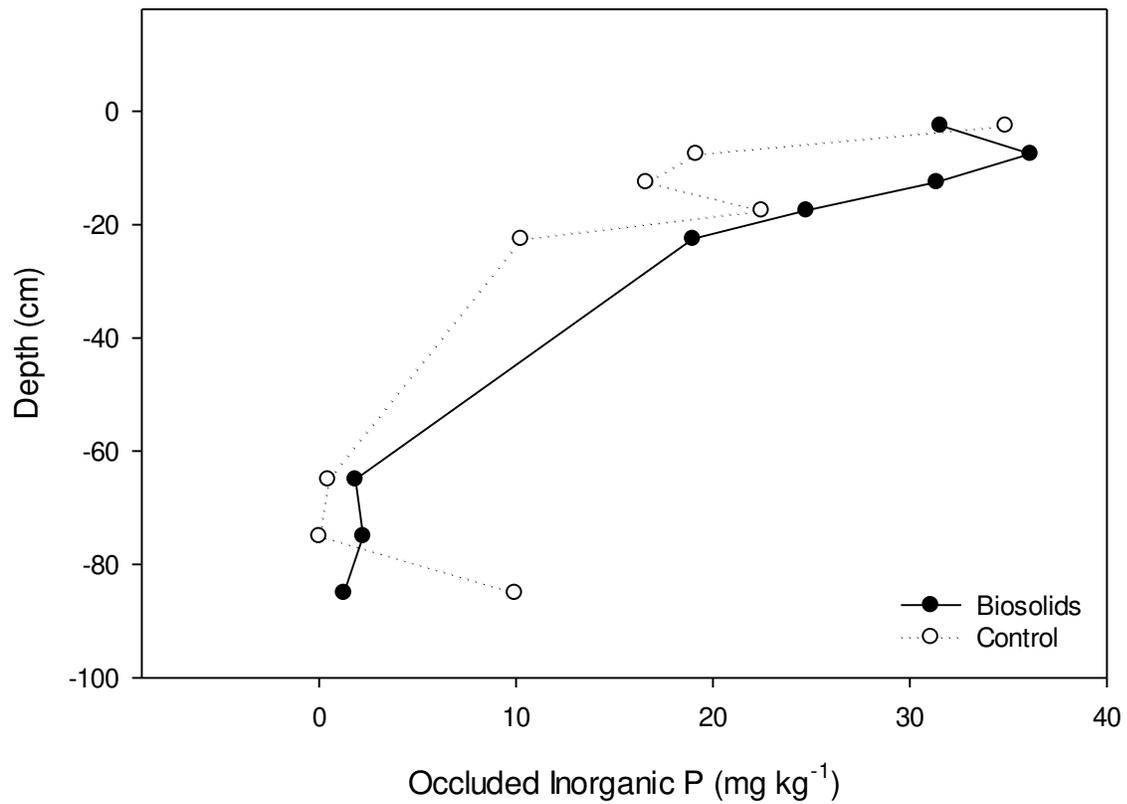


Figure 3.26. Interaction between occluded inorganic P and depth at the production location. The interaction was not significant, but there was a significant depth effect. There was a higher concentration at the soil surface most likely due to the higher amount of oxidation and air flow.

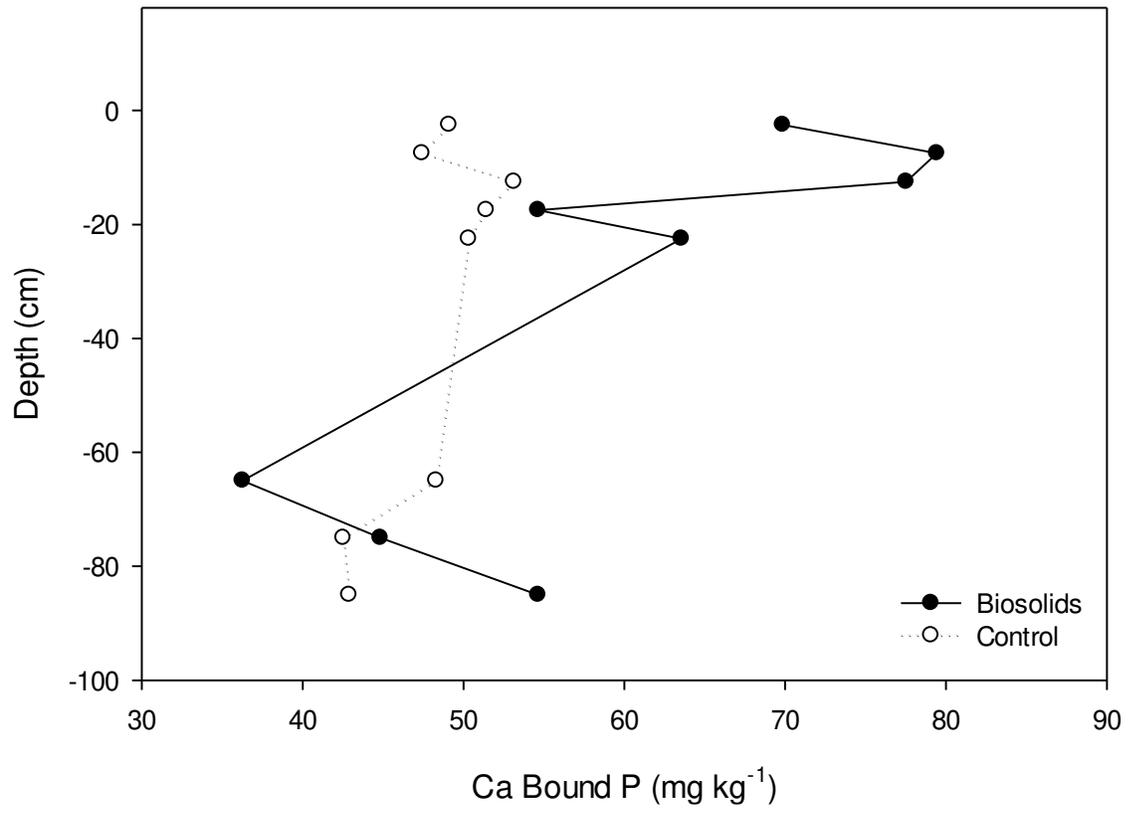


Figure 3.27. Interaction between Ca bound P and depth at the production location. There were not any significance differences for depth, biosolids application, or the interaction.

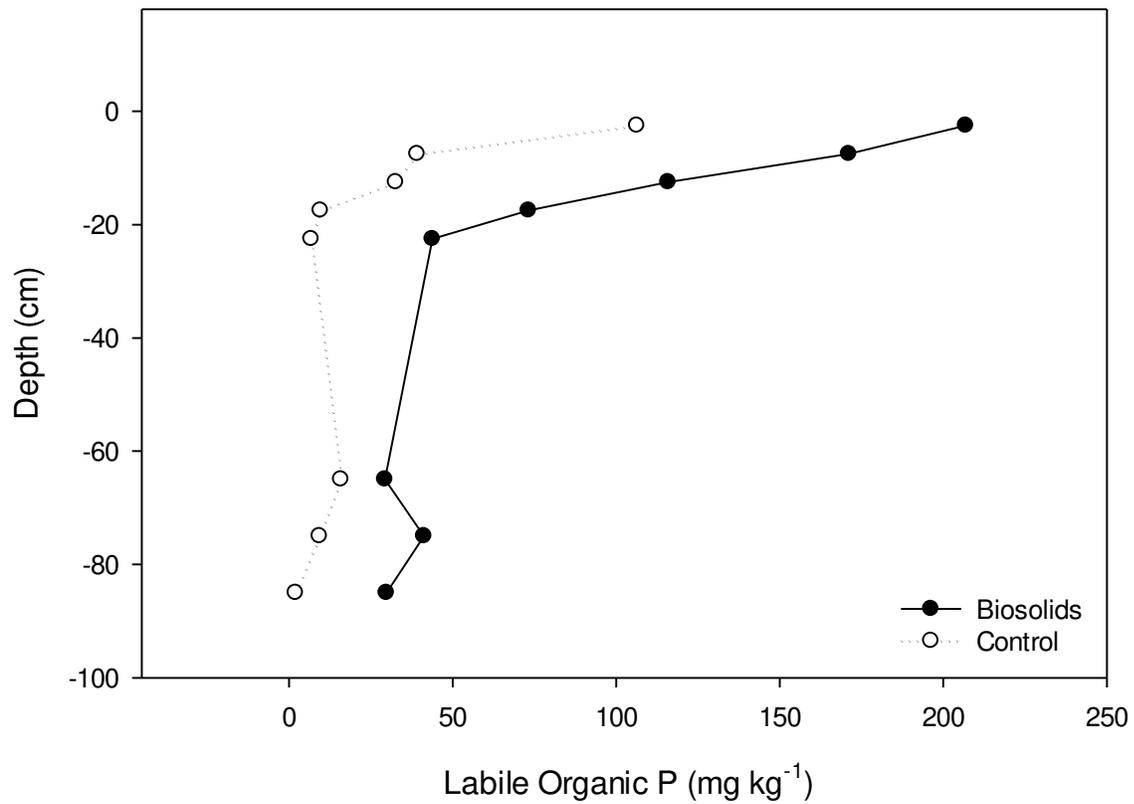


Figure 3.28. Interaction between labile organic P and depth at the production location. Both main effects were significant, but the interaction was not.

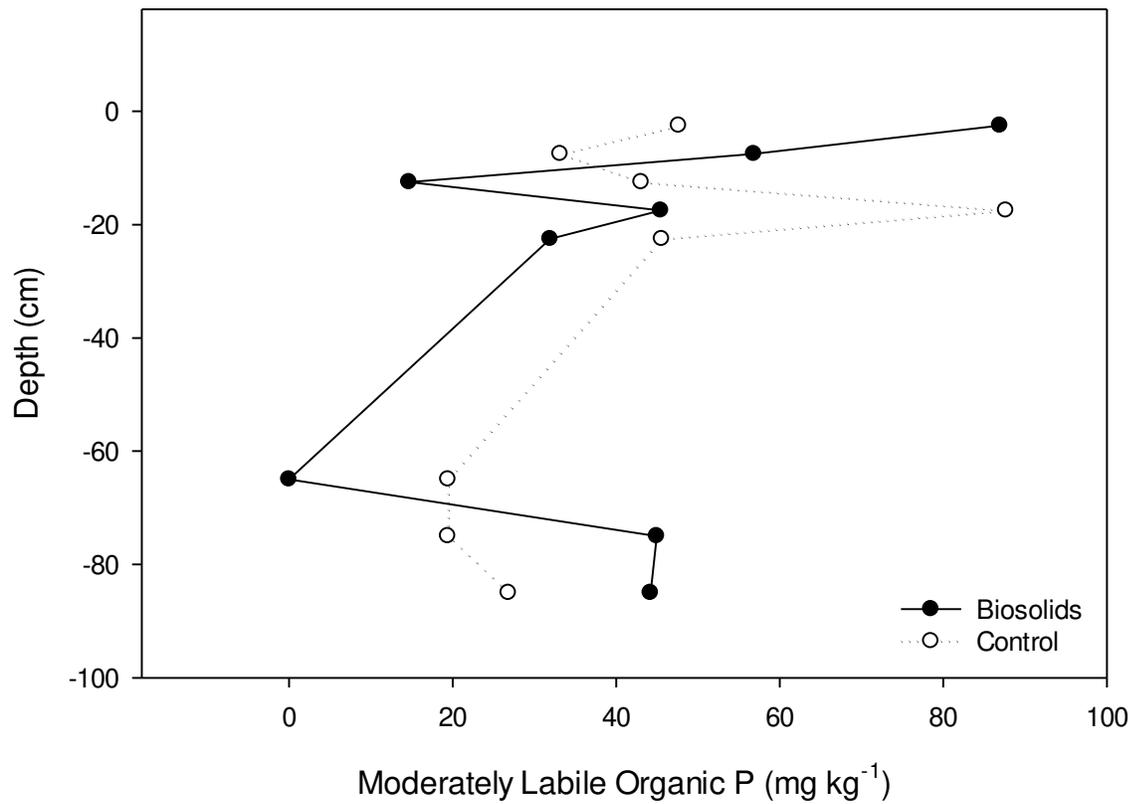


Figure 3.29. Interaction of moderately labile organic P and depth at the production location. The interaction was significant (P=0.024).

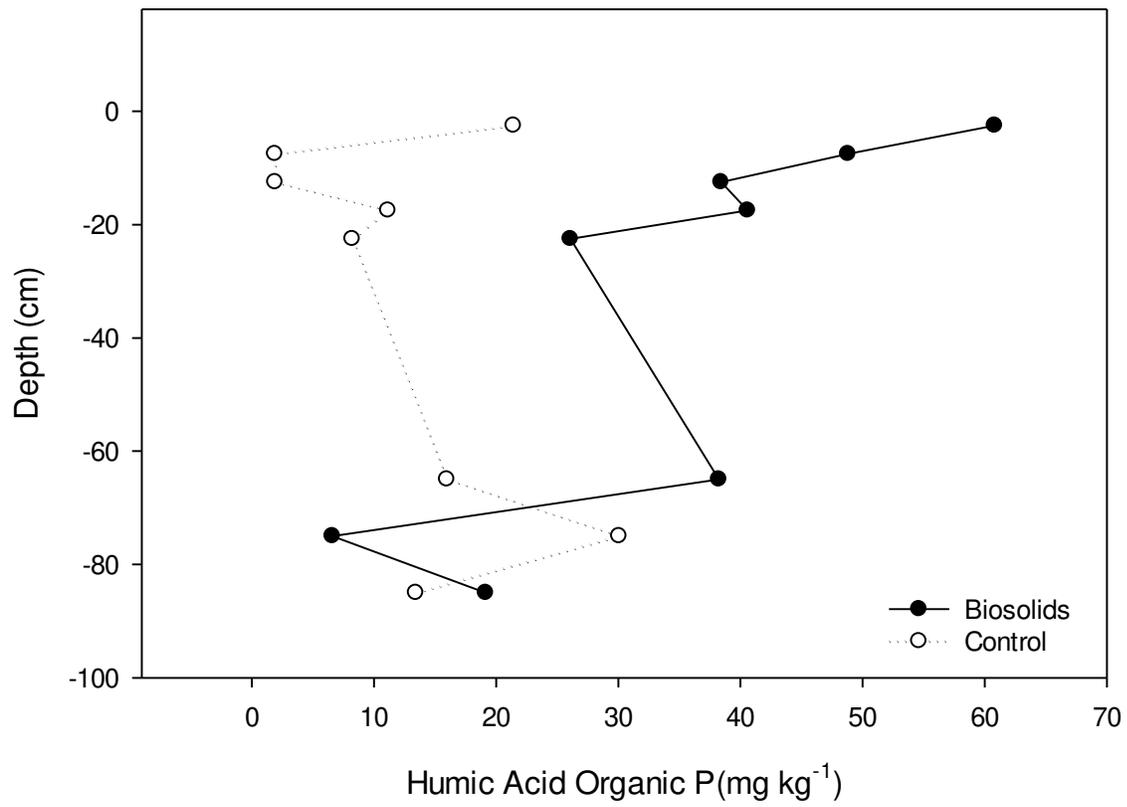


Figure 3.30. Interaction between humic acid organic P and depth at the production location. There was a significant treatment effect ($P=0.031$), but depth and the interaction were not significant.

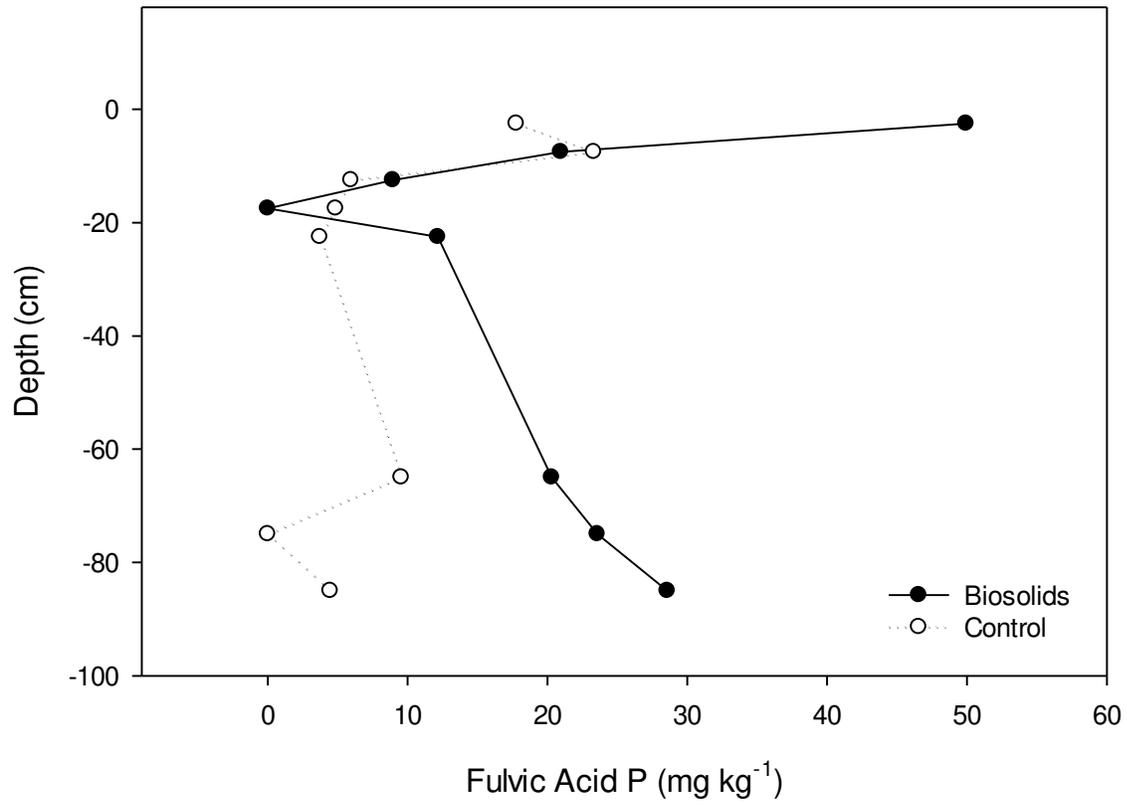


Figure 3.31. Interaction between fulvic acid organic P and depth at the production location. There were no differences seen between the control and biosolids application for fulvic acid.

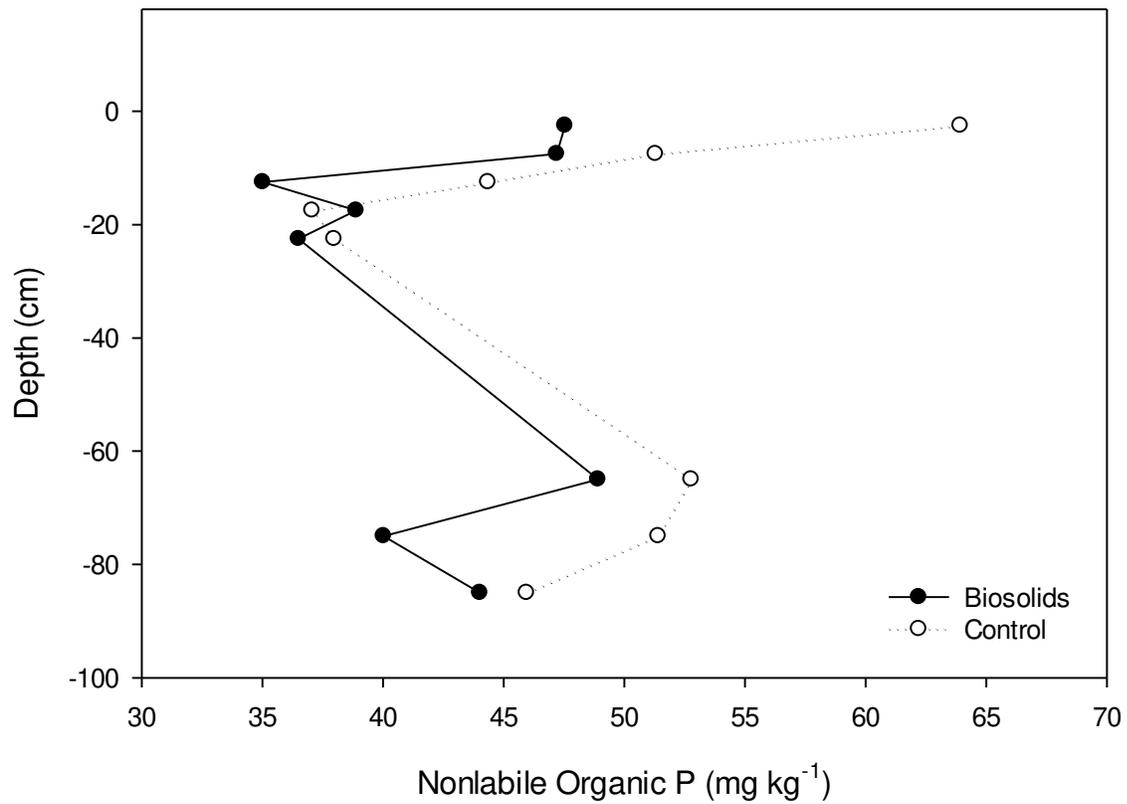


Figure 3.32. Interaction between nonlabile organic P and depth at the production location. There was not a difference seen between the control and the addition of biosolids.

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CHAPTER 4: SEASONAL CHANGES TO SOIL PHOSPHORUS FRACTIONS

Introduction:

Soil P cycles between different forms during the growing season because of biological activity and changes in environmental conditions. These changes can be expected to affect soil test values used for determining fertilizer application rates. Most soil tests that are used for fertilizer recommendations quantify the amount of P available and compare the value to an index (Kleinman, 2005). If there were changes in the forms of soil P, but these changes did not result in the soil P test value changing, then there would be little impact on management decisions. However, a change in form may affect the ability of plants to take up P and change the availability of P to the environment. In addition to changes due to time of year, soil type would influence the transformations that take place and agricultural practices may have a small impact.

Many factors contribute to the cycling of P in soil. Pierzynski (2005) developed a conceptual model for how P moves in and out of the system and between forms (Figure 4.1). The cycling of P within the system can be separated into two main mechanistic categories: chemical equilibrium reactions and biologically facilitated transformations. Chemical reactions include P sorption-desorption, P precipitation-dissolution, and P oxidation-reduction. The biologically driven portions are dominated by immobilization-mineralization and plant uptake. In this model, the only plant-available forms of P are from the soil solution, even though many studies have shown that organic P plays a significant role in supplying P (Chen et al., 2002, Firsching and Claassen, 1996, Oehl et al., 2001). The model also makes a clear separation between the organic and inorganic fractions and has the only pathway for transformation from one pool to the other going through the soil solution P pool. There are two ways that the P is considered to leave the system (1) uptake and subsequent removal of plant tissues, or (2) removal by water in the form of runoff/erosion or leaching. This model does allow for the

transformation of P between sorbed forms (less stable) and more stable secondary minerals without having to become part of the soil solution first.

The pathways described in this model provide a solid framework for the P cycle. However, it has been shown that many of the transformations that take place in the soil are very time dependent and rarely match the thermodynamic equilibrium (Pierzynski and McDowell, 2005). This is due to the large number of other elements that are in the soil solution and the tendency for amorphous P forms to develop (Kleinman, 2005, Lindsay, 1979, Olsen and Khasawneh, 1980, Sample et al., 1980). In soils that have had large amounts of P added with the addition of biosolids, manure or inorganic fertilizer (Kleinman, 2005) the systems generally are out of equilibrium for longer periods of time.

The objective of this study is to investigate how the forms of soil P change over a growing season under different agricultural practices and different application rates. Three different studies were conducted including cycling under 1) research conditions with varying application rates in a wheat-fallow rotation, 2) wheat-fallow under commercial production, and 3) continuous maize under irrigation. The sites are analyzed separately, and the trends are compared.

Materials and Methods:

Field locations:

The research location was established 20 years prior to sampling in a randomized complete block design with four replications. This site is located near Bennett, Colorado, and is a wheat (*Triticum aestivum* L.) -fallow system, where the soil is a Weld loam (fine, smectitic, mesic Aridic Argiustoll) (Soil Survey Staff, 2012). At this site plots were established in two locations to allow for a wheat crop each year, and both sets of plots were sampled.

The dryland production fields were located nears Byers, Colorado and were in a wheat (*Triticum aestivum L.*) - fallow system. The selected fields had an eight-year history of biosolids application and contained multiple soil series. The two soils sampled were Weld loam (fine, smectitic, mesic Aridic Argiustoll) and Colby-Adena (complex of fine-silty, mixed, superactive, calcareous, mesic Aridic Ustorthents and fine-loamy, mixed, active, mesic Ustic Paleargids) (Soil Survey Staff, 2012).

The four irrigated field sites selected had at least a five-year history of biosolids application for irrigated crop production. Two of the fields were fine-textured soils that contained the soil series of Heldt (fine, smectitic, mesic Ustertic Haplocambrids) and Nunn (fine, smectitic, mesic Aridic Argiustolls) (Soil Survey Staff, 2012). The other two fields contained two sandy textured soils, Bijou (Coarse-loamy, mixed, superactive, mesic Ustic Haplargids) and Valent (mixed, mesic Ustic Torripsamments) (Soil Survey Staff, 2012).

Soil sampling and analysis:

The three study sites involved in this investigation were sampled on different schedules. The research sites were targeted for sampling every other month for two years. Unfortunately, several months were missed due to the inability to sample frozen soil. The production dryland locations were sampled for the growing season of winter wheat (*Triricum aestivum L.*) starting in August through July. For the irrigated locations, soil samples were collected in June following the establishment of maize and then sampled in July, September, and November.

Soil samples (20cm sampling depth) were air dried and ground to pass through a 2-mm sieve. At each of the sampling points four composite samples were collected from each soil within each field. Soil samples were analyzed to determine chemical properties at a commercial laboratory (Table 4.1). The composited soil samples were then analyzed to determine the concentration of both inorganic and

organic forms of P (Kuo, 1996). For the irrigated sites only, Olsen extractable P (Kuo, 1996) was measured at each sampling time.

Statistical analysis:

Each of the three studies were analyzed separately and the overall trends were then compared. The data was analyzed in SAS 9.4 (SAS Institute, 2012) using a repeated measures mixed model using Proc Glimmix. The data were checked for normality, and a log transformation was used when necessary. Several models were fitted to the data to determine which best represent the covariance structure of the repeated sampling times. The final model was chosen based on the covariance structure that produced the lowest Akaike information criterion (AIC) value. For the irrigated locations, due to large differences in the soils, each of the soils was analyzed separately and then the trends were compared between each of the different soils.

Due to the differences in management history between fields, the data were also analyzed by looking at the percentage of P in each of the fractions (soluble, Al bound, Fe bound, Occluded, Ca bound, labile organic, microbial, moderately labile organic, fulvic acid, humic acid, and nonlabile). The percentage data was also checked for homogeneity of variance, and a log transformation was used when needed.

Results and discussion:

Irrigated Maize:

Olsen Extractable P:

Olsen extractable P is often used as an index to determine the application rate of fertilizer and is used to determine environmental risks based on the Colorado P Risk Assessment. For the fine-textured soils (Heldt and Valent) there was a statistically significant interaction between sampling month and the field that was sampled ($P < 0.001$, and $P < 0.001$ respectively) for Olsen extractable inorganic P (Table 4.2).

The coarse-textured soils did not show a consistent trend (Figure 4.2). Bijou had an interaction between month and field ($P=0.003$), but only the main effects were significant for Valent (field $P<0.001$, month $P<0.001$, Table 4.2). There was a low concentration of Olsen extractable P in the Valent soil series in June and then it increased and maintained a relatively consistent concentration (Figure 4.3) through the observation period. There were no additions of P during this time suggesting that transformations have increased the forms of P that are extractable by the Olsen method.

The data shows a trend for increasing Olsen extractable P with time during the growing season, with the trend being less dramatic for the coarse-textured soils (Figure 4.2). There were slight changes but much of that may have been due to sampling variation in the field (Beegle, 2005). For the coarse-textured soils, the amount of Olsen extractable P stayed mostly constant. The fine-textured soil had a trend for increasing Olsen extractable P during the growing season. In soils that are higher in clay there is more surface area for reactions to take place (Havlin, 2005). With the larger surface area of the clayey soils, and higher amounts of chemical activity, more transformations occur, thus increasing Olsen P. In addition, the transformations of organic P to inorganic P may be higher due to the higher organic matter content of the soils (Table 4.1).

Soluble Inorganic P:

Soluble inorganic P showed a significant interaction between field and sampling month for Bijou, Heldt, and Valent soils ($P=0.013$, $P<0.001$, and $P=0.044$ respectively, Table 4.3). Nunn only had a significant month effect ($P<0.001$). Overall, the trend was for an increase in soluble P between June and July and then a decrease from September to November (Figure 4.4). The increase may be due to an increase in the amount of biological activity in the soil by both soil microbes and roots that could increase the soluble forms of P (Sylvia, 2005). The increased activity will also lead to the turnover of organic P into inorganic forms through mineralization. In addition, many of the forms of P that are

present in soils are inorganic. During the warmer months and with increased moisture content in the soil from irrigation, the chemical processes that cause the transformations of less available forms of P to more available forms become favored. Also during times of plant growth there are changes in respiration in the soil that result in higher amounts of organic acids being formed in the soil leading to release of P, especially in higher pH soils. The concentration of soluble P observed may be under-representative of the amount of P solubilization because of plant uptake of P from the solubilized pool and transformations of solubilized P to other forms (Figure 4.1).

Al, Fe, and Ca bound P:

Based on the high pH of the soils, it would not be expected that there would be a very large impact of Al on the forms of soil P, but the data does show that there are still some interactions in the finer textured soils. Aluminum bound P had a significant interaction between field and month for the fine-textured soils ($P < 0.001$), and both main effects were significant for the coarse-textured soils, but there was not an interaction (Table 4.4). There was a slight increase in Al bound P in the coarse-textured soils, but there was not a clear trend for the fine-textured soils (Figure 4.5). In the coarse-textured soil there is less chemical reactivity and less cation exchange (Table 4.1).

The interaction between field and month was significant for all the soils for Fe bound inorganic P (Table 4.5). There was an increase in the concentration for all soils from June to July. The large amount of Fe in the biosolids would bind some of the soil P (Lindsay, 1979). The data also suggest that when biosolids are applied they are not at chemical equilibrium and there is a lag time for P to bind with Fe. The results had more variability in the data for the fine-textured soil than the coarse-textured soils (Figure 4.6) probably due to the more heterogeneous flow of water and the resulting distribution of nutrients (Gardiner, 2008).

Bijou and Nunn showed an interaction of month and field for occluded P, and Heldt and Valent had statistically significant effects of month and field, without an interaction (Table 4.6). The general trend for the data was that there was a decrease in the concentration of occluded P until September and then it began to rise again (Figure 4.7). One explanation for this trend is soil water content related; in addition to irrigation, this year of the study had atypically high precipitation during the sampling period. Occluded P is P that is encapsulated in Fe. If the soil was excessively wet, it would likely reduce the Fe and therefore reduce the concentration of occluded P (Patrick and Khalid, 1974). At several of the sampling times there was gleying present in the fine-textured soils which helps to support there were redox changes in the soil.

There was a decrease in the concentration of Ca bound P from July to September, and then it recovered by November in most of the fields (Figure 4.8). There was a significant field by month interaction for fine-textured soils and a month effect for the coarse-textured soils (Table 4.7). A possible explanation may be due to increase in microbial and plant respiration increased the concentration of carbonic acid that would dissolve some of the Ca bound P. At the September sampling, the level had risen back to the initial conditions, suggesting that some of the P may have rebound to the freed Ca.

Labile Organic P, moderately labile Organic P, and microbial P:

The concentration of labile organic P decreased from June to September for all the soils and then started to increase by November (Figure 4.9). There was a significant interaction between field and month for the coarse-textured soils and only a significant month effect for the fine-textured soils (Table 4.8). The application of biosolids added organic P to the soil and transformations to more stable forms could explain the reduction in labile organic P during the growing season. These transformations include generation of inorganic forms that are then taken up by the growing corn plants. During this time, there

would also be increased biological activity that could help in reducing the concentration of a more available pool. The end of season increase in labile organic P may be due to the breakdown of the roots and other organic matter in the soil after the corn had completed its vegetative growth.

Microbial biomass P increased from June to September and then decreased rapidly in November for Bijou and Valent soils. For the Heldt plots, the microbial biomass P stayed statistically constant during the growing season and then decreased in November. There were no statically significant results for the Nunn plots (Table 4.9). Generally, microbial biomass increases during the growing season and as a result there is more soil P associated with the microbial biomass. These finding also help to support the concept that some of the labile organic P was transformed by microbial activity.

There were no statistically significant differences for the moderately labile P in the Bijou soil. There was a month by field interaction for Heldt and Valent and only a month effect for Nunn (Table 4.10). For Nunn, Heldt, and Valent there was only a slight trend for a decrease across the growing season (Figure 4.10). The significante effects seen did not impact soil textures the same and were small changes with a large amount of variability suggesting that the differences may have been due to field variability. Since this fraction is a more stable form of P, it is possible that over the time scale of a growing season, this fraction does not change, and changes will only be seen over multiple-year studies. Previous work investigating the kinetics of P transformations between labile and moderately labile P pools with the addition of organic P fertilizer (compost) found that the transformation has a half-life of ~385 days (Audette et al., 2016).

Fulvic acid P, humic acid P, and non-labile P:

Fulvic acid associated P had a significant month effect for Bijou, Valent, and Heldt (Table 4.11). There was a month by field interaction for Nunn (Table 4.11). The fulvic acid P decreased from June to July and increased from September to November for most soils (Figure 4.11). Humic acid associated P

had a significant month effect for Bijou, Heldt, and Valent (Table 4.12). There was a month by field interaction for Nunn (Table 4.12). All the soils followed the same trend for an increase from June to July and then a decrease in September that held constant through November (Figure 4.12). Humic and fulvic acid fractions are affected by the concentration and turnover of organic matter (Wright, 2009). The trends for the two fractions moved in the reverse direction of each other, suggesting that the application of biosolids added fulvic acid associated P and that the P was then transformed during the growing season.

Non-labile P had a significant month by field interaction for the fine-textured soils and only a month effect on the sandy-textured soils (Table 4.13). For the sandy soils, the concentration stayed fairly constant across the growing season, with a slight decrease in July (Figure 4.13). For the fine-textured soil there was a decrease from June to September and then it began to increase again. Except for Heldt, there were small changes that were seen in the concentration of non-labile P. This pool of P is one of the most stable forms, taking many years to form (Audette et al., 2016) so large changes are not expected during a single growing season. The larger differences seen in the concentration were in the fine-textured soils and may be a result of the heterogeneity of the soil.

Percent of total:

To account for management differences between fields, the data were converted to a percent of the total P (Tables 4.14-25). Again, the soil types were not directly compared but the trends were compared against each other.

The trends in percent soluble inorganic P (Figures 4.14-26) are like those observed with the actual concentration values. All the significant interactions between month and field observed in the concentration data are absent for the percent P analysis except for in the Nunn soil. There were

not any changes in the interaction of month and field for Al bound P. For Fe bound P the interaction was no longer significant for Nunn and Valent.

Dryland Production Field:

There was a significantly ($P=0.010$) higher percentage of soluble inorganic P for Colby-Adena than Weld (Table 4.26). There was also a significant month effect for soluble inorganic P ($P<0.001$) when the data was average over soil series. Initially, there was a higher percentage of soluble inorganic P in August, September, and November before decreasing and then remaining constant (Figure 4.28). When biosolids were added prior to the start of sampling, many forms of P (Huang et al., 2008, Shober et al., 2006) were introduced, and these would lead to new equilibrium concentrations of the various P-pools. This would result in the inability to separate the effect of the addition of biosolids and time on the early changes.

There was a significant interaction between soil series and month ($P=0.004$) for Al bound inorganic P (Table 4.26). The percent Al bound P was higher for Colby-Adena than it was higher for Weld (Figure 4.27). The percent of Al bound P for the Weld samples was higher in September and November (Table 4.26). Aluminum bound P increased from August to September for Colby-Adena and from August to November for Weld. These results suggest that the transformations are more dependent on soil properties than on the time of year.

There was a significant interaction between month and soil series for Fe bound P ($P=0.005$). The Weld soil series had a significant increase from August to November and then returned to the August levels (Table 4.26). The increase was likely due to the addition of Fe with the biosolids. With increasing time since addition of the biosolids, P was transformed into more stable forms such as Ca bound P which increased at the later sampling times. Based on the pH of the soil, Ca bound P would be more favorable than Fe and Al bound P (Lindsay, 1979). There was a significant interaction of month and soil series for

Ca bound P ($P=0.001$). There was also a significantly higher percentage of occluded P for Colby-Adena ($P=0.008$), but the reason for this difference is unknown. Both soils should have similar amounts of occluded P since they were in the same field, under the same management and have similar amounts of Fe.

The interaction of month and soil series was significant for labile organic P ($P=0.036$) (Table 4.27). The percent labile P was mostly constant but there was an increase for Colby-Adena in November. This was likely due the breakdown of organic matter. There was a significant interaction of month and soil series for humic acid associated P ($P=0.022$). There was an increase for both soil series starting in March due to the soil warming leading to increased biological activity. Humic acid associated P is a fraction that is related to biological activity, and it would be expected to increase when the conditions favor increased biological activity.

The changes in the microbial biomass percent P followed the expected trend that when there was increased microbial activity in the soil there would be a larger percent of the soil P in microbial biomass. Fulvic acid associated P followed a similar pattern. In November, there was a significant increase in the percent moderately labile P. This is a fraction that would not be expected to change due to the amount of time that it takes for labile P to transform into moderately labile P (Audette et al., 2016). There were also some variations in the percent of non-labile P over the year. There was a significant increase in March that then declined later in the season, the reason for this change is unknown and may be due to field variability. However, during all the sampling times there was not a significant change in the total soil P suggesting that there was not sampling variability that resulted in changes in concentration.

There were statistically higher percentages of fulvic, humic, and nonlabile organic P in the Weld soil series. The Colby-Adena showed higher levels of soluble, Al, and occluded P. The data suggests that

Weld favors higher percentages of organic P and Colby-Adena favors higher percentages of inorganic P. One explanation of the difference is that Weld has a higher yield potential for winter wheat (Soil Survey Staff, 2012) resulting in more biological activity in the soil increasing organic forms of soil P. Different soil series have different abilities to produce specific crops based on their chemical and physical properties and landscape position. Therefore, it would be expected that there would be more biological activity thus increasing the pools of organic P in the Weld soil.

Dryland Research Field:

Inorganic:

Overall, the amount of soluble P never accounted for more than 1.5% of the total soil P. Even though there was a significant ($P < 0.001$) interaction between month and site for soluble P (Table 4.28), there was not a consistent trend (Figure 4.29).

Aluminum bound inorganic P also showed a significant interaction between month and site ($P = 0.002$); however, there was not a consistent difference between the two sites with month (Figure 4.30). There was an increase in the percent of Al bound P at both sites over both years from January into March. This was most likely due to the increase in inorganic forms of P in the early spring as the soil began to warm and P became bound to Al.

Iron-bound P exhibited a significant site by month interaction ($P < 0.001$). For both locations, there was a trend for increasing Fe bound P from the summer into late fall (Figure 4.31). This may be due to the biological activity in the soil resulting in acidification of the root zone favoring the formation of Fe bound P. Even though the soil is basic and the soil P chemistry is expected to be dominated by Ca, it has been shown that even in calcareous soils Fe bound P still plays an important role (Ippolito et al., 2007). There was also a significant effect of application rate ($P = 0.036$). The control treatments had a

lower percentage of Fe bound P. The addition of biosolids added not only P to the soil but also a large amount of Fe (Ippolito et al., 2007).

Occluded P also had a significant month by site interaction ($P < 0.001$). However, the two sites had a very similar trend (Figure 4.32). The percentage started out low for the first six months and then began to increase slowly in January and continued to stay elevated over the initial concentration. This form of P is Fe encapsulated which takes longer to form than soluble or Fe and Al bound P.

There was a significant interaction between month and site for Ca bound P ($P < 0.001$), and the west plots had a higher percentage of Ca bound P than the east plots (Figure 4.33). Since the two sites have had very similar management, the difference is mostly caused by differences in parent material.

For all the organic P fractions, there was a significant interaction between site and month. Labile organic P made up a larger percentage of organic P in the east field in September (Figure 4.34). This may have been due to differences in the amount of plant growth. Even though there was a significant interaction between site and month for microbial biomass P, there was not a clear trend (Figure 4.35). Also, this fraction had a lot of variability in the data. The location of this study had many dry periods and low levels of productivity that would result in stressful environments for soil microorganisms.

Moderately labile P also had a fair amount of variability in the data (Figure 4.36). There was a lot of change that took place, and it would not be expected for this fraction to change since it is a more stable fraction. The variability was mostly due to sample variation and the variability of P in the field. Care was taken to sample in the same location each time; however, the distribution of P is not uniform across a field.

During the first season of sampling there was a steady trend at both locations for a decrease in the percentage of fluvic acid associated P (Figure 4.37). During the second year there was an increase at both locations in May and September. These two times coincide with changes to the biology of the soil where in May the soil temperature is rising and there is an increase in root activity. In the fall when the soils are starting to cool and the plants are beginning to die, there would be an increase in the amount of decomposing roots and plant matter. Humic acid associated P did not change (Figure 4.38). Similarly, moderately labile organic P, and nonlabile P did not change (Figure 4.39). There appeared to be an increase from July to January, but it was only an increase of approximately 6% of the total soil P.

Conclusion:

When the results between the three different studies are compared, there are many similarities but also some differences in how P was transformed. There were three main factors that drove the transformations that were seen: soil properties, water availability and climate, and application rates. When there was greater ability for the soil to absorb P due to the chemical properties (Ca concentration), there were lower concentrations of P in the soluble fraction. Also, for the soils that had a high amount of parent material Ca (Soil Survey Staff, 2012) there was a larger percentage of P that was found in the Ca fraction. However, even Fe and Al fractions played an important role in soils that had high amounts of Ca and a basic pH. At most of the locations there was a significant interaction observed between month and the Fe and Al fractions.

Water availability and climate impacted changes in the inorganic fractions by affecting redox conditions. There was a lower concentration of occluded P when soils were saturated and showed gleying. The environmental factors affected biological activity thus leading to changes in the organic P pools. When conditions were favorable for increased biological activity, such as during the growing season, the pools of organic P increased.

The application rate of biosolids also had an impact on the P transformations; however, the apparent effect may have been due to the variability of soil P in the field. At the dryland research location, six application rates were sampled, and soluble, Fe, and microbial biomass P were significantly affected by application rate. Addition of biosolids would make additional changes other than the addition of P. The biosolids that were applied had a large amount of Fe, thus increasing the potential for Fe bound P species to be present. The addition of organic matter and the total increase of nutrients will lead to better crop production and microbial activity, increasing both microbial biomass P and soluble P.

Overall, the results of the study showed important transformations that take place following the application of biosolids and that the changes vary with the time of the year. The increase in fractions such as labile and soluble P in the spring are important for providing P to growing plants. The increase in these fractions in the spring also suggest that soil samples taken in the fall to determine fertilizer application may underestimate the amount of plant available P during the growing season.

The effect of soil properties on the timing of transformation was also seen and highlights the potential need to look at P fertility differently depending on soil properties (texture, pH, and CaCO_3 concentration). Due to the variability of P forms during the year and plants only taking up one form of P, there is also a need to monitor plant P concentration in season through scouting and tissue sampling. This study also points out that following low application rates of biosolids there is more variation in the soil P and thus more samples are required to estimate the mean soil P in fertility assessment.

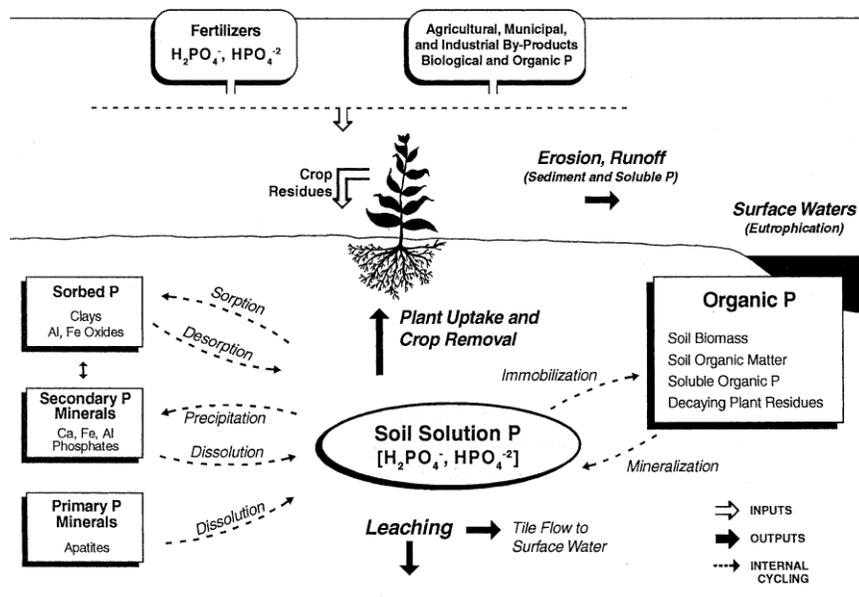


Figure 4.1. Conceptual model developed by Pierzynski (2005) to show the transformations and cycling of phosphorus.

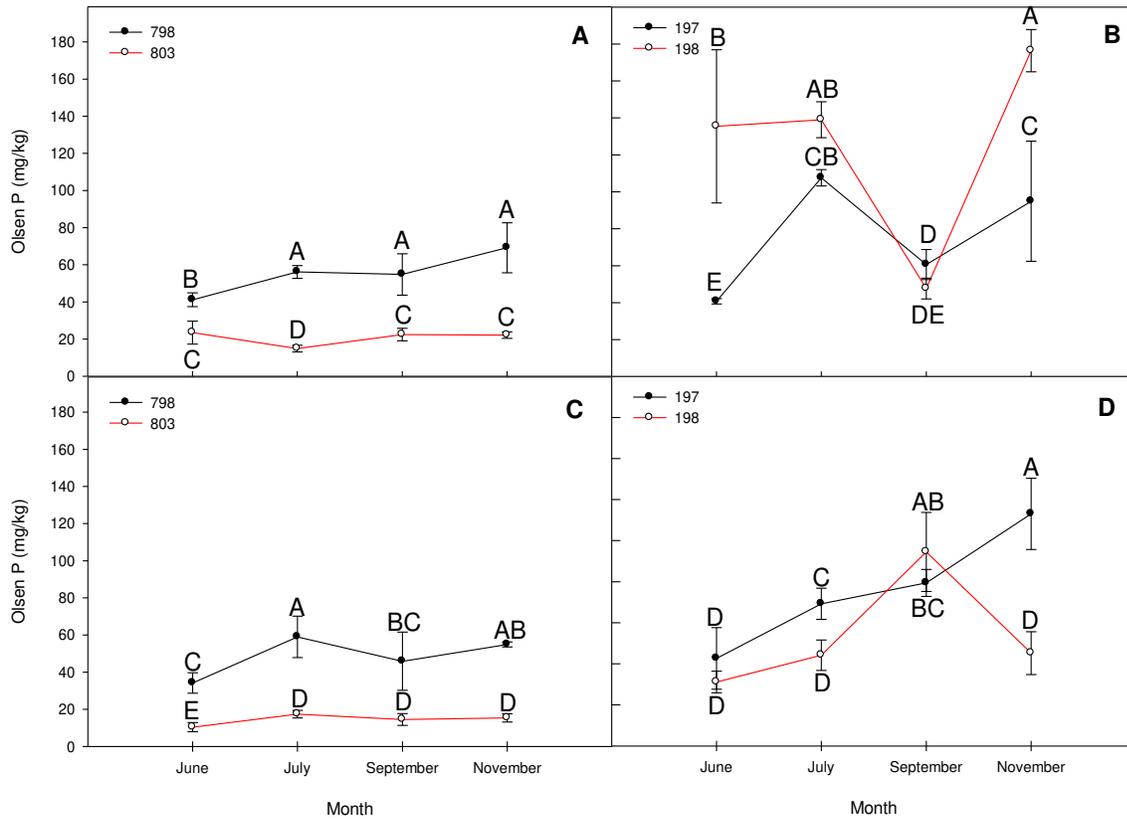


Figure 4.2. Plots of the interaction of month and field on Olsen P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation.

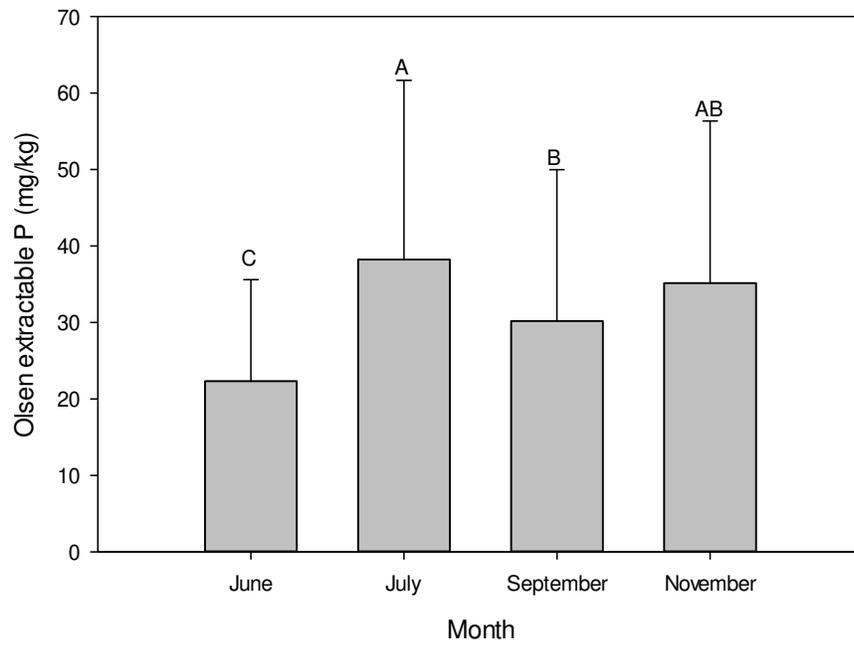


Figure 4.3 Olsen extractable P from Valent soil series averaged over field. The data from the multiple locations were averaged due to there only being a significant month effect. There was a lower concentration in June and then it began to rise and stayed relatively constant.

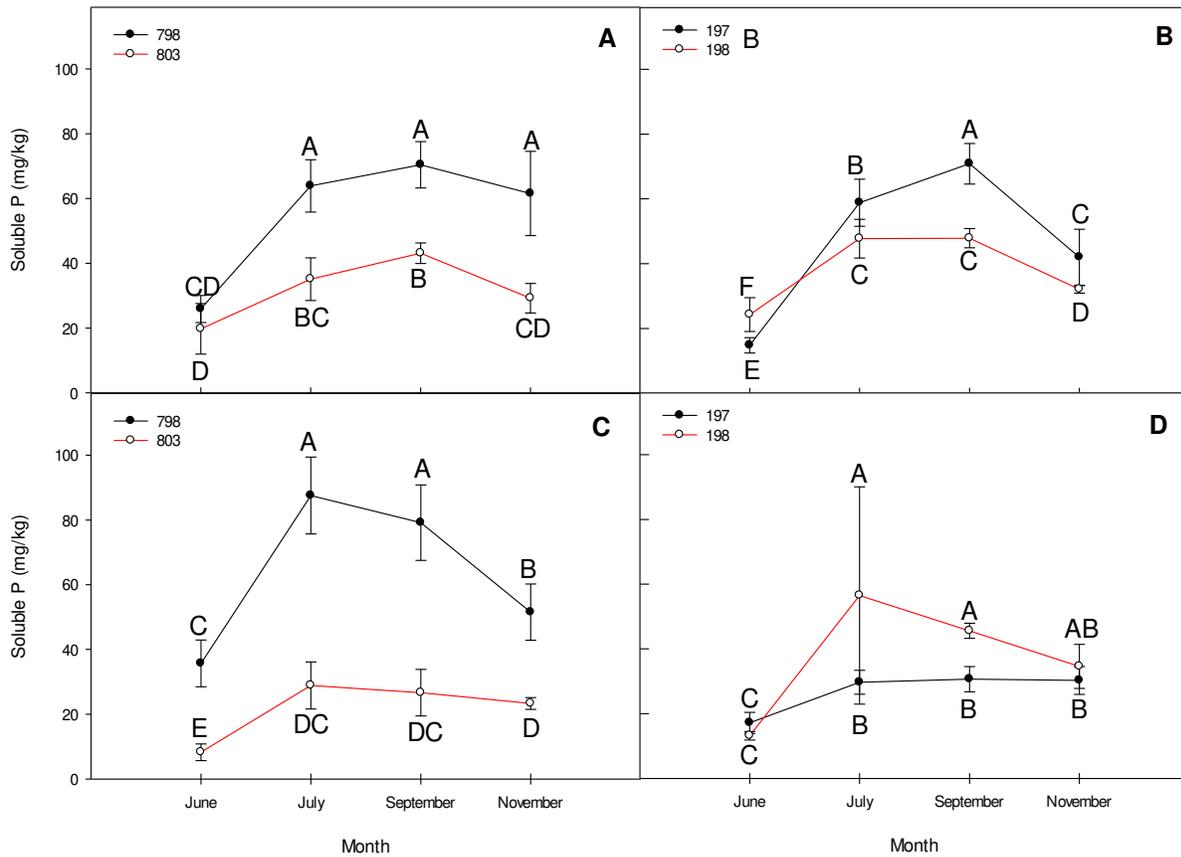


Figure 4.4 Plots of the interaction of month and field on soluble inorganic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

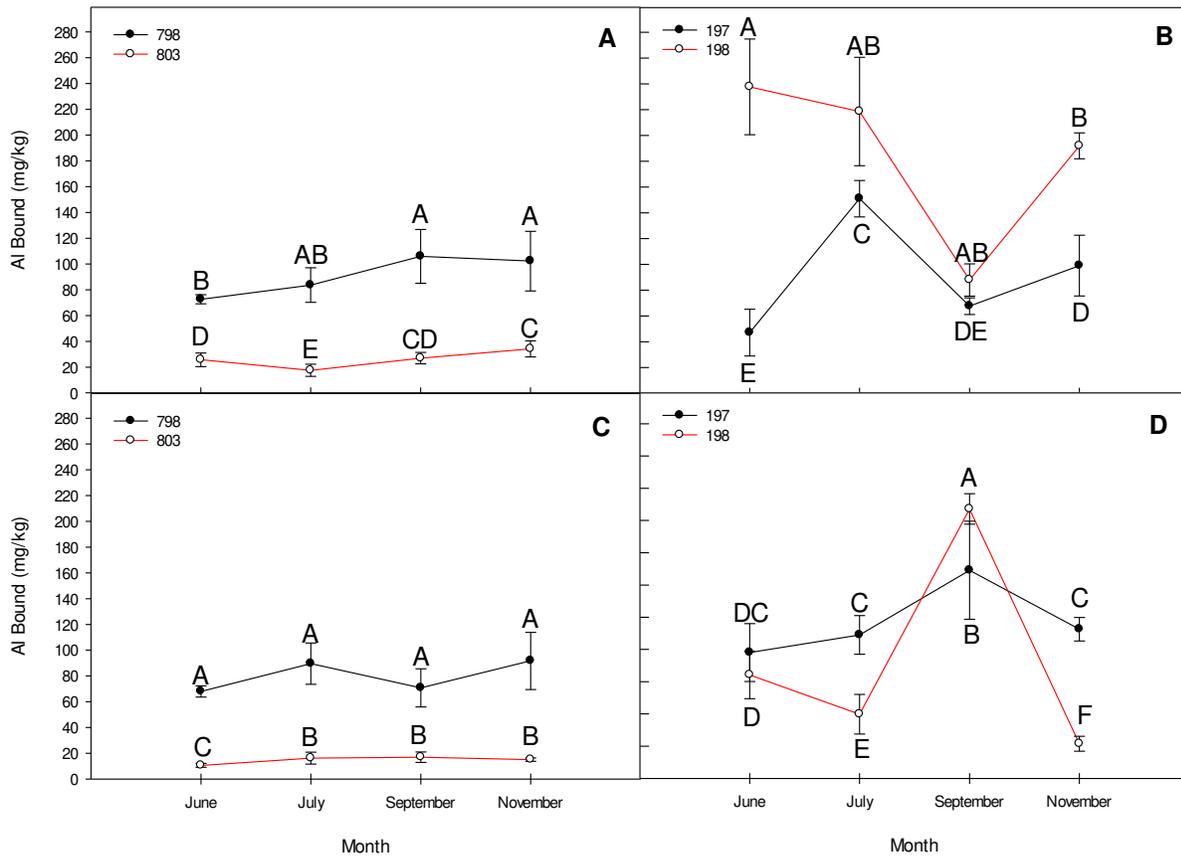


Figure 4.5 Plots of the interaction of month and field on Al bound inorganic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

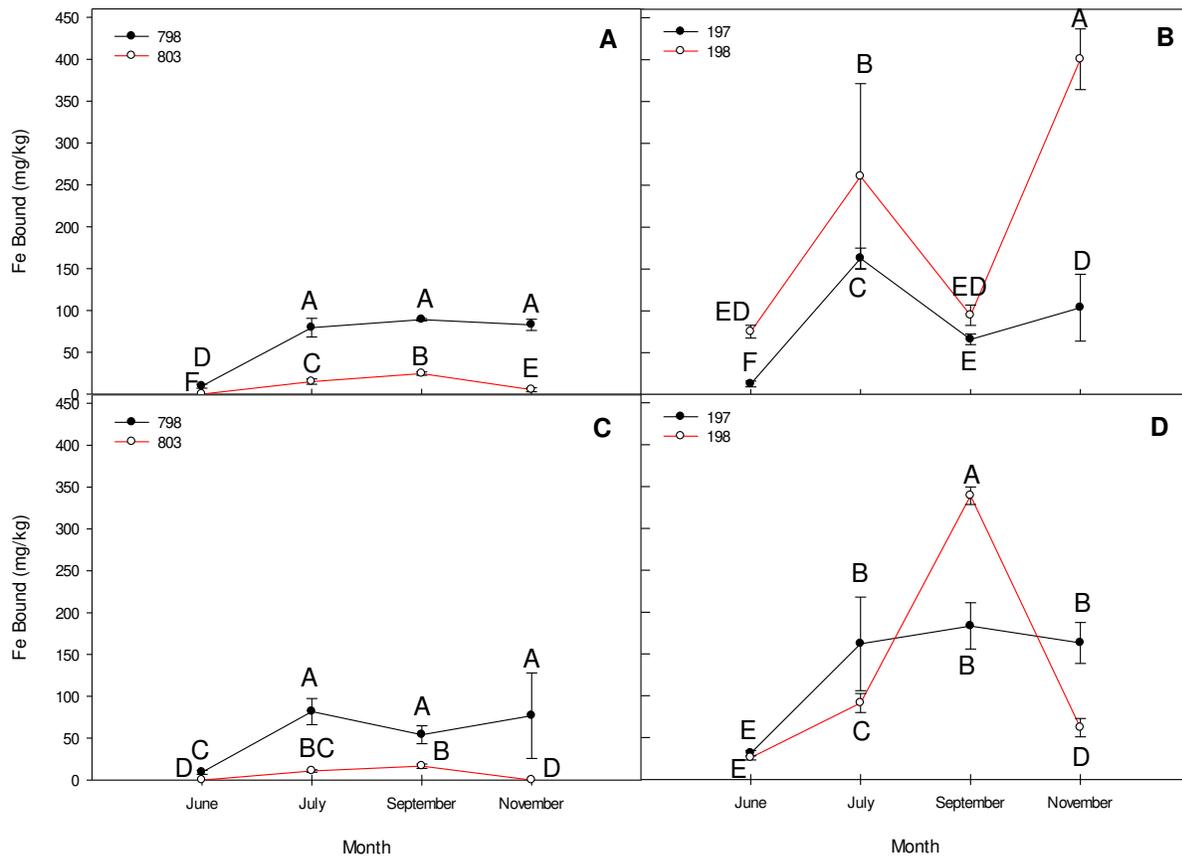


Figure 4.6 Plots of the interaction of month and field on Fe bound inorganic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

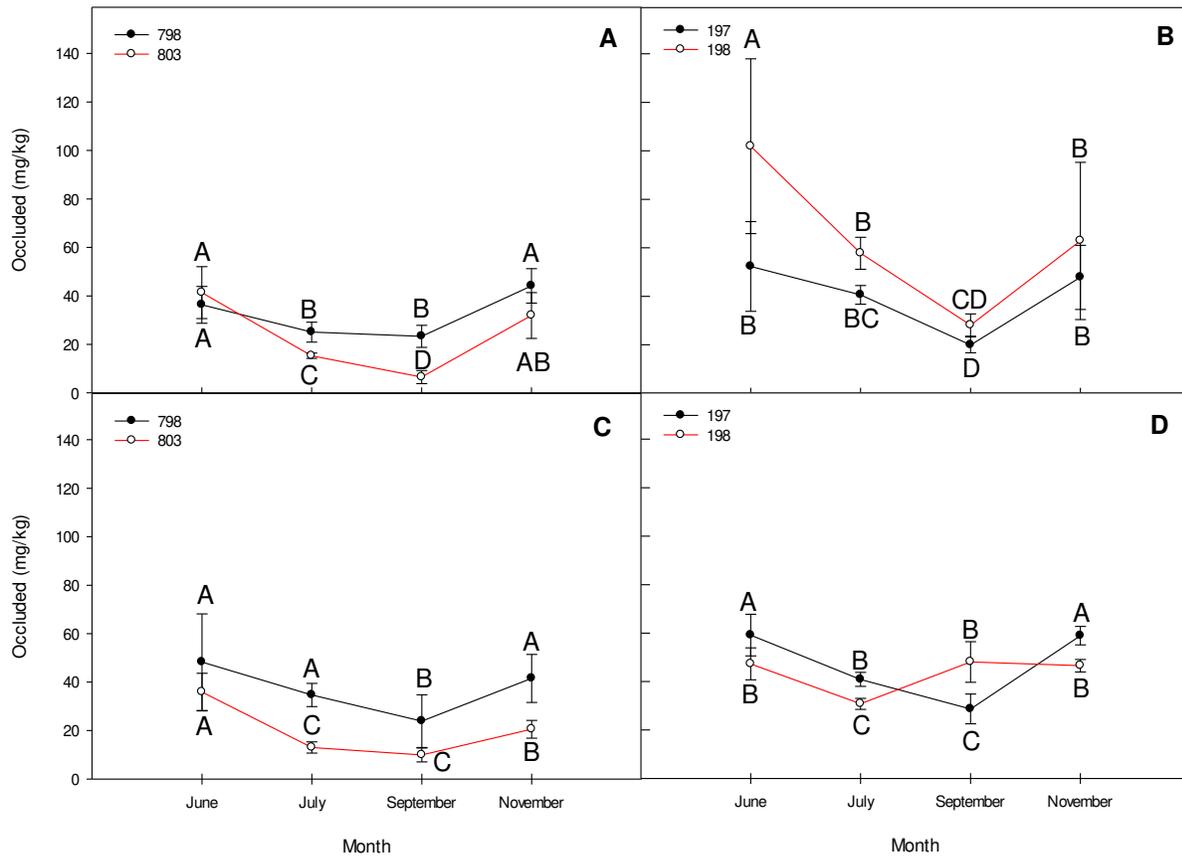


Figure 4.7 Plots of the interaction of month and field on occluded inorganic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

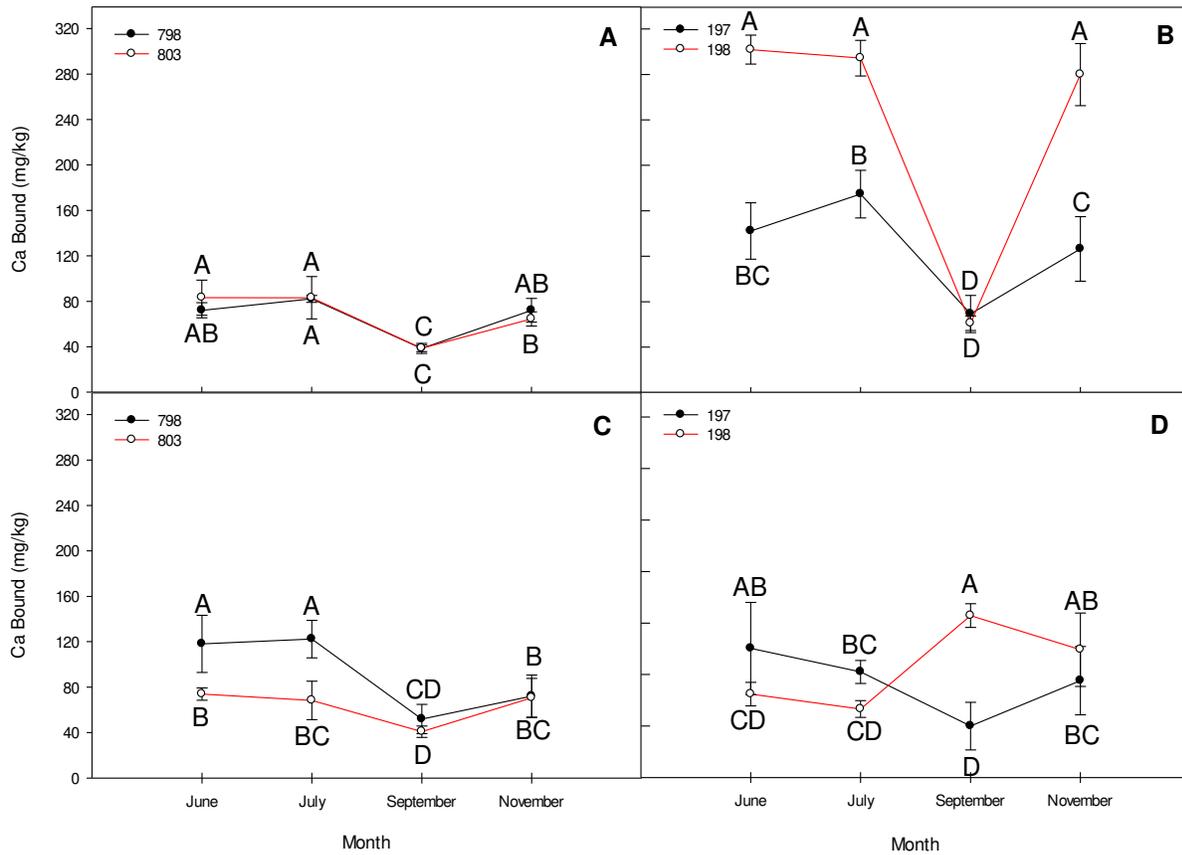


Figure 4.8 Plots of the interaction of month and field on Ca bound inorganic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

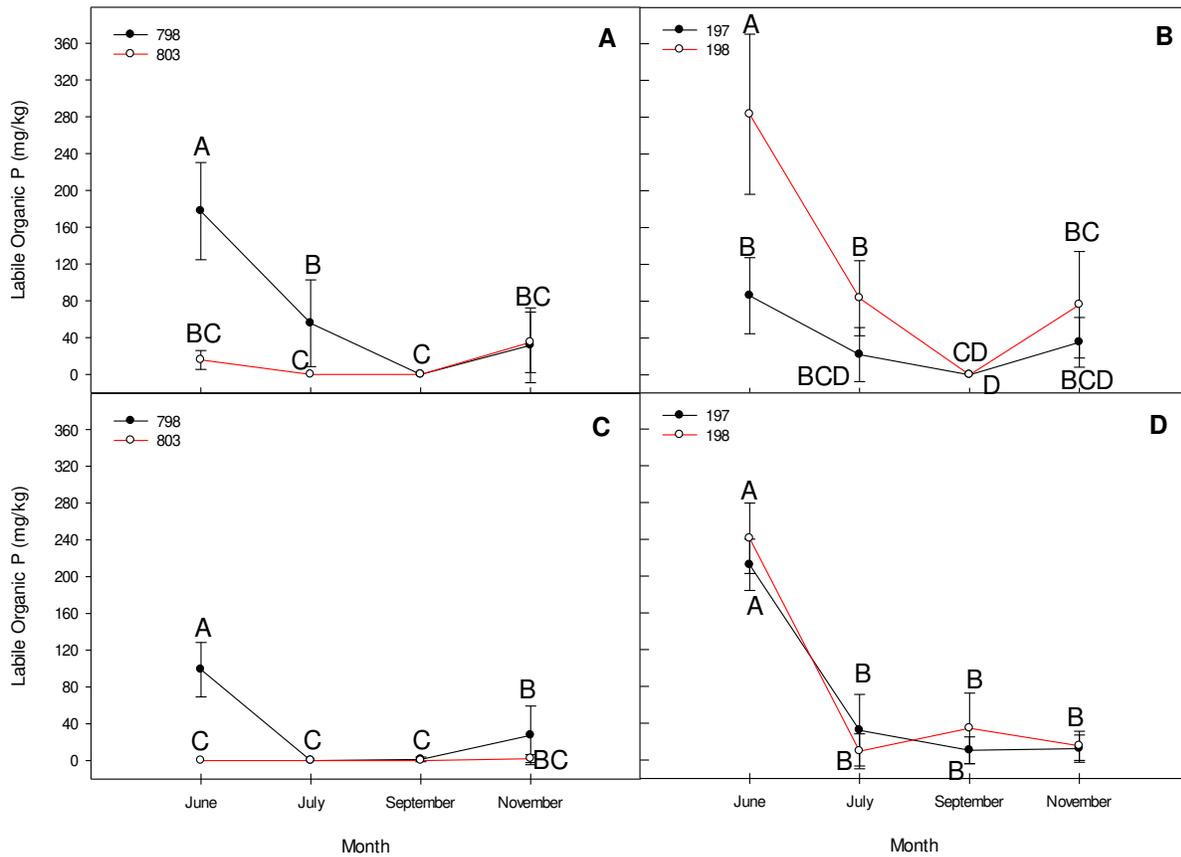


Figure 4.9 Plots of the interaction of month and field on labile organic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

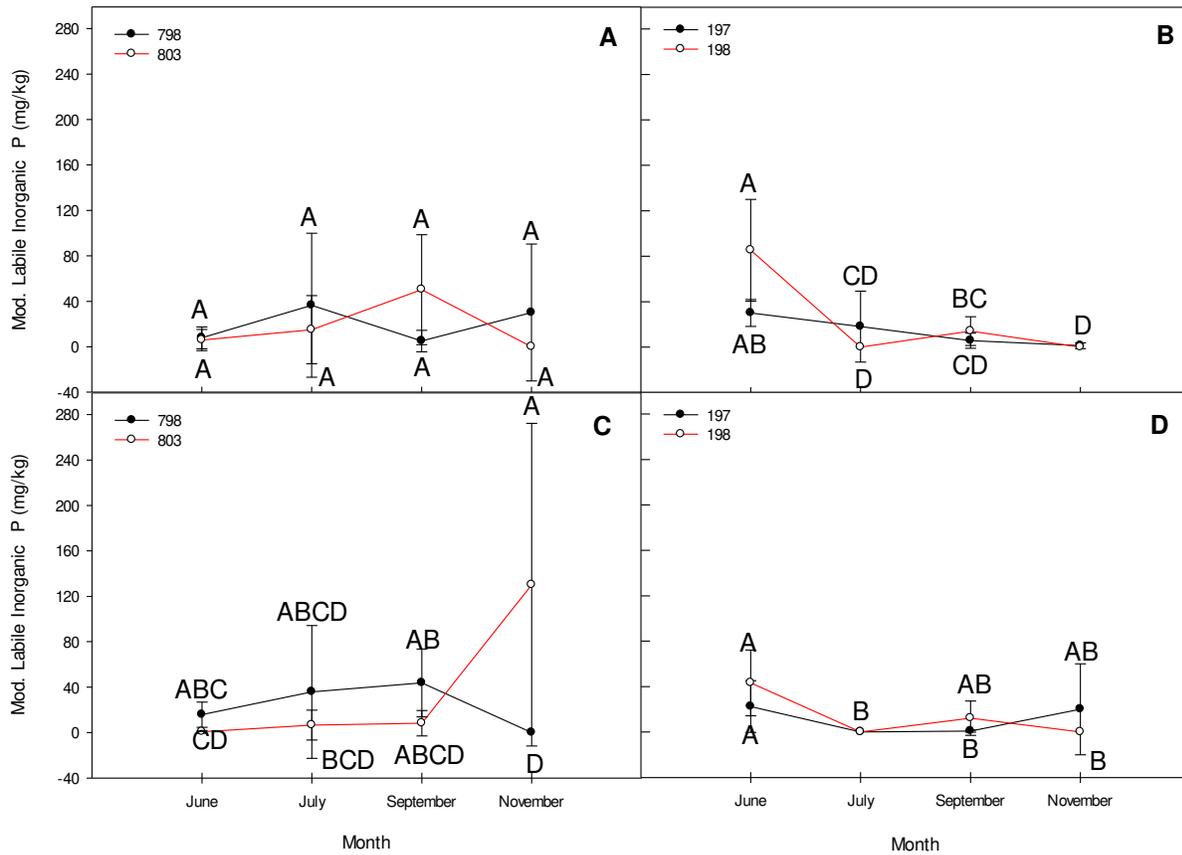


Figure 4.10 Plots of the interaction of month and field on moderately labile organic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

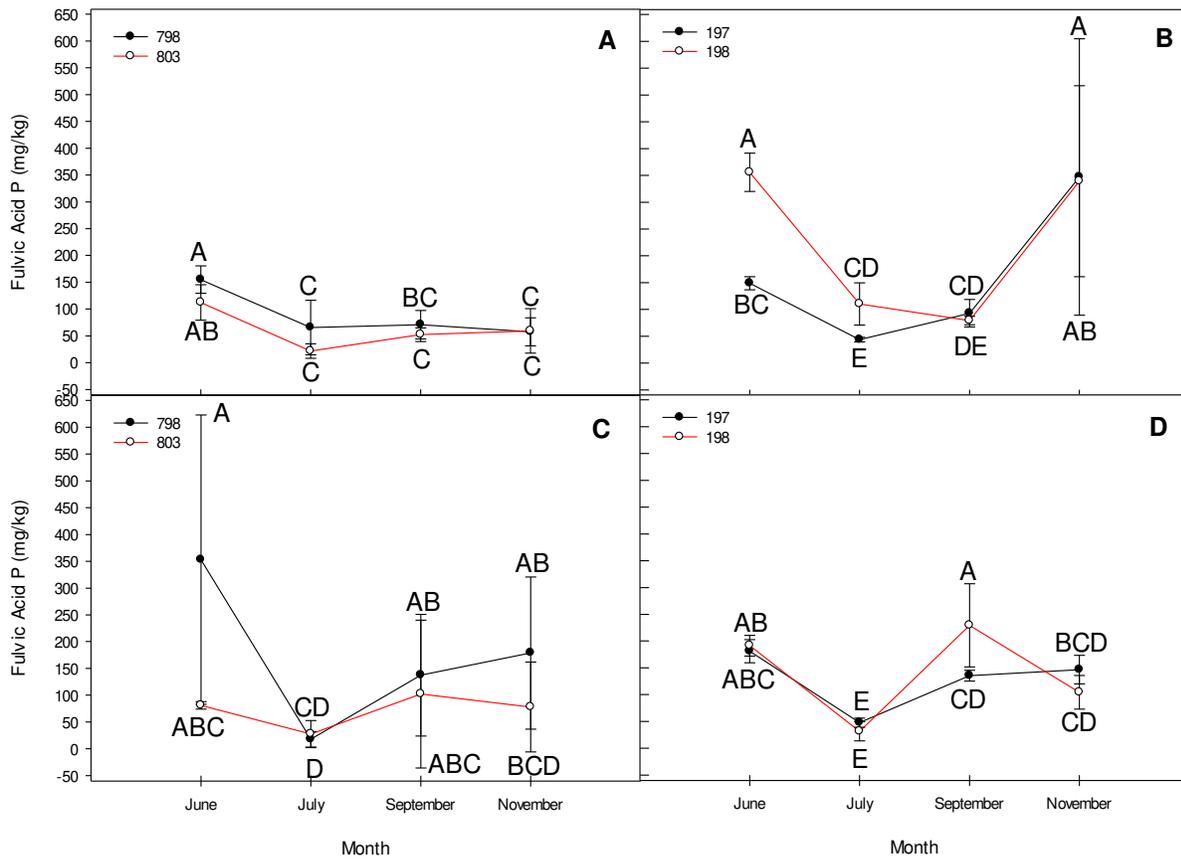


Figure 4.11 Plots of the interaction of month and field on fulvic acid organic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

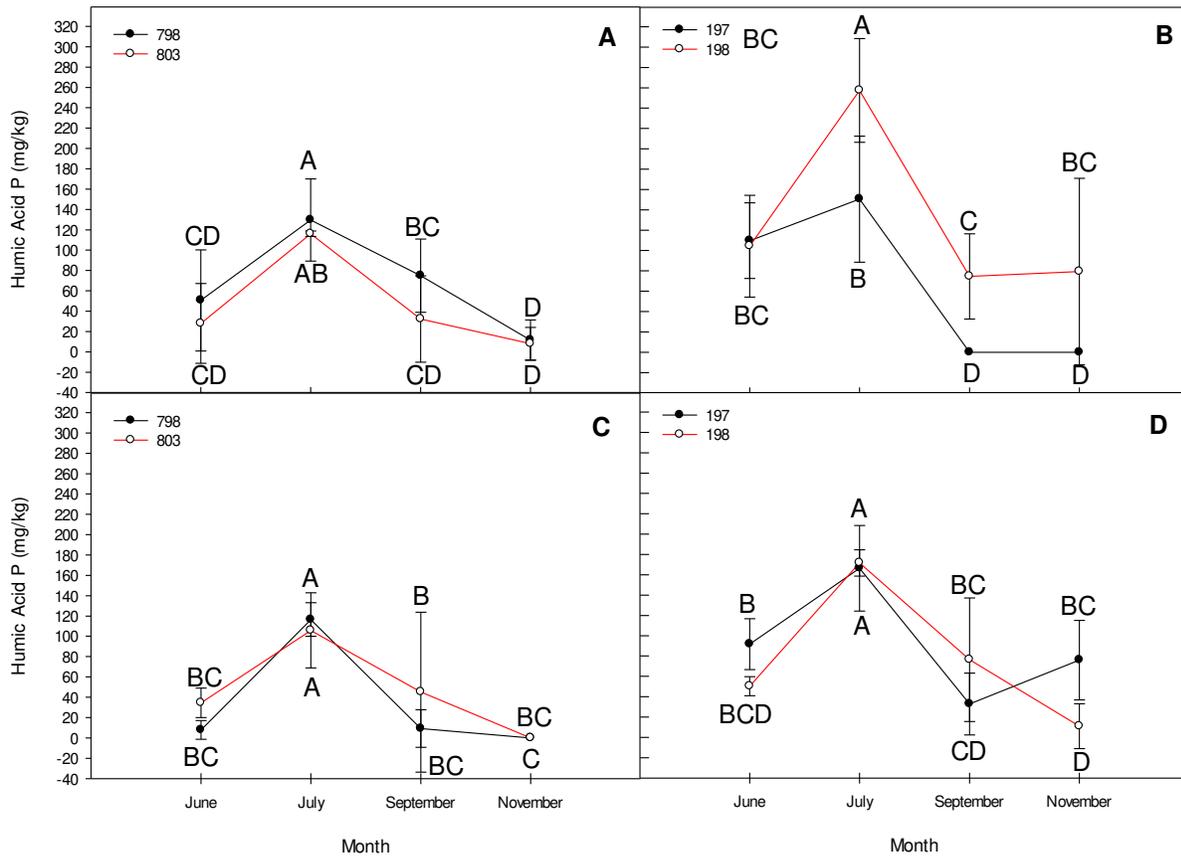


Figure 4.12 Plots of the interaction of month and field on humic acid organic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

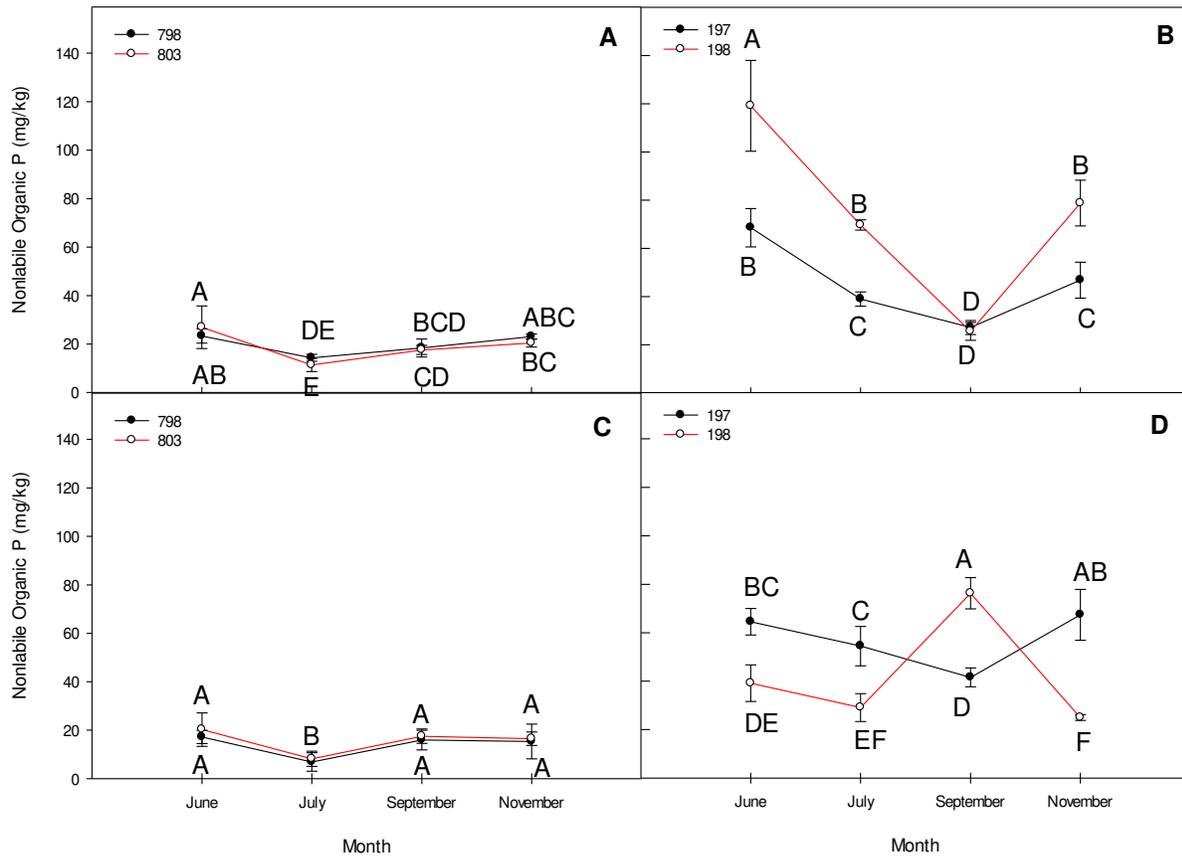


Figure 4.13 Plots of the interaction of month and field on non-labile organic P. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

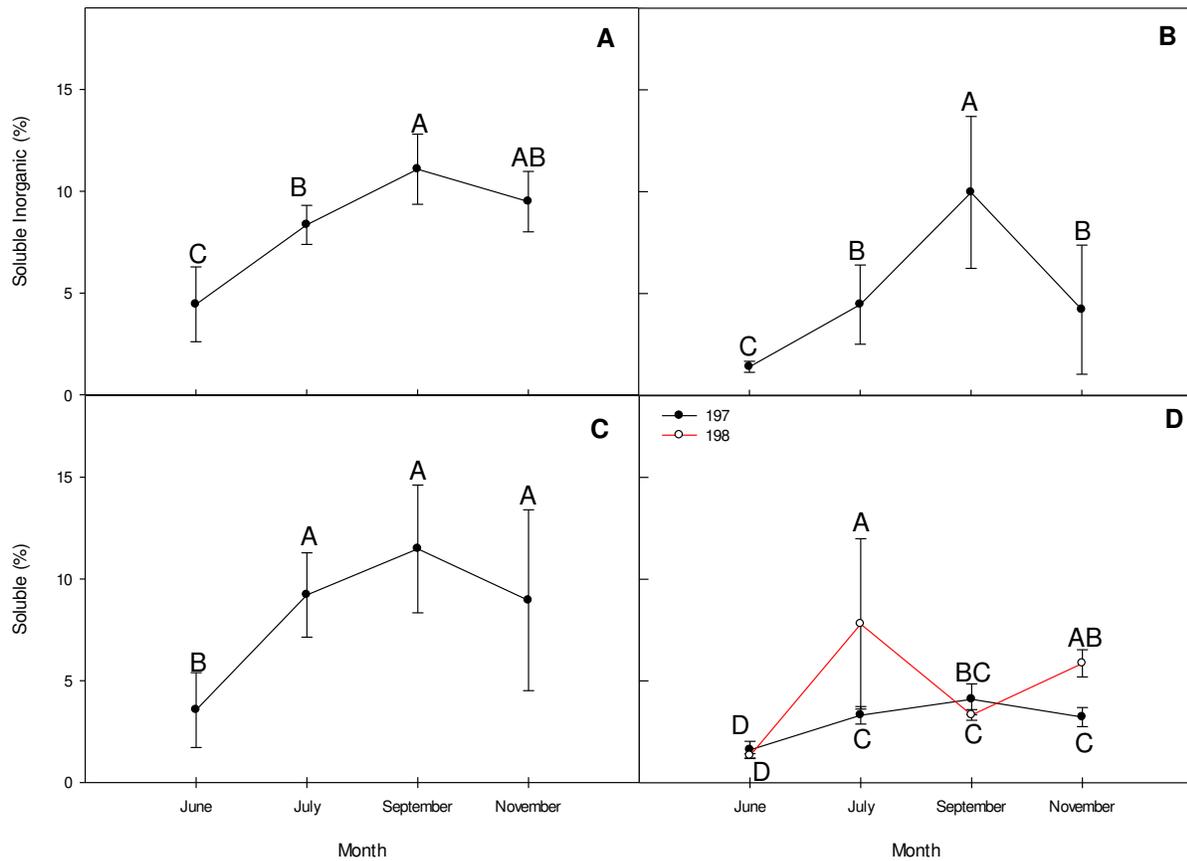


Figure 4.14 Plots of the percent of the total soil P that soluble inorganic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

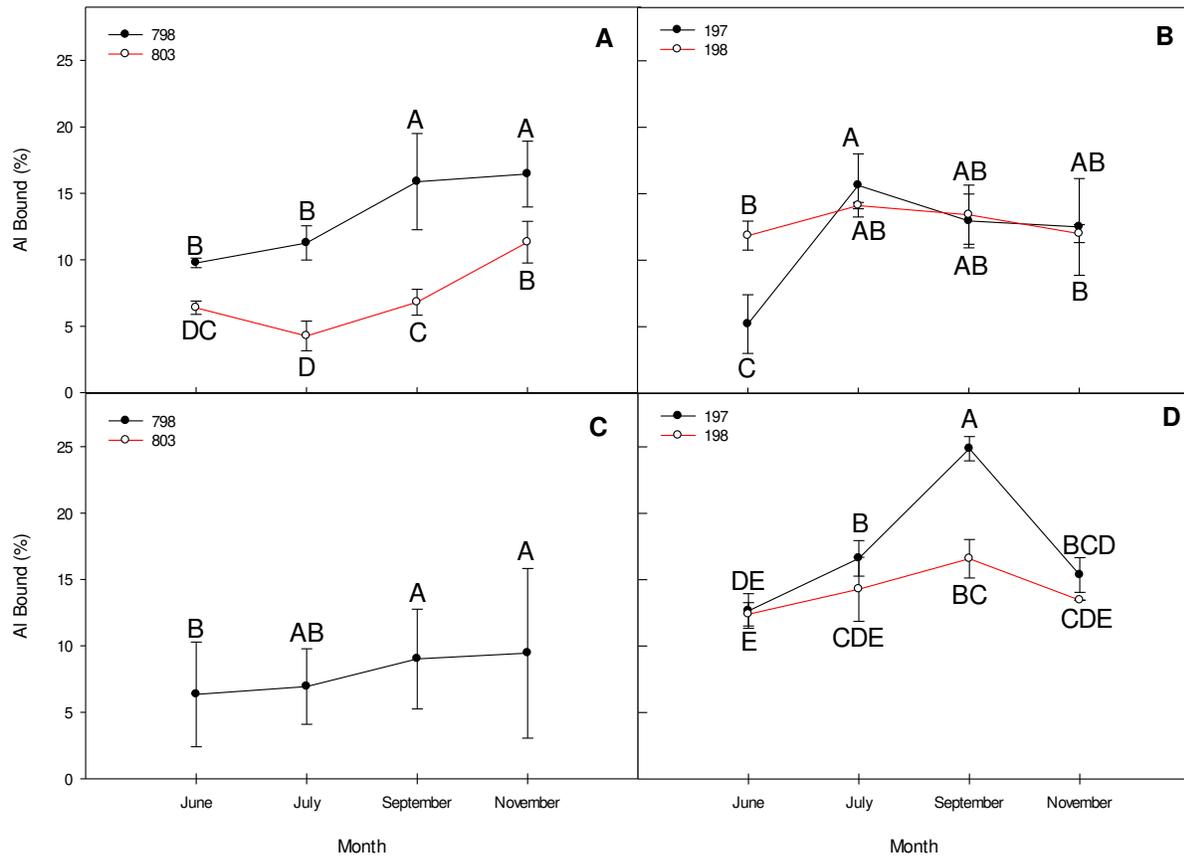


Figure 4.15 Plots of the percent of the total soil P that Al bound inorganic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

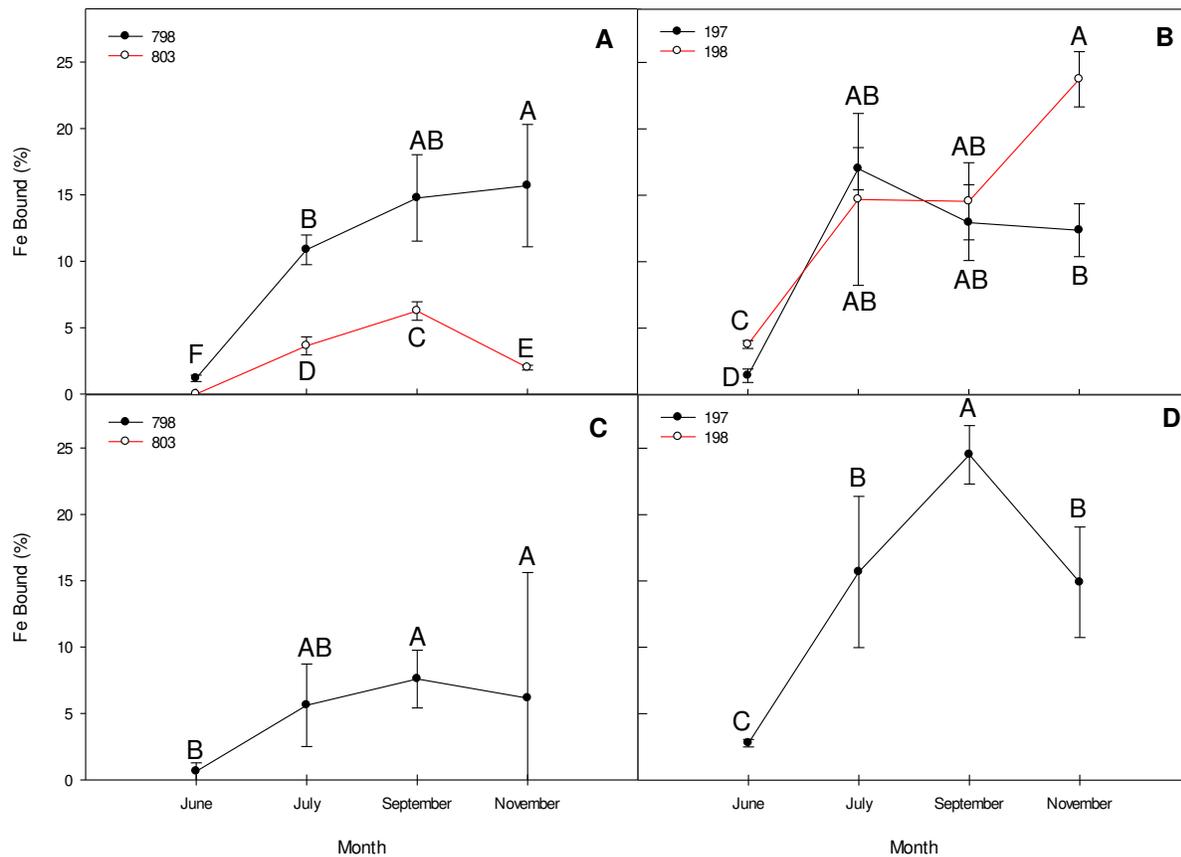


Figure 4.16 Plots of the percent of the total soil P that Fe bound inorganic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

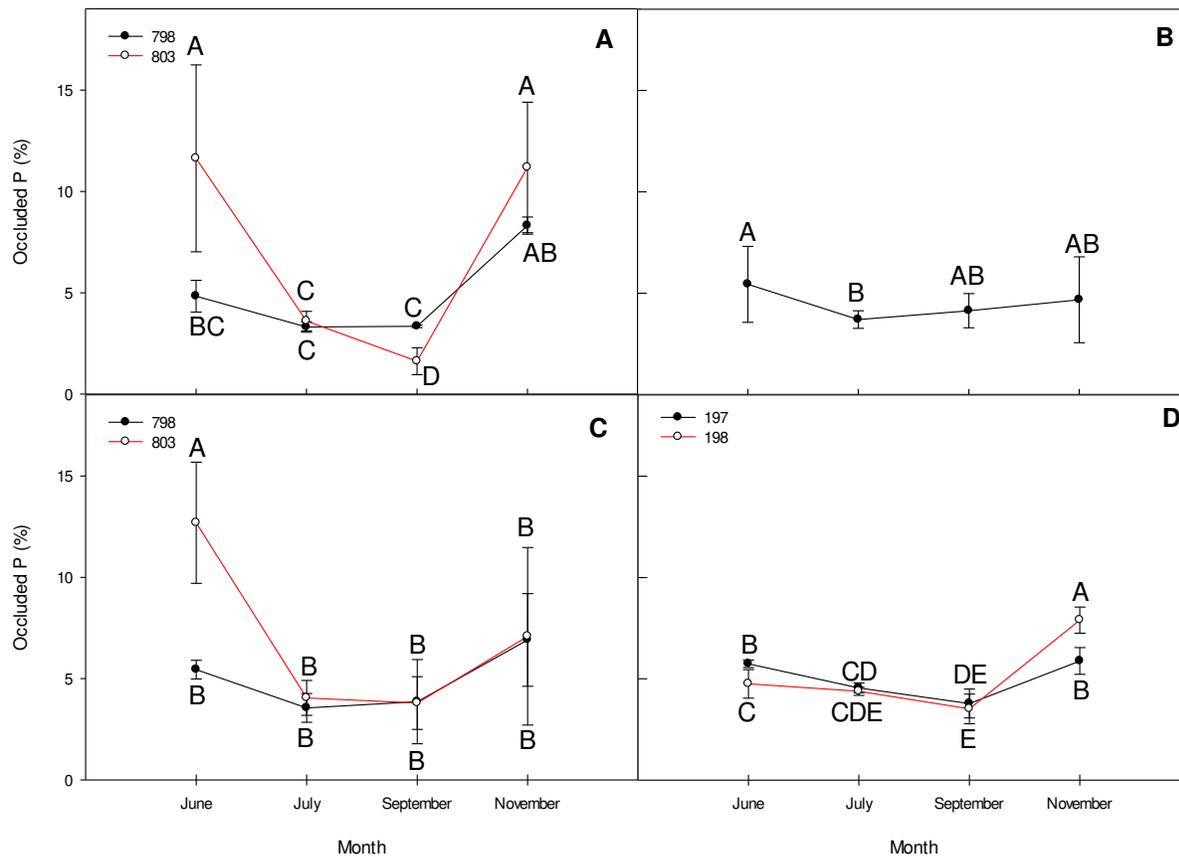


Figure 4.17 Plots of the percent of the total soil P that occluded inorganic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

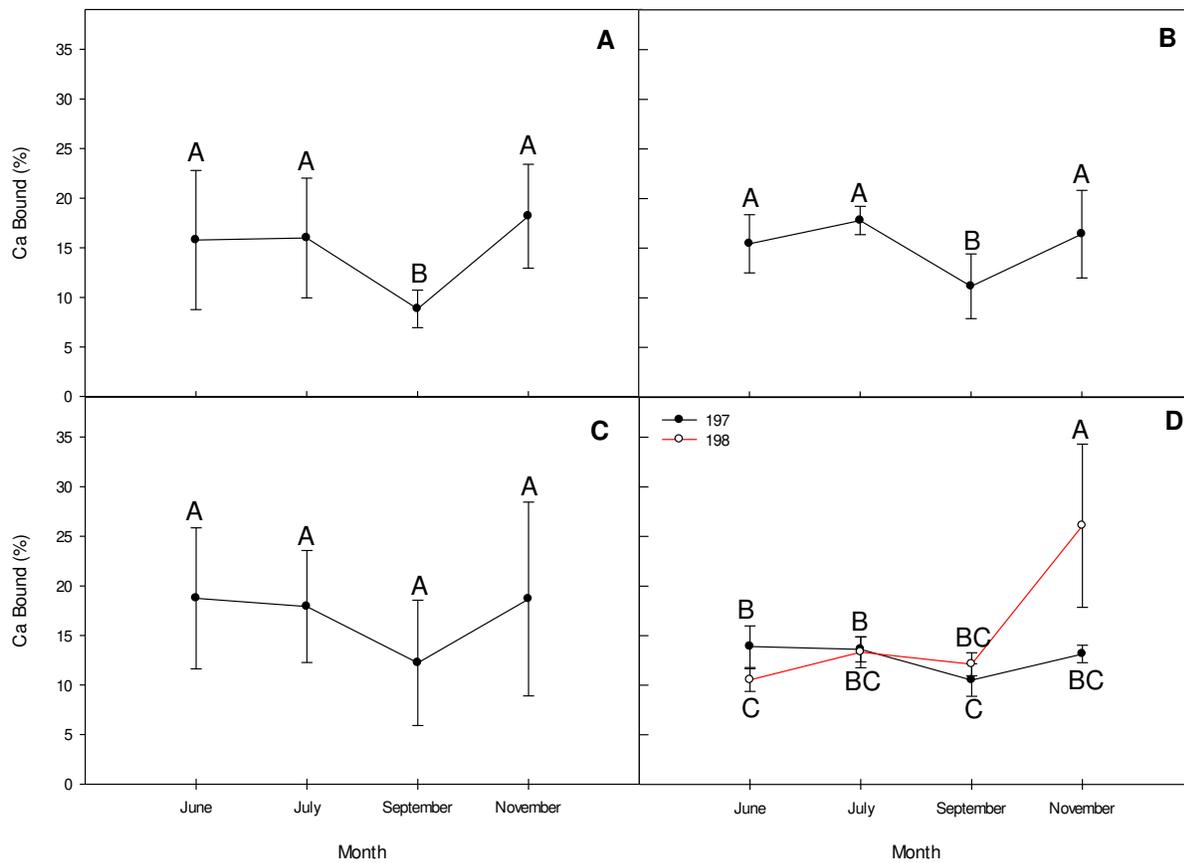


Figure 4.18 Plots of the percent of the total soil P that Ca bound inorganic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

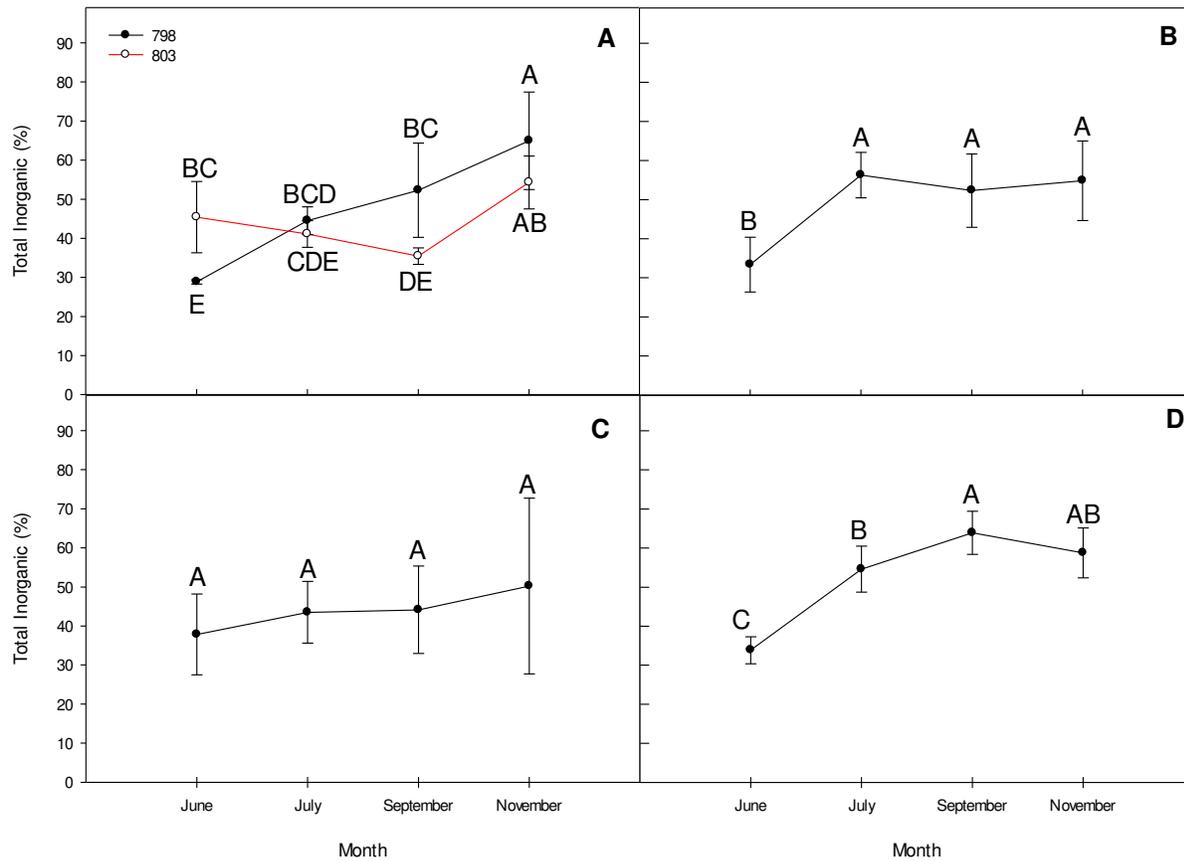


Figure 4.19 Plots of the percent of the total soil P that total inorganic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

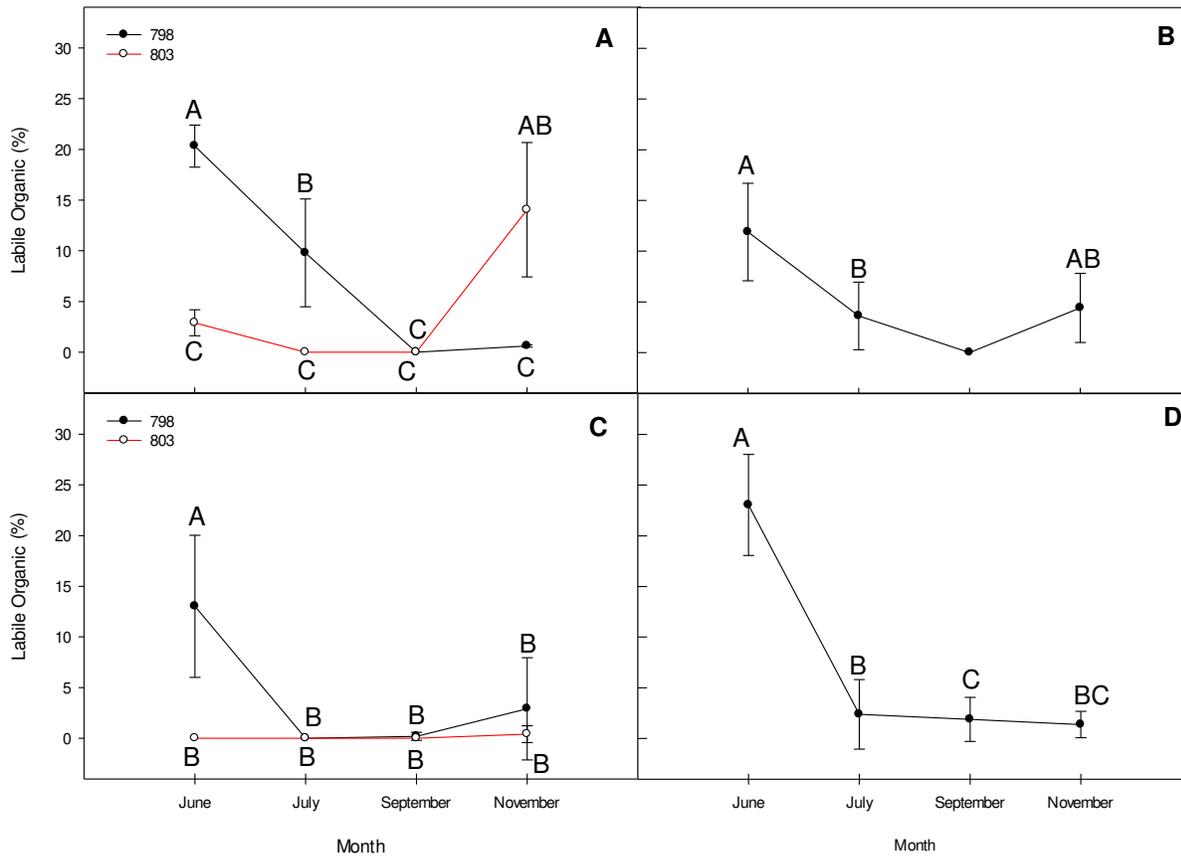


Figure 4.20 Plots of the percent of the total soil P that labile organic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

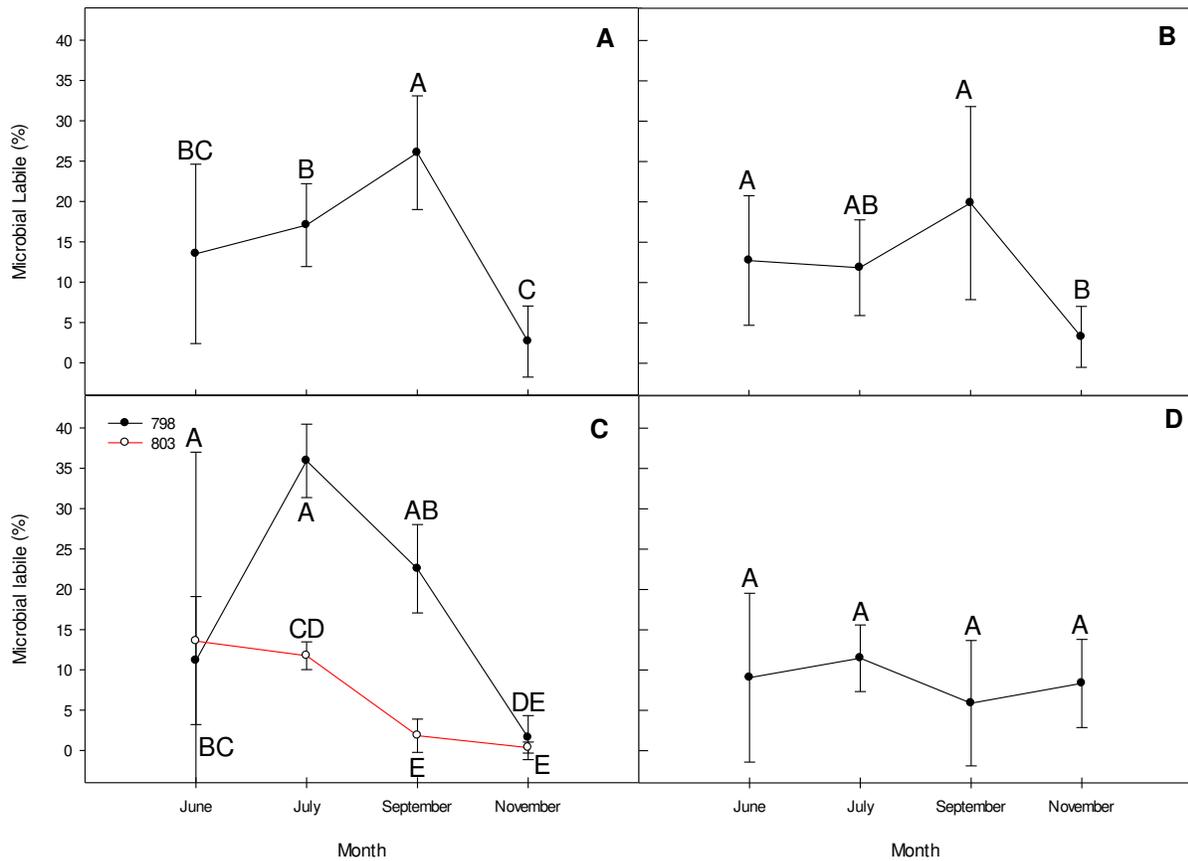


Figure 4.21 Plots of the percent of the total soil P that microbial biomass P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

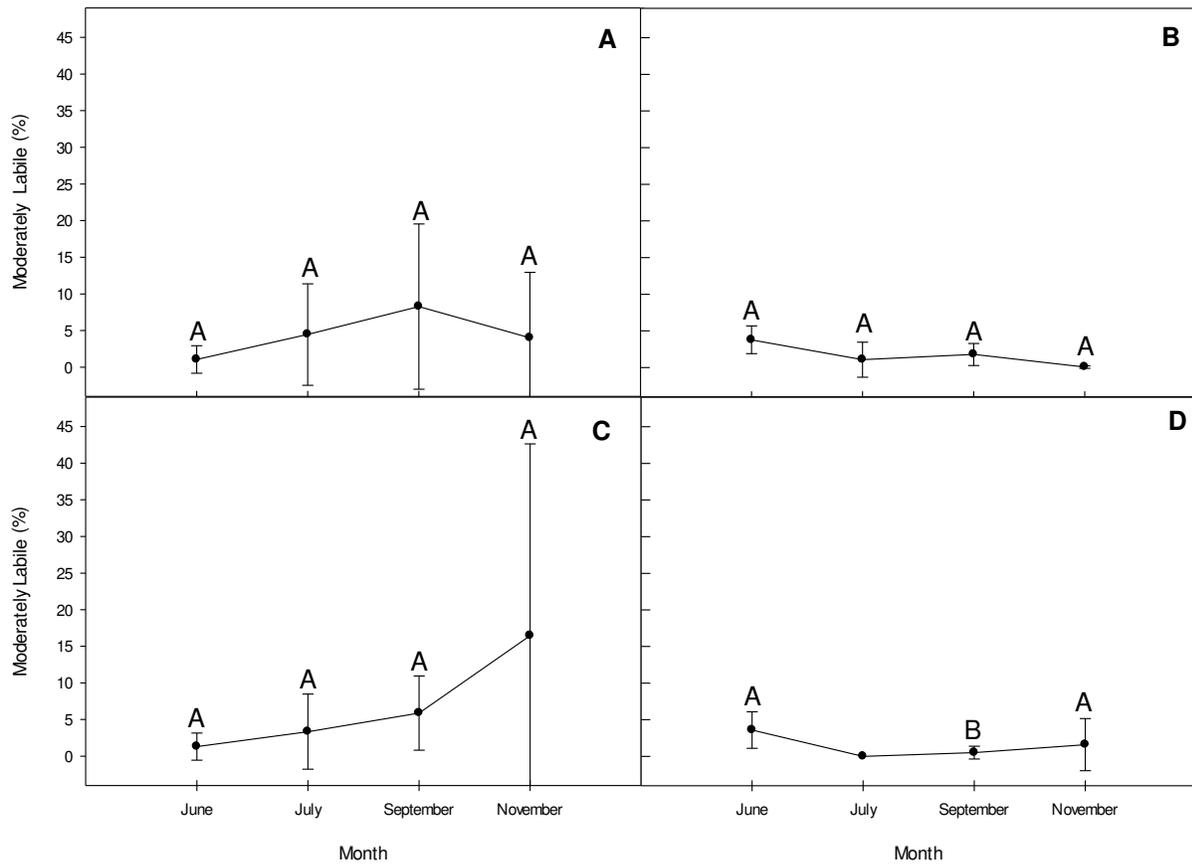


Figure 4.22 Plots of the percent of the total soil P that moderately labile organic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

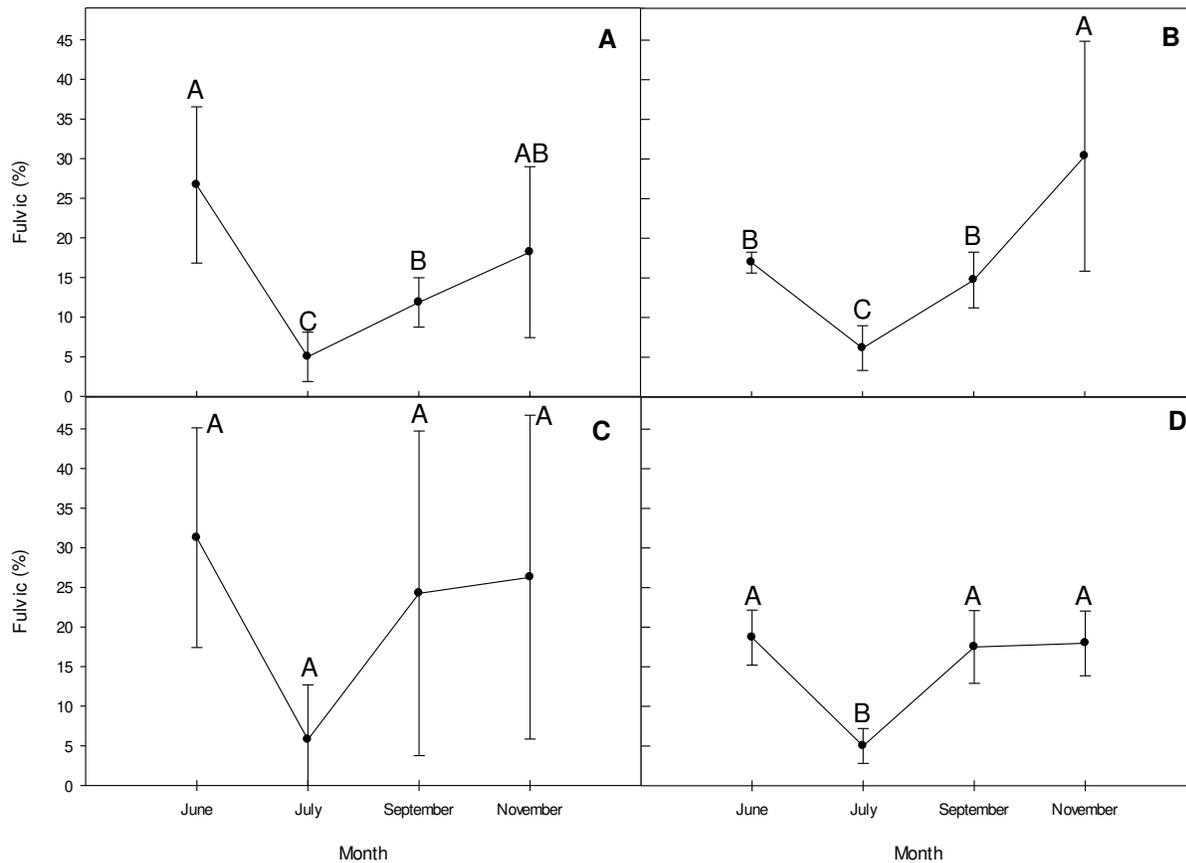


Figure 4.23 Plots of the percent of the total soil P that fulvic acid organic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

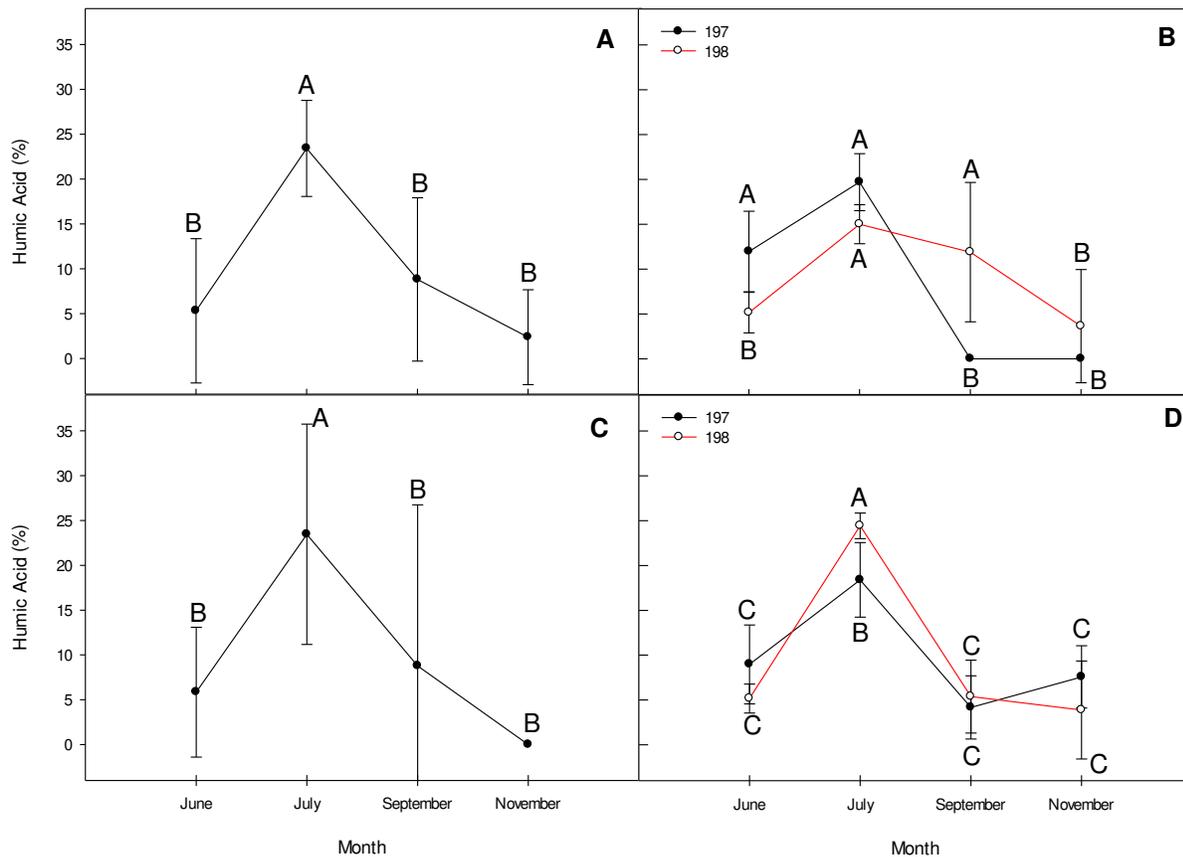


Figure 4.24 Plots of the percent of the total soil P that humic acid organic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

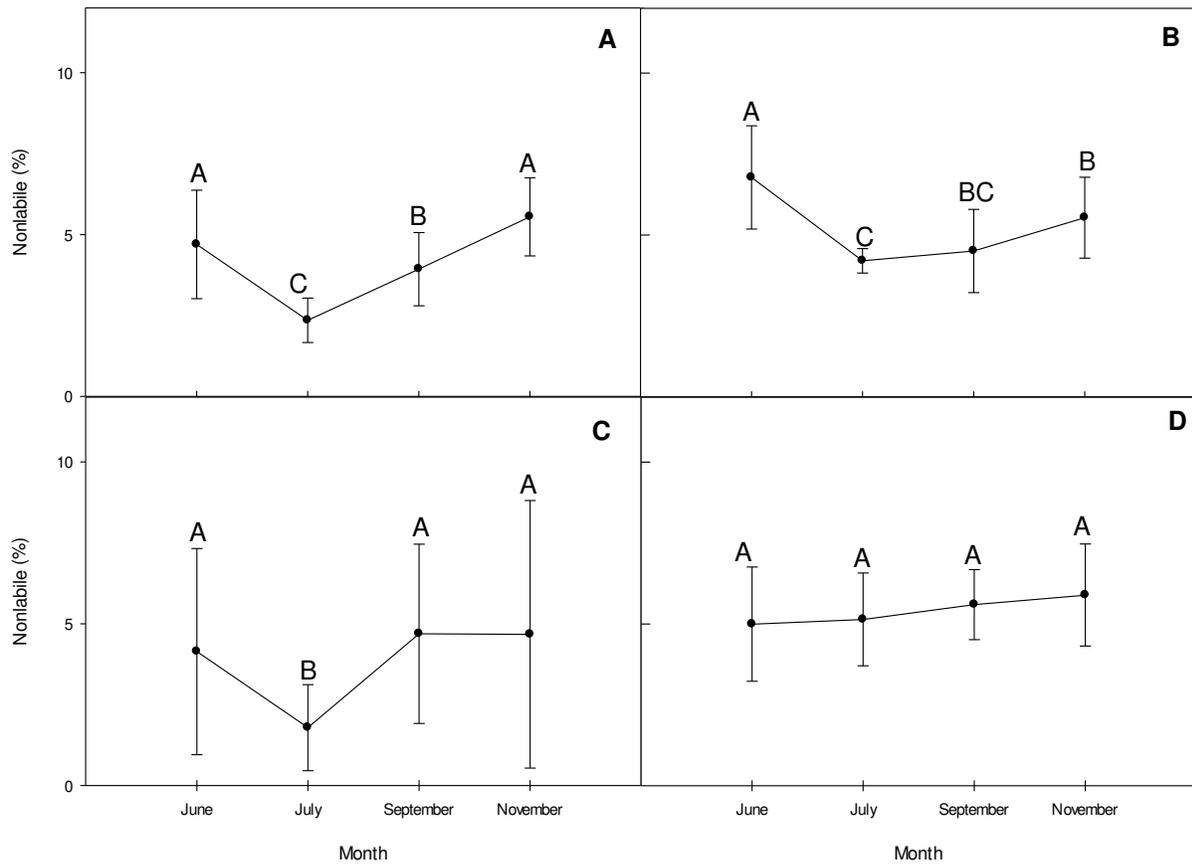


Figure 4.25 Plots of the percent of the total soil P that nonlabile organic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

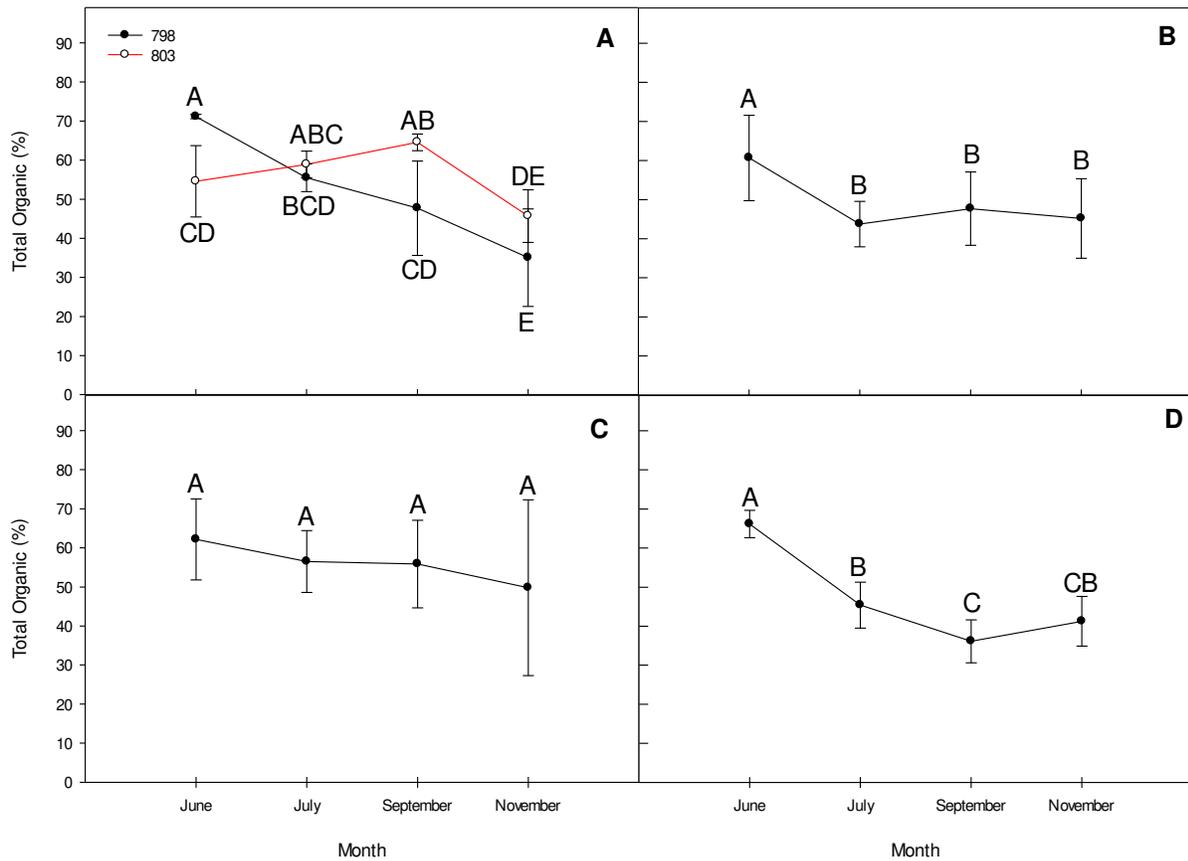


Figure 4.26 Plots of the percent of the total soil P that total organic P contributed. Each of the soils is shown in a separate window (A – Bijou, B – Heldt, C – Valent, and D – Nunn). Means that have a different letter for the same soil are statistically different at an alpha value of 0.05. Error bars represent one standard deviation of the mean. Statistical comparisons were not made between soils. The data shown is for the untransformed data but the statistics were completed using a log transformation when needed.

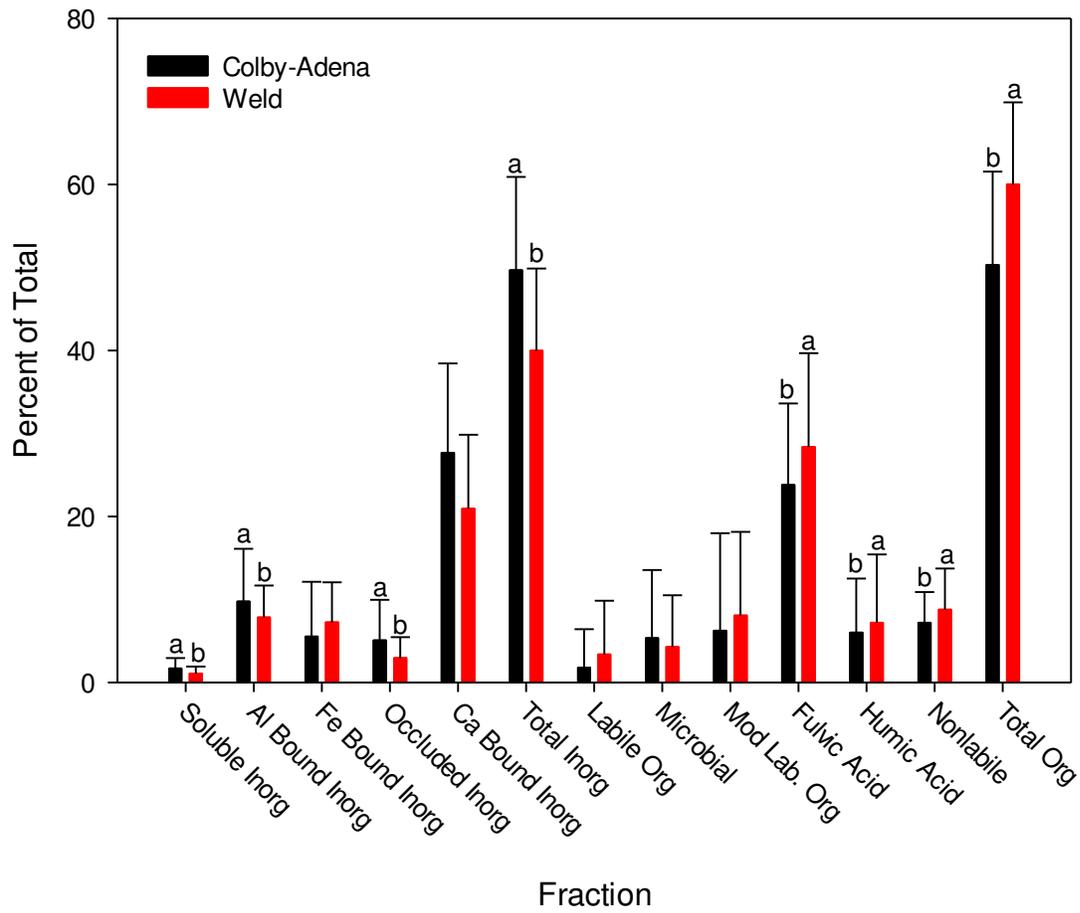


Figure 4.27. Effect of soil series on percent P from dryland winter wheat. Soil samples were collected over the growing season and the data was averaged over sampling time.

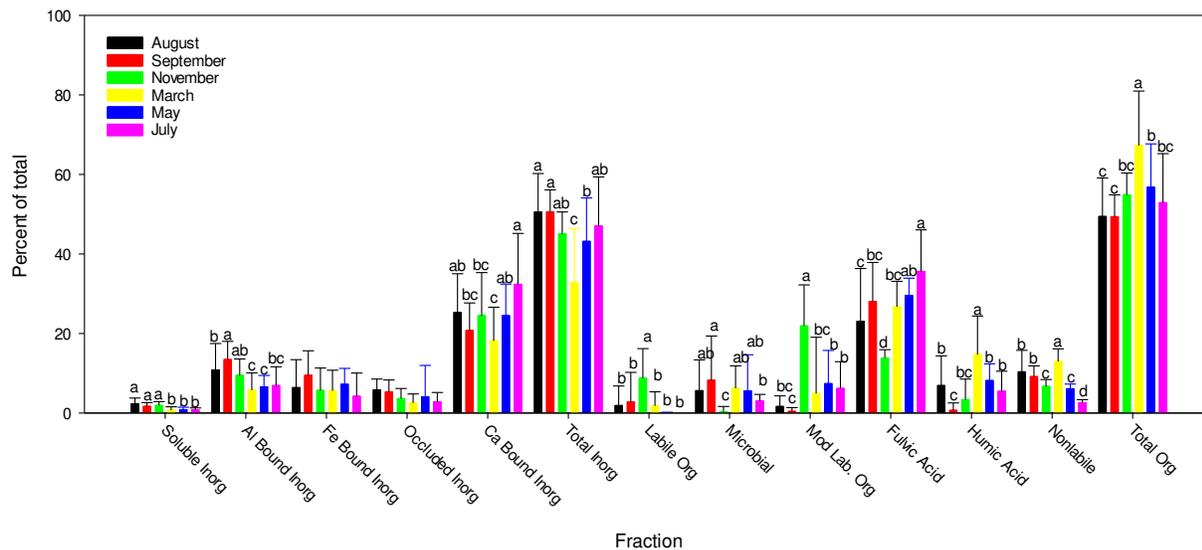


Figure 4.28. Effect of month on percent P from dryland winter wheat. Soil samples were collected over the growing season and the data was averaged over soil series.

Soluble

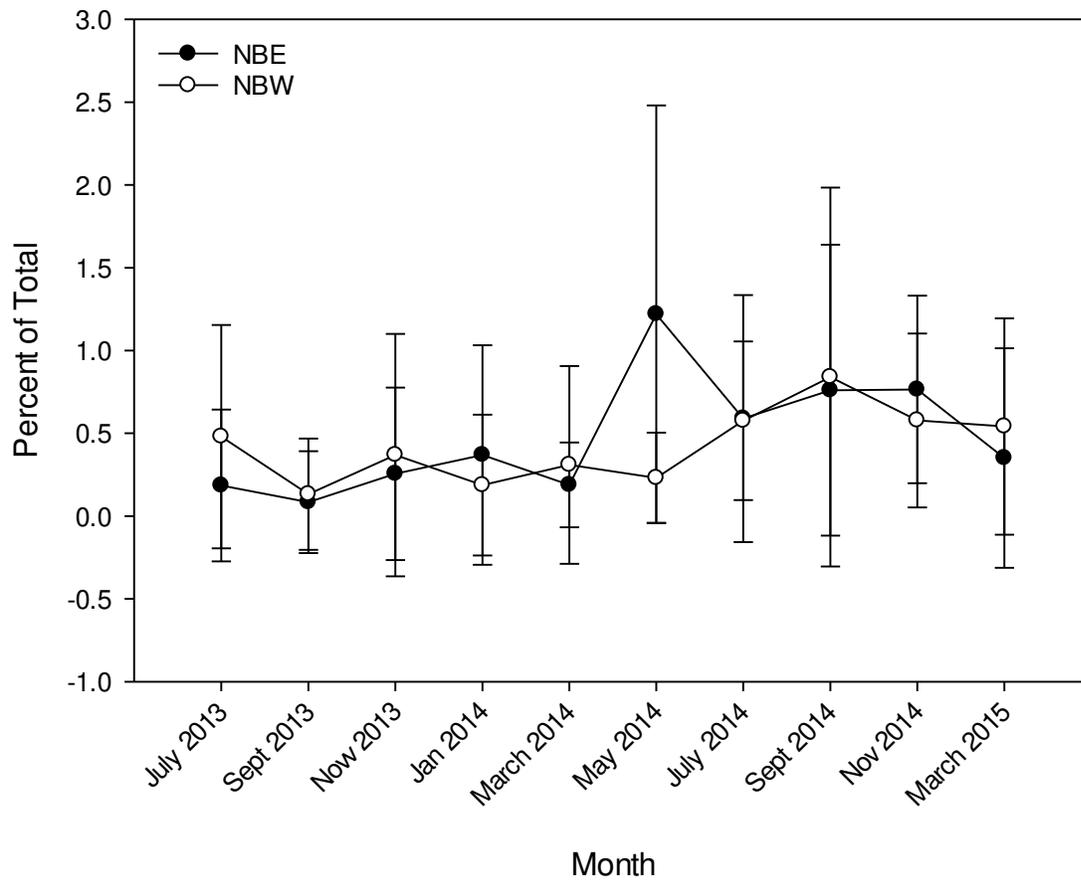


Figure 4.29. Soluble inorganic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Al Bound

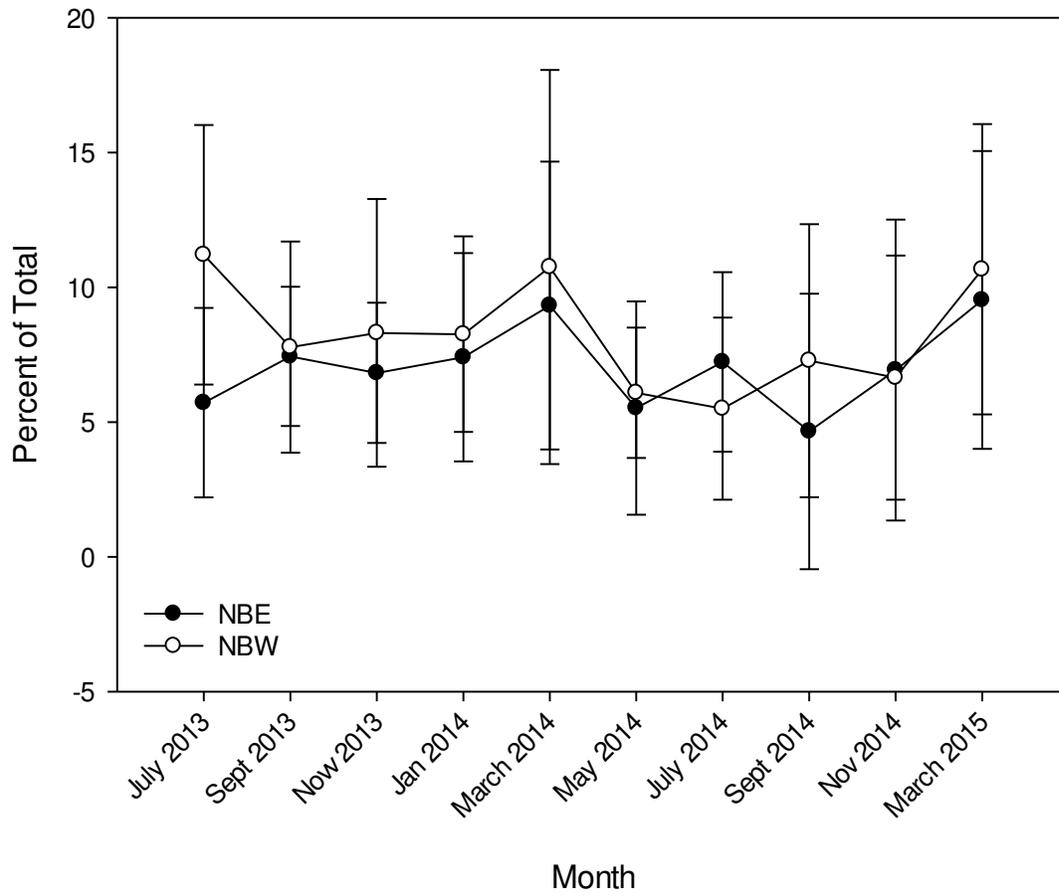


Figure 4.30 Aluminum bound inorganic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Fe Bound

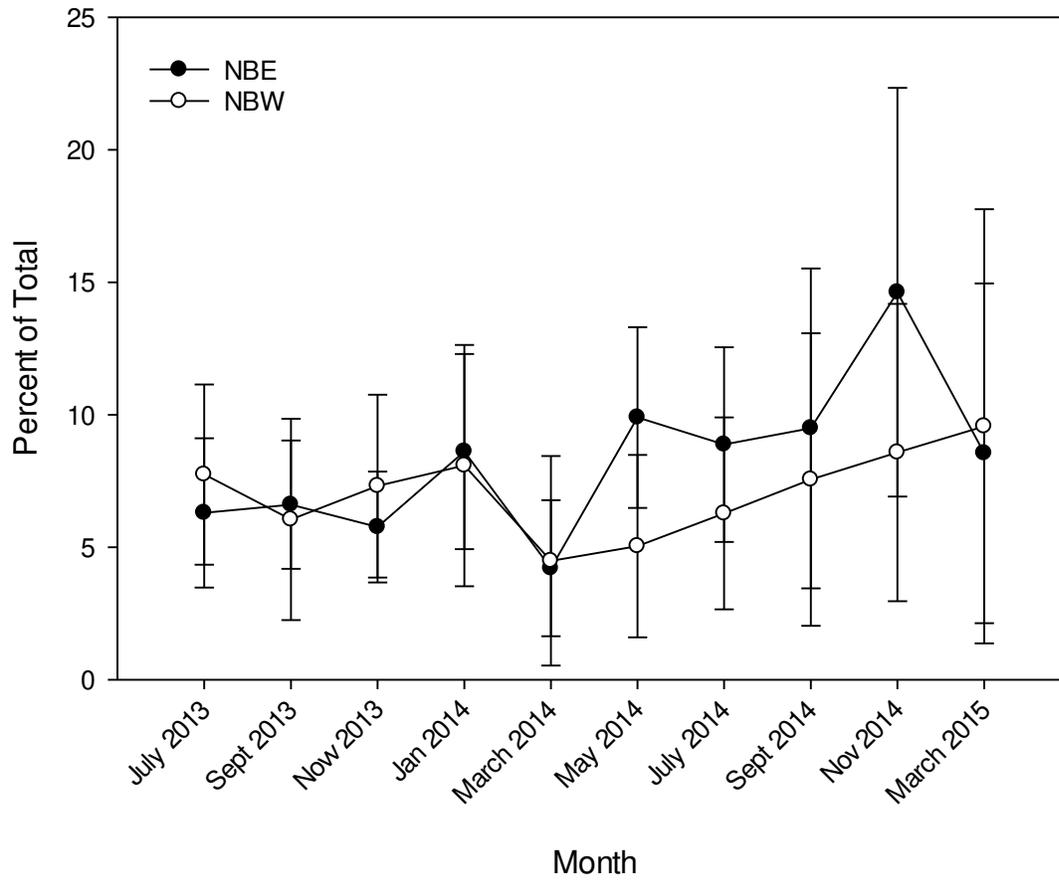


Figure 4.31. Iron bound inorganic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Occluded

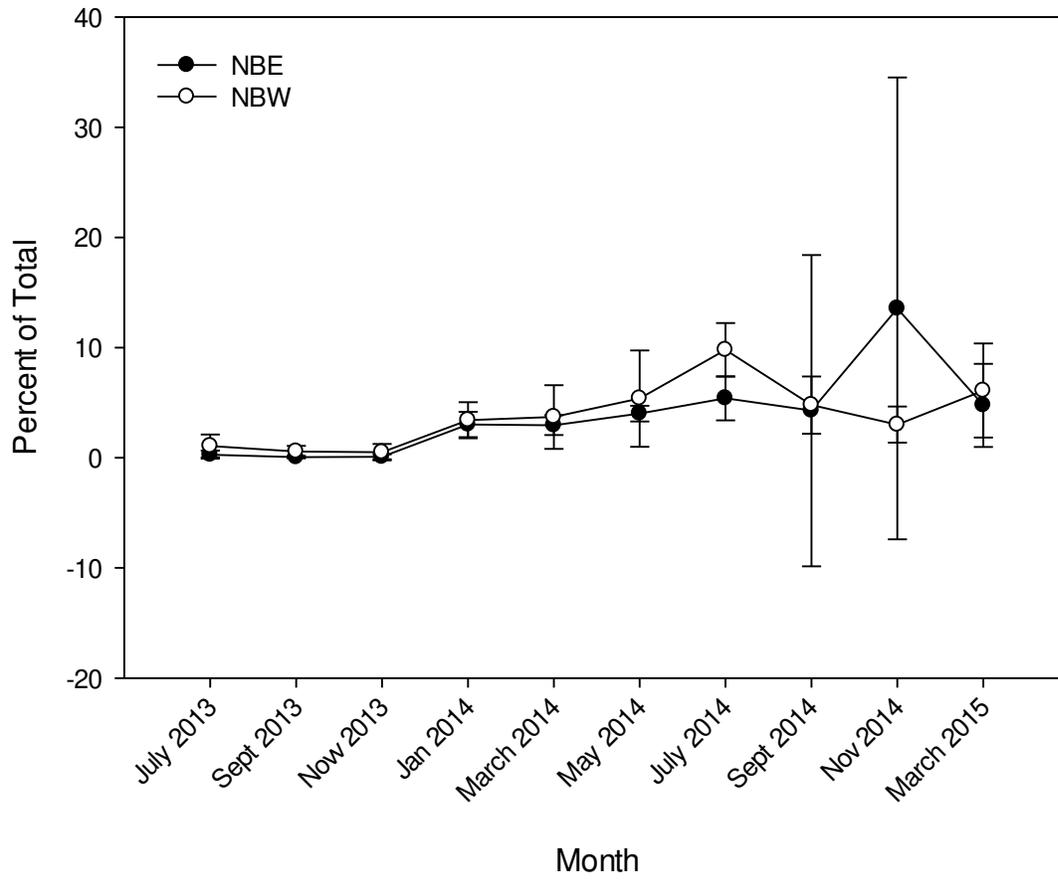


Figure 4.32. Occluded inorganic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Ca Bound

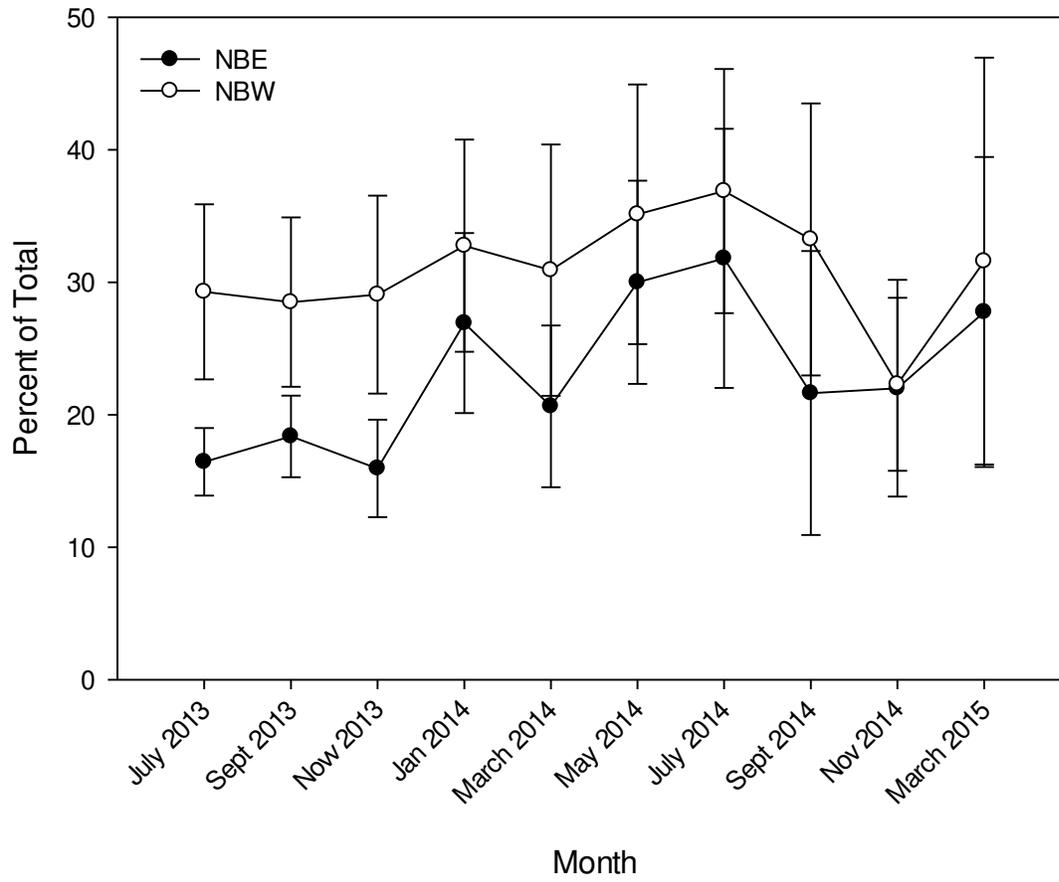


Figure 4.33. Calcium bound inorganic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Labile Organic

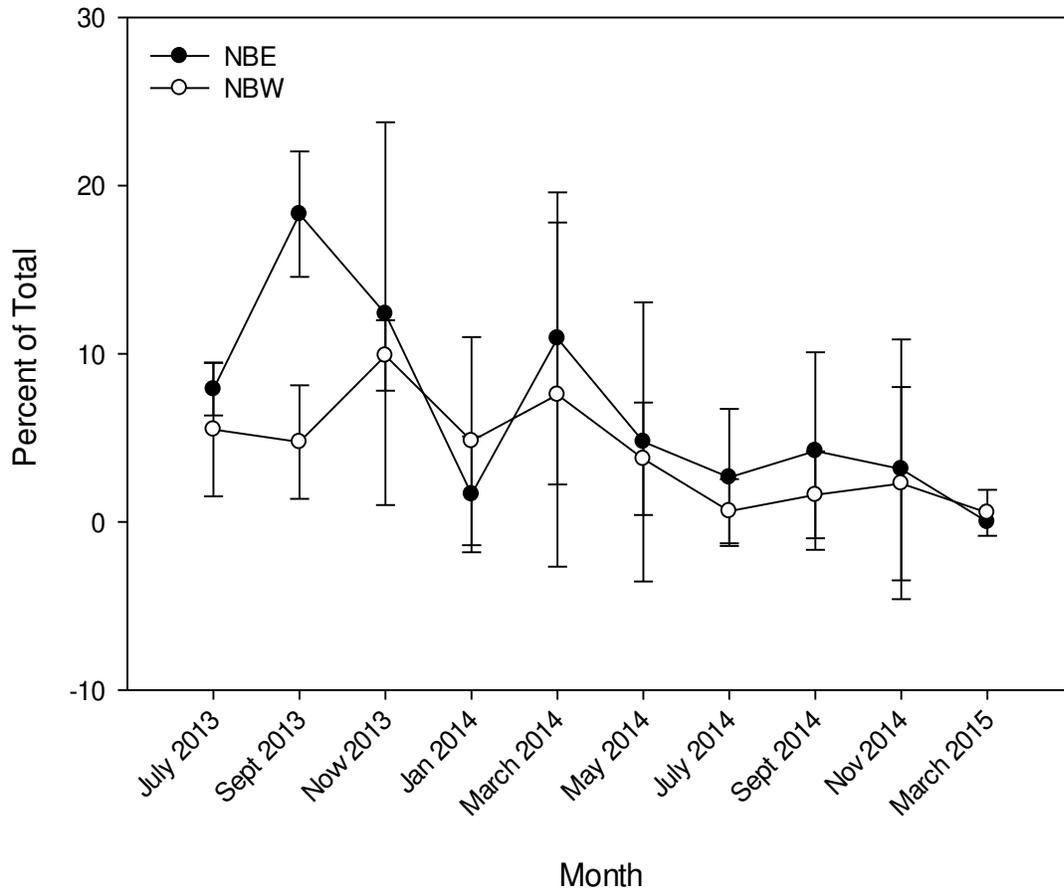


Figure 4.34. Labile organic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Microbial P

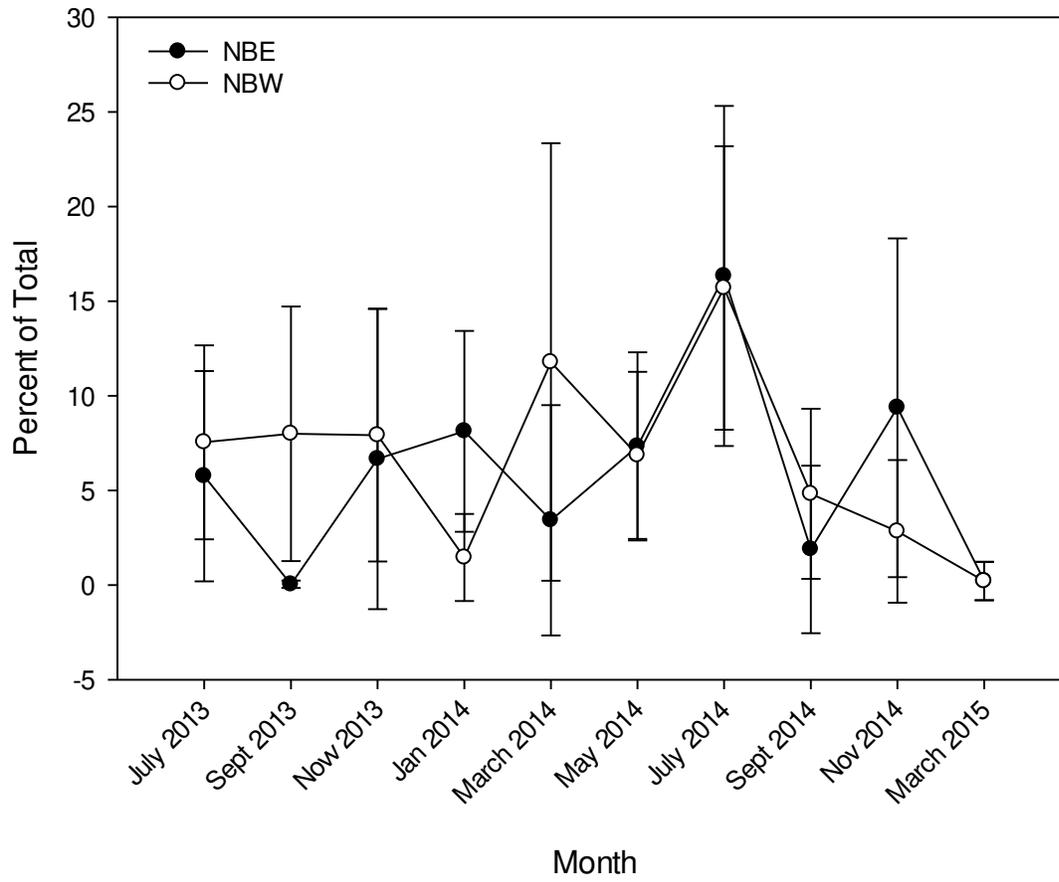


Figure 4.35. Microbial biomass P at the dryland research location. Phosphorus data was averaged over the different application rates.

Mod. Labile Organic

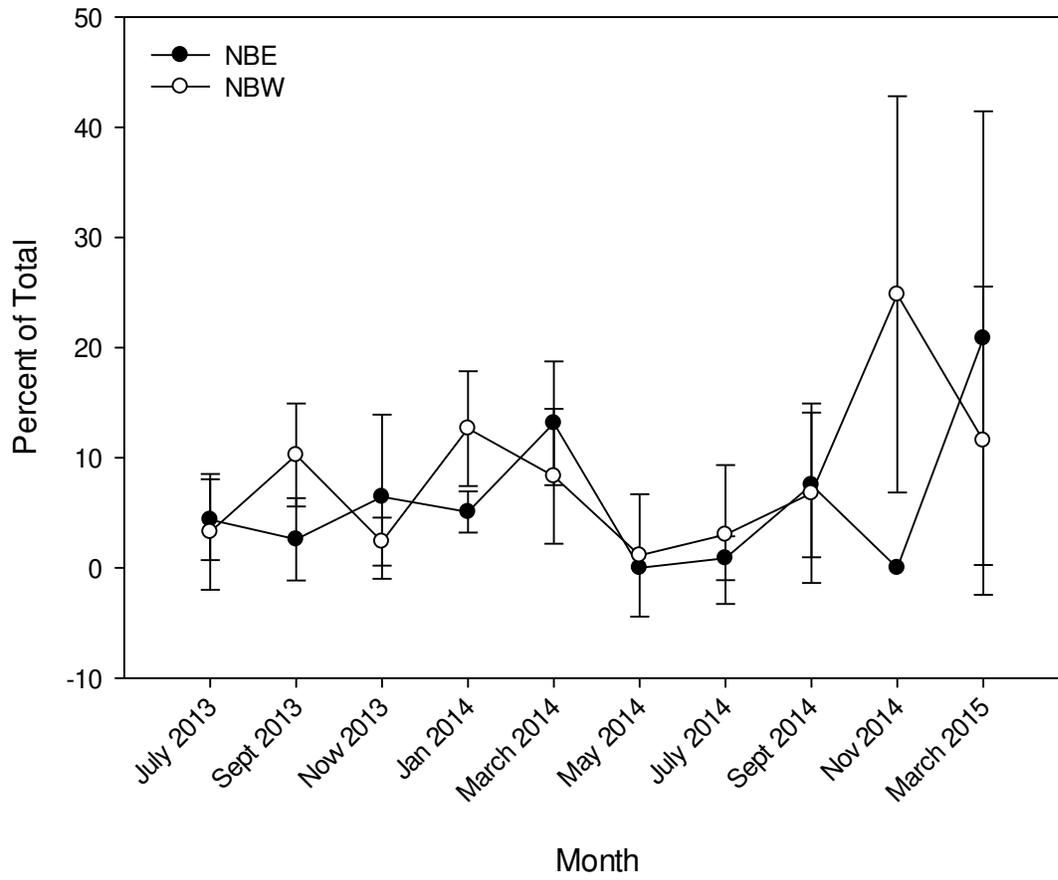


Figure 4.36. Moderately labile organic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Fulvic

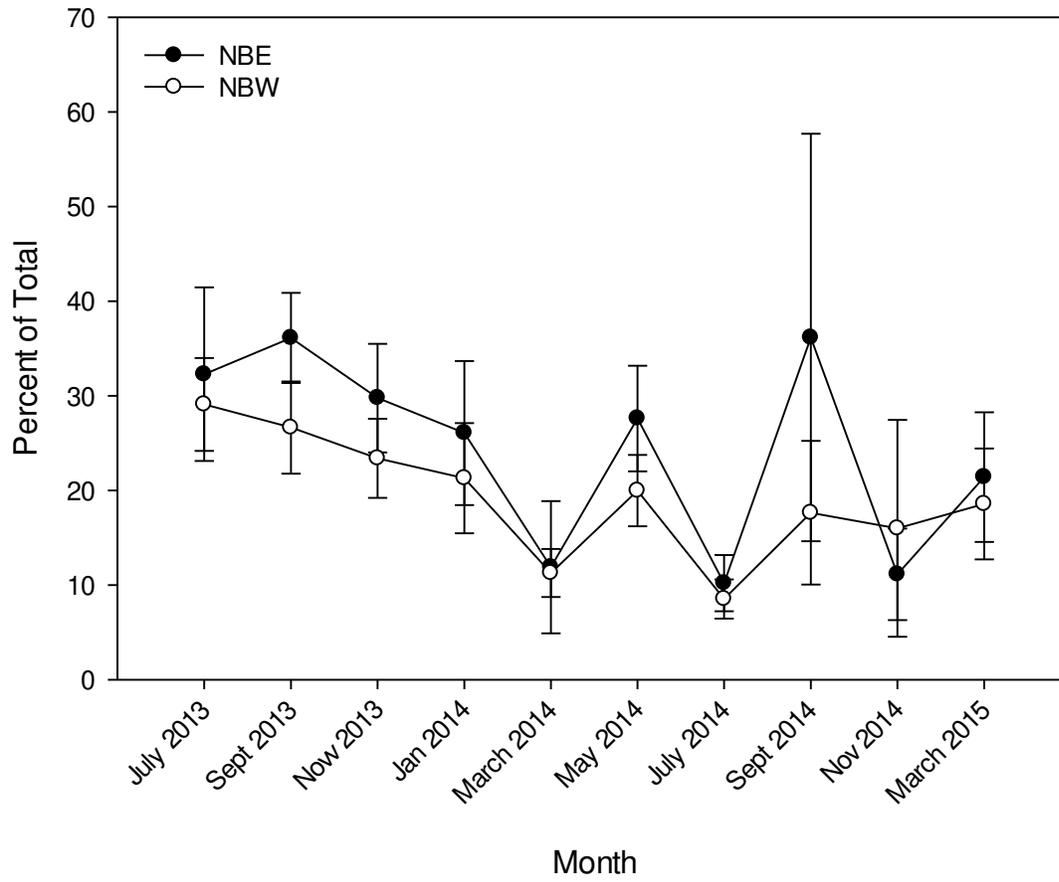


Figure 4.37 Fulvic acid associated organic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Humic Acid

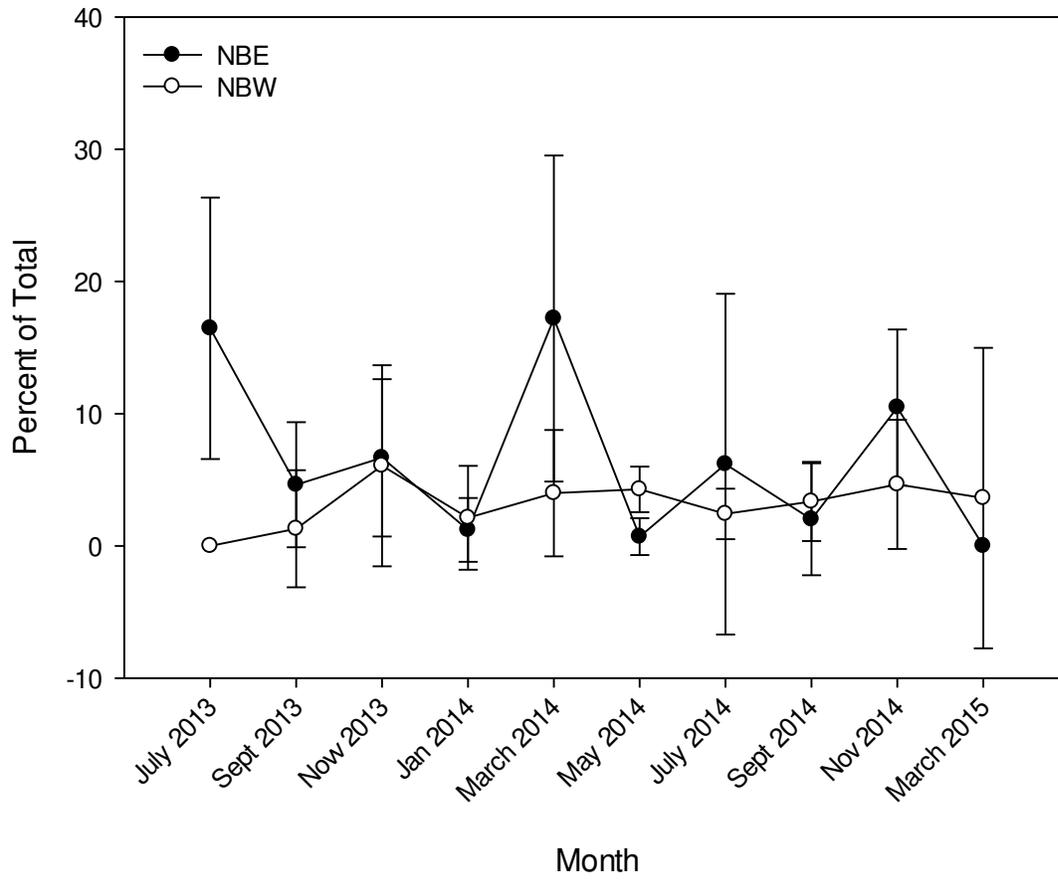


Figure 4.38. Humic acid associated organic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Non-Labile Organic P

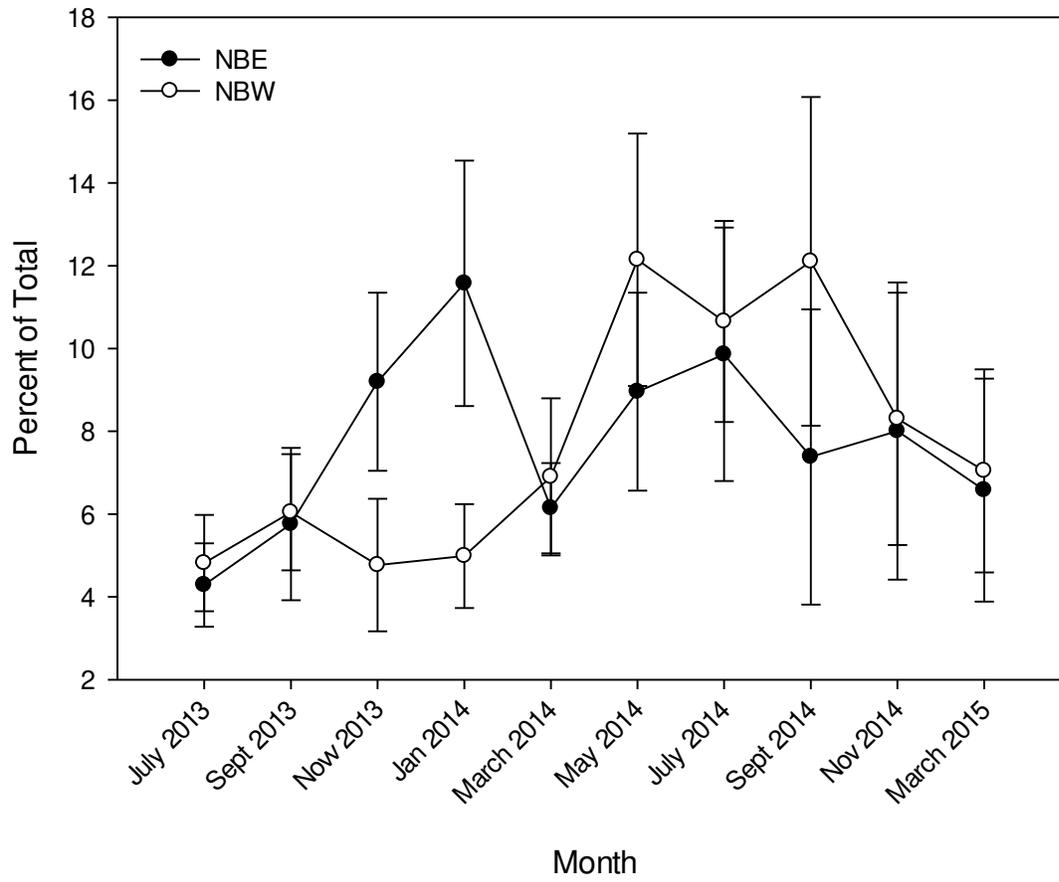


Figure 4.39. Nonlabile organic P at the dryland research location. Phosphorus data was averaged over the different application rates.

Table 4.1. Properties of the irrigated corn field soils. Composite soil samples were analyzed to determine the chemical properties of the four soil series that were analyzed in this study.

	Bijou	Heldt	Nunn	Valent
1:1 Soil pH	7.0	7.0	6.9	7.1
WDRF Buffer pH	7.2	7.2	7.2	7.2
1:1 S Salts mmho/cm	0.75	0.77	0.54	0.95
Organic Matter LOI %	1.4	2.9	1.9	1.4
Nitrate-N ppm N	44.3	36.5	28.1	42.9
lbs N/A	106	88.0	67.5	103
Potassium ppm K	199	383	335	147
Sulfate-S ppm S	43.5	40.5	16.5	54.0
Zinc ppm Zn	6.08	6.07	4.01	5.07
Iron ppm Fe	16.5	47.8	34.9	16.1
Manganese ppm Mn	19.1	26.3	24.5	15.3
Copper ppm Cu	4.18	7.03	4.58	3.85
Calcium ppm Ca	1610	3169	2200	1536
Magnesium ppm Mg	186	408	265	166
Sodium ppm Na	64	92	56	66
CEC/Sum of Cations me/100g	10	21	14	9.7
%H Sat	0	0	0	0
%K Sat	5	5	6	4
%Ca Sat	78	77	77	79
%Mg Sat	15	16	16	15
%Na Sat	3	2	2	3
Aluminum ppm Al	0.3	0.3	0.1	0.3
Mehlich P-III ppm P	175	310	262	150

Table 4.2. Type III ANOVA for the Olsen extractable P data using Proc Glimmix in SAS 9.4. Significant difference are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	6.04	<0.001
	Month	3	12.51	0.002
	Field X Month	3	12.51	0.003
Heldt	Field	1	5.96	<0.001
	Month	3	10.38	<0.001
	Field X Month	3	10.38	<0.001
Nunn	Field	1	5.69	0.002
	Month	3	11.7	<0.001
	Field X Month	3	11.7	0.001
Valent	Field	1	8.69	<0.001
	Month	3	18.01	<0.001
	Field X Month	3	18.01	0.836

Table 4.3 Type III ANOVA for the soluble inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	12.02	<0.001
	Month	3	15.21	<0.001
	Field X Month	3	15.21	0.013
Heldt	Field	1	6.00	0.016
	Month	3	12.24	<0.001
	Field X Month	3	12.24	<0.001
Nunn	Field	1	5.62	0.099
	Month	3	12.4	<0.001
	Field X Month	3	12.4	0.073
Valent	Field	1	5.98	<0.001
	Month	2	13.7	<0.001
	Field X Month	3	13.7	0.044

Table 4.4 Type III ANOVA for the Al bound inorganic P data using Proc Glimmix in SAS 9.4. Significant difference are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	13.67	<0.001
	Month	3	16.34	0.001
	Field X Month	3	16.34	0.067
Heldt	Field	1	5.72	<0.001
	Month	3	9.05	<0.001
	Field X Month	3	9.05	<0.001
Nunn	Field	1	13.79	<0.001
	Month	3	16.59	<0.001
	Field X Month	3	16.59	<0.001
Valent	Field	1	5.63	<0.001
	Month	2	12.79	0.014
	Field X Month	3	12.79	0.203

Table 4.5 Type III ANOVA for the Fe bound inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	7.32	<0.001
	Month	3	14.26	<0.001
	Field X Month	3	14.26	<0.001
Heldt	Field	1	8.98	<0.001
	Month	3	17.28	<0.001
	Field X Month	3	17.28	<0.001
Nunn	Field	1	10.27	0.001
	Month	3	17.63	<0.001
	Field X Month	3	17.63	<0.001
Valent	Field	1	9.33	<0.001
	Month	2	13.24	<0.001
	Field X Month	3	13.24	<0.001

Table 4.6 Type III ANOVA for the occluded inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	7.42	0.003
	Month	3	16.14	<0.001
	Field X Month	3	16.14	<0.001
Heldt	Field	1	7.46	0.006
	Month	3	12.62	<0.001
	Field X Month	3	12.62	0.499
Nunn	Field	1	5.79	0.139
	Month	3	12.01	<0.001
	Field X Month	3	12.01	<0.001
Valent	Field	1	9.95	<0.001
	Month	2	16.50	<0.001
	Field X Month	3	16.50	0.131

Table 4.7 Type III ANOVA for the Ca bound inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	10.32	0.899
	Month	3	16.55	<0.001
	Field X Month	3	16.55	0.419
Heldt	Field	1	5.58	<0.001
	Month	3	9.17	<0.001
	Field X Month	3	9.17	<0.001
Nunn	Field	1	5.54	0.256
	Month	3	10.72	0.265
	Field X Month	3	10.72	<0.001
Valent	Field	1	5.91	0.010
	Month	2	13.56	<0.001
	Field X Month	3	13.56	0.057

Table 4.8 Type III ANOVA for the labile organic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	8.99	0.012
	Month	3	16.23	<0.001
	Field X Month	3	16.23	0.049
Heldt	Field	1	6.01	0.066
	Month	3	11.62	<0.001
	Field X Month	3	11.62	0.364
Nunn	Field	1	12.23	0.353
	Month	3	16.73	<0.001
	Field X Month	3	16.73	0.368
Valent	Field	1	4.41	0.023
	Month	2	13.07	0.001
	Field X Month	3	13.07	0.002

Table 4.9 Type III ANOVA for the microbial biomass P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	6.12	0.075
	Month	3	12.30	0.001
	Field X Month	3	12.30	0.442
Heldt	Field	1	6.00	0.032
	Month	3	11.88	0.033
	Field X Month	3	11.88	0.285
Nunn	Field	1	6.13	0.629
	Month	3	8.66	0.383
	Field X Month	3	8.66	0.163
Valent	Field	1	4.52	0.022
	Month	2	12.39	0.011
	Field X Month	3	12.39	0.535

Table 4.10 Type III ANOVA for the moderately labile organic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	5.96	0.995
	Month	3	10.70	0.256
	Field X Month	3	10.70	0.140
Heldt	Field	1	8.17	0.857
	Month	3	16.66	<0.001
	Field X Month	3	16.66	0.015
Nunn	Field	1	6.06	0.680
	Month	3	10.21	0.011
	Field X Month	3	10.21	0.420
Valent	Field	1	6.20	0.686
	Month	2	12.86	0.503
	Field X Month	3	12.86	0.006

Table 4.11 Type III ANOVA for the fulvic acid P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	8.87	0.043
	Month	3	15.64	<0.001
	Field X Month	3	15.64	0.473
Heldt	Field	1	5.29	0.032
	Month	3	13.24	<0.001
	Field X Month	3	13.24	0.081
Nunn	Field	1	9.22	0.428
	Month	3	18.31	<0.001
	Field X Month	3	18.31	0.001
Valent	Field	1	13.68	0.063
	Month	2	17.62	0.007
	Field X Month	3	17.62	0.545

Table 4.12 Type III ANOVA for the humic acid P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	7.35	0.160
	Month	3	14.29	<0.001
	Field X Month	3	14.29	0.751
Heldt	Field	1	5.68	0.026
	Month	3	13.57	<0.001
	Field X Month	3	13.57	0.148
Nunn	Field	1	9.34	0.294
	Month	3	17.38	<0.001
	Field X Month	3	17.38	0.017
Valent	Field	1	5.73	0.209
	Month	2	11.83	<0.001
	Field X Month	3	11.83	0.272

Table 4.13 Type III ANOVA for the non-labile P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	5.09	0.630
	Month	3	12.26	<0.001
	Field X Month	3	12.26	0.322
Heldt	Field	1	7.45	<0.001
	Month	3	15.06	<0.001
	Field X Month	3	15.06	<0.001
Nunn	Field	1	6.01	0.001
	Month	3	11.8	0.002
	Field X Month	3	11.8	<0.001
Valent	Field	1	6.12	0.454
	Month	2	11.6	0.001
	Field X Month	3	11.6	0.982

Table 4.14 Type III ANOVA for the percent of total P, soluble inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	4.60	0.871
	Month	3	7.99	0.001
	Field X Month	3	7.99	0.468
Heldt	Field	1	5.26	0.004
	Month	3	6.00	<0.001
	Field X Month	3	6.00	0.279
Nunn	Field	1	11.54	0.037
	Month	3	16.03	<0.001
	Field X Month	3	16.03	0.0036
Valent	Field	1	5.89	0.052
	Month	3	10.35	<0.001
	Field X Month	3	10.35	0.542

Table 4.15 Type III ANOVA for the percent of total P, Al bound inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	6.37	<0.001
	Month	3	10.57	<0.001
	Field X Month	3	10.57	0.031
Heldt	Field	1	5.84	0.227
	Month	3	9.48	<0.001
	Field X Month	3	9.48	<0.001
Nunn	Field	1	2.82	0.003
	Month	3	6.16	0.001
	Field X Month	3	6.16	0.027
Valent	Field	1	6.03	0.003
	Month	3	10.09	0.036
	Field X Month	3	10.09	0.192

Table 4.16 Type III ANOVA for the percent of total P, Fe bound inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	6.09	<0.001
	Month	3	8.89	<0.001
	Field X Month	2	7.93	0.001
Heldt	Field	1	5.67	0.066
	Month	3	7.31	<0.001
	Field X Month	3	7.31	0.009
Nunn	Field	1	5.67	0.097
	Month	3	6.26	<0.001
	Field X Month	3	6.26	0.157
Valent	Field	1	8.40	0.015
	Month	3	11.23	<0.001
	Field X Month	1	8.45	0.236

Table 4.17 Type III ANOVA for the percent of total P, occluded inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	8.17	0.434
	Month	3	11.86	<0.001
	Field X Month	3	11.86	0.007
Heldt	Field	1	5.04	0.347
	Month	3	10.4	0.111
	Field X Month	3	10.4	0.063
Nunn	Field	1	10.29	0.525
	Month	3	13.45	<0.001
	Field X Month	3	13.45	0.006
Valent	Field	1	7.22	0.051
	Month	3	13.46	0.002
	Field X Month	3	13.46	0.035

Table 4.18 Type III ANOVA for the percent of total P, Ca bound inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	5.42	0.001
	Month	3	7.67	<0.001
	Field X Month	3	7.67	0.075
Heldt	Field	1	5.88	0.777
	Month	3	9.22	0.008
	Field X Month	3	9.22	0.081
Nunn	Field	1	9.42	0.028
	Month	3	12.66	<0.001
	Field X Month	3	12.66	0.001
Valent	Field	1	6.32	0.005
	Month	3	12.32	0.104
	Field X Month	3	12.32	0.951

Table 4.19 Type III ANOVA for the percent of total P, total inorganic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	5.65	0.399
	Month	3	8.38	0.001
	Field X Month	3	8.38	0.010
Heldt	Field	1	5.72	0.916
	Month	3	8.24	<0.001
	Field X Month	3	8.24	0.061
Nunn	Field	1	11.02	0.314
	Month	3	15.11	<0.001
	Field X Month	3	15.11	0.052
Valent	Field	1	9.62	0.554
	Month	3	13.56	0.438
	Field X Month	3	13.56	0.566

Table 4.20 Type III ANOVA for the percent of total P, labile organic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	3.83	0.078
	Month	3	7.12	0.009
	Field X Month	3	7.12	0.001
Heldt	Field	1	4.58	0.355
	Month	2	5.42	0.045
	Field X Month	2	5.42	0.800
Nunn	Field	1	6.42	0.737
	Month	3	6.19	0.002
	Field X Month	3	6.19	0.403
Valent	Field	1	3.03	0.060
	Month	3	9.42	0.017
	Field X Month	3	9.42	0.017

Table 4.21 Type III ANOVA for the percent of total P, microbial biomass P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	4.587	0.375
	Month	3	5.034	0.003
	Field X Month	3	5.034	0.308
Heldt	Field	1	4.487	0.214
	Month	3	10.34	0.041
	Field X Month	3	10.34	0.951
Nunn	Field	1	7.718	0.555
	Month	3	14.14	0.507
	Field X Month	3	14.14	0.747
Valent	Field	1	7.39	0.003
	Month	3	8.511	<0.001
	Field X Month	3	8.511	0.002

Table 4.21 Type III ANOVA for the percent of total P, moderately labile organic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	1	0.224
	Month	3	2	0.192
	Field X Month	2	2	0.201
Heldt	Field	1	6.01	0.594
	Month	3	2.09	0.067
	Field X Month	1	2.94	0.935
Nunn	Field	1	2.75	0.111
	Month	2	4.10	0.017
	Field X Month	1	2.54	0.518
Valent	Field	1	7.09	0.905
	Month	3	5.21	0.314
	Field X Month	1	4.60	0.651

Table 4.22 Type III ANOVA for the percent of total P, fulvic acid P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	6.28	0.557
	Month	3	10.70	0.002
	Field X Month	3	10.70	0.797
Heldt	Field	1	10.46	0.382
	Month	3	15.20	<0.001
	Field X Month	3	15.20	0.143
Nunn	Field	1	9.16	0.968
	Month	3	16.06	<0.001
	Field X Month	3	16.06	0.391
Valent	Field	1	5.79	0.797
	Month	3	10.27	0.112
	Field X Month	3	10.27	0.640

Table 4.23 Type III ANOVA for the percent of total P, humic acid P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	6.24	0.424
	Month	3	7.55	0.001
	Field X Month	3	7.55	0.782
Heldt	Field	1	6.14	0.551
	Month	3	10.73	<0.001
	Field X Month	3	10.73	0.005
Nunn	Field	1	7.93	0.916
	Month	3	14.78	<0.001
	Field X Month	3	14.78	0.031
Valent	Field	1	4.67	0.093
	Month	3	7.76	0.017
	Field X Month	3	7.76	0.304

Table 4.24 Type III ANOVA for the percent of total P, non-labile P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	5.14	0.001
	Month	3	7.29	0.001
	Field X Month	3	7.29	0.292
Heldt	Field	1	4.71	0.287
	Month	3	9.00	<0.001
	Field X Month	3	9.00	0.425
Nunn	Field	1	8.31	0.010
	Month	3	14.84	0.787
	Field X Month	3	14.84	0.170
Valent	Field	1	6.08	0.010
	Month	3	11.03	0.003
	Field X Month	3	11.03	0.480

Table 4.25 Type III ANOVA for the percent of total P, total organic P data using Proc Glimmix in SAS 9.4. Significant differences are in bold. For some of the soils the main effects were also significant when looking at only the P value (0.05). However, since there was an interaction between the field and the month the main effects should not be investigated separately and instead the interaction should be studied. Soils that are in red had a log transformation to the data to correct for heterogeneity of variance.

Soil	Effect	Num DF	Den DF	P Value
Bijou	Field	1	5.65	0.399
	Month	3	8.38	0.001
	Field X Month	3	8.38	0.010
Heldt	Field	1	6.35	0.413
	Month	3	12.00	0.013
	Field X Month	3	12.00	0.590
Nunn	Field	1	11.02	0.314
	Month	3	15.11	<0.001
	Field X Month	3	15.11	0.052
Valent	Field	1	9.62	0.554
	Month	3	13.56	0.438
	Field X Month	3	13.56	0.566

Table 4.26 Production Dryland wheat fields inorganic P fractions. Each fraction was analyzed separately and statically different at an alpha value of 0.5 are shown by the subscripts.

Soil	Month	Soluble Mean	Al Bound Mean	Fe Bound Mean	Occluded Mean	Ca Bound Mean	tot inorganic Mean				
Colby-Adena	August	3.02%	13.05%	ab	8.08%	abc	5.34%	26.06%	bcde	55.56%	
	September	1.87%	15.89%	ab	10.53%	a	5.56%	17.77%	f	51.62%	
	November	1.81%	7.30%	cde	2.10%	cd	5.16%	31.80%	ab	48.17%	
	March	1.02%	5.81%	cde	3.39%	abcd	3.39%	23.86%	cdef	37.48%	
	May	1.16%	7.60%	cde	7.44%	abcd	6.86%	26.45%	cdef	49.51%	
	July	1.12%	8.62%	bcd	1.85%	d	4.28%	39.87%	ab	55.75%	
	August	1.59%	8.56%	cde	4.67%	d	6.24%	24.51%	abc	45.56%	
Weld	September	1.42%	10.65%	bc	8.28%	abcd	4.95%	24.19%	bc	49.49%	
	November	1.98%	11.62%	ab	9.25%	ab	2.05%	17.14%	def	42.05%	
	March	0.46%	5.67%	de	7.70%	abcd	1.68%	12.47%	ef	27.97%	
	May	0.56%	5.68%	e	7.03%	bcd	1.66%	22.75%	bc	37.68%	
Colby-Adena Weld	July	0.46%	5.19%	cde	6.58%	abcd	1.33%	24.85%	c	38.40%	
	August	1.68%	a	9.76%	a	5.53%	5.06%	a	27.66%	49.68%	a
	September	1.07%	b	7.84%	b	7.23%	2.94%	b	20.92%	39.99%	b
	November	2.31%	a	10.81%	b	6.37%	5.79%	25.28%	ab	50.56%	a
	March	1.66%	a	13.44%	a	9.48%	5.27%	20.77%	bc	50.62%	a
	May	1.89%	a	9.46%	ab	5.68%	3.61%	24.47%	bc	45.11%	ab
	July	0.74%	b	5.74%	c	5.55%	2.54%	18.16%	c	32.72%	c
Soil	August	0.84%	b	6.58%	c	7.22%	4.08%	24.48%	ab	43.20%	b
	September	0.79%	b	6.91%	bc	4.22%	2.80%	32.36%	a	47.07%	ab
	Month	0.010	0.020	0.651	0.008	0.490	<0.001				
	Soil x Month	<0.001	0.002	0.459	0.068	0.003	<0.001				
Soil x Month		0.069	0.004	0.005	0.314	0.001	0.319				

Table 4.27 Dryland production wheat field organic P fractions. Each fraction was analyzed separately and statically different at an alpha value of 0.5 are shown by the subscripts.

Soil	Month	Labile organic	Microbial		Moderately organic		Fulvic	Humic		Nonlabile	Tot Organic					
		Mean		Mean		Mean		Mean		Mean		Mean				
Colby-Adena	August	3.01%	bc	4.11%		0.74%		20.65%		7.22%	bcd	8.71%		44.44%		
	September	2.45%	bc	13.07%		0.40%		24.80%		0.41%	e	7.25%		48.38%		
	November	4.67%	b	0.66%		24.07%		13.60%		1.46%	de	7.37%		51.83%		
	March	0.25%	bc	6.35%		6.45%		27.11%		10.55%	b	11.82%		62.52%		
	May	0.08%	bc	4.68%		1.97%		28.00%		9.93%	b	5.84%		50.49%		
Weld	July	0.00%	c	3.02%		3.20%		29.08%		6.92%	bc	2.04%		44.25%		
	August	0.75%	bc	7.14%		2.54%		25.44%		6.59%	bcd	11.98%		54.44%		
	September	3.20%	bc	2.70%		0.44%		31.83%		0.99%	cde	11.36%		50.51%		
	November	12.92%	a	0.00%		19.72%		14.01%		5.27%	cba	6.03%		57.95%		
	March	3.32%	bc	6.11%		3.36%		26.29%		18.85%	a	14.11%		72.03%		
Colby-Adena Weld	May	0.00%	c	6.32%		12.15%		30.90%		6.62%	cb	6.33%		62.32%		
	July	0.00%	c	3.09%		9.18%		42.15%		4.04%	bcde	3.13%		61.60%		
		1.78%		5.33%		6.23%		23.79%	b	6.00%	b	7.20%	b	50.32%	b	
		3.37%		4.26%		8.06%		28.36%	a	7.19%	a	8.77%	a	60.01%	a	
	August	1.88%	b	5.62%	ab	1.64%	bc	23.05%	c	6.90%	b	10.34%	b	49.44%	c	
	September	2.80%	b	8.23%	a	0.42%	c	28.08%	bc	0.68%	c	9.16%	b	49.38%	c	
	November	8.79%	a	0.33%	c	21.90%	a	13.81%	d	3.36%	bc	6.70%	c	54.89%	bc	
	March	1.78%	b	6.23%	ab	4.90%	bc	26.70%	bc	14.70%	a	12.96%	a	67.28%	a	
	May	0.04%	b	5.56%	ab	7.40%	b	29.55%	ab	8.16%	b	6.10%	c	56.80%	b	
	July	0.00%	b	3.06%	b	6.19%	b	35.61%	a	5.48%	b	2.58%	d	52.93%	bc	
	Soil		0.108		0.597		0.431		0.022		0.039		0.021		<0.001	
	Month		<0.001		0.005		<0.001		<0.001		<0.001		<0.001		<0.001	
	Soil x Month		0.036		0.225		0.094		0.253		0.022		0.234		0.246	

Table 4.28 Research dryland field inorganic P fractions significant differences. Fractions in red had a log transformation to correct for heterogeneity of variance.

	SOLUBLE	AL	FE	OCCLUDED	CA	INORG TOT
	P Value					
SITE	<0.001	0.123	0.059	0.652	<0.001	<0.001
TRT	0.031	0.070	0.036	0.844	0.092	0.032
SITE*TRT	0.486	0.560	0.474	0.630	0.774	0.492
MONTH	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SITE*MONTH	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
MONTH*TRT	0.136	0.697	0.548	0.842	0.729	0.137
SITE*MONTH*TRT	0.898	0.983	0.326	0.754	0.608	0.887

Table 4.29 Research dryland field organic P fractions significant differences. Fractions in red had a log transformation to correct for heterogeneity of variance.

	lab org P Value	Microb P Value	mod org P Value	Fulvic P Value	Humic P Value	nonlab P Value	tot org P Value
Site	<0.001	0.124	0.001	<0.001	<0.001	0.995	<0.001
trt	0.287	0.002	0.385	0.490	0.224	0.070	0.031
Site*trt	0.431	0.917	0.219	0.734	0.898	0.662	0.486
month	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Site*month	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
month*trt	0.551	0.922	0.780	0.203	0.505	0.272	0.136
Site*month*trt	0.464	0.299	0.558	0.617	0.839	0.688	0.898

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CHAPTER 5: PLANT UPTAKE OF P FROM SOILS IN THE WESTERN UNITED STATES

Introduction:

For many years, biosolids have been beneficially recycled through land application. Biosolids have been shown to provide both macro and micronutrients (Barbarick et al., 2012), and their application has been based on supplying the crop with the agronomic rate of nitrogen (N). However, there has been a growing concern with the over application of phosphorus (P) and the resulting environmental impacts such as algae blooms and eutrophication of waterways. This problem has been addressed with changes to regulations that require P risk assessments and in some cases application at the agronomic rate of P instead of N.

However, much of the work completed on this topic was performed in the eastern United States (O'Connor et al., 2004) where the soils have very different properties than soils in the western parts of the country. Differences in western soil pH result in different mechanisms that control soil P availability. Phosphorus availability can be separated into aspects such as how much of the P is on potentially available to plants and how much is a potential environmental contaminant. The focus of this paper is determining how much biosolids P is available to plants. Other studies that investigated the uptake of P have looked at biosolids that were from wastewater treatment plants in the eastern United States and applied to soils in that region (O'Connor et al., 2004). This study focuses on the western United States.

The objectives of this study were to determine differences in the availability of P from different amendments and to estimate the amount of P available to plants prior to land application.

Methods and Materials:

Design of study:

A greenhouse study was established that consisted of five fertilizer sources of importance to the Front Range of Colorado (Metro Wastewater biosolids, Littleton/Englewood biosolids, Fort Collins biosolids, raw cattle manure, and composted cattle manure) (Table 5.1) applied at three rates (agronomic rate of N, agronomic rate of P assuming 50% available, and agronomic rate of P assuming 100% available). The application rate of the biosolids was based on their average P and N content (determined from the annual reports for the treatment plants where the biosolids were obtained). The rates for the application of the composted and raw manures were based on average N and P content as reported in the literature (Reddy et al., 2005). Three soils were used to represent a variety of soil textures, pH, and calcium carbonate (CaCO₃) concentrations (Table 5.2). The soils series selected account for 26.8 million hectares (Soil Survey Staff, 2012) in the western United States.

The 15 by 15 cm pots used in the study were lined with a plastic bag to prevent leaching during the study. The pots were filled with 2.3 L of sieved (1 cm), field moist soil to a bulk density of 1.2 g cm⁻³. The soil was then poured out of each pot individually, and the fertilizer amendment was added to the soil and thoroughly mixed (to simulated incorporation of the amendment). The amended soil was put back into the pot and repacked to a bulk density of 1.2 g cm⁻³. After the treatments had been set up, urea was added to the treatments that had not received the full amount of N that would be needed by the crop based on the fertilizer recommendation guide (Mortvedt et al., 1996).

The pots were arranged on a bench in the greenhouse in a randomized complete block design with four replications of each treatment. To help reduce edge effects there was a row of pots placed around the treatment pots to provide a buffer. The border pots were fertilized with commercial greenhouse fertilizer approximately every three weeks. The row of border pots was used to reduce the

amount of heating from the sides of the pots due to direct exposure to sunlight and to standardize the amount of competition of different pots for light.

The pots were watered to bring them to 60% water filled pore space and allowed to sit overnight before being seeded with eight Sorghum Sudan grass (*Sorghum bicolor* (L.) Moench ssp. drummondii) seeds. The pots were watered nearly daily to ensure proper moisture conditions in the top 5 cm of soil (each pot was weighed and water was added for mass lost). A drip irrigation system was added and used throughout the course of the study to maintain proper soil water conditions.

Analysis of biomass and soil:

Eighteen days after seeding, the plants were thinned to leave the three most viable plants per pot. The plants were allowed to continue growing for four weeks before the first harvest. The plants were then harvested every three weeks for a total of seven harvests. Since Sorghum Sudan grass can be harvested as a forage crop, a 2-cm ring was placed at the soil surface and all biomass above the ring was harvested. The biomass was rinsed with distilled water and oven dried in paper bags at 60° C for 48 hours.

The oven dry biomass was found, and the plant tissue was ground with a Wiley Mill. The biomass was then digested with concentrated nitric acid (Huang and Schulte, 1985) and analyzed on an inductively coupled plasma atomic emission spectroscopy (ICP-AES) to determine the concentration of P.

With each harvest, a small soil sample (10 cm deep) was collected from each pot. The soil was air dried, ground with a mortar and pestle, and analyzed for Olsen Extractable P (Kuo, 1996). After the first, third, fifth, and seventh harvests the soil was also analyzed for NO₃-N and NH₄-N using a 2 M KCl extraction (Mulvaney, 1996) and an Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station, TX). Nitrogen fertilizer (0.43M ammonium nitrate) was added to all

treatments following the fourth and sixth harvest to reduce the chances of N stress. The average amount of N needed for each treatment was based on the soil test value and the fertilizer recommendation guide (Mortvedt et al., 1996). All replications in the same treatment received the same amount of supplemental N.

At the completion of the study the electrical conductivity (EC) of the soils was measured with a 1:1 extraction (Rhoades, 1996). This was done to check if there was an increase in the salt concentration of the soil. The irrigation water used was the domestic water supply for the greenhouse and was considered to be high quality.

Root analysis:

The roots were sampled destructively at the conclusion of the study and cleaned by hyperwashing the soil/root balls. Since the pots were lined with a plastic bag to prevent leaching during the study, the pots were filled with water to saturate the soil. The soil was then dumped onto a 1 cm wire mesh and the root-ball was washed until the water ran clear. The roots were then placed in a 60° C oven for 48 hours. The dried root balls were ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). The ground samples were digested with concentrated nitric acid (HNO₃) to determine the amount of P in the roots and the elemental makeup (Huang and Schulte, 1985). Extracted samples were then analyzed on an ICP-AES to determine total P.

Amendment P extraction:

The concentration of P for each organic amendment was determined to calculate application rates. The moisture content of each of the biosolids, manure, and compost was determined by oven drying samples. Two grams of oven dry equivalent material was added to a 500 mL Nalgene bottle. An extracting solution was then added at a ratio of 1:100 (m:v) or 1:200 (m:v). Three extracting solutions were used: deionized distilled water, 0.5 M NaHCO₃ (pH 8.5), and 0.5 M NH₄Cl. Three replications of

each sample were shaken for 1 hour on a horizontal shaker before being filtered through Whatman #42 filter paper. Samples were then analyzed colorimetrically to determine P concentration (Rodriguez et al., 1994).

Soil Isotherms:

Soil isotherms were determined for P sorption to better understand the ability of the soil to make P less available. Ten grams of air-dried ground soil was placed into a 50mL centrifuge tube and 25 mL of varying concentrations of P in 0.01M KCl was added to each tube. P concentrations of 0, 0.2 0.5, 1, 3, 6, 10, 30, 60, 100, 200, and 400 mg L⁻¹ P were used to develop the sorption curves. The three soils that were used for the greenhouse study were analyzed in triplet. At the time that the samples were setup, a soil sample was oven dried to determine the current water content and the data was adjusted to correct for the water added with the soil. Following the shaking of the samples the tubes were centrifuged and a subsample was removed and passed through a 0.45um filter. The filtered sample was then analyzed colorimetrically to determine the concentration of P (Rodriguez et al., 1994).

Statistical Analysis:

All of the data from the study was analyzed using SAS version 9.4 (SAS Institute, 2012) using Proc Glimmix. For all the parameters that were sampled multiple times the data was analyzed as a repeated measure. An autoregressive type I structure was used to characterize the covariance structure of the data. This allowed for the covariance of data that was collected closer together in time to have a higher correlation than samples farther apart in time. The structure used also has an assumption that that the samples are evenly space (for this study all sampling was conducted every three weeks). Each of the data sets were also checked for homogeneity of variance and when necessary a transformation was performed on the data.

Results and Discussion:

Biomass Production:

The biomass data did not show a significant effect of fertilizer type, application rate, or soil type (Table 5.3); however, there was a difference in biomass production (Figure 5.1) for the different harvest dates. The largest biomass production was at the seventh harvest and was approximately a third larger than the rest of the harvest. Throughout the study, there was an intermittent problem with aphids (Aphidoidea) and they were controlled with two applications of insecticides. The aphid populations were also reduced by removal of the biomass at harvest. At the seventh sampling time, the entire plant was harvested down to the soil surface unlike the other sampling times.

Over the course of the study, there was also a trend for decreasing yield from the harvest prior to supplemental N fertilizer additions, after the 4th and 6th harvests. For the N application rate, there was a mineralization rate that was used to determine the amount of amendment to add but the data shows that there may not have been enough N released. All the other treatments had inorganic N fertilizer added at the start of the study to provide N to the plants. Under greenhouse conditions, the moisture and temperature are favorable for volatilization of N to take place, resulting in nitrogen deficiencies for the plants (Gardiner, 2008).

Soil Isotherms:

For all the soils used in this study the Langmuir isotherm fit the P adsorption data the best of the forms tested (produced the lowest sum of squares, Table 5.4). The sandy soil (Bijou, Coarse-loamy, mixed, superactive, mesic Ustic Haplargids) had the lowest amount of sorption and remained low until the solution P concentration was increased to 400 mg L⁻¹ P (Figure 5.2). Colby (Fine-silty, mixed, superactive, calcareous, mesic Aridic Ustorthents) was in the middle (Figure 5.3) and Nunn (Fine, smectitic, mesic Aridic Argiustolls) (Figure 5.4) had the highest amount of adsorption of P. Nunn and

Colby both are fine textured soils and therefore have a larger surface area and a higher cation exchange capacity. The larger surface area provides more area for reactions to take place and for the P to bind to the soil particles.

The isotherms for Bijou showed a very small amount of P sorbed (0.01 mg g^{-1}) with a solution concentration of approximately 25 mg mL^{-1} and after this point the amount of P sorbed began to rapidly rise. There are several reasons why this could be taking place. For some chemicals the initial sorption is low but after some of the chemical is on the soil particle the amount of apparent sorption increases sharply, perhaps through cooperative sorption, or there is precipitation taking place (Bohn, 1979). It is believed that in this case that there was precipitation due to the relatively high concentration of P and the presence of Ca and Fe in the soils that provide binding sites for precipitation (Lindsay, 1979). If precipitation is taking place then the assumptions for isotherm analysis are violated (Bohn, 1979) because when an isotherm is fit to the data it expected that P is either in solution or sorbed to the solids, and does not have a way to account for P that formed a precipitate. When the data was analyzed with all the data points included, the isotherm appears linear. When the analysis was repeated excluding the last three data points where precipitation is suspected the Langmuir equation fit best. Langmuir assumes that there is a limited amount of the chemical that can be absorbed and that it will make a single layer (Bohn, 1979).

The Colby soil adsorbed more P than Bijou. Colby is finer textured soil that had a larger surface area for P adsorption. The concentration of Ca, Fe, and Al were also much higher for the Colby soil than Bijou (Table 5.2). Perassi and Borgnino (2014) have shown that Fe and Al play an important role in the sorption of P when the concentration of P is low and as P concentration increases calcium carbonate becomes more important. It would be expected that due to the higher amount of P adsorption, P would

be less available in the Colby soil than in the Bijou. However, it has been shown that even though P quickly bonds to calcium carbonate it can easily be desorbed (So et al., 2011) and made available again.

The Nunn soil series absorbed the most P reaching a maximum of approximately 0.8 mg g^{-1} . The chemistry of Nunn was slightly different than Colby for the concentration of Fe and Al (Table 5.2), but the concentration of Ca was five times higher. In basic soils, P chemistry is controlled by Ca (Lindsay, 1979) and the increased in Ca allows for the formation of calcium phosphates.

The analysis of P adsorption in the soils showed the expected increase in adsorption for fine textured soils. This would help to reduce the amount of P that could cause environmental impacts but could also reduce the amount of P that the plants could absorb. If a soil has a higher adsorption of P the soil may be able to tie up a large amount of P but additional work is needed to evaluate the forms, and the stability of the absorbed P. The concern would be if the particles can adsorb a large amount of P and then the soil moves into a waterway how much of the P would be released. Also, the form of P would determine if plants are able to pull the P off the soil particle and if the adsorbed P would be detected in a soil test.

Plant Tissue P Concentrations:

After investigating the normality of variance, it was determined a transformation of the plant tissue P data was not needed. The analysis of the P concentration in the plant tissues showed a three-way interaction between soil \times rate \times harvest and a three-way interaction between soil \times fertilizer \times rate (Table 5.5). These interactions made it extremely difficult to draw conclusions from the data; therefore, the data was separated by soil type to reduce statistical complexity.

There was an interaction of fertilizer source \times application rate for all the soils that were used in this study (Tables 5.6-8). There was also an application rate \times harvest interaction for Bijou (Table 5.6). Nunn had a second interaction of fertilizer \times harvest (Table 5.8).

The fine-textured soils that had a higher sorption of P, as shown by the isotherm data, had a trend of increasing concentration of P over time in the plant tissue. Plant in the sandy-textured soil started at a higher concentration, approximately 3000 mg kg⁻¹, for the first four harvests and then it began to rapidly decline (Figure 5.5). This trend is also supported by the isotherm data where very little P is adsorbed to the soil particles so that there is a larger amount of P available early. The initial concentration of plant tissue P for the fine-textured soils was approximately 1000 mg kg⁻¹ for Nunn and steadily increased with each harvest (Figure 5.6). Colby started at approximately 2000 mg kg⁻¹ and stayed mostly constant for the first six harvests (Figure 5.7). For all the soils, there was a decrease in the concentration for the seventh harvest. At this harvest the plants had been affected by aphids and many of the plants were unhealthy and growing much slower. It is also possible that at this point the plants were stressed due to the limited space for root growth.

Since the fine-textured soils adsorb more P (isotherm data above), there is less P available in the soil solution for plant uptake than in the coarse textured soils. As the plants continued to grow during the study, the root mass was increasing, more soil was in contact with the roots and the root exudates (organic acids) that allow the plants to extract more P. Increasing root mass is important for nutrient uptake since it is estimated that roots are only in contact with 1% of the soil and the zone of influence around each root is small (Havlin et al., 2005).

There was a significant interaction between the fertilizer source and the application rate for each of the soils ($P=0.002$ for the sandy soil and $P<0.001$ for the fine textured soils, Tables 5.6-8). The plant tissues concentrations did not show a constant decline with a decrease in P application rate (Figures 5.8-10). This was due to variations in biomass production and the mass removed will be discussed below. For the Nunn soil, there was a very slight trend for increased P concentration in the plant tissues with an application of manure. It was expected that there would be more P available from

the manure due to the manure having a higher percentage of organic P (Gungor et al., 2007) than biosolids (Huang et al., 2008). Kovar and Claassen (2005) showed that even though plants do not take up organic P directly, microbial populations in the soils are able to more quickly transform organic P into a form that plants can use.

Above Ground Plant P Tissue Mass:

In an attempt to correct for the variation in biomass produced by different treatments at different times the concentration of a given sample was multiplied by the biomass that was produced to obtain the mass of P removed at each harvest. This data was then analyzed by each soil separately and a transformation was not needed on the data. It is expected that there were differences in the amount of shoot and root P, but without destructive sampling it was not possible to determine the root P until the conclusion of the study.

Colby had an interaction of both fertilizer × rate ($P < 0.001$) and rate × harvest date ($P = 0.001$) for the mass of P removed with the plant biomass (Table 5.9). For all the amendments except for Metro there was a trend for decreasing P mass removed with lower amounts of P application (Figure 5.11). The application rate over time also showed a higher P mass removed with higher application rate (Figure 5.12) and the fluctuation in the data followed the data for nitrogen concentration and yield.

Bijou followed similar trends as Colby with the same interactions of fertilizer × rate ($P = 0.013$, Figure 5.13) and rate × harvest date ($P = 0.003$, Figure 5.14, Table 5.10). However, for Bijou there was initially a higher amount of P being removed by the plants than in the Colby soil.

Nunn only had a significant rate × harvest interaction ($P = 0.011$, Figure 5.15, Table 5.11). Nunn followed a very similar pattern to the concentrations of P in the biomass as observed in the other soils. Again, there were large increases seen immediately following the addition of nitrogen fertilizer.

Root Mass P:

The mass of dry roots required a log transformation before the statistical analysis could be completed. There was a significant effect of soil ($P < 0.001$, Figure 5.16) and an interaction for amendment \times rate ($P = 0.038$, Figure 5.17, Table 5.12). The effect of soil type on root mass showed the Colby and Bijou were statistically the same and greater than Nunn. Colby and Bijou have less of an ability to absorb P than Nunn (isotherm data present above); and therefore, there is more likely to be times that the Nunn soil will be deficient in P. When plants experience a deficiency in P they will begin to take P from the older roots and shoots and translocate the P to newer growth (Schachtman et al., 1998). As a result, the older roots may be at a higher risk of dying thus reducing the root mass. The only significant difference in the root mass was that compost at the agronomic rate of N and raw manure at the lowest application rate had smaller root balls than the other treatments.

A log transformation was need for root P concentration. The roots had a significant difference in the concentration of P due to fertilizer source ($P < 0.001$) and an interaction of rate \times soil ($P < 0.001$, Table 5.13). The concentration of root P had a trend following the application rate of P (Figure 5.18). The highest concentration of root P was the application rate based on the agronomic need of N, the second highest was assuming that 50% of the P was available, and the lowest was where it was assumed that 100% of the P was available. There was not a discernable trend for one soil to have a consistently higher root P concentration, though the biosolids additions had a higher root P concentration than manure or compost (Figure 5.19). Biosolids generally have a higher concentration of inorganic P than manure, resulting in the plants being able to take in more P.

Final Soil Electrical Conductivity:

There was a statistically significant difference in the log transformed data for the ending EC for both the soil ($P < 0.001$) and rate ($P < 0.001$) effects (Table 5.14). The fine-textured soils (Nunn 0.61 dS m^{-1}

and Colby 0.66 dS m^{-1}) had a higher EC than the sandy-textured soil (Bijou 0.29 dS m^{-1}). All the soils that were used in this study were collected from field locations. The Colby and Nunn soils were collected from dryland fields and the Bijou soil was collected from an irrigated field. In addition, the higher percentage of sand in Bijou would allow for easier leaching of salts. The EC also tended to increase with higher application rates of amendment but only the highest application rate (agronomic rate of N 0.68 dS m^{-1}) was significantly different than the other two rates (0.45 dS m^{-1}). This suggests that the amendments increased the salinity. Even with the slight increases in EC in the soils, these values are not high enough to have had a negative effect on the growth of the plants.

Olsen Extractable P:

There was not a three- or four-way interaction, but there were three, two-way interactions of soil \times harvest, fertilizer \times rate, and soil \times fertilizer (Table 5.15). The fertilizer \times rate plot (Figure 5.20) showed that there was higher Olsen Extractable P for composted manure and raw manure especially at the application rate of N. Since the ratio of total N to total P was approximately the same for all the amendments, this suggests that the forms of P in manures are more available than P in biosolids. Manures have a higher amount of water extractable P than biosolids (Garcia-Albacete et al., 2012).

Since Olsen extractable P is an index of the amount of P that is in the soil solution, water extractable P would also be in this pool. Much of the P that is in biosolids is found in inorganic forms (Shober et al., 2006); and therefore, there is a slower process for P release to the soil solution. The biosolids contain higher amounts of metals that can bind with P and tie it up. There was a slight trend for a decrease in Olsen Extractable P with decreasing rates but it stayed fairly constant. Even though there was a significant interaction of harvest date \times soil series (Table 5.15) there was not a clear trend in the data (Figure 5.21). It was expected that there would be a difference between the soils due to the large differences seen in both the isotherm data and plant tissue concentrations.

The interaction plot of soil × fertilizer (Figure 5.22) showed that in general there were lower concentrations of Olsen Extractable P for the Colby soil than Nunn or Bijou. For all the soils, there was higher P concentration for the composted and raw manure treatment than for the biosolids. The compost treatment also had a higher concentration of Olsen P for the Colby soil than the other two soils potentially due to the changes in forms of nutrients during composting. Microorganisms will break down some of the more complex forms of nutrients into simpler forms and the addition of biosolids has been shown to increase microbial activity (Roig et al., 2012).

Soil Nitrate-Nitrogen and Ammonium-Nitrogen:

The soils were initially fertilized with N assuming that 20% of the organic N would mineralize and if needed additional N was added in the form of urea. There was a reduction in soil NO₃-N from harvest one to three and harvest five to seven for Bijou (Figure 5.23). Following the third harvest, N fertilizer was recalculated, and it was added after the fourth harvest. This resulted in an elevated concentration at harvest five, but this was still below the optimal concentration so additional fertilizer was added after the sixth harvest.

There was a large decrease in soil NO₃-N in the Colby soils after the first harvest (Figure 5.24). The initial sampling shows that there was little difference between the treatments, indicating that the adjustments to soil nitrogen were correct. It would be expected that with rapid growth there would be a reduction in soil N, and the data show this. This also helps to show that there was a need for continuous additions of N during the study to reduce N stress. This result highlights the added complexity of managing a fertility program if the application rate of biosolids is based on the agronomic rate of P.

For the Nunn soil series, there was an interaction between application rate × harvest date (Figure 5.25). At the first harvest, there was lower soil NO₃-N for the application rate of amendments at

the agronomic rate of N. Mineralization of the amendments did not provide the required N. In addition, the conditions in pots were optimal for microbial activity and it is possible that the microbial populations were immobilizing a large amount of the inorganic nitrogen (Havlin et al., 2005).

Ammonium-nitrogen followed a similar trend as $\text{NO}_3\text{-N}$ except there was a smaller decrease in the concentration from harvest five to harvest seven (Figure 5.26). The concentrations were also much lower than the concentrations of $\text{NO}_3\text{-N}$. The soils were fertilized during the study with ammonium nitrate and it is likely that the microbial populations used much of the ammonium (Sylvia, 2005).

Amendment P extraction:

All the amendments used in the study were extracted to determine the P concentration in an effort to correlate the concentration of P to the amount of P that the plants absorbed. There was an interaction of amendment \times extraction solution ($P < 0.001$), amendment \times ratio ($P < 0.001$), and extraction solution \times ratio ($P < 0.001$, Table 5.16). The interaction of extraction ratio and extraction solution always had a significantly higher extraction with the 1:200 ratio (Figure 5.29). There was also a significantly higher extraction with the 1:200 ratio for all of the biosolids but not for the manures (Figure 5.30). The extraction methods create a liquid phase (extraction solution) at equilibrium with the solid phase (the amendment). As a result, by having a large amount of extracting solution it would be possible to extract more P on a mass basis. No consistent trends were observed for an extraction solution that extracted the most P (Figure 5.31). These results may indicate that there is a difference in the P forms present in the different amendments.

For the manures, there was a smaller amount of P extracted overall, but the amount of P extracted was the same with the different ratios for the same amendment. Since biosolids have a larger $P_{\text{inorganic}}:P_{\text{organic}}$ ratio than manure (Garcia-Albacete et al., 2012) the extraction ratio had less of an effect on manure. The method used to analyze P did not determine organic P so it does not have an effect on

the P measured. Also, with a lower concentration of inorganic P there is less chance to saturate the amount of P in the extraction solution. The majority of the soluble P forms have a relatively high solubility constant (Tunesi et al., 1999) leading to it to be more possible to saturate the extraction solution with the high P amendments.

Conclusions:

It was expected that there would be a difference in plant uptake between fertilizer sources due to the results of the extraction of the amendments showing that there was a difference between the amendments. There were also a trends showing differences in uptake of P between fertilizer sources. This may lead one to believe that different amendments should be treated differently. However, the plant uptake data does not support this theory. The results of the tissue analysis did not show a fertilizer effect on the plant P concentrations. The data shows that soil properties are more important in predicting P uptake and specifically a soil's ability to adsorb P.

The interaction between soil adsorption or transformation of P and the amount of P that plants can remove needs to be determined prior to amendment application. However, the design of this study was not set up in a way to develop a conclusion about how this interaction works. It was expected that the differences in the amount of P supplied by amendments would be greater than the soil-type effect. Previous work with biosolids from the eastern United States (O'Connor et al., 2004) shows that biosolids produced from different wastewater treatment plants act differently and it was expected that similar results would be seen in this study. However, in this study there was not a significant effect of amendment as seen in the eastern United States. This suggest that it may be due to the high amounts of Ca in the soils used. Many of the differences seen in plant uptake of P showed that there was a difference in the amount of soluble P between the soils that was due to texture, but this could have also been related to the amount of Ca in the soils (Table 5.2). When comparing the P uptake by harvest

between soils (Figures 5.5-6), there were three very different uptake patterns between the different soils. Bijou had a rapid increase and then it began to decline, Colby was fairly constant, and Nunn continued to increase with each harvest. There are probably many factors that were controlling these different uptake patterns including surface area, Ca concentration, P sorption. Further work will be needed to evaluate a larger number of soils that have a range of Ca concentrations.

Table 5.1. Properties of the amendments that were used in a greenhouse study to investigate plant uptake of P from different amendments.

	Composted Manure	Manure	Fort Collins	Littleton/ Englewood	Metro Wastewater
Organic N, % N	1.10	1.67	5.55	5.19	5.63
Organic N, dry basis	11000	16700	55500	51900	56300
Ammonium, % N	0.014	0.043	1.03	0.589	0.890
Ammonium N, mg kg ⁻¹ dry basis	150	450	10400	5900	8900
Nitrate N, mg kg ⁻¹ dry basis	650	0	0	0	0
Total N, %	1.18	1.72	6.58	5.78	6.52
Phosphorus, % P ₂ O ₅	1.20	1.16	6.72	6.49	5.12
Phosphorus P ₂ O ₅ , mg kg ⁻¹ dry basis	12100	11600	67200	64900	51200
K, % K ₂ O	2.13	2.49	0.33	0.20	0.30
K, K ₂ O mg kg ⁻¹ dry basis	21300	24900	3250	1950	3050
Sulfur, %	0.36	0.45	1.15	1.47	1.35
Sulfur, mg kg ⁻¹ dry basis	3550	4450	11600	14700	13500
Calcium, mg kg ⁻¹ dry basis	21600	24200	28000	48700	29200
Magnesium, mg kg ⁻¹ dry basis	8650	7750	10900	4600	4350
Sodium, mg kg ⁻¹ dry basis	4000	2150	550	1000	1000
Zinc, mg kg ⁻¹ dry basis	200	150	550	850	600
Iron, mg kg ⁻¹ dry basis	9900	6900	11500	17600	11500
Manganese, mg kg ⁻¹ dry basis	300	250	150	450	250
Copper, mg kg ⁻¹ dry basis	50	50	500	800	600
Aluminum, mg kg ⁻¹ dry basis	2500	2250	1500	1700	1700
Soluble Salts, dS m ⁻¹	30.02	45.00	34.97	23.76	33.79
pH	9.2	7.2	6.5	7.1	6.5

Table 5.2. Properties of the soils that were used in a greenhouse study to investigate plant uptake of different amendments.

	Colby	Nunn	Bijou
1:1 Soil pH	7.50	8.07	6.70
1:1 S Salts mmho cm ⁻¹	0.24	0.61	0.31
Excess CaCO ₃	None	High	None
Organic Matter, LOI %	2.13	3.40	1.17
Nitrate-N, mg kg ⁻¹ N	9.93	23.03	3.00
Olsen P, mg kg ⁻¹ P	4.35	6.50	8.45
Potassium, mg kg ⁻¹ K	571	526	509
Sulfate, mg kg ⁻¹ S	9.67	17.7	10.7
Zinc, mg kg ⁻¹ Zn	1.27	2.14	2.02
Iron, mg kg ⁻¹ Fe	6.83	10.8	41.2
Manganese, mg kg ⁻¹ Mn	7.37	14.1	22.6
Copper, mg kg ⁻¹ Cu	2.80	1.83	1.62
Calcium, mg kg ⁻¹ Ca	2240	5610	709
Magnesium, mg kg ⁻¹ Mg	306	772	154
Sodium, mg kg ⁻¹ Na	5.33	53.3	11.0
CEC, Sum of Cations meq 100g ⁻¹	15.2	36.1	6.23
%H Sat	0.00	0.00	1.00
%K Sat	9.67	4.00	21.0
%Ca Sat	73.3	77.0	56.3
%Mg Sat	17.0	18.0	20.7
%Na Sat	0.00	1.00	1.00

Table 5.3. ANOVA table for biomass produced from a greenhouse study to investigate plant uptake of P from different fertilizer sources and rates.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Soil	2	1	46.9	0.103
Fertilizer	4	1	1.54	0.535
Soil*Fertilizer	8	1	1.77	0.526
Rate	2	1	0.99	0.580
Soil*Rate	4	1	3.06	0.402
Fertilizer*Rate	8	1	2.89	0.427
Soil*Fertilizer*Rate	16	1	1.90	0.521
Harvest	6	1	651	0.030
Soil*Harvest	12	1	22.2	0.165
Fertilizer*Harvest	24	1	1.69	0.551
Soil*Fertili*Harvest	48	1	0.97	0.685
Rate*Harvest	12	1	2.01	0.506
Soil*Rate*Harvest	24	1	1.86	0.523
Fertili*Rate*Harvest	48	1	1.36	0.605
Soil*Fert*Rate*Harve	96	1	0.85	0.719

Table 5.4. Fit parameters of the sorption isotherms for three different soils that were used in a greenhouse study investigating plant available P from five different amendments at varying rates.

Isotherm	Bijou		Colby		Nunn	
	SSQ	R ²	SSQ	R ²	SSQ	R ²
Langmuir	0.004	0.982	0.001	0.995	0.011	0.983
Freundlich	0.028	0.970	0.040	0.880	0.188	0.787
Linear	0.004	0.982	0.002	0.995	0.049	0.945

Table 5.5. Type III ANOVA table for plant tissue P concentration. Results that were significant at a P value of 0.05 are bolded.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Soil	2	937	600	<0.001
Fertilizer	4	937	11.5	<0.001
Soil*Fertilizer	8	937	8.33	<0.001
Rate	2	937	68.7	<0.001
Soil*Rate	4	937	18.1	<0.001
Fertilizer*Rate	8	937	6.94	<0.001
Soil*Fertilizer*Rate	16	937	2.13	0.006
Harvest	6	937	35.6	<0.001
Soil*Harvest	12	937	58.7	<0.001
Fertilizer*Harvest	24	937	1.53	0.050
Soil*Fertili*Harvest	48	937	1.06	0.362
Rate*Harvest	12	937	1.77	0.048
Soil*Rate*Harvest	24	937	2.05	0.002
Fertili*Rate*Harvest	48	937	1.08	0.338
Soil*Fert*Rate*Harve	96	937	0.79	0.926

Table 5.6. Type III ANOVA table for plant tissue P concentration for Bijou soil. Results that were significant at a P value of 0.05 are bolded.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Fertilizer	4	310	2.59	0.037
Rate	2	310	21.8	<0.001
Fertilizer*Rate	8	310	3.09	0.002
Harvest	6	310	44.8	<0.001
Fertilizer*Harvest	24	310	1.24	0.207
Rate*Harvest	12	310	3.31	<0.001
Fertili*Rate*Harvest	48	310	0.66	0.959

Table 5.7. Type III ANOVA table for plant tissue P concentration for Colby soil. Results that were significant at a P value of 0.05 are bolded.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Fertilizer	4	312	6.83	<0.001
Rate	2	312	89.0	<0.001
Fertilizer*Rate	8	312	3.45	0.001
Harvest	6	312	22.7	<0.001
Fertilizer*Harvest	24	312	0.62	0.921
Rate*Harvest	12	312	0.61	0.831
Fertili*Rate*Harvest	48	312	1.17	0.214

Table 5.8. Type III ANOVA table for plant tissue P concentration for Nunn soil. Results that were significant at a P value of 0.05 are bolded.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Fertilizer	4	309	21.8	<0.001
Rate	2	309	10.3	<0.001
Fertilizer*Rate	8	309	5.14	<0.001
Harvest	6	309	88.9	<0.001
Fertilizer*Harvest	24	309	1.78	0.015
Rate*Harvest	12	309	1.30	0.215
Fertili*Rate*Harvest	48	309	1.04	0.401

Table 5.9. Type III ANOVA table for mass of P taken up by plants grown in Colby soil. Bold effects were significant at an alpha value of 0.05

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Fertilizer	4	78.0	1.46	0.223
Rate	2	101	26.5	<0.001
Fertilizer*Rate	8	97.0	4.83	<0.001
Harvest	6	256	44.1	<0.001
Fertilizer*Harvest	24	275	0.94	0.553
Rate*Harvest	12	273	2.79	0.001
Fertili*Rate*Harvest	48	273	0.95	0.566

Table 5.10. Type III ANOVA table for mass of P taken up by plants grown in Nunn soil. Bold effects were significant at an alpha value of 0.05

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Fertilizer	4	58.2	1.43	0.237
Rate	2	82.3	2.53	0.086
Fertilizer*Rate	8	77.8	1.13	0.353
Harvest	6	255	146	<0.001
Fertilizer*Harvest	24	271	1.42	0.096
Rate*Harvest	12	272	2.23	0.011
Fertili*Rate*Harvest	48	271	0.78	0.853

Table 5.11. Type III ANOVA table for mass of P taken up by plants grown in Bijou soil. Bold effects were significant at an alpha value of 0.05

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Fertilizer	4	62.6	1.02	0.402
Rate	2	87	21.0	<0.001
Fertilizer*Rate	8	82.5	2.62	0.013
Harvest	6	258	75.9	<0.001
Fertilizer*Harvest	24	274	1.54	0.056
Rate*Harvest	12	275	2.55	0.003
Fertili*Rate*Harvest	48	273	0.74	0.891

Table 5.12. Type III results for the log transformed data of root mass following a greenhouse study with different rates of application of five different fertilizer sources.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Soil	2	135	15.9	<0.001
Fertilizer	4	135	1.33	0.261
Soil*Fertilizer	8	135	1.85	0.074
Rate	2	135	1.05	0.352
Soil*Rate	4	135	0.70	0.593
Fertilizer*Rate	8	135	2.12	0.038
Soil*Fertilizer*Rate	16	135	1.15	0.315

Table 5.13. Type III results for the log transformed data of root P concentration following a greenhouse study with different rates of application of five different fertilizer sources.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Soil	2	135	16.32	<0.001
Fertilizer	4	135	23.6	<0.001
Soil*Fertilizer	8	135	1.03	0.415
Rate	2	135	157	<0.001
Soil*Rate	4	135	7.75	<0.001
Fertilizer*Rate	8	135	1.04	0.407
Soil*Fertilizer*Rate	16	135	0.39	0.982

Table 5.14. Type III results for the log transformed data of ending soil EC following a greenhouse study with different rates of application of five different fertilizer sources.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Soil	2	133	26.7	<0.001
Fertilizer	4	133	1.52	0.201
Soil*Fertilizer	8	133	1.38	0.210
Rate	2	133	8.36	0.004
Soil*Rate	4	133	1.72	0.149
Fertilizer*Rate	8	133	0.46	0.880
Soil*Fertilizer*Rate	16	133	0.94	0.524

Table 5.15. ANOVA table for the results of the log transformed Olsen Extractable data following the completion of a greenhouse study on plant available P from 5 amendments at 3 different application rates.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Soil	2	234	80.8	<0.001
Fertilizer	4	250	99.5	<0.001
Soil*Fertilizer	8	249	6.59	<0.001
Rate	2	322	307	<0.001
Soil*Rate	4	319	17.0	<0.001
Fertilizer*Rate	8	307	36.7	<0.001
Soil*Fertilizer*Rate	16	308	1.59	0.069
Harvest	6	767	25.1	<0.001
Soil*Harvest	12	809	2.62	0.002
Fertilizer*Harvest	24	845	0.67	0.883
Soil*Fertili*Harvest	48	850	1.02	0.437
Rate*Harvest	12	827	0.83	0.618
Soil*Rate*Harvest	24	857	0.58	0.948
Fertili*Rate*Harvest	48	864	0.54	0.996
Soil*Fert*Rate*Harve	96	851	0.72	0.978

Table 5.16. Type III ANOVA for the extraction of P from the amendments used in the greenhouse study.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Source	4	60	601	<0.001
Extractant	2	60	211	<0.001
Source*Extractant	8	60	39.7	<0.001
Ratio	1	60	68.5	<0.001
Source*Ratio	4	60	9.59	<0.001
Ratio*Extractant	2	60	4.73	0.012
Source*Ratio*Extract	8	60	0.87	0.550

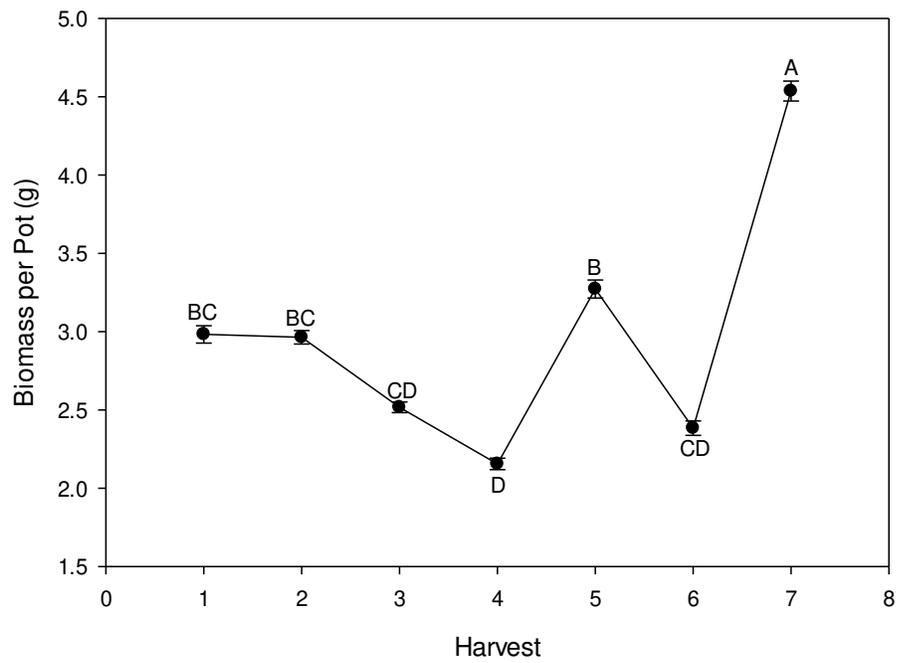


Figure 5.1. Biomass production from each harvest during a greenhouse study to investigate plant uptake of P from different fertilizers at different application rates.

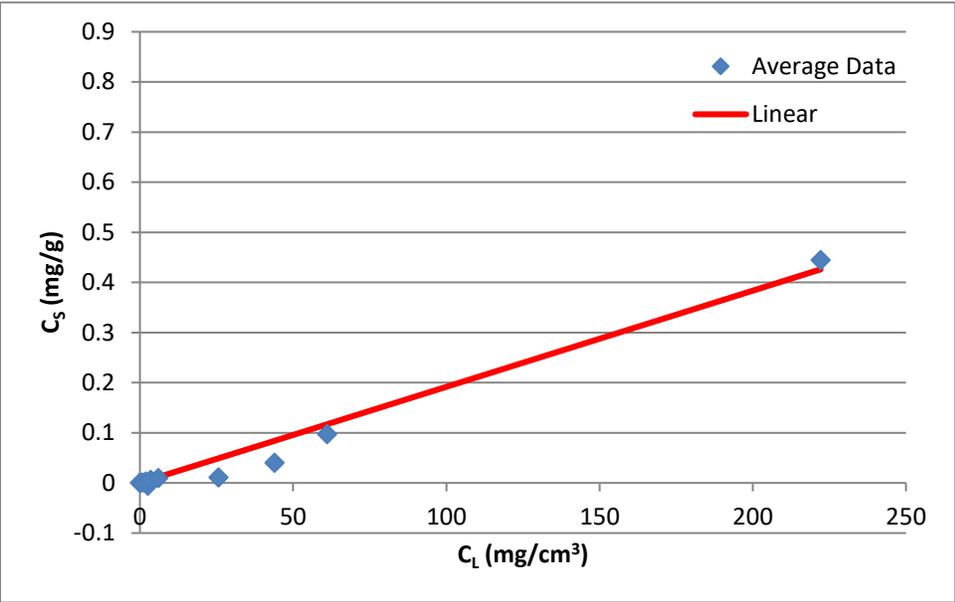


Figure 5.2. Linear isotherm of P sorption to the Bijou soil.

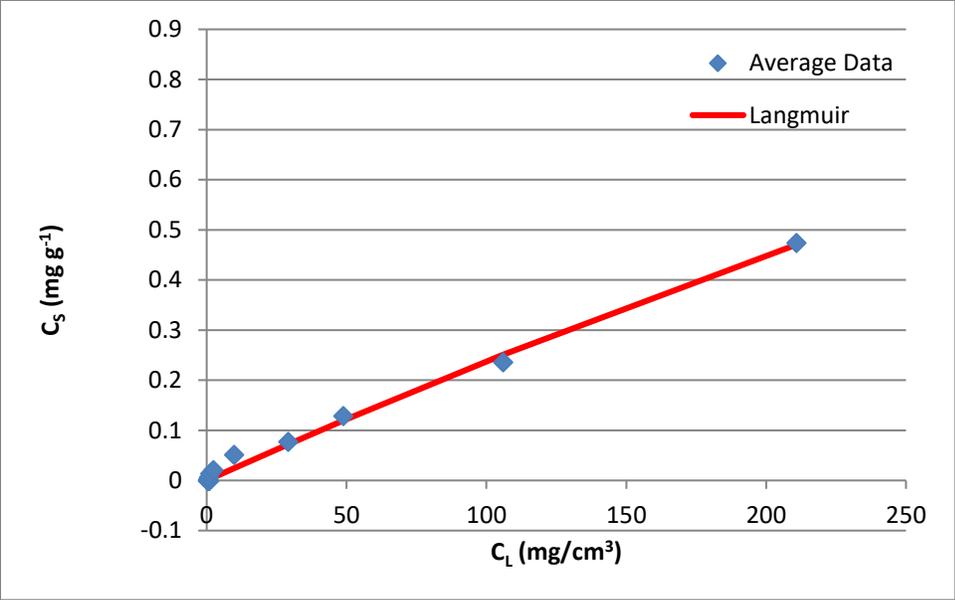


Figure 5.3. Langmuir isotherm of P sorption to Colby soil.

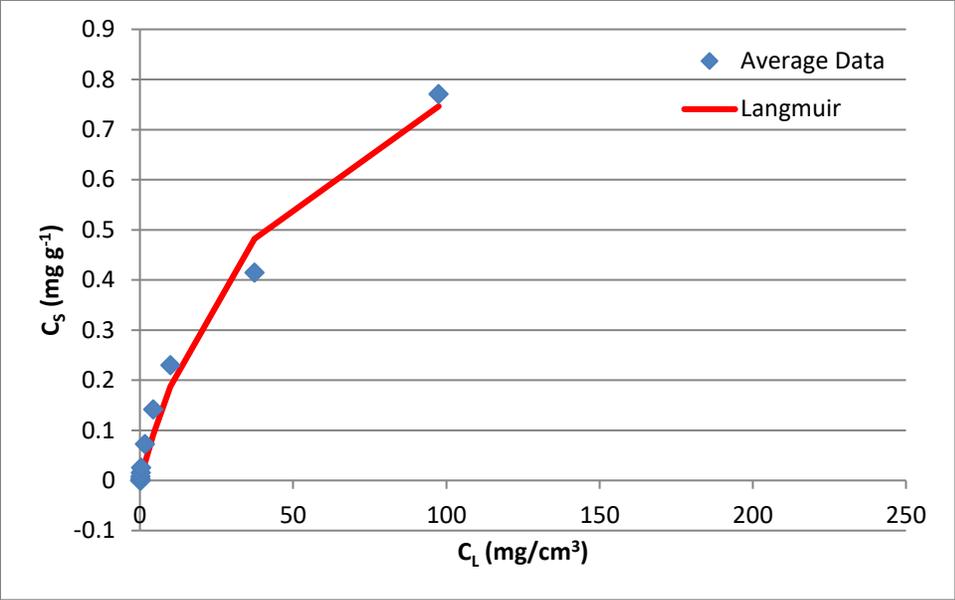


Figure 5.4. Isotherm of P sorption to the Nunn soil.

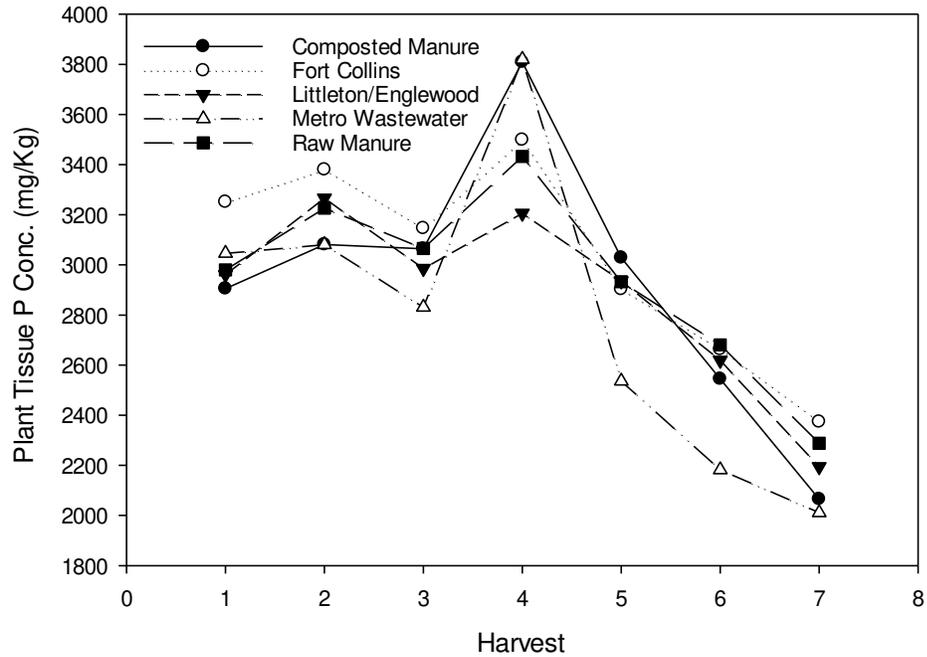


Figure 5.5. Interaction plot of amendment source and harvest on plant tissue P concentration grown in soil from the Bijou soil series. The harvest occurred three weeks apart in a study to investigate the uptake of P by sorghum-sudan grass.

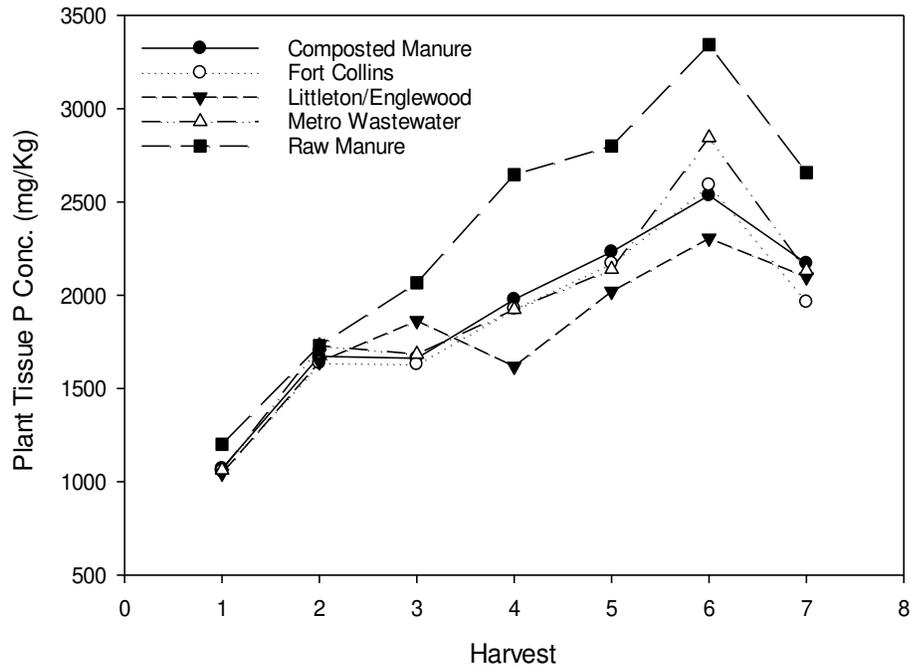


Figure 5.6. Interaction plot of amendment source and harvest on plant tissue P concentration grown in soil from the Nunn soil series. The harvest occurred three weeks apart in a study to investigate the uptake of P by sorghum-sudan grass.

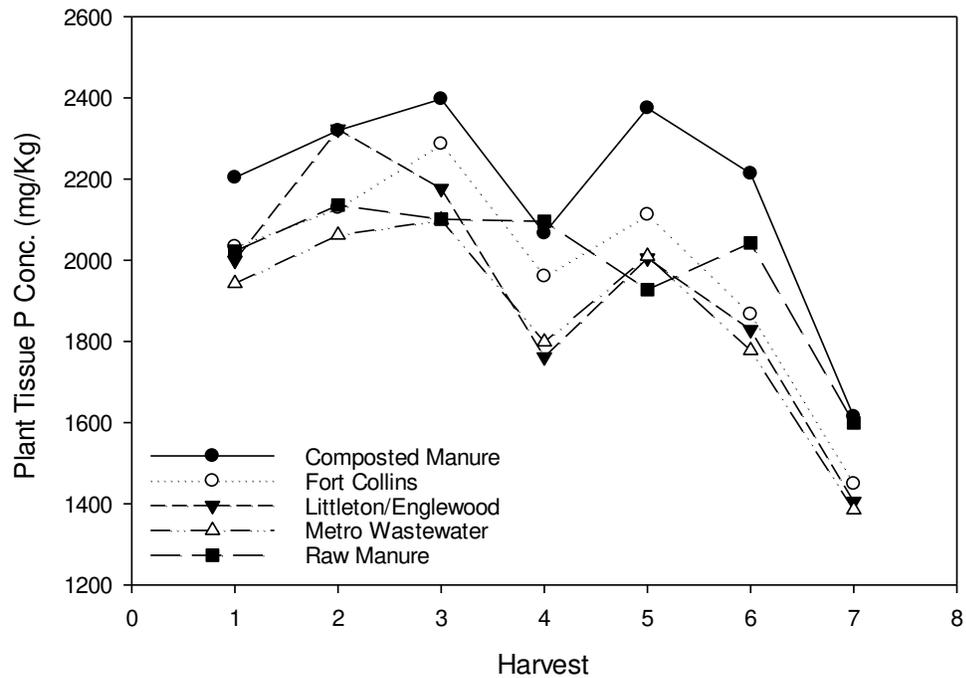


Figure 5.7. Interaction plot of amendment source and harvest on plant tissue P concentration grown in soil from the Colby soil series. The harvest occurred three weeks apart in a study to investigate the uptake of P by sorghum-sudan grass.

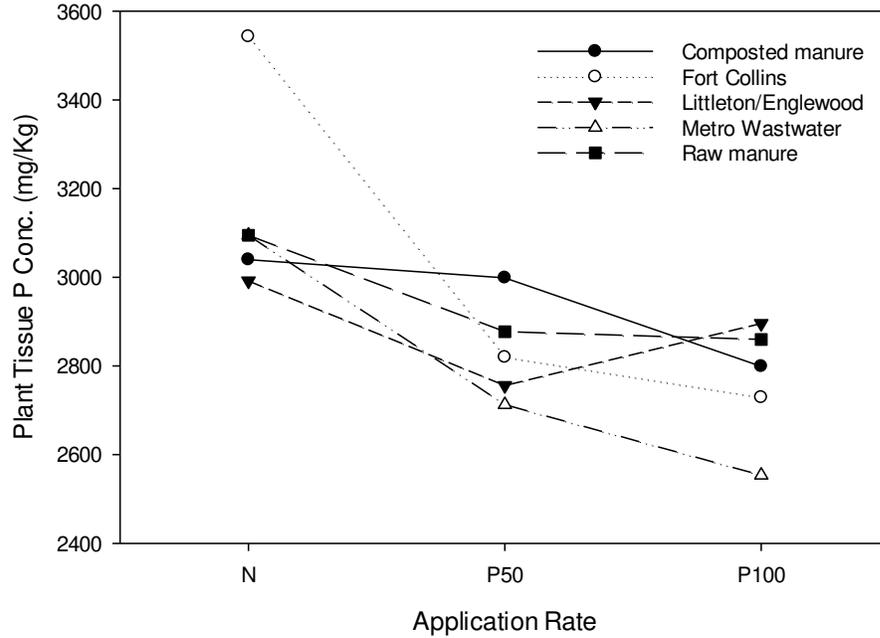


Figure 5.8. Interaction plot of amendment source and application rate on plant tissue P concentration grown in soil from the Bijou soil series. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

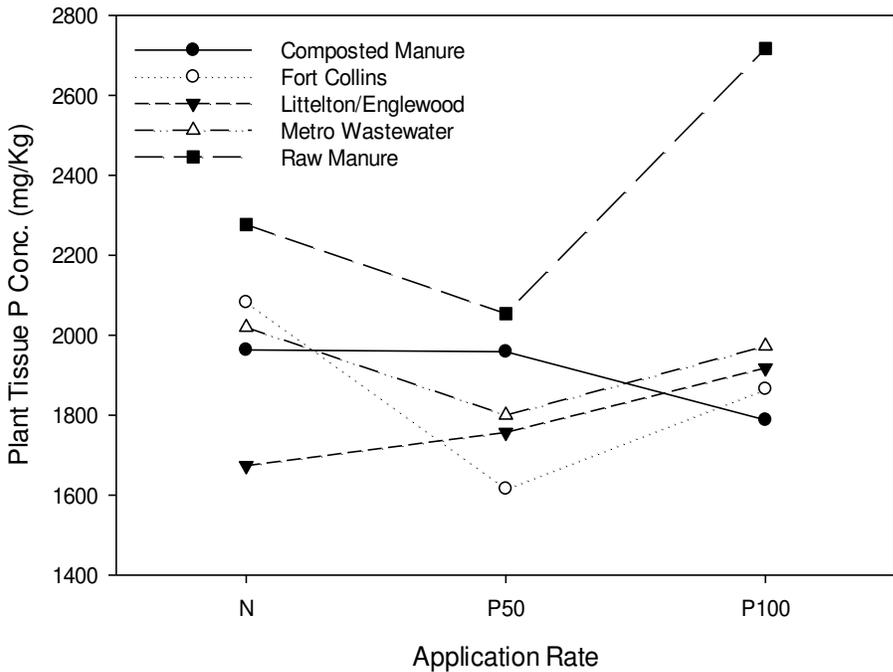


Figure 5.9. Interaction plot of amendment source and application rate on plant tissue P concentration grown in soil from the Nunn soil series. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

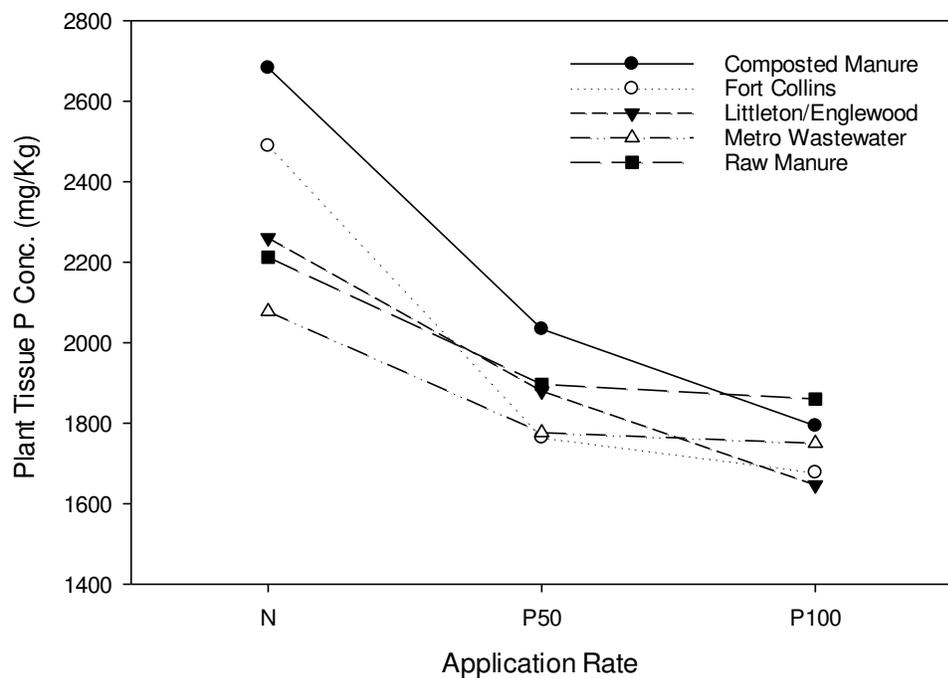


Figure 5.10. Interaction plot of amendment source and application rate on plant tissue P concentration grown in soil from the Colby soil series. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

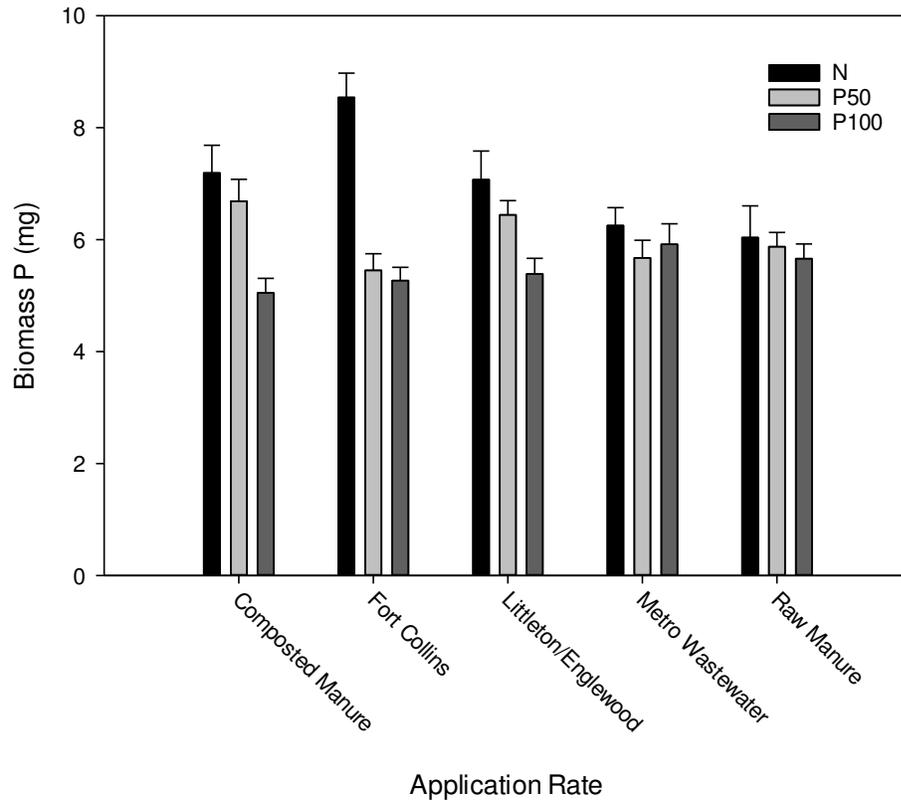


Figure 5.11. Interaction of application rate and amendment sources for Colby. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

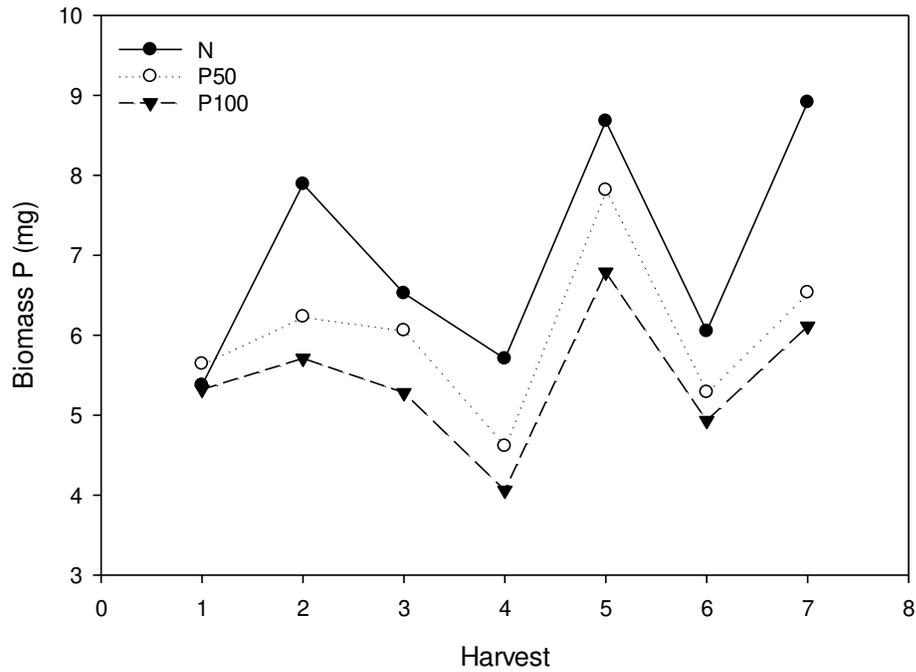


Figure 5.12. Interaction of application rate across harvest averaged over all fertilizer sources for Colby. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

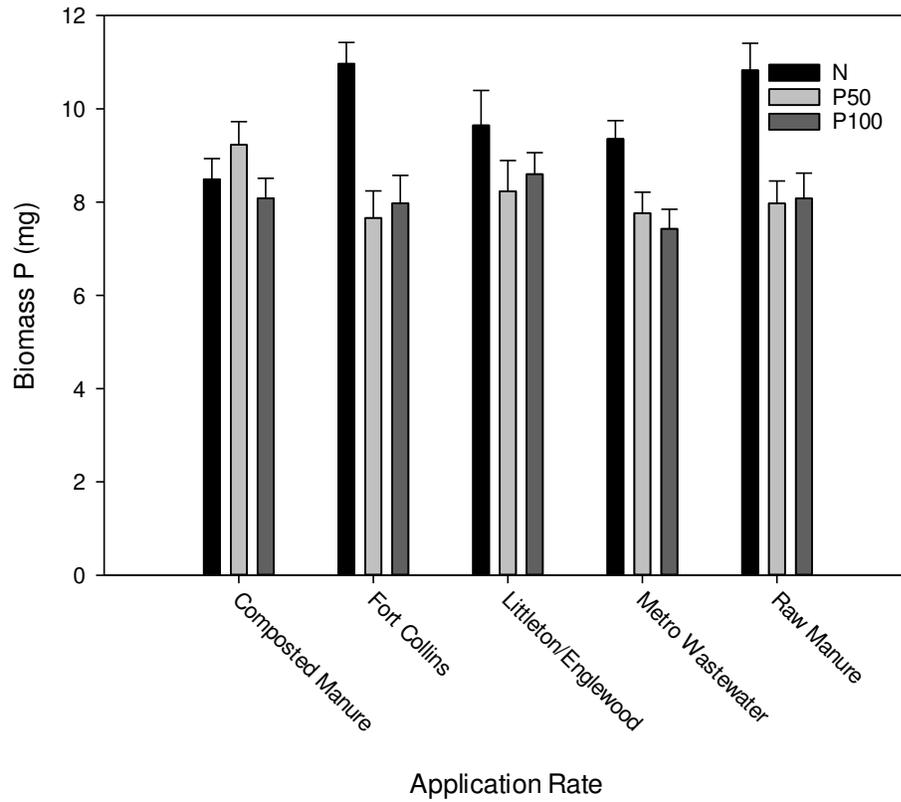


Figure 5.13. Interaction of application rate and fertilizer sources for Bijou soil. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

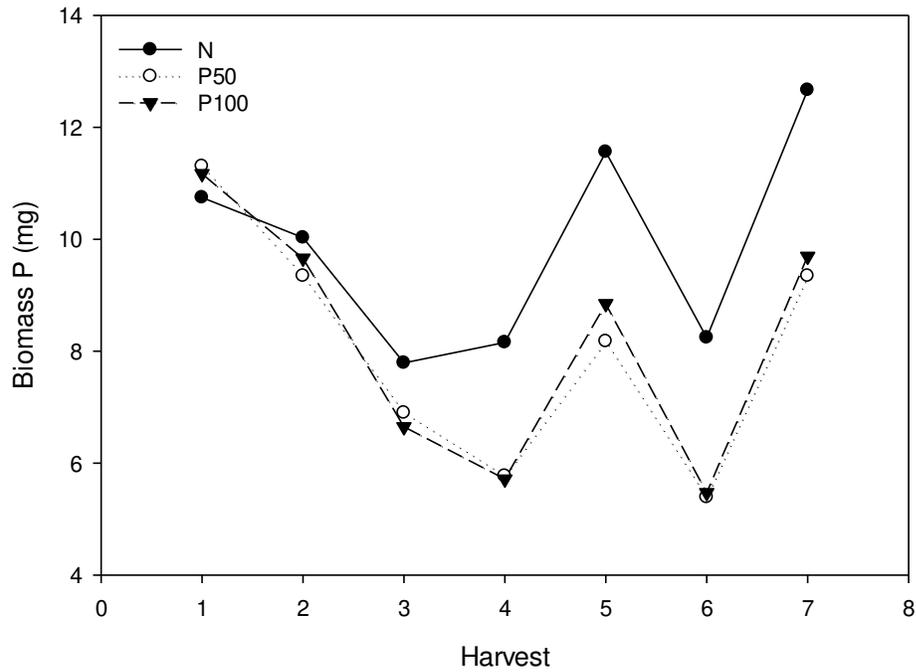


Figure 5.14. Interaction of application rate and harvest for Bijou soil. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

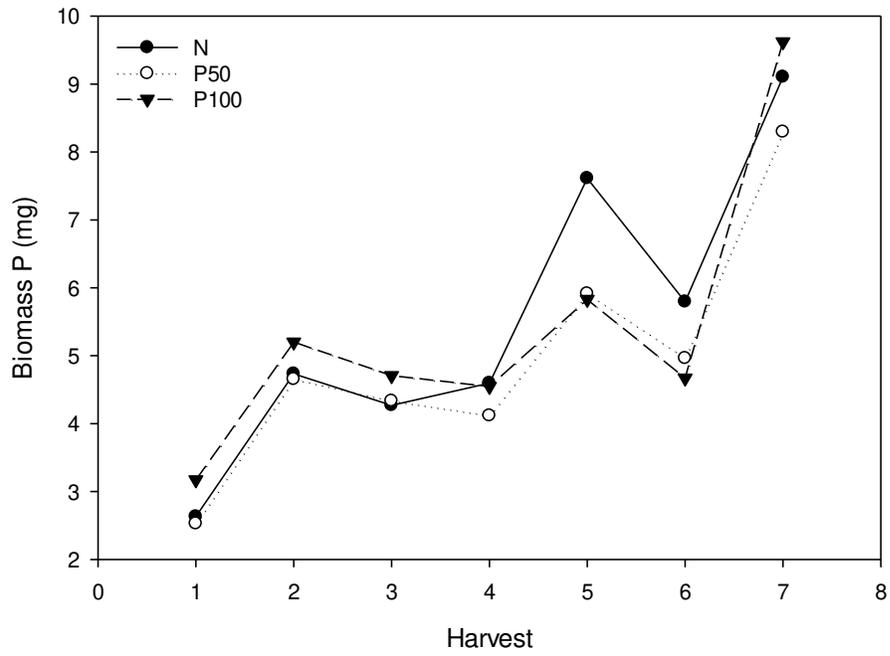


Figure 5.15. Nunn biomass P harvest by rate. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

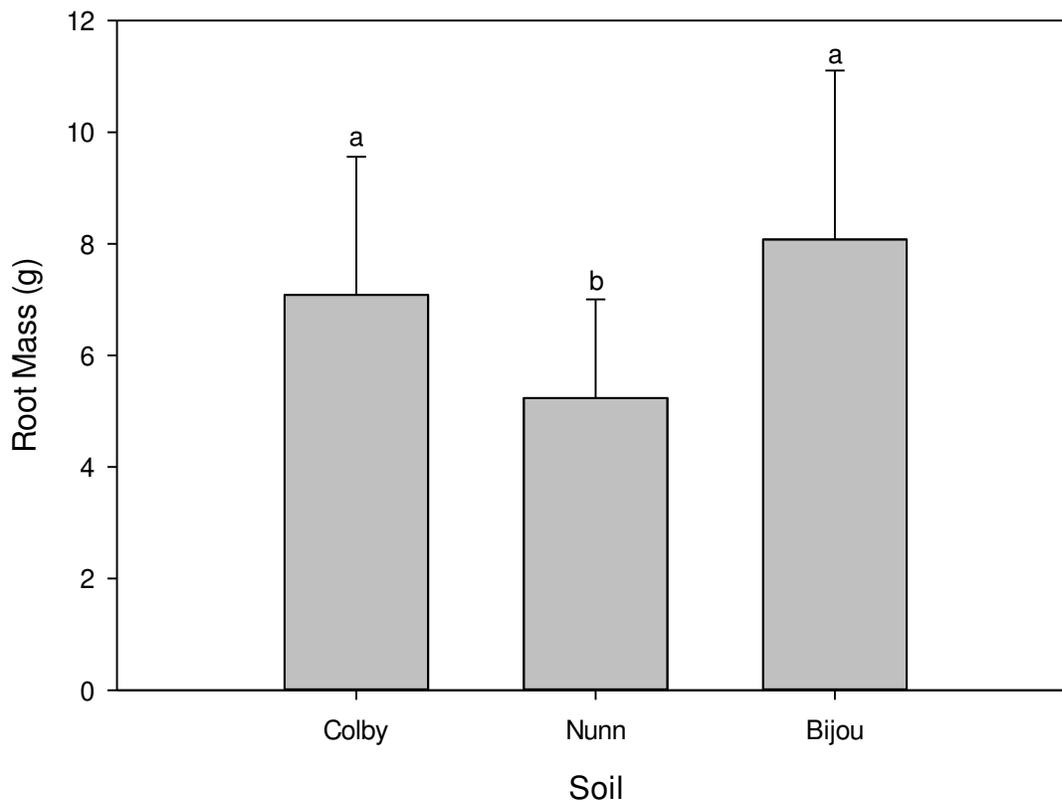


Figure 5.16. Effect of soil series on root mass at the end of the study. Colby and Bijou produced the largest amount of root mass per pot at the end of the study. Soils with different letters are significantly different at an alpha value of 0.5.

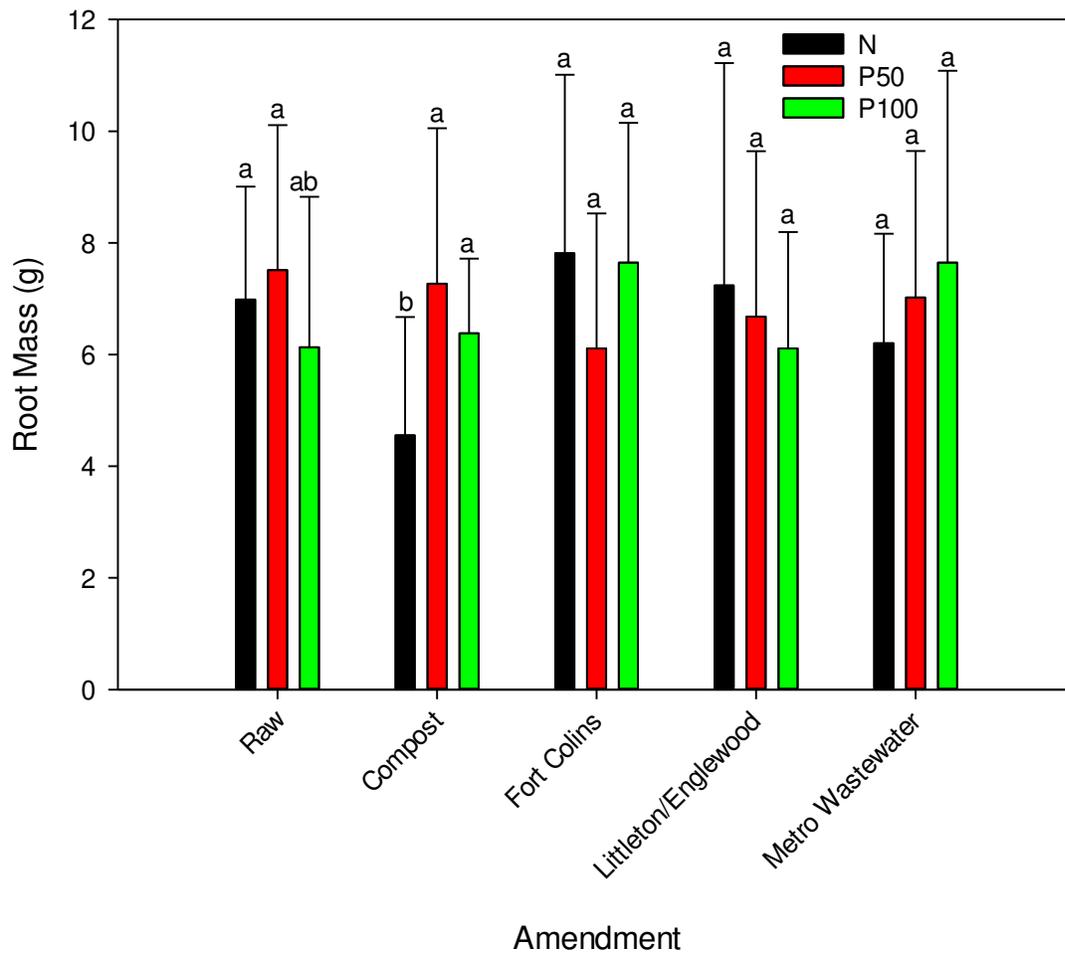


Figure 5.17. Interaction of amendment source and application rate on root mass per pot. Amendments with different letters are significantly different at an alpha value of 0.5. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

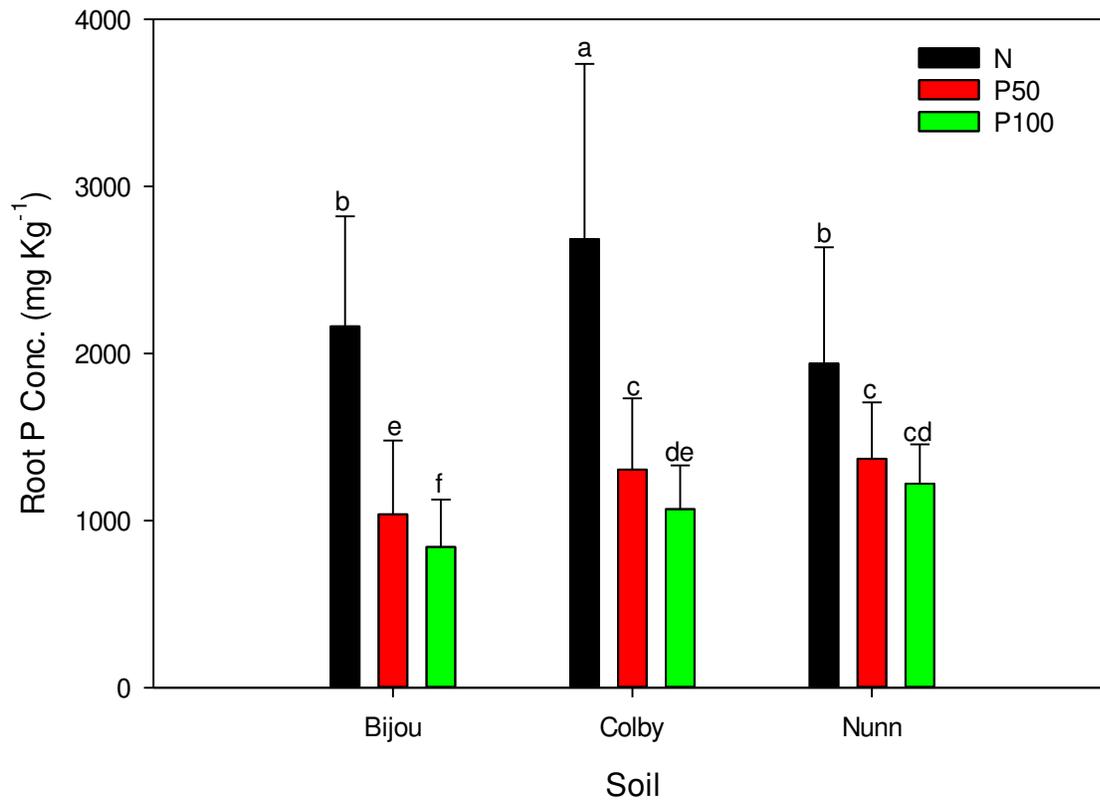


Figure 5.18. Interaction of soil series and application rate on root P concentration. Soils with different letters are significantly different at an alpha value of 0.5. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

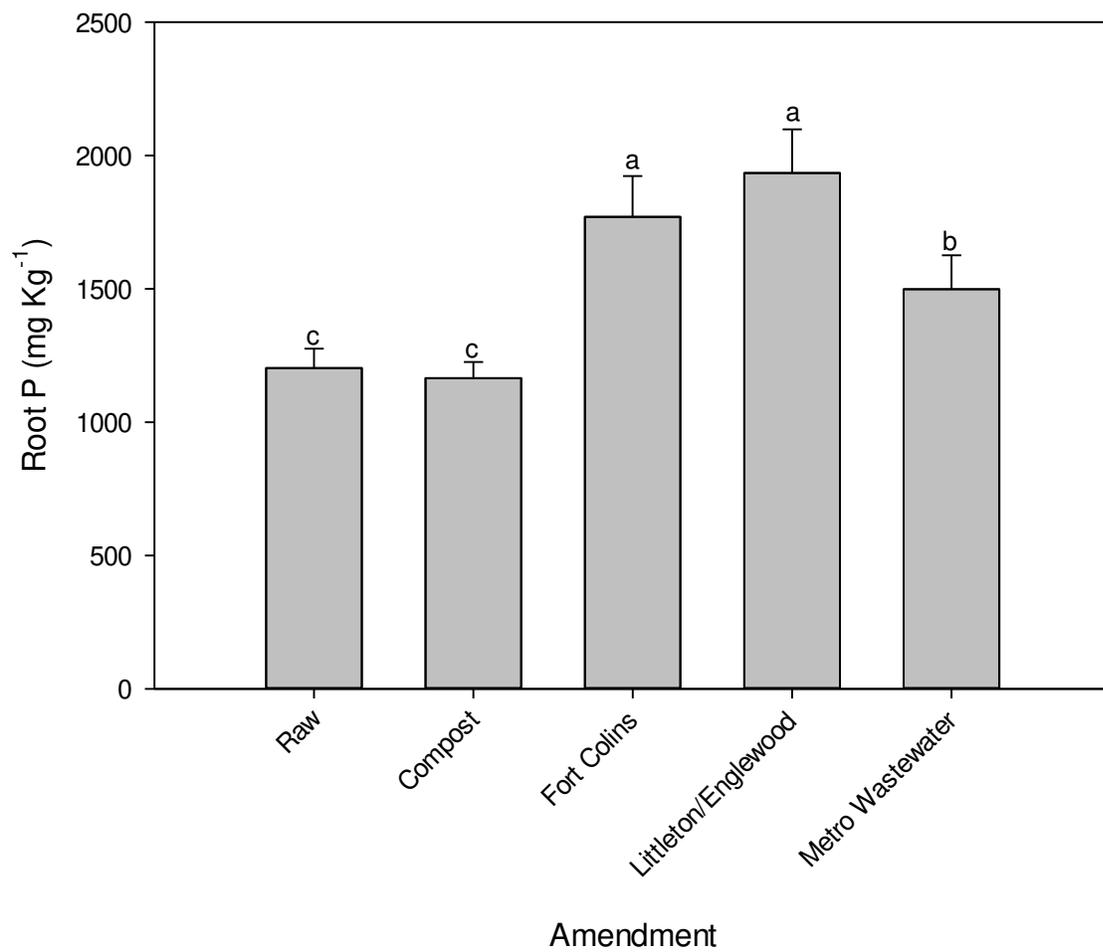


Figure 5.19. Effect of amendment source on root P concentration. Amendments with different letters are significantly different at an alpha value of 0.5.

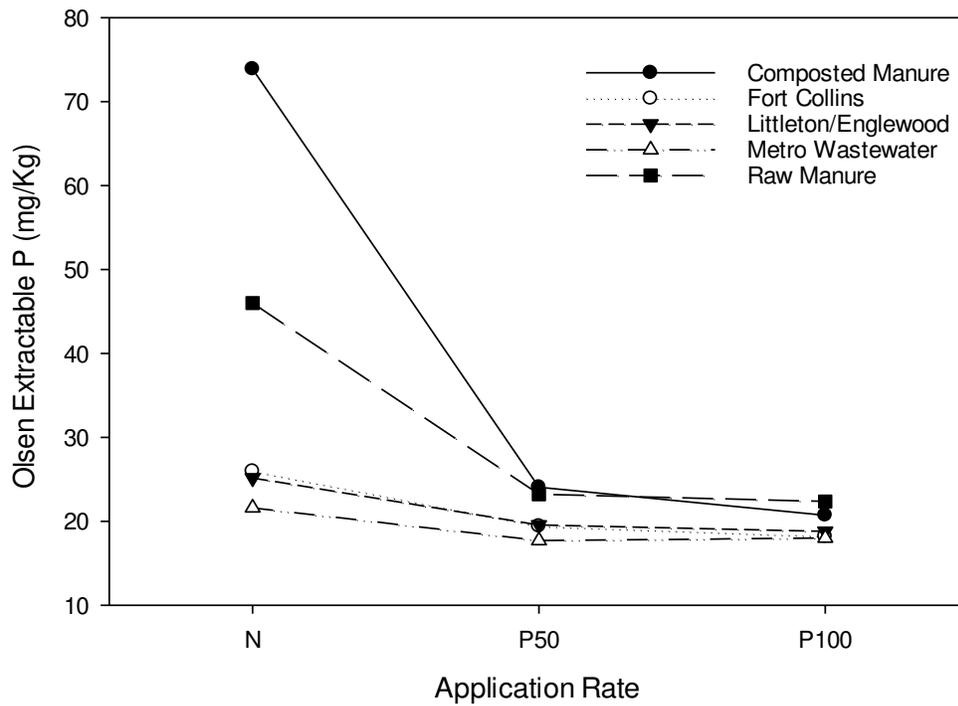


Figure 5.20. Interaction of application rate and fertilizer sources on Olsen Extractable P from a greenhouse study. There were five fertilizer sources that were added at three rates to investigate what effect on plant availability. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

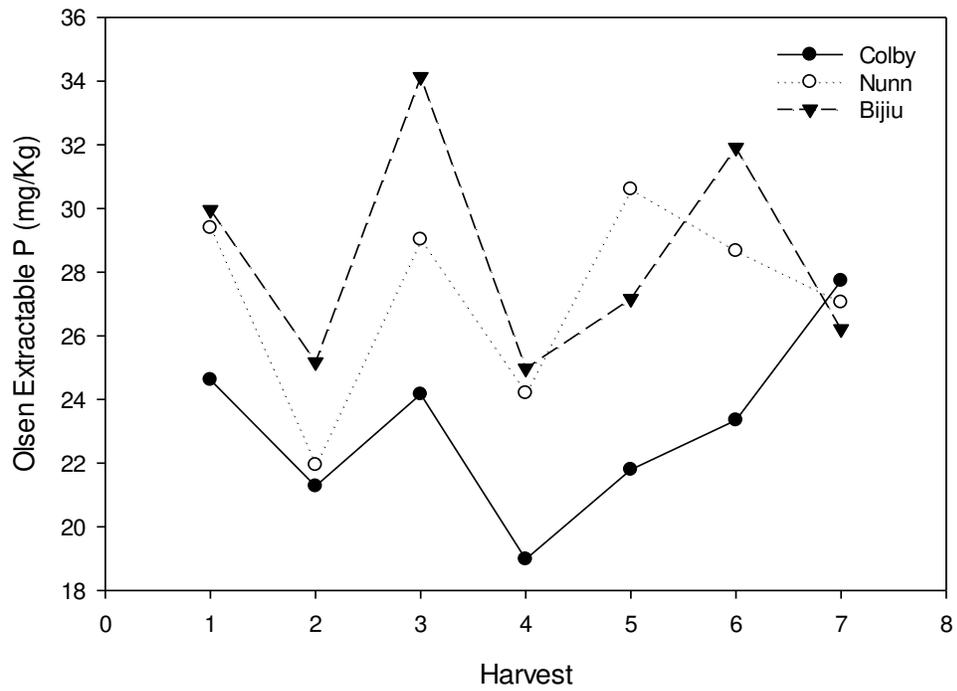


Figure 5.21. Interaction of soil series and harvest on Olsen Extractable P from a greenhouse study. There were five fertilizer sources that were added at three rates to investigate what effect on plant availability. Each of the harvest occurred 3 weeks apart.

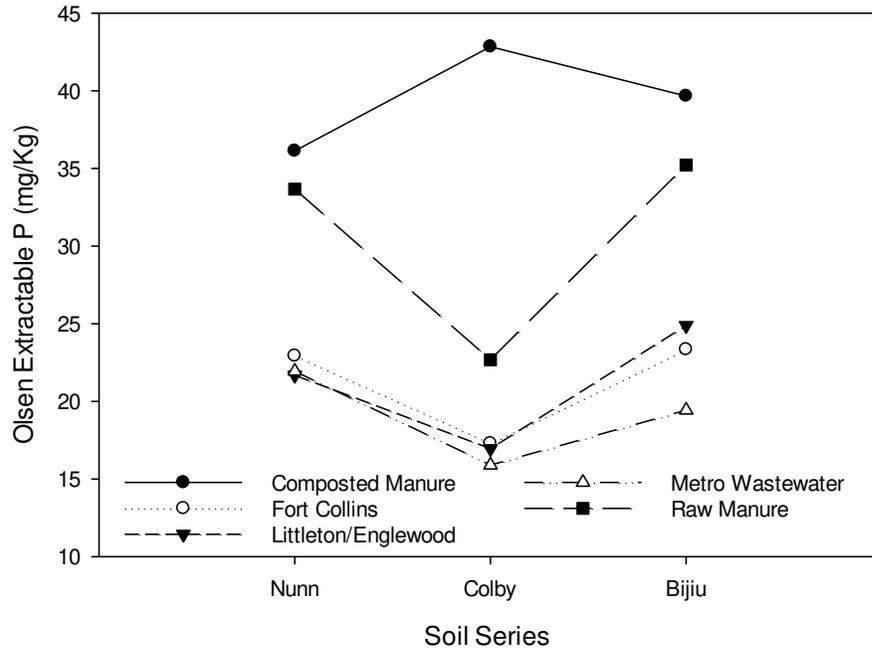


Figure 5.22. Interaction of soil series and harvest on Olsen Extractable P from a greenhouse study. There were five fertilizer sources that were added at three rates to investigate what effect on plant availability.

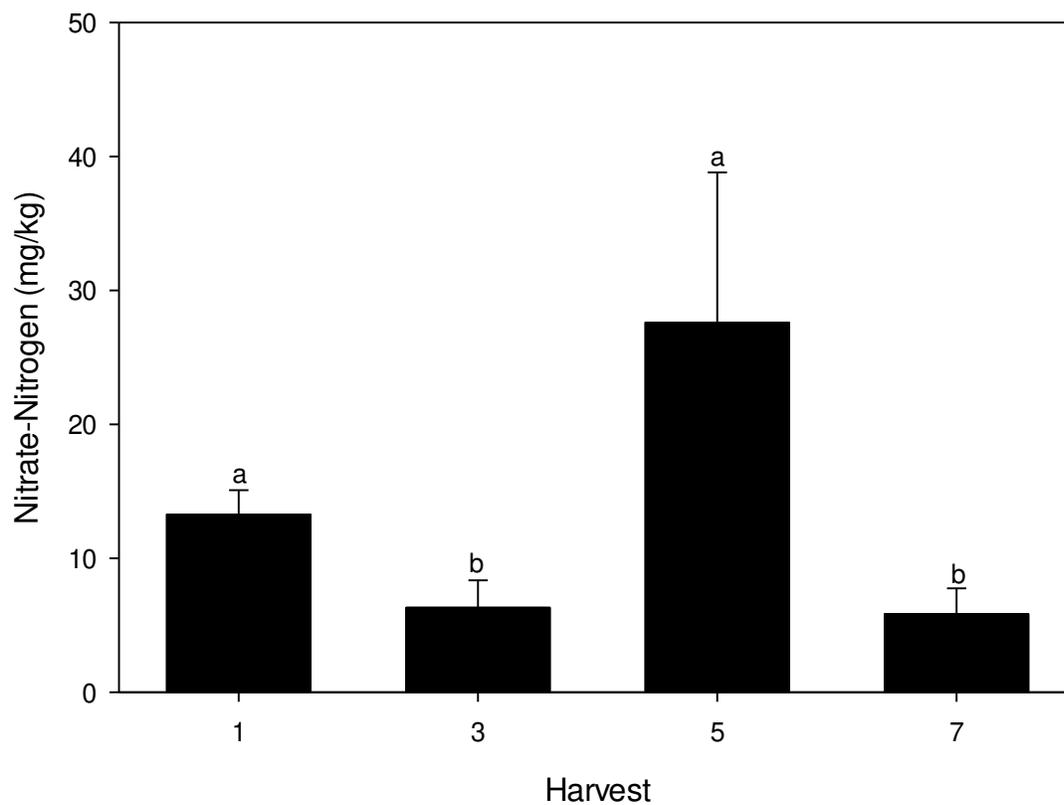


Figure 5.23. Soil nitrate-nitrogen concentration for the Bijou soils series over 7 harvests of biomass from Sorghum-Sudan grass from a greenhouse study. Values with different letters are significantly different within a particular soil at an alpha value of 0.05.

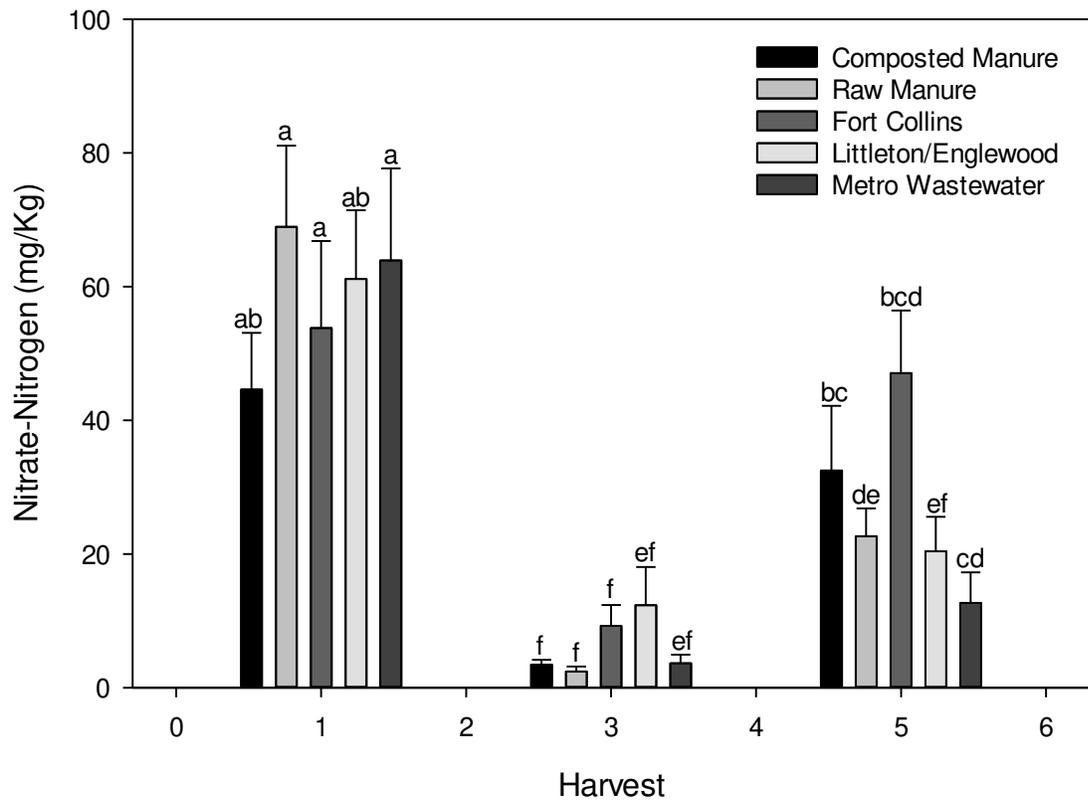


Figure 5.24. Interaction of harvest and amendment source on soil nitrate-nitrogen concentration for the Colby soils series over 7 harvests of biomass from Sorghum-Sudan grass from a greenhouse study. Values with different letters are significantly different within a particular soil at an alpha value of 0.05.

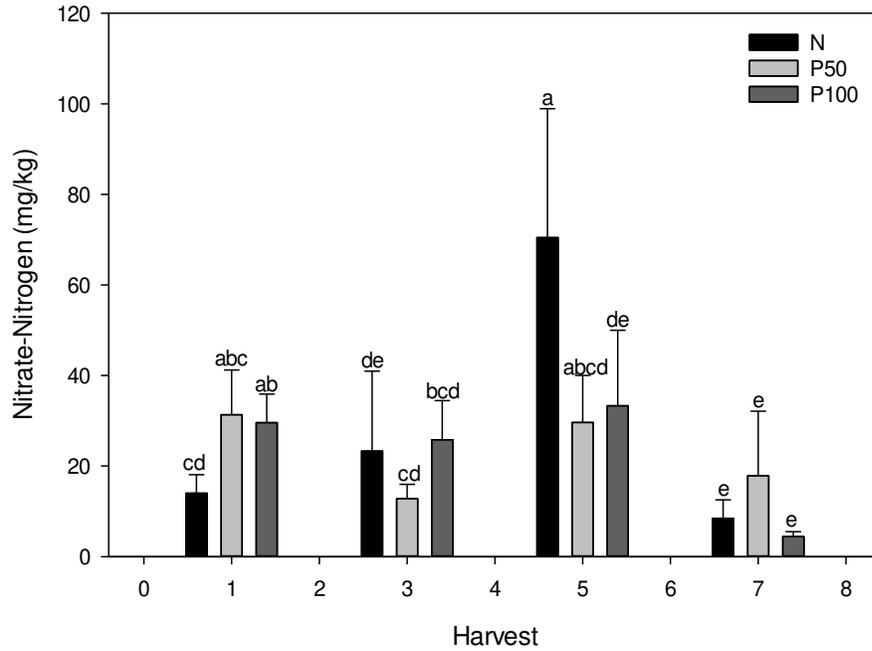


Figure 5.25. Soil nitrate-nitrogen concentration for Nunn soil at 3 application rates over 7 harvests of biomass from Sorghum-Sudan grass from a greenhouse study. Values with different letters are significantly different at an alpha value of 0.05. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

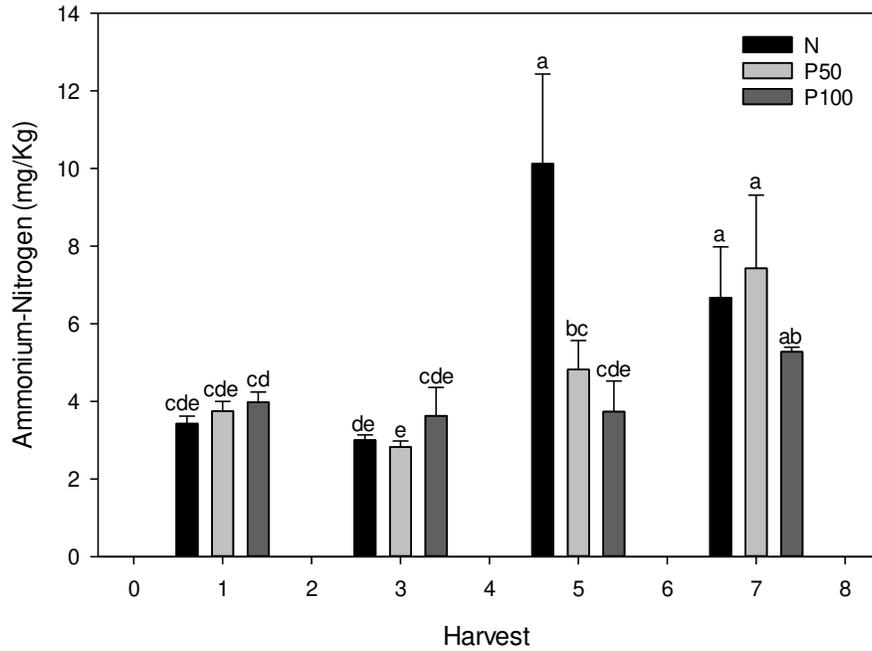


Figure 5.26. Soil ammonium-nitrogen concentration for Nunn soil at 3 application rates over 7 harvests of biomass from Sorghum-Sudan grass from a greenhouse study. Values with different letters are significantly different at an alpha value of 0.05. The three application rates were based on applying fertilizer at the agronomic rate of N (N), the agronomic rate of P assuming 50% of total P is available (P50) or 100% of total P is available (P100).

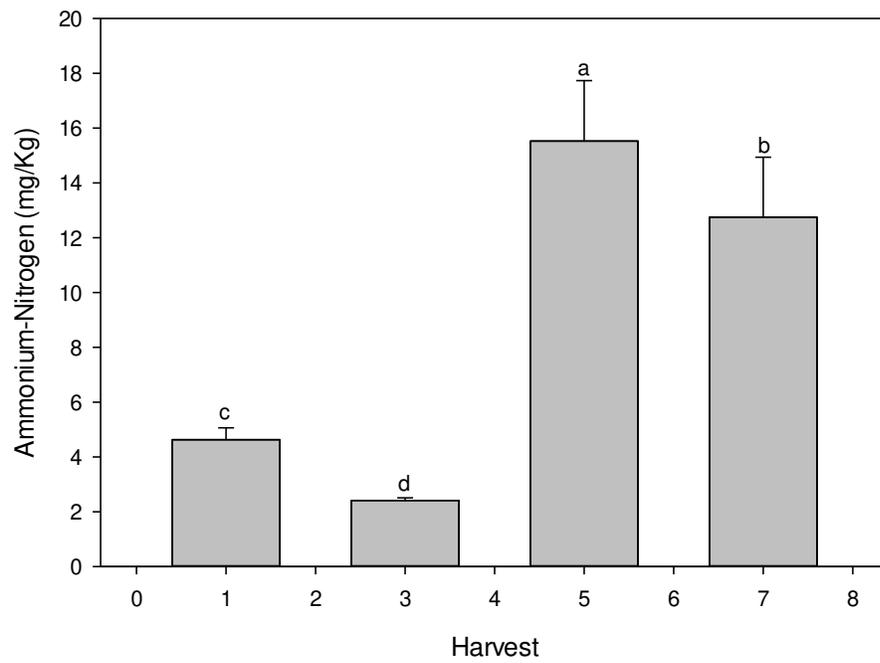


Figure 5.27. Soil ammonium-nitrogen concentration for Colby over 7 harvests of biomass from Sorghum-Sudan grass from a greenhouse study. Values with different letters are significantly different at an alpha value of 0.05.

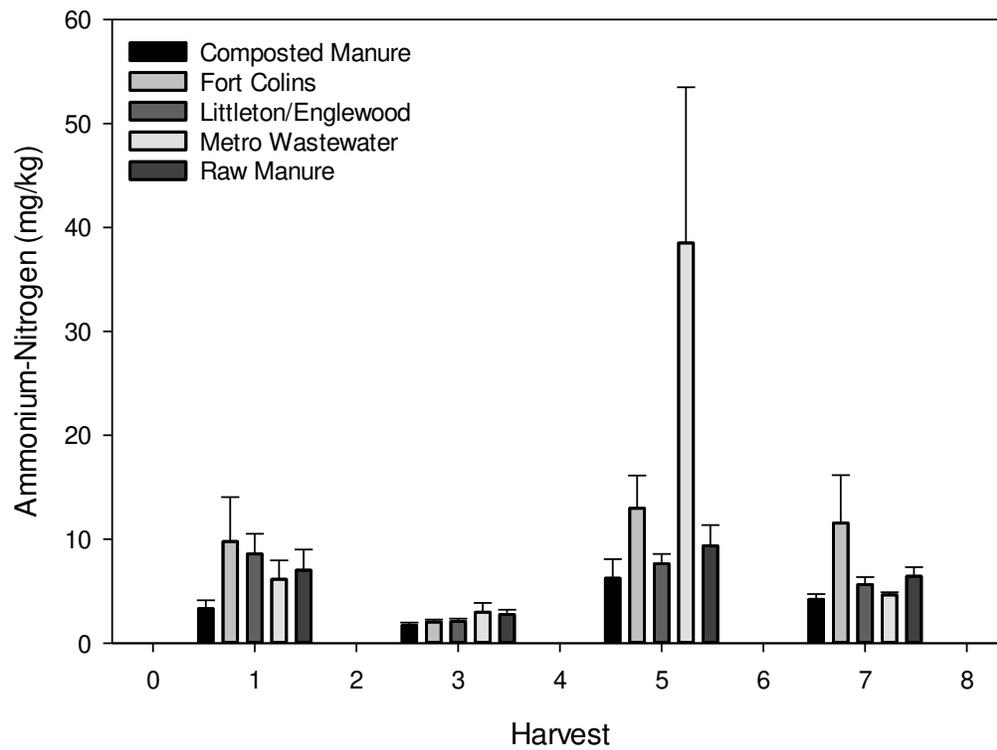


Figure 5.28. Soil ammonium-nitrogen concentration for Bijou over 7 harvests of biomass from Sorghum-Sudan grass from a greenhouse study. Values with different letters are significantly different at an alpha value of 0.05.

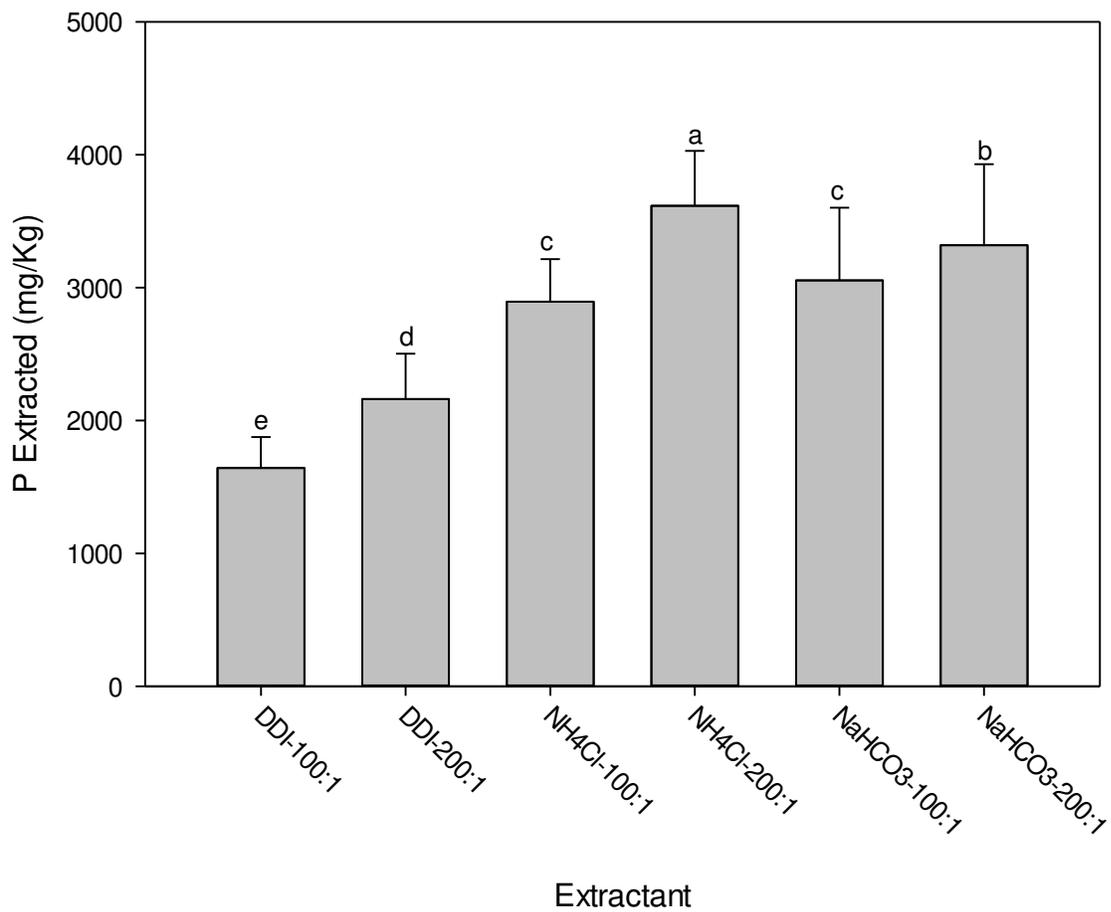


Figure 5.29. Amendment P extracted with different extraction solutions at varying extraction ratios. Values with different letters are significantly different at an alpha value of 0.05.

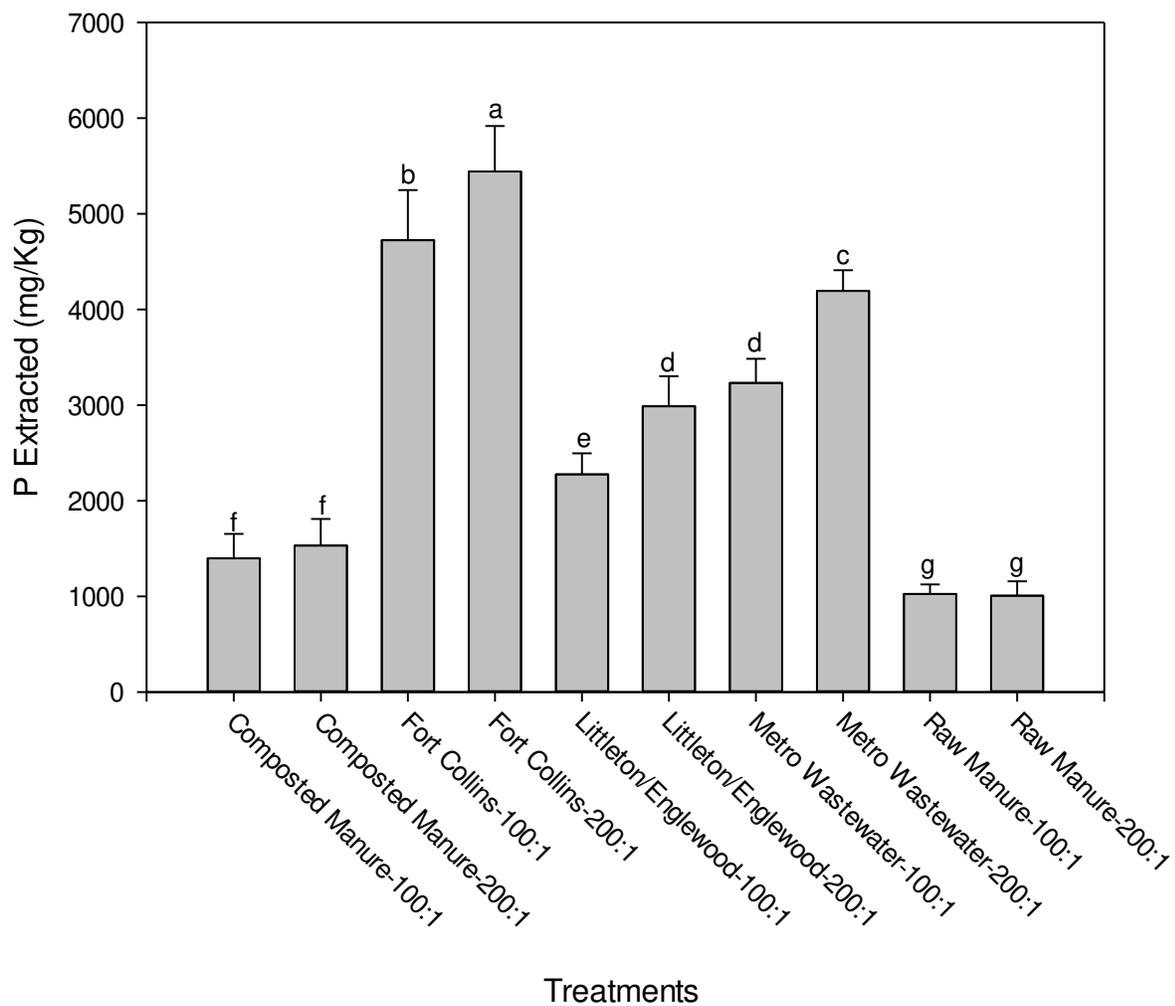


Figure 5.30. Interaction of amendment and extraction ratio on P extracted. Values with different letters are significantly different at an alpha value of 0.05.

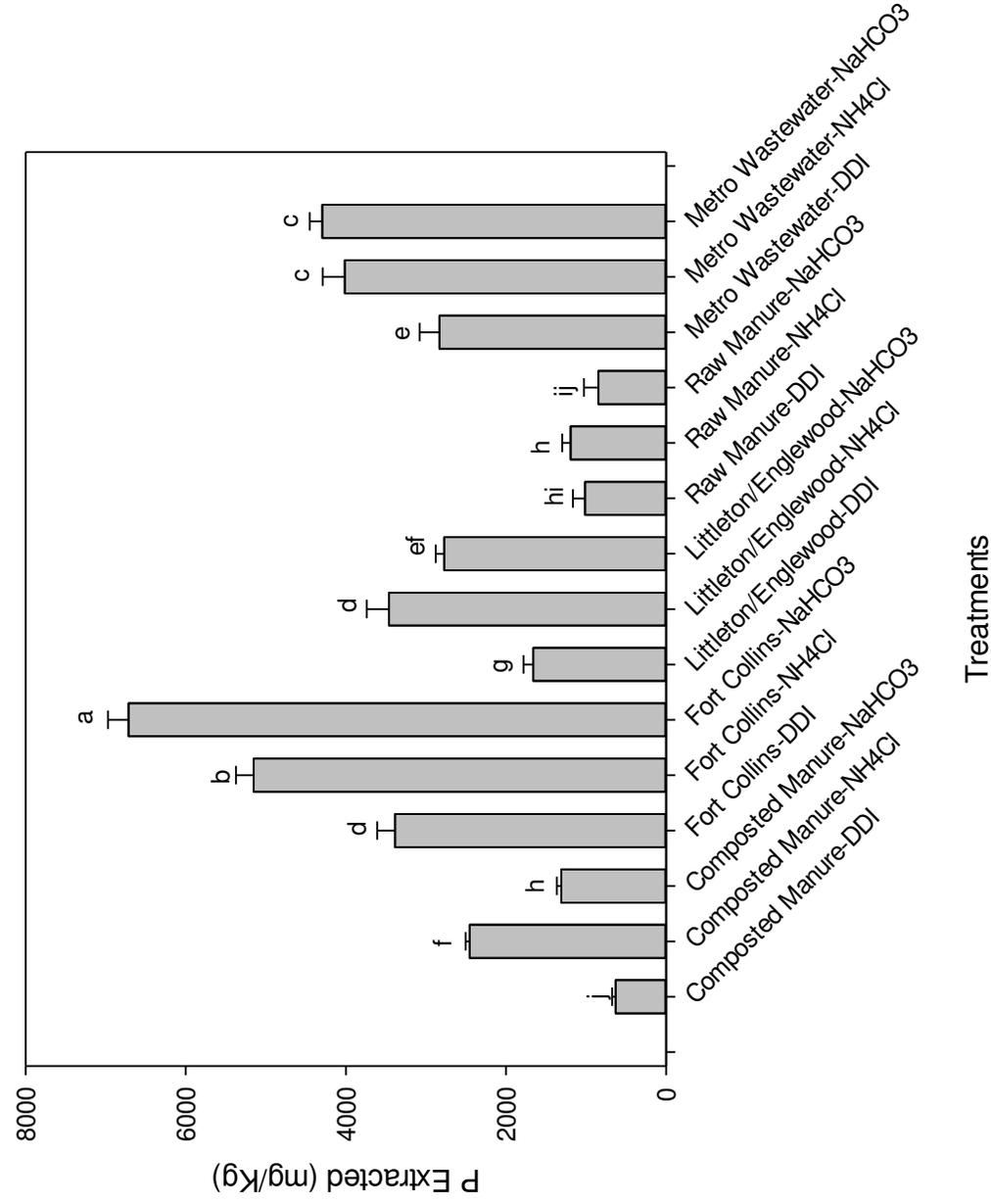


Figure 5.31. Interaction of amendment and extraction solution on P extracted. Values with different letters are significantly different at an alpha value of 0.05.

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CHAPTER 6: CONCLUSIONS FOR SOIL PHOSPHORUS AVAILABILITY AND TRANSFORMATIONS FOLLOWING BIOSOLIDS APPLICATIONS

The application of biosolids to soils in the western United States did lead to differences from what have been reported for the eastern United States. The main factors that were affecting the changes to soil phosphorus (P) were biological effects in the short term (within a season) but the long-term effects were driven by chemical activity. Also changes in water content, soil chemical properties, and application rate had impacts on the availability and forms of P.

The work from the studies reported here focused on quantifying the differences with biosolids applications relative to a control and determining if the results were similar to what has been seen in the eastern United States. This study was able to show there were differences and that the assumptions that are used for the management of biosolids in the East do not necessarily apply. The greenhouse study on plant uptake showed that soils had a very large effect on P availability, and the seasonal changes study showed differences due to soil. The main soil properties that consistently had an effect on P were calcium carbonate (CaCO_3), calcium (Ca), iron (Fe), aluminum (Al), sorption ability of the soil (soil isotherm data), and texture. The effect of soil pH is speculated to also be important but could not be separated from CaCO_3 effects in this study.

During the analysis of the data in the plant uptake study, the soil isotherm data appeared to be the factor that most closely correlated to plant uptake. The ability of soil to sorb P should be looked at more closely in future studies to see if a relationship can be developed that could be used to guide the application rate of P. In this study, biosolids were applied at three applications rates, but there was very little difference in uptake in the above ground plant tissue. However, there was a large difference with the sorption data for the three soils: one resulted in excess P concentration in the plant biomass, one

was right at the optimal concentration, and the third was significantly below for most of the study. With having three points a curve could start to be developed that would show a relationship between sorption rate and plant uptake. However, due to interactions between soil properties (Ca concentration, Fe concentration, pH, texture, CaCO₃ concentration) that drive sorption and affect plant growth and uptake, more soils would need to be analyzed to develop a relationship.

It would also be important to look at soil pH and biological effects more closely. It is hard to separate if pH is driving the fractions that P is partitioning into or if it is strictly due to the availability of cations in the soil. The seasonal changes in P distribution between fractions was believed to be driven by changes in biological activity during the growing season with biological activity driving the changes directly. However, it is possible that there were seasonal changes in soil pH, and this was the main driver to P fractionation. To be able to separate these changes, a study should collect data during the growing season on soil pH changes in the field as well as the distribution of P between soil fractions. Then a laboratory batch study could be conducted to impose similar pH changes to the soil in the absence of changes in biological activity to see if similar changes are observed. Both pH and biological activity are closely related in season, but it would be helpful to be able to further separate them to understand the role of higher pH that is seen in the West.

There were also some limitations to the design of studies that were identified during the data analysis that should be considered when others are doing work in this field. During the development of a fractionation method for P in biosolids, repeated extractions were used for the same extraction solution and this led to the method becoming very long and time consuming to complete. Another option that should be considered is to include a rinsing procedure after each step. Washing the residual with a saturated sodium chloride (NaCl) solution may help to remove excess P that was released but was not removed due to the extraction solution being saturated.

The results from the season changes study may have been better explained if additional data on environmental conditions were collected. Many of the changes that were seen are believed to have taken place as a result of biological activity or changes in environmental conditions. To better understand the effect of environmental conditions, it would have been helpful to have installed temperature, soil moisture, and pH probes. This data may have allowed for the ability to say what factor was driving the changes that were taking place opposed to speculating on the driving factor. The changes that were seen in the Fe and occluded fractions were believed to be due to saturated conditions and that was based on observational data at the time of sampling, but being able to quantify water content may have helped to explain variability between sites and soil series.

Overall, the distribution of P that was seen in both the seasonal changes study and vertical distribution showed that the forms and distribution of P in the soil were in forms that had low availability to the environment. This information as well as the distance to surface water for most agricultural fields in the West would support the continued application of biosolids at the agronomic rate of N. However, due to P being a limited resource, the economics and market opportunities should be explored for the recovery of P either in the wastewater treatment plant or from biosolids in the West.

Overall the results of the studies showed that when biosolids are applied at the agronomic rate of N to soils from the western United States there are not negative effects or large increases in the forms of soil P that are at a high risk for environmental impacts. The results did show that there is the potential for under fertilizing plants with the application of organic amendments at the agronomic rate of N or P in soils with a high ability to sorb P. The results did show that there was an increase in uptake over time but early in the season there could be early season P deficiencies. Also, the seasonal change study showed that many of the transformations are driven by biological activity and environmental

conditions. These effects may also be limiting the availability of P early in the growing season. With these factors in mind, caution should be taken if regulations are changed to apply biosolids at the agronomic rate of P so that there is not a system set up that would lead to systemic under application of P leading to early season P deficiency.