SEQUENTIAL FRACTIONATION AND WATER SOLUBLE PHOSPHORUS METHODS TO INVESTIGATE SOIL PHOSPHORUS IN A LONG-TERM MANURE APPLICATION

By

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CHAPTER I

INTRODUCTION

Intensive confined livestock production has enlarged over the years due to importance of those animals for the food production. Thereby, number of confined animal feeding operations (CAFO) and poultry production operations have increased and resulted in localized concentration of animals in certain regions. Accumulation of animal manure can enhance crop production if properly managed, as a source of plant nutrients and organic matter. However, if mismanaged, manure applications may be detrimental to environment quality. A significant concern about manure application is the increase of soil phosphorus (P) as well as soluble P transport to surface waters that may also contributing to water eutrophication.

Zhang et al. (2009) demonstrated that manure applied at rates designed to fulfill crop nitrogen (N) requirements, often result in a buildup of soil P. The increase of total soil phosphorus (TP) also increases P in soil solutions, which can be subsequently transported to waterways via erosion or runoff. The study with chicken manure and swine slurry conducted by vonWandrsuka (2006) shows that they are apt to provide readily mobile, water soluble phosphorus (WSP) to soil, likely to lead to runoff and eutrophication problems.

Phosphorus containing compounds in manures vary greatly in solubility, availability for plant uptake, and absorption potential in soils (Wienhold and Miller, 2004). Total P content in manures is an important tool for monitoring loading rates and long-term repeated applications. However, it is the amount of plant available P that is significant, because crop response is directly related to the inorganic P (Pi) forms in manure (Bromfield, 1960). According to Barnett (1994), P forms vary in their availability to plants and therefore it is important to evaluate their relative abundance when animal manure is used to fertilize crops.

Generally, 10-20% of P applied is available for crop uptake in the year of application, whereas the remaining 80-90% soil P (residual P) is adsorbed on the soil constituents and builds up as the soil P capital. The residual P is partitioned into different fractions and pools (Gikonyo et al., 2008).

Several fractionation schemes have been developed for analyzing the P composition of manure (Wienhold and Miller, 2004). Soil phosphorus fractionation procedure modified from Hedley et al. (1982) and Tiessen and Moir (1993) was used to determine the various P forms found in soil where several different types of animal manure were used. In this procedure, sequential extraction using H_2O, $NaHCO_3$, NaOH, HCl and concentrated HCl were combined to analyze total and inorganic P. Organic P is determined by the difference between total P and inorganic P in each extract.

Most previous studies (Castilho et al., 1993; Charistie and Beattie, 1989; Gao and Chang, 1996; Sharpley et al., 1993; Sommerfeldt and Chang, 1987; Stadelmann and Furrer, 1985) on the long-term application of animal manure were conducted in higher effective precipitation regions. There is research gap in semiarid agroecosystems on long-term, repeated applications of animal manures in irrigated maize production systems. Therefore, the effects of long-term animal manure applications in low rainfall (<500mm) agricultural environment need to be evaluated. Research on types of animal manure used in the Oklahoma Panhandle, such as SE and BM applied on irrigated maize, was conducted to assess their environment benefits in relation to each other and to chemical fertilizers at different nitrogen rates (NR).

Phosphorus

History

In 1669, a German merchant called Henning Brand obtained elementary phosphorus through the distillation of urine even though in the 12th century Arabian alchemists may have discovered it, the credit belongs to Brand (Savica et al., 2009). Phosphorus was the first element documented in a historical register. The name phosphorus was derived from the Greek words *phôs* meaning light and *phoros* meaning bearer due to its property of shining in the darkness when exposed to the air (Huminicki and Hawthorne, 2002).

Further investigations revealed that addition of sand or coal to urine helped the freeing of phosphorus. About one century after its original work, researchers discovered that phosphorus is an important constituent of the bones, introducing a new method of industrial production of phosphorus. The first method of phosphorus commercial production was a reaction of the bone with nitric or sulfuric acid produces phosphoric acid that, when heated with coal, produces elemental phosphorus. The first effective phosphorus fertilizer was produced in 1808 in Ireland by converting phosphorus obtained from bones to phosphate, which plants can absorb. Later, it was discovered that rock phosphate could be used in this same process. In 1851, John Jay Mapes of Long Island, New York, built the first phosphate manufacturing plant in the United States. Thus, he earned the title of “Father of the American Fertilizer Industry.” By 1889, America produced 90 percent of the world’s phosphate fertilizer and continues to produce 30 percent of the fertilizer produced today (CFAITC, 2009).

Plant Utilization

Phosphorus is a macronutrient and, a component of certain enzymes and proteins; adenosine diphosphate (ADP), adenosine triphosphate (ATP), ribonucleic acids (RNA), deoxyribonucleic acids (DNA), phospholipids and phytin. Adenosine diphosphate and ATP provide the energy required for all biochemical processes such as uptake of nutrients and their movement through cell membranes, as well as their assimilation into different biomolecules. RNA directs protein synthesis in both plant and animals; DNA contains the genetic information. Phospholipids are a major and vital component of all biological membranes and play a key role in processes such as signal transduction, cytoskeletal rearrangement, and in membrane trafficking (Cowan, 2006). Playing an important role for the plant nutrition, phosphorus deficiency seriously affects plant development and yield.

Deficiency of Phosphorus

A major visual symptom of phosphorus deficiency in plants is dwarfed or stunted growth. Symptoms of P deficiency vary with the plant type, but generally leaves and stems turn dark green with a purple tint, beginning with lower, older leaves. Phosphorus deficient plants develop very slowly in relation to other plants growing under similar environmental conditions. Phosphorus deficient plants are often mistaken for unstressed, much younger plants (Taiz and Zeiger, 2006). In older leaves under very severe deficiency conditions a brown netted veining of the leaves may develop (Taiz and Zeiger, 2006).

Under severe deficiency conditions there is also a tendency for leaves to develop a blue-gray luster. According to Alkema and Seager (1982), anthocyanins are responsible for most red, blue, and purple colors in higher plants. However, cold temperature can also induce purple pigmentation inhibiting P uptake. A phosphorus shortage may delay maturity of several crops, including corn, cotton, soybeans, and others.

Forms of Phosphorus in Soil

Phosphorus importance for plant development is well known. Therefore, maintenance of adequate amounts of soil P through application of inorganic and /or organic P sources is critical for the long-term sustainability of cropping systems (Sharpley et al., 1994). However, accumulated soil P in surface soils from long-term, continuous agricultural fertilization is a major source of soluble and particulate forms of P contained in runoff that is entering water resources and degrading water quality throughout the USA (Daniel et al., 1998).

The relative distribution of soil inorganic and organic P pools may be influenced by initial soil chemical characteristics due to soil type (Tiessen et al., 1984), climate, and management practices (Montavalli et al., 2002).

Phosphorus is found in the soil in several forms that vary according to the chemical nature of the compounds and bound energy (Gatiboni et al., 2005). According to Reed et al. (2010) early in soil development, the majority of P is in primary mineral forms, mostly as apatite. As apatite is weathered, it releases biologically available forms of P (as PO_4^{-3}). Some P is taken up by plants and microbes and is ultimately returned to inorganic P (Pi) pools in the soil via mineralization, or remains within the soil in organic forms (Po). However, during each turn of this cycle, some P may also be sorbed by secondary soil minerals, precipitated, or leached in organic or inorganic forms, slowly depleting the total and available P pools. Brady and Weil (2008) determined concentration of phosphorus in the soil solution is very low ranging from 0.001 mg/L in very infertile soils to about 1 mg/L in rich, heavily fertilized soils.

Knowing the species of phosphorus determines its retention in the soil profile when animal manure is applied. Crouse et al. (2002) determined the orthophosphate content of the soil increases during mineralization, while organic phosphorus (Po) decreases. The sorption of P_o (nucleotides and inositol hexaphosphate) is positively correlated with both organic matter, and Fe and Al content of the soil (Leytem et al., 2002).

Once applied, P has multiple reaction pathways; it may be taken up by the crop and incorporated into organic P (McLaughlin et al., 1988), may become weakly (physisorption) or strongly (chemisorption) adsorbed on to Al, Fe and Ca surfaces (Syers and Curtin, 1988). The increase in available soil P is a function of physical and chemical soil properties, including clay, organic C, Fe, Al and calcium carbonate ($CaCO_3$) content (Sharpley and Rekolainen, 1997).

Understanding the environmental fate of manure phosphorus requires robust procedures for determining its composition and solubility (Turner and Leytem, 2004). Sequential fractionation involves extraction of phosphorus from manure with increasingly strong chemical solutions to quantify pools of phosphorus with varying degrees of solubility (Turner and Leytem, 2004). The technique is commonly used because it provides information on manure phosphorus using standard laboratory procedures, which is important given that most laboratories do not have access to the advanced analytical equipment required for detailed chemical speciation (Turner and Leytem, 2004). All those compounds are released by the sequential P fractionation procedure that, according to Huang et al. (2008) yield: a loosely bound fraction (also referred to as exchangeable); fractions associated with Al, Fe, and Mn oxides and hydroxides; the Ca- and Mg-bound fraction; and mineral and organic fractions resistant to previous extracts. The procedures often estimate organic as well inorganic.

Phosphate can be unavailable for plant growth; phosphate being an insoluble form that is highly affected by soil pH where reactions can fix it to soil surfaces. Fixation prevents the leaching of phosphorus, but also changes it to a form that plants cannot use (CFAITC, 2009). Generally, in strong acidic soil (pH 3.5- 4.5) there is an insoluble iron phosphate form. Aluminum is the dominant ion that will react with phosphate between pH 4.0-6.5 (Busman et al., 2002). In these soils, the first products formed would be amorphous Al and Fe phosphates, as well as some Ca phosphates (Busman et al., 2002), only at high concentration of calcium. The amorphous Al and Fe phosphates gradually change into compounds that resemble crystalline variscite (Al phosphate) and strengite (Fe phosphate) (Busman et al., 2002). Each of these reactions will result in very insoluble compounds of phosphate that are generally not available to plants (Busman et al., 2002). They also determined that maintaining soil pH between 6 and 7 will generally result in the most efficient use of phosphate; calcium phosphate occurs in alkaline soil (pH 7.0-9.0), a general sequence of reactions is the formation of dibasic calcium phosphate dihydrate, octocalcium phosphate, and hydroxyapatite.

Basically, phosphorus forms and amounts as well as manure’s residual effect are considerable important for the availability of P for crop uptake, environmental risk assessment and manure management strategies.

Anhydrous Ammonia

The most extensive use of anhydrous ammonia is in the field of agriculture, by means of fertilization. Anhydrous ammonia is quickly attracted to any form of moisture. Soil moisture absorbs the fertilizer as rapidly as human tissue; therefore when handling NH_3 safety clothes and equipment are required. Under normal temperatures NH_3 is a gas and to prevent its escape according to Tisdale et al. (1999), it is stored under pressure and/or refrigerated (-28°F).

Sequential P extraction of long-term conventional agricultural systems receiving manufactured fertilizers also reveals changes in forms of inorganic and organic P which may contribute to P nutrition of crops and which are not measured by traditional tests for plant-available P (Richards et al., 1995).

Urea

When urea or ammonium sulphate is used as fertilizer on calcareous soils, N is lost through the volatilization of ammonia because of the high pH and high calcium content of the soil (Fenn and Hossner, 1985).

Nitrogen applied as urea or NH_4^+ undergoes chemical transformation to produce either ammonia (NH_3) or nitrate (NO_3^-), depending on soil pH, moisture conditions, application methods (He et al., 2002) as well temperature.

Calcareous Soil

As defined by FAO (2000), calcareous soils are characterized by the presence of calcium carbonate in the parent material and by a calcic horizon, a layer of secondary accumulation of carbonates (usually Ca or Mg) in excess of 15% calcium carbonate equivalent and at least 5% more carbonate than an underlying layer.

In acid soils available P is present in a large part as the H_2PO_4 ion while in alkaline soils HPO_4 is the important phosphate ion (Samin, 1971).

McGeorge and Breazeale (1931) believed that phosphate fixation in calcareous soils is due in many cases to formation of a compound more basic than tricalcium phosphate and additional calcium as calcium carbonate is a definite part of the calcium phosphate molecule.

Manure is a good form of fertilizer on calcareous soil due to slow release of nutrients and chelating of ions (Mathers et al., 1980). Based on other studies, Sharpley et al. (2004) determined that the extent to which Ca in manure adds to the pool of exchangeable Ca in soil depends to a large degree on the nature of the bond between the organic molecule and Ca in added manure. As organic compounds in fresh manures are of low molecular weight, the added Ca can readily contribute to the pool of exchangeable soil Ca (Siddique and Robinson, 2003).

Manure characteristics

Animal manure as defined by the Oklahoma Concentrated Animal Feeding Operations Act (2007), paragraph 20-41 part 4 “means animal excrement, animal carcasses, feed wastes, process wastewaters or any other waste associated with the confinement of animals from an animal feeding operation.”

Manure characteristics are based on dietary feed, animal performance, nutrient intake specific to an individual situation, and also external effects as weather conditions and manure storage. Equations have been developed for estimating manure excretion to facilitate its management by the farmer. They are all based on levels commonly observed in commercial production.

According to Hue et al. (1986) and Iyamuremye (1996), the affinity constants and sorption capacities of soils for P are reduced by organic amendments, especially manure. This can be due to competition for P fixation sites by organic acids, and/or the complexing of exchangeable Al and Fe by components of manure. The latter may, at least partially, be ascribed to the release of sulfates and fluorides by the manure, both of which are strong complexing agents for Al and Fe (von Wandruszka, 2006).

Problems of Manure

Manure can be used as a source of fertilizer that benefits crops due to the beneficial amounts of nutrients, mainly nitrogen, potassium and phosphorus. However, it can also cause environment problems if nutrients from manure are over applied becoming a source of water pollution. Furthermore, excess nutrients in surface waters causes eutrophication and algae blooms, which increases the biological oxygen demand (BOD) and turbidity of surface waters. Additionally, the generation then deposition of gaseous air pollutants and odors to surface waters may increase the deleterious effects to animals that live in the water.

Excessive applications of manure to the soil increases the amount of phosphorus available for transport into surface waters increasing phosphate levels, even though manure is not high in P content. Adequate application of manure depends on the soil type, manure content and crop thus, the correct amount should not exceed nutrient needs of the crop.

Not only do excess nutrients impact the environment but animal manure also contain pathogens. According to Gerba and Smith (2005), each year more than 300 million tons of animal manure is spread in the United States, and more than 150 pathogens have been found in manure. Thus, disease organisms are a problem in animal manure. However, incorporation of manure into the soil can dramatically reduce pathogens.

On the other hand, according to Fuentes et al. (2008), an economic alternative to stabilize organic wastes through the action of microorganisms is aerobic degradation. During degradation of organic wastes, diverse changes take place in the characteristic physicochemical properties of the waste mixture and in the microbial populations (Smith and Collins, 2007).

Kahleel et al. (1980) have suggested that a high density of intensive animal rearing facilities could facilitate the contamination of surface water, resulting in large reservoirs for pathogenic bacteria. Johnson et al. (2003) suggest a management practice to minimize the risk of water contamination within the region: CAFOs should not have direct access to surface water. Moreover, the authors recommend valuable agricultural management practices to mitigate surface-water contamination within agricultural regions.

However, as determined by Mohanty et al. (2006), organic sources of P are known to increase P availability more than inorganic P fertilizers, which may involve prevention of reactions converting available P to slowly soluble inorganic forms and enhance efficient use of applied P fertilizer.

On the other hand, P availability is also affected by texture where most fine-medium texture soils have larger capacity to adsorb phosphate than coarse textured soils. Likewise, calcareous soils fix substantial amounts of P due to their high content of sesquioxides and calcite (Wang and Tzou, 1994), which is a stable form of calcium carbonate.

Land application of manure generated under livestock production can improve soil fertility and tilth, but can also resulting elevated concentrations of P in runoff (Sharpley et al., 1994). Water-extractable P in manure has been linked to dissolved P ($< 0.45\mu m$) concentrations in runoff from manure-amended soils (Kleinman et al., 2005). Withers et al. (2001) found that concentrations of dissolved P in runoff from soils amended with mineral fertilizer, cattle manure, and biosolids were proportional to the concentration of water-extractable P (WEP).

Kleinman et al. (2005) determined that WEP in manure can also serve as a quantitative predictor of dissolved P in runoff when expressed as a concentration on a dry weight equivalent basis.

Content of Manure

According to Xavier et al. (2009) organic manures are a vital resource for supplying plant nutrients and replenishing organic matter content. Zhang (2009) found the approximate fertilizer nutrient content for different type of manures an that feedlot manure has a greater nutrient content than lagoon effluent.

Manure composition depends on several factors, such as animal species, dietary options, animal genetics and performance. The manure composition also depends on management functions: collection, storage, treatment, transportation and utilization.

Based on other studies, Motavalli et al. (2002) determined that factors influencing the magnitude of P availability are the composition of organic amendment, the rate of application, the soil type, climate, the method of application and incorporation, and the amount of reaction time with soil after application.

Manure Nutrient Availability for Crops

Based on other studies Hao et al. (2006) determine that most manure P is in inorganic form so availability following application to soil should be high. Dou et al. (2000) found that most (up to 84%) manure P was in available forms and consequently susceptible to runoff loss after land application. According to Xavier et al. (2009) mineralization of organic P is responsible for the most P plant supply. Additionally, orthophosphate and calcium phosphate compounds are generally considered crop available during the first-year of application.

The organic P fractions depend on organic matter inputs whereas inorganic forms are mainly related to soil chemical weathering stage, which is influenced by soil properties as mineralogical composition, pH and others (Xavier et al., 2009). The composition of manure varies widely due to external and internal factors as climate and feed composition.

Long-term application of animal manures and other organic amendments, which is often a major component of sustainable agricultural systems, may have several effects on soil P availability and losses (Montavalli et al., 2002). In general, such applications have been shown to increase soil total, available, and soluble P levels in both the surface and subsurface horizons, to reduce soil P adsorption capacity, and to increase rates of biologically-mediated turnover of organic P due to stimulation of microbial and enzyme activities (Montavalli et al., 2002).

Potassium is present in inorganic forms readily plant available. As plants can only acquire K^+ from solution, its availability is dependent upon the nutrient dynamics as well as on total K content (Ashley et al., 2006).

Basically, the manure application rate is based on crop fertilization requirements; crop availability of manure N, P and K, and adjusting estimates based on N volatilization and mineralization rates.

Nutrient Losses from Manure

Nutrient loss continues after manure is spread in the field. Nitrogen is the nutrient that if not incorporated into the soil, can be highly lost due to ammonia volatilization as discussed before. The ammonium may be released as gaseous ammonia under favorable temperature, moisture, and pH conditions (Van Horn et al., 1996).

Differently from nitrogen, phosphorus losses are due to the relationship between soil test P (STP) concentrations and dissolved P losses (DRP) in runoff which vary with soil type due to differences in soil properties (Penn et al., 2005). Decrease in availability of P is suggested to be a complex function of several factors such as: soil chemical composition, amount and reactivity of silicate clays, $CaCO_3$, Fe oxides, P addition rates and time (Afif et al., 1993).

CHAPTER II

OBJECTIVES

The southern Great Plains region is a semi-arid moisture regime receiving 250-500mm of annual precipitation (Turner, 2004). Soils in low rainfall areas that are amended with animal manure require investigation, specifically in soil phosphorus accumulation and potential environmental problems associated with P source application based on N requirements.

This study is based on following hypothesis;

1. Beef manure amended soils have higher soil phosphorus concentration than the other nitrogen sources due to its composition varying significantly from the control.
2. Phosphorus concentrations are highest in the HCl extractable pool due to the calcareous clay loam.
3. Risk of phosphorus leaching and runoff losses are minimized due to the type of soil and climate in Western Oklahoma.

The objectives of this study were to assess phosphorus movement and fate among long-term established animal manure management plots in semiarid ecosystems, using sequential fractionation of bioavailable P and water soluble P (WSP) levels at multiple depths (0-120 cm) in the soil when different N sources (beef manure, swine effluent and anhydrous ammonia) are utilized as sources of plant nutrient.

CHAPTER III

SOIL PHOSPHORUS FRACTIONATION METHODS

Organic Phosphorus

Organic forms of P are found in humus and other organic material. Phosphorus in organic materials is released by a mineralization processes involving soil organisms. The activity of these microbes is highly influenced by soil moisture and temperature.

The forms of organic P applied to soil in manure will depend on the nature of the animal manure (Hansen et al., 2004). According to the authors, manure from different animal species is known to contain different P forms and concentrations. In addition, the handling and storage of manure before land application can also affect P forms and concentrations due to differences in microbial species, oxygen, pH, and temperature.

According to Stewart and Tiessen (1987) for an adequate description of the dynamics of Po in soils requires an understanding of the interactions of microbes, fauna and plants upon which the processes involved in the rapid cycling of P-compounds depend.

Organic P (Po) usually represents between 20% and 80% of the total P in soils (Anderson, 1980). Transformation of P via the immobilization-mineralization process plays an important role in the cycling and bioavailability of P in soils. Phosphate ions released by mineralization of Po can make a contribution (albeit a poorly defined contribution) to the supply of plant-available P (Sharpley 1985; Stewart and Sharpley 1987).

According to Williams and Steinbergs (1958) soils derived from basalt and basic igneous parent material contain higher amounts of organic phosphorus than those derived from granite, although a lower percentage of their total phosphorus is present as organic P.

Walker and Adams (1958) showed that the P content of the organic matter decreased as the rainfall and mean temperature increased. Based on another study, Dalal (1977), found some other factors that affect the organic P; organic P content generally increases as the soil pH decreases; cultivation which decreases organic P content because of greater mineralization of organic matter; and sulfur content in areas of low atmospheric returns. In general, long-term studies have observed a decline in organic P pools with continuous cultivation and no fertility inputs (Montavalli et al., 2001).

The low plant availability of organic phosphorus in soil may be due to the sorption as well as fixation of these compounds by soil colloids and, possibly, by formation of insoluble Fe and Al complexes (Anderson et al., 1974). Many studies have demonstrated the availability of organic P compounds. According to Dalal (1977) organic P in the soil solution may be important to P nutrition of plants; where phosphatase enzymes excreted by the plant roots could hydrolyze this fraction thus releasing inorganic P.

Inorganic Phosphorus

In most agricultural soils, 50-75% of the P is inorganic, although this fraction can vary from 10 to 90% (Sharpley, 1995). Inorganic P forms are dominated by hydrous sesquioxides, amorphous and crystalline Al and Fe compounds in acidic, non-calcareous soils and by Ca compounds in alkaline and calcareous soils (Sharpley, 1995). Inorganic P occurs in the soil in two forms: “fixed” P and “labile” P (Eck and Stewart, 1995). Based on their studies, fixed P is tightly adsorbed on or within soil particles while labile P is loosely bound to soil particles.

Total inorganic P is divided into active and inactive forms, the former consists of Fe-P, Al-P and Ca-P, occluded P, reductant soluble P and residual P (Chang and Jackson, 1957).

Other factor that can affect inorganic P availability is immobilization and mineralization. Immobilization of inorganic phosphorus in mineral soils affects the plant available P where inorganic P is converted biologically into organic P and is not readily available for plant uptake (von Wandruska, 2006).

Immobilization of inorganic P is affected by microbial activity such as, temperature, moisture, aeration, soil pH, cultivation, presence of growing plants and fertilizer P additions (Stevenson and Cole, 1999). Net immobilization will occur when the C/organic P is 300 or more; net mineralization will result when the ratio is 200 or less (Stevenson and Cole, 1999).

Application of manure and other organic wastes to the soil invariably lead to changes in available P, but not in a predictable manner (Stevenson and Cole, 1999). Following their study, an initial immobilization of P can occur, followed by mineralization of biomass P, which releases inorganic P.

According to Bhatti (1998), some organic P forms excreted in manure may displace sorbed inorganic P and increase inorganic P leaching and/or runoff in the soil which depends on rainfall intensity, soil type, topography, soil moisture content, crop cover, and the form, rate, timing, and method of P application. Conservation management can minimize the risks of erosion and surface runoff hence, reducing the risk of P loss from soil.

Methods to Extract Phosphorus

Phosphorus (P) fractionation is a method developed to estimate both the size of the readily phyto-available P pool and the soils ability to replenish it (Guppy et al., 2000). The method has been used to examine the decline of soil P under cultivation; the effect of different management practices on the distribution of soil P; the influence of microbiological activity on soil P processes; and the pathways of P movement and transformation in soil (Guppy et al., 2000). Phosphorus forms found in the soil have different desorption capacities and soil supply due to both the P chemistry nature and energy bound.

Organic and inorganic P fractions occur in the soil at different degrees of lability as follows: a labile fraction (promptly available to plant and microbial biomass), a moderate labile fraction (weakly adsorbed P), a restrict labile fraction (associated with Fe and Al hydrous oxide being medium term plant available) and a non labile fraction (stable Fe- and Al-associated inorganic P and stable organic P) (Tiessen and Moir, 1993), which is largely Ca bound P.

Tiessen and Moir (1993) developed a routine method for use by Canadian soil chemists adding a hot concentrated acid extraction, which removes P_o that may be able to take part in short-term transformations. According to Tiessen and Moir (1993), the original fractionation introduced by Hedley et al. (1982), left between 20 to 60% of the P in the soil unextracted. This residue often contained significant amounts of Po that sometimes participated in relatively short-term transformations.

On relatively young Ca-dominated soils, this residual Po can be extracted by NaOH, while on more weathered soils, hot HCl (Metha et al., 1954) extracts most of organic and inorganic residual P (Tiessen and Moir, 1993). The authors determined that hot HCl solution appears to work satisfactorily on most soils, and is presented below as part of an extensive soil P fractionation.

This study used a sequential fractionation method based on Hedley et al. (1982), and Tiesen and Moir (1993) procedures modified by Warren (2007). The sequential fractionation of soil P into forms of differing availability allows further evaluation of the disposition of P in soils treated with manure, releasing P by basic and acidic extraction.

The method determines total P content in manure, which is important for monitoring loading rates and long-term applications (Barnett, 1994). However, it is the amount of plant-available P that is significant, because crop response is directly related to the inorganic P forms in manure (Bromfield, 1960).Moreover, inorganic P is obtained by Murphy and Riley method in the soil extract based on the reduction of the ammonium molybdiphosphate complex by ascorbic acid in the presence of antimony.

The qualitative and quantitative information provided by the fractionation data is useful to investigations on the forms of native Pi and Po and their transformations, as well as of the P forms available to plants (Huang et al., 2008).

While there is much work to be done to identify specific P compounds present in manure, fractionation is an inexpensive way to rapidly estimate P solubilities and labilities (Sharpley and Moyer, 2000).

CHAPTER IV

MATERIALS AND METHODS

This study conducted on a Gruver clay loam from plots established in 1995 at the Oklahoma Panhandle Research and Extension Center (OPREC) located in Goodwell, Oklahoma (36º35 N, 101º37 W; elevation 992 m). Gruver soils are classified as very deep, well drained, moderately slowly permeable calcareous soils rich in calcium phosphate (Park et al., 2010). The calcareous nature of this soil increases risks associated with N losses due to ammonia volatilization that occurs under increased pH levels found in Gruver soils (Wu et al., 2003a, 2003b). Soil characteristics at the study site prior to initiation of the study are given in Table 1.

Temperatures of 32° C or greater occur, on average, about 60-65 days per year in the Western panhandle and the average annual precipitation is about 432 mm (Source: Oklahoma Mesonet). A continuous wheat (*Triticum* spp.) routine test was conducted in the experimental area for several years before the implementation of the current study. Micronutrients (Mg, Ca, S, Fe, and Mn) and macronutrients (P and K) were found to meet or exceed plant requirements, so these nutrients were not added.

Before the start of the experiment in 1995, soil P was sufficient, with an initial value of 73 kg ha^{-1}, which exceeded the recommended P level of 32 kg ha^{-1}, and remained above this level throughout the experiment (Zhang and Raun, 2006). Soil N

Table 1. Initial soil characteristics of a continuously cropped, conventionally tilled corn system, measured prior to manure applications in 1995.

Study	Depth	pH†	NH4-N	NO3-N	P	K	Mg	Ca	TN	TC
	cm		………………….....………...		mg kg^{-1}	………..………………………..…….			………....g kg^{-1}	………....
Continuously cropped conventionally tilled corn system	0-15	7.18	10.7	55.4	34.3	634	747	2512	1.2	12.3
	15-30	7.60	9.9	51.1	14.0	525	867	2628	1.0	8.7
	30-45	7.76	6.7	24.6	25.2	534	867	2980	0.9	6.7
	45-60	7.70	7.1	25.2	25.4	510	833	7938	0.6	12.1
	60-120	8.14	5.2	34.6	22.9	394	720	16708	0.6	19.9

† Soil pH using glass electrode with a 1:2 soil: H_2O ratio; NH_4-N and NO_3-N from 2M KCl extracts; P, K, Mg, and Ca from Mehlich III extracts; Leco CN2000 dry-combustion analysis at 1350°; Soil electrical conductivity for forage cropping system used a 1:2 soil: H_2O ratio; all others used a saturated paste extract. Adapted from Turner (2004).

levels were 141 kg ha^{-1} before the start of the experiment, which were about 50 kg ha^{-1} below the recommended soil N level of 190 kgha^{-1} (Zhang and Raun, 2006). Soil pH levels were not adjusted because they would interfere with one of the long-term objectives of the experiment, which was to evaluate the cumulative effects of repeated nutrient applications on crop yields and soil properties (including pH) across different NS (Park et al., 2010).

Soils samples were collected in 2008 using Giddings probe following 13 annual manure applications consisting of four equivalent N applications rates of beef manure, swine effluent and anhydrous ammonia (0, 56, 168 and 504 kg N ha^{-1}) applied to the surface (0 cm) each year beginning in 1994 under continuously cropped corn with conventional tillage.

Corn (*Zea mays* L.) planting date, cultivar selection, seeding rate, and other management information are presented in the study conducted by Park et al. (2010) for each year of the experiment. Study plots were irrigated under a center pivot system using the low-energy precision application system (Table 2).

Nitrogen application levels were selected based on the maximum amount of swine effluent applied at 0.0205 ha-m yr^{-1} as part of the waste management system for swine confined animal feeding operation units in the region, which supplied approximately 504 kg N ha^{-1} yr^{-1} (Park et al., 2010). Equivalent N rates of 504 kg N ha^{-1} yr^{-1} for AA and BM were also included in the experiment to maintain a balanced design, even though they are higher than recommended application rates (Park et al., 2010). The second highest N rate of 168 kg ha^{-1} yr^{-1} was included to be consistent with recommended N rates to satisfy yield goals in the region (Zhang and Raun, 2006) A low N rate of 56 kg N ha^{-1} yr^{-1} was included to provide additional NS comparisons (Park et al., 2010).

Table 2. Soil moisture additions from rainfall, irrigation, and swine effluent applications, 1995-2007.

	Year													
Moisture event	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**Average**
	………………… mm ha^{-1} ……………………….……………….													
Precipitation (P)	419	343	342	489	400	473	336	494	376	535	380	482	306	414
Irrigation (I)	413	318	413	413	413	191	445	413	381	381	381	413	413	383
Total (P+I)	831	661	755	902	813	664	780	907	757	916	761	895	718	797
Events, n	13	10	13	13	13	6	14	13	12	12	12	13	13	12
SE x 56	5.6	5.6	5.6	5.6	8.4	6.1	8.4	8.4	5.6	8.4	8.4	8.4	8.4	7.2
SE x 168	16.9	16.9	16.9	16.9	16.9	18.4	16.9	16.9	16.9	16.9	16.9	16.9	16.9	17.0
SE x 504	50.6	50.6	50.6	50.6	50.6	55.2	50.6	50.6	48.6	50.6	50.6	50.6	50.6	50.8

Adapted from Park et al. (2010).

Manure samples were collected during plot applications and stored at 4°C before nutrient analysis. Swine effluent was obtained from a commercial nursery lagoon, whereas BM was obtained from a local feedlot. The quantities of BM and SE applied were adjusted each year to meet the target level of N so that all three sources applied equivalent rates of N.

Anhydrous ammonia, with approximately 82% N content, was soil injected each year on dates that varied between 9 January and 19 April. Feedlot beef manure was broadcast followed by incorporation to the top 15 cm depth of soil in March each year (approximately 1 month before planting). Swine effluent was surface applied by flood irrigation when corn was at six-leaf stage (approximately the 1st week of May). The entire experiment was irrigated after SE application to minimize the effect of additional soil moisture supplied by SE.

The average chemical composition of some characteristics of beef manure and swine effluent (Table 3) was, respectively, as follows: total N (%), 2 and 0.1; total C (%) 21 and 0.2; EC (dS m^{-1}), 15 and 10, N-NH_4 (mg l^{-1}), 18 and 774. Table 3 also shows information about other nutrients found in the animal manure. Anhydrous ammonia was applied until 2005, since then fertilizer urea was used as a nitrogen source.

Table 3. Selected characteristics of beef manure (BM) and swine effluent (SE) used over thirteen years on experiments located at OPREC, Goodwell, OK. †

		BM(32)‡		SE(59)	
pH		7.84	±0.53	8.00	±0.44
EC	dS m-1	15.81	±6.97	9.38	±3
Dry matter	%	70	±11.17	1	±0.7
Na	mg L^{-1}	3,776	±2,340	270	±74
Ca	mg L^{-1}	47,418	±31,561	97	±46
Mg	mg L^{-1}	7,832	±4,428	27	±20
K	mg L^{-1}	19,818	±10,478	970	±385
TN	%	2	±0.62	0.1	±0.02
TC	%	21	±8	0.2	±0.05
NH_4	mg L^{-1}	18	±2.5	774	±314
Mn	mg L^{-1}	194	±70.5	0.4	±0.23
S	mg L^{-1}	11,170	±11,828	165	±458
B	mg L^{-1}	86	±58	2.5	±1.25
Fe	mg L^{-1}	4,615	±1,982	8.6	±5
Zn	mg L^{-1}	290	±391	23	±36
Cu	mg L^{-1}	34	±17	2.2	±3

†Numbers based on manure dry-weight

‡Number of samples

A total of 180 soil samples from soil cores were subdivided by depth into five segments (0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm and 60-120 cm), composited, air-dried, crushed, and sieved (2 mm) prior to sequential P extraction method modified by

Warren (2007). Sequential extraction procedure provides relevant information to mobility, bioavailability and distribution of P in animal manure-amended soils (Houtin et al., 2000). This fractionation technique uses a series of extractants to identify labile inorganic (Pi) and organic (Po) fractions followed by the more stable forms (Houtin et al., 2000).

In the fractionation, 0.5g of air-dried soil was placed in a 50 ml centrifuge tube after shaking overnight (16h) at room temperature, was first extracted with 30 mL each of deionized water. After shaking overnight, the extraction solution was centrifuged at 10,000 rpm for 15 min at room temperature. Then, the supernatant was vacuum-filtered through a 0.45 µm filter paper and the material retained on the filter was rinsed back into the centrifuge tube and extracted with the next extractant. The residues were then sequentially extracted by 0.5 M $NaHCO_3$ (pH=8.5), 0.1 M NaOH, 1.0 M HCl and 11.3 M concentrated HCl.

The $NaHCO_3$ extraction was followed by 0.1 M NaOH to remove the Po associated with humic compounds (Bowman and Cole, 1978) and Pi more strongly bound to Fe and Al compounds. Sonication of NaOH extracts promotes extraction of inorganic and organic P physically protected in aggregates (Hedley et al., 1982). The HCl extractant dissolves P minerals and possible fertilizer reaction products (Okalebo et al., 1993). The fractionation scheme involves a sequence of extractions that separates soil Po into labile, moderately labile, and nonlabile fractions.

Residual phosphorus which contains fairly insoluble Pi and Po forms was determined using microwave digestion (HNO_3/HCl) (Warren, 2007). According to He et al. (2003) animal manure must contain a considerable amount of soluble chemicals, including P compounds, and lesser amounts of recalcitrant (residual) P. Extracted total P

(Pt) was determined using inductively coupled plasma atomic emission spectroscopy (ICP) (Warren, 2007).

The concentration of inorganic phosphorus (Pi) in all extracts was determined colorimetrically by the molybdenum-blue method (Murphy and Riley, 1962) on a spectrophotometer at 880 nm using ascorbic acid as a reducing agent. Acid or alkali filtered extracts were neutralized prior to P determination. The organic P was represented by difference between total P and inorganic P in the extracts.

All statistical analyses were performed using the Statistical Analysis System (SAS) software package. The 36 plots were arranged in a randomized, complete block design with three replications of each of the main treatments effects, NS and NR. Analysis of variance (ANOVA) using the PROC MIXED routine was used to evaluate the main effects of manure application rates and soil depth, and their interactions to determine differences in P concentrations for the various extraction methods. Fixed effects included in the ANOVA model were NS and NR.

Type III least-square means obtained from the PROC MIXED routine were used for mean comparison tests using the PDIFF option (SAS Institute, 2002). Model parameters and treatment differences were considered significant at the $p < 0.05$ level. PROC GLM was implemented in order to analyze the effect of treatments on P concentration and means were separated using least significance difference (LSD). Regression analysis was also performed to demonstrate how WSP responded to the net phosphorus applied for both beef manure and swine effluent

CHAPTER V

RESULTS AND DISCUSSION

Soil P Pools

The pools of soil P were significantly affected by NS. Nitrogen rate and soil depth with an increase of soil P primarily in the top two depths sampled. Much of the difference in changes to the soil P pools can be explained by manure composition (Table 4). The distribution of P within each fraction provides an indication of the potential of P forms in the soil to be affected by various land management practices (Castillo and Wright, 2008). It has been reported that manure applications have a greater effect on the retention of Pi than the retention of Po (Lehman et al., 2005) as shown by this research where Pi represented greater differences for most of the treatments than Po. The Po fraction most impacted by P additions was the NaOH extraction.

After 13 years of organic fertilization of corn, the soil P pools were significantly affected by animal manure treatments when compared to the check (Table 5). At each depth in the soil profile, the WSP fraction was least for both beef manure (3.8%) and swine effluent (1.5%), whereas the largest soil P fraction was HCl-P for both beef manure (41.5%) and swine effluent (48.9%) when total P was summed within all depths and rates.

Table 4. Summary of analysis of variance (significance of *F* values) to determine the effect of nitrogen rate, nitrogen source and soil layer depth and their interactions on the concentrations of inorganic and organic soil P fractions in a Gruver soil in maize production which had received animal manure for 13 consecutive years.

		H_2O		**$NaHCO_3$** (0.5 M)		**NaOH** (0.1 M)		**HCl** (1.0M	**HCl** (11.3 M)		**Residual-P**
Source	df	Pi‡	Po	Pi	Po	Pi	Po	Pi	Source	df	Pi‡
NR	3	<0.0001	0.8657	<0.0001	0.2941	<0.0001	<0.0001	<0.0001	0.6141	0.8442	0.6670
NS	3	<0.0001	0.9440	<0.0001	0.7505	<0.0001	0.1037	0.7828	0.2000	0.7092	0.0939
Depth (D)	4	<0.0001	0.9354	<0.0001	0.0350	<0.0001	<0.0001	0.0001	0.7339	0.5980	<0.0001
NRxNS	6	<0.0001	0.7616	<0.0001	0.8420	<0.0001	0.0031	0.5843	0.0135	0.0509	0.1889
NRxD	12	<0.0001	0.9970	<0.0001	0.6026	<0.0001	<0.0001	0.0004	0.8213	0.7070	0.7043
NSxD	8	<0.0001	0.6467	<0.0001	0.9895	0.0004	<0.0001	0.0464	0.9991	0.9542	0.8794
NRxNSxD	24	<0.0001	0.9996	<0.0001	0.9997	<0.0001	0.0002	0.2270	0.9043	0.8445	0.9937

‡ Pi=inorganic phosphorus and Po= organic phosphorus.

Labile P

The labile P pool consists of the summation of the WSP and $NaHCO_3$ fractions, which evaluates portion of P that is most soluble. Water-soluble and NaOH P increases were greatest in the BM treatments and increased with increasing NR in the 0-15cm depth (Table 5). However the only SE at 504 was greater than the check for either extraction at the 0-15cm depth. This is attributed to greater quantity of P applied in with BM as an NS versus SE. In this study, plots receiving animal manure showed that inorganic phosphorus extracted with water was present in higher amount than organic P at the surface for beef manure (Table 5). Inorganic P increased from 5.50 mg P kg^{-1} (control) to 124.60 mg P kg^{-1} (504 kg ha^{-1}) for BM at the soil surface (0-15 cm depth) (Table 4). On the other hand, swine effluent showed a great amount of inorganic P at the surface (Table 5) and the 15-30 cm (Table 6) and 45-60 cm (Table 7). Control is significantly different from the highest rate for swine effluent at the surface only (Table 5).

Water-soluble P for the BM application decreased with depth and NR for BM treatments (Figure 1). For BM at 504, there as also a significant increase in WSP at the 15-30cm depth relative to BM at 0 or the lower soil depths indicating translocation of WSP to subsequent depths in the soil profile. Water-soluble P has been associated with plant-available P and $NaHCO_3$-extractable P with labile inorganic and organic P. Water-soluble soil P has been studied in great detail because it represents the amount of P readily available to plants (Kuo 1996) and may be the most appropriate environmental estimator of P concentrations in runoff compared with other soil test methods developed for crop production (Pote et al. 1996).

Based on other studies, Wienhold and Miller (2004) reported that water-soluble P comprises the largest fraction of manure P and most extracted P is in inorganic form. Dormaar and Chang (1995) reported 15-46% of total P as water-extractable Pi+Po and resin-extractable Pi after 20 years of cattle manure addition. However, in this study WSP was the smallest fraction, on average 3% of total P for SE and the second smallest fraction for BM with 10%, considering all rates at the surface when compared with the total P for all fractions. MnKeni and MacKenzie (1985) suggested that this is due to an important cumulative effect of manure additions and a decrease in the soil P adsorption as fixation sites become saturated.

Based on other studies, Qian and Schoenau (2000) determined that long-term manure addition has been known to increase the Pi forms in the labile and moderately labile fractions and also to increase to total P content.

According to Houtin et al. (2000) the potential environmental P hazard of swine manure depends on many factors among which are the application rate and the mobility of bioavailable P. The bioavailability of P is controlled by its chemical pools indicating that it is desirable to determine the amount of P pools in a soil profile rather than in a surface layer because P form may move downward along with the effluent moisture.

A reduction in water-extractable P compared to previous study for SE has been reported to be highly correlated with total and inorganic P leached down from manure (Sharpley and Moyer, 2000). Application of P into the soil may lead to surface adsorption and precipitation of P, which depresses the availability of applied P (Samadi and Gikes, 1999).

The two highest rates of animal manure application over 13 years resulted in an inorganic P concentration almost 10 and 23 times greater for soils amended with swine effluent (Figure 2), and beef manure (Figure 1) respectively, than for control soils at the surface. The P concentration in the surface increased significantly with manure application rates, only for the inorganic P form (Table 5).

Similar trends were also observed the $NaHCO_3$ P pool where P content increased with NR for the BM for both Pi and Po. This is slightly different from the WSP which did not exhibit an increase in Po with increasing NR (Table 5). Applications of SE did not significantly alter the $NaHCO_3$ P pool at the 0-15cm depth in this study.

The $NaHCO_3$ was the second smallest fraction for both animal manure sources considering all depths and rates when compared with the total P for all fractions. The $NaHCO_3$-Pi was influenced by animal manure source, rate of N fertilization, and depth, whereas $NaHCO_3$-Po was only significantly influenced by rate at the 15-30 cm depth. About half of the Po in the H_2O fraction is enzymatically hydrolysable – mainly as phytate in pig manure (He and Honeycutt, 2001). In contrast, a major portion of Po in the $NaHCO_3$ fraction is not hydrolysable by either wheat phytase, alkaline phosphatase, nuclease P1, or nucleotide pyrophosphatase. This indicates that Po extracted from manure with $NaHCO_3$ is not especially labile (von Wandruszka, 2006).

After 13 years, the moderate available P forms concentration ($NaHCO_3$) increased to 28.69 mg kg^{-1} at the lowest manuring rate (56 kg ha^{-1}) and reached up to 254.86 mg kg^{-1} at the highest manuring rate (504 kg ha^{-1}) for beef manure compared with roughly 11 mg kg^{-1} at the control for inorganic P form, which was significantly different among

Table 5. Soil phosphorus fractionation (mg kg^{-1}) for thirteen years amended with commercial fertilizer, beef manure and swine effluent at 0-15 cm.

		H_2O		**$NaHCO_3$** (0.5 M)		**NaOH** (0.1 M)		**HCl** (1.0M)	**HCl** (11.3 M)		**Residual-P**
Source[†]	Rate	Pi[‡]	Po	Pi	Po	Pi	Po	Pi	Pi	Po	
	kg ha^{-1}	………………….………………….………………..... mg kg^{-1} …..………..…..…...…………………………………….									
N fertilizer	0	1.68d	5.50a	9.19c	9.54a	12.56de	37.22b	152.19b	17.93a	9.86ab	93.60ab
	56	6.51d	0a	7.47c	6.43a	12.45de	34.60abc	150.11b	20.16a	8.70ab	92.70ab
	168	2.84d	5.94a	14.85c	14.44a	20.21bcd	20.84cd	155.30b	15.19a	11.12ab	110.98ab
	504	3.40d	6.15a	10.90c	22.83a	21.53bcd	20.18cd	252.31a	19.10a	2.92ab	113.34ab
Beef manure	0	5.50d	0a	10.39c	6.24a	14.83cde	55.20a	176.79b	27.14a	0b	98.56ab
	56	40.46c	0a	28.69bc	6.86a	21.51bcd	37.22b	153.13b	23.57a	5.38ab	93.60ab
	168	76.81b	0a	55.04b	15.36a	28.48b	40.99ab	159.21b	21.51a	10.05ab	104.06ab
	504	124.60a	3.69a	254.86a	46.72a	6.31a	37.54b	276.02a	19.78a	20.24a	107.94ab
Swine effluent	0	3.26d	0.73a	4.83c	4.53a	8.58e	39.25ab	149.59b	13.72a	14.74ab	82.00b
	56	0d	4.70a	8.78c	10.70a	14.98cde	12.81d	163.62b	30.42a	0b	120.12ab
	168	0d	5.72a	8.78c	10.38a	15.20cde	15.79d	159.55b	19.95a	4.69ab	99.10ab
	504	32.26c	11.08a	0.09c	17.30a	24.55bc	8.22d	179.90b	14.05a	10.82ab	127.90a

†Treatments are control (0), commercial fertilizer, beef manure and swine effluent

‡ Pi=inorganic phosphorus and Po= organic phosphorus.

§Means followed by the same letters are not significantly different at the 0.05 alpha level among treatments.

Table 6. Soil phosphorus fractionation (mg kg^{-1}) for thirteen years amended with commercial fertilizer, beef manure and swine effluent at 15-30 cm depth.

		H_2O		**$NaHCO_3$** (0.5 M)		**NaOH** (0.1 M)		**HCl** (1.0M)	**HCl** (11.3 M)		**Residual-P**
Source[†]	Rate	Pi[‡]	Po	Pi	Po	Pi	Po	Pi	Pi	Po	
	kg ha^{-1}	…………………	…………………	…………………	…………………	........mg kg^{-1}……	…………	…………	…………	…………	…………
N fertilizer	0	0.82c	2.23b	5.05cd	6.26abc	8.90de	32.61b	152.19b	15.40a	10.75a	105.58ab
	56	2.09c	2.40b	4.25d	5.86bc	8.32de	33.75b	138.10b	22.57a	3.33a	99.88ab
	168	0.90c	5.47b	10.09cd	10.74abc	17.12bcde	15.11c	140.17b	15.25a	8.39a	99.22ab
	504	30.88b	0c	19.84b	13.48ab	19.13bcd	34.29b	132.48b	14.77a	9.82a	113.48a
Beef manure	0	11.56c	0bc	6.94cd	12.73abc	19.32bc	52.96a	152.56b	21.62a	0.74a	93.60ab
	56	0c	3.94b	11.21cd	17.83a	17.27bcde	8.19c	162.91ab	14.68a	9.03a	104.40ab
	168	0.95c	5.44b	12.73bc	0.89c	20.063b	17.03c	157.42b	24.14a	0a	96.20ab
	504	44.67a	28.06a	32.78a	13.34ab	46.05a	17.83c	209.56a	18.22a	8.82a	95.42ab
Swine effluent	0	0.9c	1.40b	3.59d	6.10abc	7.21e	33.15b	147.09b	20.87a	5.26a	82.24b
	56	0c	3.90b	5.64cd	10.19abc	14.98bcde	15.37c	154.86b	15.13a	6.15a	119.46a
	168	0c	4.52b	5.74cd	10.49abc	12.41cde	16.09c	144.33b	14.51a	8.38a	106.16ab
	504	6.60c	0.13b	6.74cd	8.18abc	14.10bcde	31.17b	156.51b	14.51a	10.83a	101.22ab

†Treatments are control (0), commercial fertilizer, beef manure and swine effluent.

‡ Pi=inorganic phosphorus and Po= organic phosphorus.

§Means followed by the same letters are not significantly different at the 0.05 alpha level among treatments.

Table 7. Soil phosphorus fractionation (mg kg^{-1}) after thirteen years amended with commercial fertilizer, beef manure and swine effluent at 45-60 cm depth.

		H_2O		**$NaHCO_3$** (0.5 M)		**NaOH** (0.1 M)		**HCl** (1.0M)	**HCl** (11.3 M)		**Residual-P**
Source[†]	Rate	Pi[‡]	Po	Pi	Po	Pi	Po	Pi	Pi	Po	
	kg ha^{-1}					……………mg kg^{-1}……………					
N fertilizer	0	0.56ab	3.36ab	5.23b	5.30a	16.02b	18.19a	197.43a	12.81a	9.53ab	93.14ab
	56	0b	3.20ab	3.01b	2.56a	11.60bcd	13.15a	211.85a	17.63a	7.27ab	90.60ab
	168	0.47ab	2.38abc	5.75b	8.29a	12.85bcd	12.06a	166.98a	15.64a	5.93ab	107.84ab
	504	1.66ab	0.71bc	3.74b	6.38a	5.25d	20.93a	187.45a	21.19a	6.98ab	130.86a
Beef manure	0	3.27ab	2.68abc	10.51ab	10.16a	25.97a	23.04a	210.85a	26.18a	14.38a	84.88b
	56	5.54b	0bc	7.23ab	3.94a	16.16b	21.59a	151.98a	14.67a	9.55ab	93.26ab
	168	0.28ab	3.42ab	4.42b	2.51a	13.44bc	11.26a	185.12a	17.91a	6.05ab	83.98b
	504	1.97ab	5.46a	19.92a	2.90a	18.22b	15.69a	169.47a	17.57a	6.03ab	88.22ab
Swine effluent	0	0.80ab	2.03abc	3.37b	4.86a	7.21cd	24.47a	195.25a	16.18a	11.64ab	95.06ab
	56	0b	2.40abc	1.80b	2.77a	10.93bcd	14.50a	194.95a	16.55a	6.80ab	91.82ab
	168	0b	2.68abc	6.35b	9.33a	11.37bcd	10.76a	176.81a	14.85a	6.96ab	99.88ab
	504	6.80a	2.87abc	7.32ab	3.57a	11.51bcd	20.79a	142.10a	27.87a	0b	83.52b

†Treatments are control (0), commercial fertilizer, beef manure and swine effluent.

‡ Pi=inorganic phosphorus and Po= organic phosphorus.

§Means followed by the same letters are not significantly different at the 0.05 alpha level among treatments.

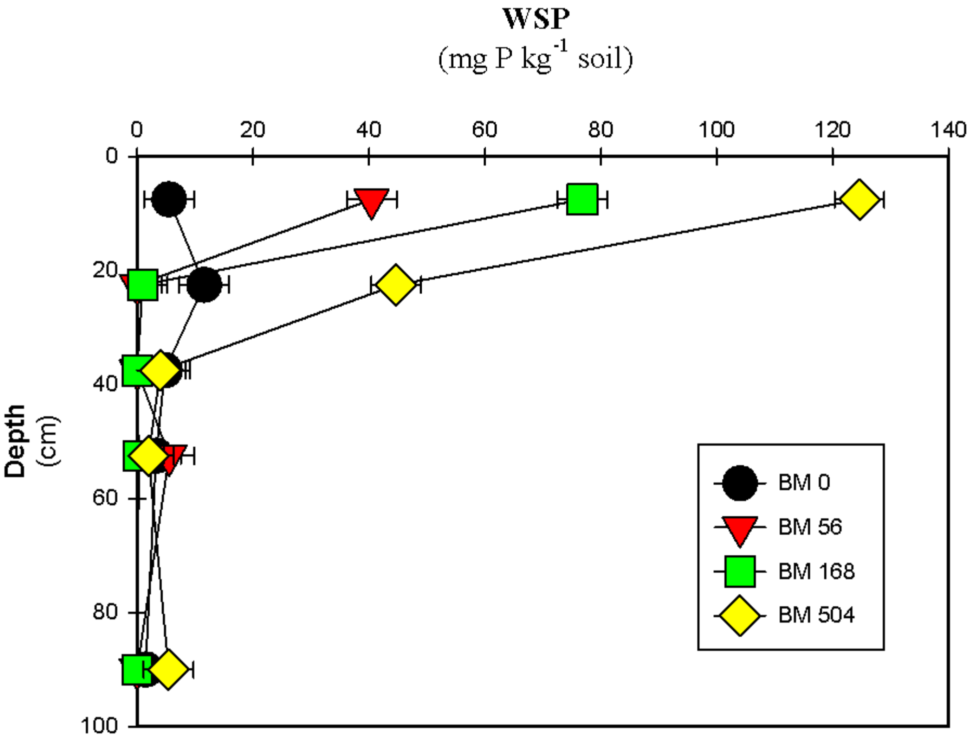

Figure 1. Distribution of water-soluble phosphorus (inorganic) with depth in Gruver clay loam soil amended with beef manure at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

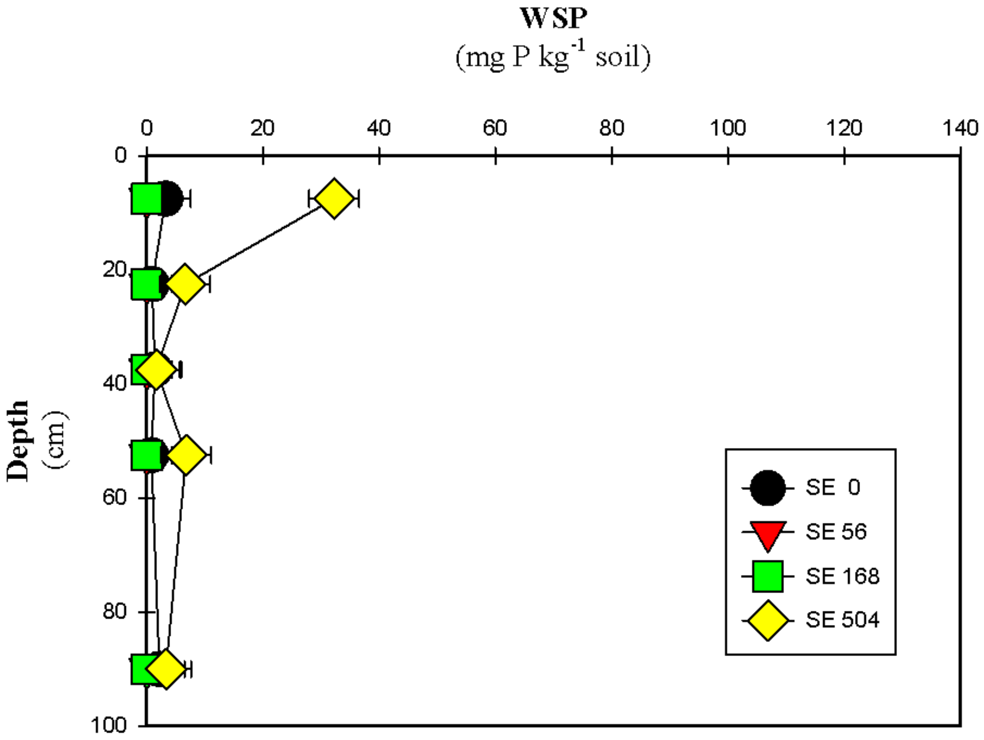

Figure 2. Distribution of water-soluble phosphorus (inorganic) with depth in Gruver clay loam soil amended with swine effluent at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

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Table 8. Soil phosphorus fractionation (mg kg^{-1}) for thirteen years amended with commercial fertilizer, beef manure and swine effluent at 30-45 cm depth.

		H_2O		**$NaHCO_3$** (0.5 M)		**NaOH** (0.1 M)		**HCl** (1.0M)	**HCl** (11.3 M)		**Residual-P**
Source[†]	Rate	Pi[‡]	Po	Pi	Po	Pi	Po	Pi	Pi	Po	
	kg ha^{-1}	…………………	…………………	…………………	…………	...... mg kg^{-1} ……	…………	…………	…………	…………	…………….
N fertilizer	0	0.75a	3.31abc	6.87ab	5.57a	15.44bc	19.56bc	180.79b	15.27ab	6.49ab	109.46a
	56	0a	2.72bc	1.60b	4.34a	11.23bc	16.783cd	156.27b	16.10ab	8.22ab	93.72a
	168	0.13a	4.12abc	6.85ab	7.30a	14.69bc	14.447cd	154.15b	15.02ab	7.98ab	113.68a
	504	1.63a	5.37ab	1.30b	11.51a	8.73c	17.85cd	239.99a	18.48ab	3.84ab	119.26a
Beef manure	0	4.79a	0c	8.86ab	10.85a	13.51bc	56.46a	150.70b	34.85a	0b	170.44a
	56	0a	4.28abc	11.11a	14.19a	18.74ab	5.90d	154.68b	14.40ab	7.72ab	105.72a
	168	0a	3.76abc	4.32ab	4.13a	15.21bc	15.13cd	155.30b	15.31ab	6.05ab	87.78a
	504	4.04a	8.35a	7.76ab	8.29a	26.98a	10.65cd	160.26b	17.63ab	5.19ab	86.52a
Swine effluent	0	1.41a	1.74bc	2.88ab	6.08a	5.56c	31.61b	144.08b	14.82ab	1.76a	89.86a
	56	0a	2.66bc	2.80ab	9.49a	9.90bc	15.82cd	154.59b	15.47ab	6.11ab	109.32a
	168	0a	3.12abc	4.94ab	9.27a	11.08bc	14.85cd	139.64b	28.27ab	0ab	93.26a
	504	1.63a	5.97ab	1.80b	12.57a	8.88bc	15.20cd	275.05a	9.50b	11.44a	91.22a

†Treatments are control (0), commercial fertilizer, beef manure and swine effluent.

‡ Pi=inorganic phosphorus and Po= organic phosphorus.

§Means followed by the same letters are not significantly different at the 0.05 alpha level among treatments.

amendment rates (Table 5), showing a great similarity with WSP due to swine effluent and WSP solubility.

Swine effluent does not show significantly differences for that fraction within treatments. On average, bicarbonate fraction was 14% for BM and 5% for SE of the total P at the surface, occurring mainly in the inorganic form.

Sharpley and Moyer (2000) found that organic and inorganic P in $NaHCO_3$ extracts were correlated with P leached from manure, but the correlation was weaker than that for water-extractable P. On the other hand, this study showed a greater difference for $NaHCO_3$ than for WSP for N-fertilizer and BM, which only had a significant difference at the two highest rates for the surface (Table 5). Siddique and Robinson (2003) suggested that soluble organic compounds of low molecular weight form complexes with Al, Fe, and Ca that increase soil P sorption capacity and, thus, decrease water extractability of soil P.

When summed together, the WSP and $NaHCO_3$ P pools clearly indicate in increase in the labile pools of soil P relative to the control plots as a result of applications of BM for all rates following 13 years of application. However, SE application resulted in an increase of labile P only for the 504 NR. On average, the amount of total labile Pi forms (WSP and $NaHCO_3$) was larger than the amount of labile Po forms and represented an important fraction of the total labile P pools when compared to the control of both animal manures.

Moderately Labile Fraction

The NaOH-Pi fraction varied significantly with N source, rate and depth (p=0.0001) for both inorganic and organic P (Table 4). On average, the amount of NaOH-

Pi was the highest in BM (86 mg P kg^{-1}) and lower in SE and N-fertilizer (24 mg P kg^{-1} each) at the 0-15 cm depth for the highest rate (Table 5). Control is only significantly different from the other rates at 60-120 cm for beef manure (Table 9).

The concentration of Po extracted by the two basic pools ($NaHCO_3$ and NaOH) was smaller than Pi at the highest rate for both animal manure sources (Table 5). This could be a result of hydrolysis of soil organic matter and organic molecules added as a result of BM and SE as NS. Even though, NaOH-Po for beef manure is higher than Pi at the other rates, they are not significantly different. A review by Hao et al. (2006) also shows greater increases in Pi than in Po in soils that have received long-term applications (>10 year) of different types of manure. The Po levels may be less important in soils with a long history of high manure applications than following medium- and short-term manure applications (Lehmann et al., 2005).

The moderately labile pool constituted on average, 14% for both animal manure sources at the surface, considering all rates, implying that most of the P was not associated with either Al or Fe, which are high in acid soils. At low soil pH, i.e. <4.5–5.0 additions, of P to soils can result in precipitation of Al and Fe phosphates, whilst at high pH (>6.0–6.5) insoluble calcium phosphates can form (Haynes, 1984). According to Haynes and Mokolobate (2001) highly weathered acid soils contain large quantities of Al and Fe hydrous oxides which have the ability to adsorb P onto their surfaces.

Hydroxide-extractable Pi and Po represent the moderately labile pool (Sattel and Morris, 1992). According to Wienhold and Miller (2004), concentrations of NaOH and HCl represent more recalcitrant forms of P. In extracts of the swine manure and poultry litter this was almost all phytic acid (Turner and Leytem, 2004). Phytic acid is immobile

Table 9. Soil phosphorus fractionation (mg kg^{-1}) after thirteen years amended with commercial fertilizer, beef manure and swine effluent at 60-120 cm depth.

		H$_2$O		**NaHCO$_3$** (0.5 M)		**NaOH** (0.1 M)		**HCl** (1.0M)	**HCl** (11.3 M)		**Residual-P**
Source[†]	Rate	Pi[‡]	Po	Pi	Po	Pi	Po	Pi	Pi	Po	
	kg ha^{-1}	……………………mg kg^{-1}……………………									
N fertilizer	0	0c	2.59ab	12.27a	3.97a	12.41b	6.78abc	155.13a	9.53b	6.48a	103.38a
	56	0c	2.19ab	10.29a	1.97a	9.20b	2.46c	153.54a	19.55ab	1.50ab	83.10a
	168	0c	2.56ab	6.85a	5.28a	9.76b	4.60c	128.40a	12.41b	7.42a	78.26a
	504	0.42bc	1.19abc	7.25a	4.74a	5.99b	12.24bc	146.21a	21.66ab	0.55ab	75.50a
Beef manure	0	1.46bc	2.87a	13.74a	3.05a	38.02a	0d	108.55a	35.39a	0b	86.28a
	56	0c	2.40ab	6.65a	5.66a	14.03b	1.03c	112.65a	8.90b	7.85a	74.00a
	168	0c	2.12ab	7.86a	1.19a	8.88b	3.40c	142.03a	17.46b	4.00ab	76.42a
	504	5.44a	0c	9.88a	3.52a	6.26b	12.58a	148.60a	12.93b	7.56a	70.00a
Swine effluent	0	2.19bc	0.05bc	9.52a	3.55a	5.75b	12.95a	166.33a	14.99b	7.24a	75.06a
	56	0c	1.40abc	8.17a	4.14a	9.54b	4.14c	170.61a	12.81b	7.06a	78.44a
	168	0c	1.96ab	7.66a	4.13a	9.02b	3.55c	149.20a	21.76ab	0ab	68.34a
	504	3.31ab	0c	6.59a	4.61a	6.74b	12.30ab	146.11a	11.02b	8.17a	65.88a

†Treatments are control (0), commercial fertilizer, beef manure and swine effluent.

‡ Pi=inorganic phosphorus and Po= organic phosphorus.

§Means followed by the same letters within a column are not significantly different at the 0.05 alpha level among treatments.

in soils, because it sorbs strongly to clays and reacts with metals to form insoluble precipitates (Turner et al., 2002; Celi and Barbaris, 2004). It is also difficult for organisms to access phytic acid once it is stabilized in soil (Turner et al., 2002). Organic phosphorus in the NaOH and HCl fractions can therefore be considered stable in the environment (Turner and Leytem, 2004).

Total and inorganic P concentrations in NaOH as well as $NaHCO_3$ extracts were higher for BM than SE at the surface. Tiessen and Moir (1993) reported that NaOH-extractable Pi is thought to be associated with amorphous and some crystalline Al and Fe phosphates while $NaHCO_3$-extractable Pi is thought to consist of Pi adsorbed on surfaces of more crystalline P compounds, sesquioxides and carbonates.

The NaOH-Pi forms in the manured plots were almost six times and three times higher than in the control for beef manure and swine effluent, respectively. Increases were significantly related to the rate of manure P application (Table 4), leading to increase contributions for that extract. Results for organic P were not significantly different.

Relatively Insoluble Fraction

This fraction represents 1M HCl and 11.3M HCl. The hot HCl was included because; according to (Tiessen and Moir, 1993) that fraction extracts most of the organic and inorganic residual P. The HCl-P fraction is associated with the primary minerals such as apatite (Tiessen et al., 1994) and with calcium-bound P, which is high in less weathered soils. Because organic phosphorus extracted by NaOH and HCl is considered poorly soluble, HCl fraction did not account for that form of P. In the current study, HCl-P varied significantly with depth x rate and depth x source (Table 4). Highest P concentration for all N-sources was found in the HCl fraction due to the type of soil that has high carbonate content.

The hot HCl fraction only varied significantly with the interaction of rate and source only for inorganic P. Concentrated HCl does not show any significant difference within treatments along depth. Control-Pi was only significantly different for BM at the last depth (Table 9). Phosphorus concentration for the HCl was greater than concentrated HCl.

Unlike HCl, concentrated HCl constituted roughly 7% total P for both animal manure sources considering all depths and rates when compared with the total P for all fractions.

Manure application increased HCl-P substantially, especially at the highest rate (Figure 3). On average, SE had the lowest HCl-P (180 mg P kg^{-1}) when compared with the other two N sources for the highest rate at the surface (≈250 mg P kg^{-1} each) (Table 5). At the highest rate, BM and N-fertilizer were significantly different from the other rates at the surface, whereas SE and N-fertilizer were significantly different at the 30-45 cm depth (Table 8).

When compared to the control treatment at the HCl fraction, the highest (504 kg ha^{-1}) application rate over 13 years increased: 100 mg Pi kg^{-1} soil for beef manure and 30 mg Pi kg^{-1} soil at the surface for swine effluent (Table 5) due to the higher amount of P in the beef manure content. Moreover, high concentration of Ca for beef manure at the same fraction influences the formation of Ca-phosphate and carbonate, increasing the Pi removed.

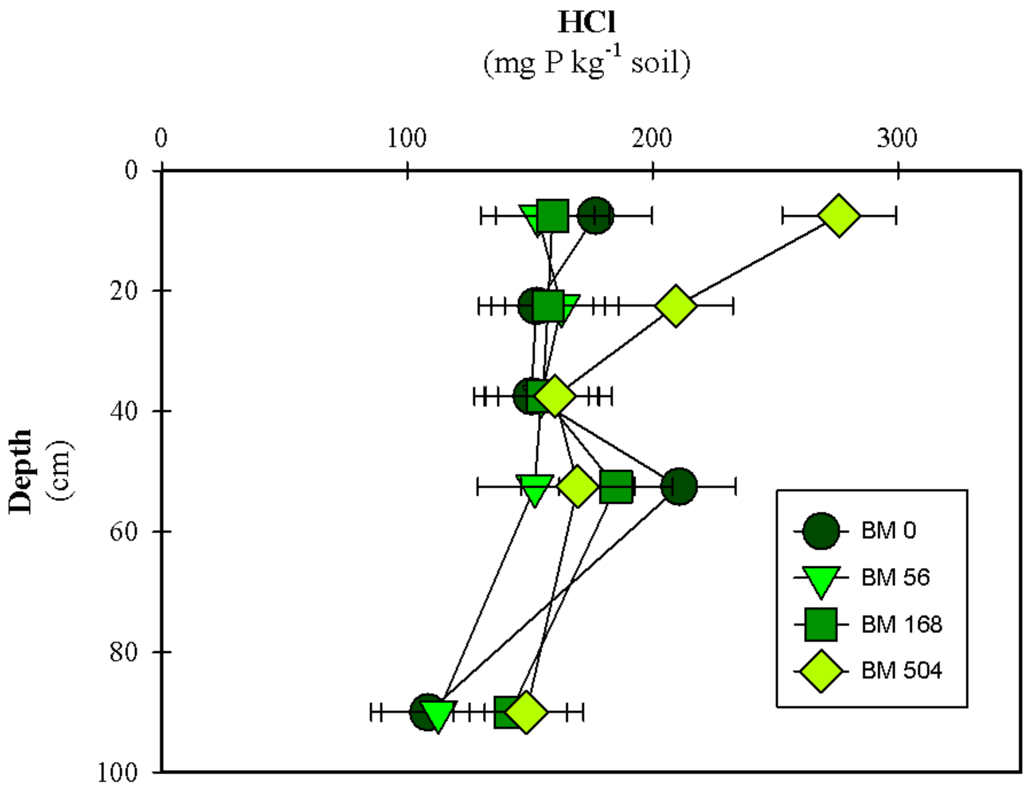

Figure 3. Distribution of 1M hydrochloric acid (HCl)-Pi with depth in Gruver clay loam soil amended with beef manure at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

The high levels of HCl-P content imply that most of P in the manured soil is associated with Ca. Carreira et al. (2006) and Harrel and Wang (2006) showed that HCl-P was the largest extractable P fraction in arid and carbonate-rich soils. Also, Yu et al. (2006) reported that HCl-P accounted for 45-60% of total soil P in neutral and alkaline soils. The relatively insoluble pool constituted about 41% total P for BM and about 50% for SE considering all depths and rates when compared with the total P for all fractions.

Sharpley et al. (1991), studying the impact of long-term swine manure application on soil and water resources in Eastern Oklahoma, reported that only small amounts of HCl-Pi accumulated in the soil profile. Unlike that study, the insoluble fraction concentration of the study presented here was higher than the other fractions because the research was conducted in Western Oklahoma, which has a different soil type.

Residual P

The residual pool constituted about 30% total P for both BM and SE considering all depths and rates when compared with the total P for all fractions, which is the second highest P concentration pool.

According to Adeli et al. (2005) residual P is considered to be a resistant mixture of occluded inorganic P covered with sesquioxides and nonextracted stable organic P (Sharpley et al., 2004). The residual fraction did not vary with any of the variables, thus manure application had no significant effect. At the surface, SE had the highest residual P (127 mg P kg^{-1}), whereas BM had the lowest (107 mg P kg^{-1}) (Table 5). Any of the sources had significantly differences through the profile.

Effects of 13 Years of Animal Manure and N-Fertilizer to the Soil

After 13 years of organic fertilization of corn (*Zea mays* L.), the increase in soil P was strongly correlated to the annual application of both animal manures treatments for all fractions. The average soil P pool contents in the various depths showed that both inorganic and organic P forms concentrations were higher in the manured soil than in the check and N-fertilizer plot. This is in agreement with the result of Dormaar and Chang (1995), where after 20 years of cattle feedlot manure (CFM) loading, the amounts of all labile Pi and Po forms in the Ap horizon of a Lethbridge loam soil generally increased with all CFM applications compared with the check.

The interactions among NS, NR and depth were significantly for labile and moderately pools. These relationships are in agreement with the known fact that available soil P pools are constantly replenished through reactions of dissolution or desorption of more stable inorganic P through the mineralization of organic P (Tiessen and Moir, 1993). Both animal manure sources had their total phosphorus for labile fraction increased as rate increased, whereas relatively insoluble fraction was decreased at the surface as shown on figures 4 and 5. Moderately and residual fraction did not have a great change as the other two pools.

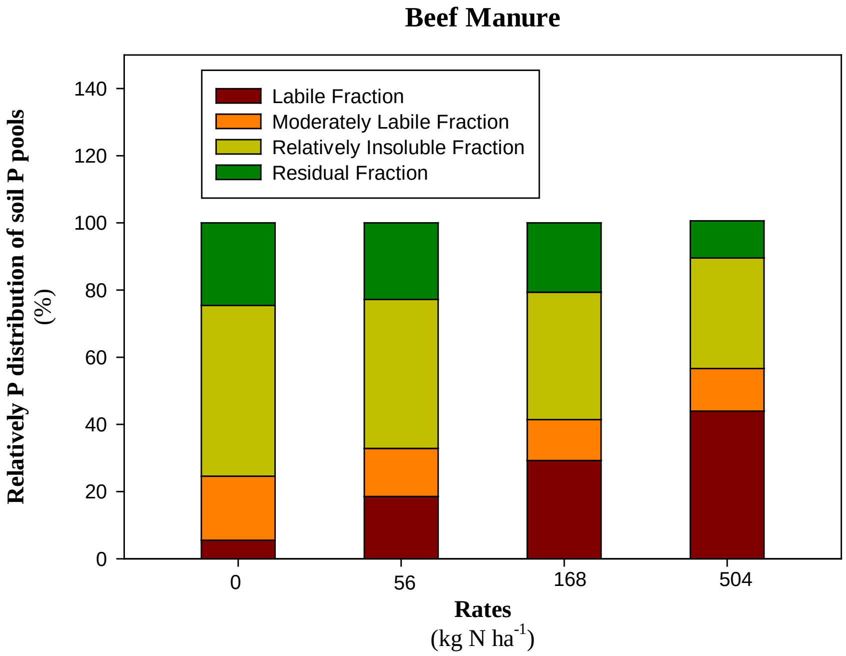

Figure 4. Relatively phosphorus distribution of soil phosphorus pools amended with beef manure at the surface according to each rate.

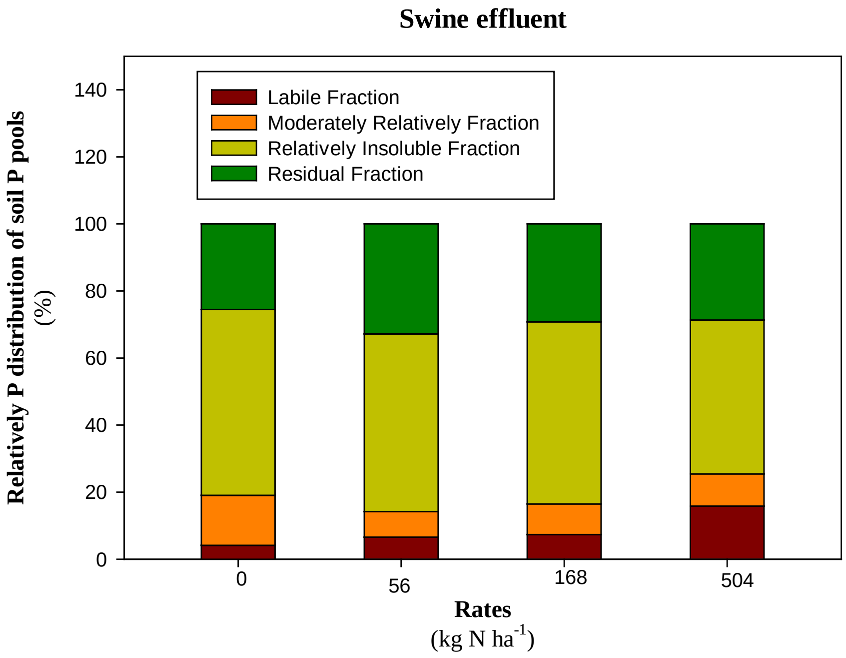

Figure 5. Relatively phosphorus distribution of soil phosphorus pools amended with swine effluent at the surface according to each rate.

Changes in the distribution of Pi and Po due to addition of P into the soil through either P fertilizer or P-containing organic by-products, are likely to occur over a long-time rather than a short time (Richards et al., 1995).

Hao et al. (2006) determined that a low rate of manure application over a longer period of time (30 years) will lead to a higher soil P than the same amount of manure applied at a higher rate over a shorter time (14 years manuring followed by 16 years no manuring). As they showed, if the purpose of manure application is to maximize the P for crop production, the manure should be applied at a lower rate over a longer period of time. However, if the purpose of manure application is to dispose of it, then the higher application over a shorter period of time should result in lower P content since the manure has a longer time to react with soil matrices.

Soil total P, as expected, was increased by the addition of manure, but not by the addition of fertilizer. Total phosphorus extracted, which includes all fractions at all depths, increased following thirteen cumulative annual animal manure applications as the net total phosphorus applied increased (Figure 6) and the P that is not taken up by plants is lost to the environment. However, addition of manure and fertilizer to the soil influenced P fractions differently. In general, there was no large effect of N-fertilizer addition on each of the fractions in either inorganic or organic forms. On the other hand, at the highest rate of that fertilizer, a significant change in P concentration was observed in the inorganic P fraction.

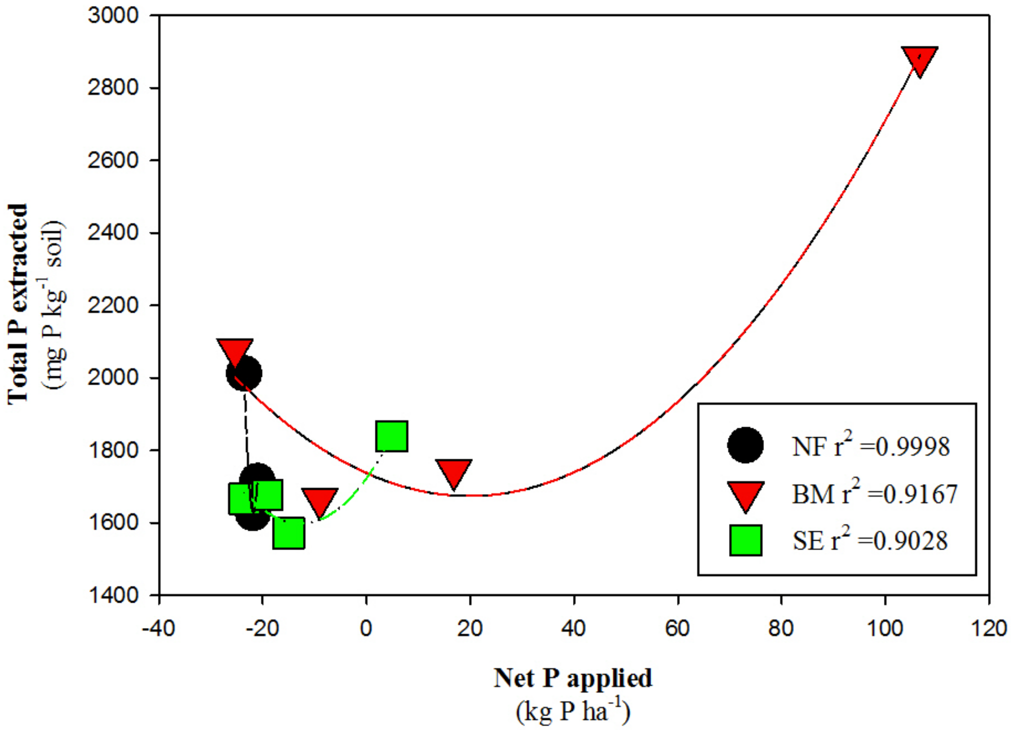

Figure 6. Total phosphorus extracted as a function of net phosphorus applications of N-fertilizer (NF), beef manure (BM), and swine effluent (SE) in a continuously cropped, conventionally tilled corn production system.

Differences in inorganic P concentrations observed in this study are consistent with those reported previously. Graetz and Nair (1995) found that the total P concentration of the surface (0-20 cm) of three Spodosols (Myakka, Immokalee, and Pomellofine sands) receiving dairy manure for 8 to 32 years (1680 mg kg^{-1}), was about 50 times greater than untreated soils (34 mg kg^{-1}). This total P increase was concomitant with a general shift from Al- and Fe-P to Ca-P forms (Graetz and Nair, 1995).

According to Zhang and Raun (2006) although BM and SE had higher P soil values throughout the experiment than N-fertilizer, sufficient P levels above 32 kg ha^{-1} remained on all of the N-fertilizer plots.

Agronomic conditions favor the build-up of P because Gruver soils are rich in calcium phosphate (Park et al., 2010). Calcareous soils typical from the experimental site, is consider an alkaline soil with high levels of calcium and magnesium carbonate. Alkaline soils subjected to long-term manure amendments have been shown to accumulate substantial quantities of P, with 50–66% in plant available forms (Whalen and Chang, 2001). Both calcareous soil and also type of manure affect the amount and specie of phosphorus present in the soil. Table 10 shows the animal manure composition for beef manure and swine effluent for each N rate and amount of manure applied to reach the rates.

As expected, the effect of lagoon effluent application rates on extractable soil P was generally in the order: high rate > medium rate > low rate (Liu et al, 1997). The initial soil characteristics (Table 1) show that the calcium concentration is very high and the P concentration decreases from the surface to the subsurface.

Irrigated plots receiving high (>60 Mg ha^{-1}) annual manure applications are considered to pose a risk of ground water contamination, as the total P

Table 10. Annually applied animal manures to a conventionally tilled, continuously cropped corn production system.

Source		Na	Ca	Mg	K	P	pH
kg ha^{-1}		……………………..…… kg ha^{-1} …..…...……………………					
BM	Mg ha^{-1}						
56	4	10	83	13	46	15	7.38
168	12	30	253	40	137	44	7.34
504	37	90	756	119	422	134	7.36
SE	m^3 ha^{-1}						
56	73	16	8	3	64	5	7.51
168	176	38	19	6	155	11	7.53
504	527	115	56	19	464	34	7.09

concentration increases with soil depth (von Wandruszka, 2006). Although irrigation is able to reduce the more significant sources of risk in the region, such as low rainfall and frequent drought, other external factors beyond experimental control were present. In some years hail damage was reported, whereas in other years pest damage was also encountered (Park et al., 2010).

The pH range for all the soil samples varied from 5.94 to 7.84, depending on treatment and soil depth. The lowest pH value is due to the high amount of anhydrous ammonia that was applied to the soil, generating more soluble calcium phosphate. Continuous application of N-fertilizer would have an acidifying effect due to nitrification within the soil, resulting in a long-term decline in soil pH (Park et al., 2010). This phenomenon has been well documented by Lindsay (1979), where phosphate solubility increases with decreasing pH as calcium phosphate minerals dissolve and release P into the soil.

Continuous application of animal waste (BM and SE) has also been shown to result in improved soil pH management, particularly compared with N-fertilizer, which can lead to acidification and resultant losses in productivity (Zhang and Raun, 2006). On average, and throughout the experiment, the BM and SE plots maintained higher soil pH levels than the corresponding N-fertilizer plots. Zhang and Raun (2006) report that, at pH 6.8, soil acidity becomes a limiting factor to plant growth, with subsequent yield declines as pH is lowered. All of the BM and SE treatments remained above pH 6.8, as did N-fertilizer for all N-rates. At the highest N rate of 504 kg N ha^{-1}, however, continuous application of N-fertilizer lowered soil pH below 6.8 in 13 year, including a pH of 4.8 in 2000, according to Park et al. (2010).

The effects of manure on P availability in various soils have been widely studied, and the general conclusion has been that it is a source of P; interacts with soil components in a manner that increases P recovery by crops; and enhances the effectiveness of inorganic P fertilizer (von Wandruska, 2006).

A major portion of soluble P in manure is determined in weak extracts such as H_2O and $NaHCO_3$, while much of the soil P requires more aggressive extract such as NaOH and HCl. This is related to the fact that soils contain *ca* 15 times as much Al, and 10 times as much Fe as manure, while manure tends to have higher Ca and Mg contents (von Wandruszka, 2006). Subramaniam and Singh (1997) showed that only Ca-P and Al-P were the major contributors for the plant available P fraction in their soils. Both animal manure sources had a larger proportion of total P in inorganic forms compared with the N-fertilizer plots, especially in the HCl extract.

Turner and Leytem (2004) caution that the presence of organic P in the HCl extract of the Hedley fractionation procedure is commonly overlooked, resulting in under-reporting. They found phytic acid to be present in HCl extracts of broiler litter and swine manure, indicating that this relatively immobile compound enters the environment from these sources. More mobile Po species in manure, such as phospholipids and simple phosphate monoesters, can, despite their relatively low abundance, become a major P component in runoff (Toor et al., 2003).

CHAPTER VI

CONCLUSION

The results showed that 13 years of animal manure application has increased soil P pools in the soil profile Gruver clay loam. The soil P fractionation results suggest that continuous application of beef manure on the basis of crop N requirements significantly increased all P fractions. The study demonstrated risk to ground water from P movement through calcareous fine-textured (clay) soils that have received annual feedlot manure applications. A possible solution to this problem is applying animal manure based on crop P rather than N requirements. However, due to the region where the experiment was located those losses are not considered a problem.

The greatest increases were observed in relatively insoluble pools of inorganic P at the surface by application of all N sources, suggesting that greatest rate and source were the major contributing factors. Application of manure at the 504 kg ha^{-1} rate also increased HCl-P fraction at lower soil depths. Manure application at the highest N rate maintained soil pH close to neutral at the subsurface compared to the N fertilizer treatments.

The order of total P distribution in all fractions at the surface for beef manure was on average: HCl>Residual P>$NaHCO_3$> NaOH > H_2O >CHCl, but HCl>Residual P> NaOH>CHCl> $NaHCO_3$>H_2O for swine effluent. Water-soluble P levels increases due to the volume of animal manure applied. Moreover, WSP increases more significantly for BM than for SE, which only had significantly increased at the surface for the highest rate.

These findings suggest that continuous application of manure on the basis of crop N needs would increase the bioavailable P at the soil surface and increase the potential for P losses from surface runoff and leaching. Thus, animal waste management practices are an important nutrient source that can be a viable alternative to a commercial fertilizer. However, soil and weather condition as well as animal manure composition should be considered for a sustainable model in the agricultural production.

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APPPENDICES

Table 11. Results of pH and phosphorus concentration after thirteen years application of beef manure from a continuously cropped, conventionally tilled corn production system.

				H_2O		$NaHCO_3$		NaOH		HCl			Residual-P
						0.5 M		0.1 M		1.0 M	11.3 M		
Source	Nrate	Depth	pH	Pi	Po	Pi	Po	Pi	Po	Pi	Pi	Po	
	kg ha^{-1}	cm		mg kg^{-1}									
Beef Manure	0	0-15	7.33	5.50	0.00	10.39	6.24	21.13	55.20	176.79	27.14	0.00	95.56
		15-30	7.41	11.57	0.00	6.94	12.73	19.34	52.96	152.56	21.62	0.74	93.60
		30-45	7.51	4.79	0.00	8.60	10.85	13.52	56.46	150.70	34.85	0.00	170.44
		45-60	7.56	3.27	2.68	10.51	10.16	25.97	23.04	210.85	26.18	14.38	84.88
		60-120	7.59	1.46	2.87	13.74	3.05	38.03	0.00	108.55	35.29	0.00	86.28
	56	0-15	7.38	40.46	0.00	28.69	6.86	21.51	37.22	153.13	23.57	5.38	93.60
		15-30	7.41	0.00	3.94	11.21	17.83	17.27	8.19	162.91	14.69	9.03	104.40
		30-45	7.53	0.00	4.28	11.11	14.19	18.74	5.90	154.68	14.40	7.72	105.72
		45-60	7.57	5.54	0.00	7.23	3.94	16.16	21.59	151.98	14.67	9.55	93.26
		60-120	7.64	0.00	2.40	6.65	5.67	14.03	1.03	112.65	8.91	7.85	74.00
	168	0-15	7.34	76.81	0.00	55.04	15.36	20.48	40.99	159.21	21.51	10.05	104.06
		15-30	7.39	0.96	5.44	12.73	0.89	20.06	17.04	157.42	24.14	0.00	96.20
		30-45	7.50	0.00	3.76	4.32	4.13	15.21	15.13	155.30	15.31	6.05	87.78
		45-60	7.48	0.28	3.42	4.43	2.51	13.44	11.26	185.12	17.91	6.05	83.98
		60-120	7.59	0.00	2.12	7.87	1.19	8.88	3.40	142.03	17.46	4.00	76.42
	504	0-15	7.36	124.60	3.69	254.86	46.72	86.31	37.54	276.02	19.78	20.24	107.94
		15-30	7.49	44.68	28.06	32.78	13.34	46.05	17.83	209.56	18.22	8.82	95.42
		30-45	7.55	4.04	8.36	7.77	8.29	26.99	10.65	160.26	17.63	5.19	86.52
		45-60	7.60	1.98	5.46	19.92	2.90	18.22	15.70	169.47	17.57	6.03	88.22
		60-120	7.63	5.45	0.00	9.89	3.52	6.26	12.58	148.60	12.93	7.57	91.64

Table 12. Results of pH and phosphorus concentration after thirteen years application of swine effluent from a continuously cropped, conventionally tilled corn production system.

				H_2O		$NaHCO_3$ 0.5 M		NaOH 0.1 M		HCl 1.0 M	HCl 11.3 M		Residual-P
Source	Nrate	Depth	pH	Pi	Po	Pi	Po	Pi	Po	Pi	Pi	Po	
	kg ha^{-1}	cm		………………………………………………..mg kg^{-1}……………….………………………………………..									
Swine Effluent	0	0-15	7.37	3.26	0.73	4.83	4.53	8.58	39.25	149.59	13.72	14.74	82.00
		15-30	7.41	0.90	1.40	3.59	6.11	7.21	33.15	147.09	20.87	5.26	82.24
		30-45	7.45	1.42	1.74	2.88	6.09	5.57	31.61	144.08	14.82	1.76	86.86
		45-60	7.58	0.80	2.04	3.37	4.87	7.21	24.47	195.25	16.18	11.64	95.06
		60-120	7.66	2.19	0.05	9.52	3.55	5.75	12.95	166.33	14.99	7.25	75.06
	56	0-15	7.51	0.00	4.70	8.78	10.70	14.98	12.81	163.62	30.42	0.00	120.12
		15-30	7.56	0.00	3.90	5.65	10.20	14.98	15.38	154.86	15.13	6.15	119.46
		30-45	7.63	0.00	2.66	2.80	9.50	9.91	15.82	154.59	15.47	6.11	109.32
		45-60	7.68	0.00	2.40	1.80	2.78	10.94	14.50	194.95	16.55	6.81	91.82
		60-120	7.70	0.00	1.40	8.17	4.15	9.54	4.14	170.61	12.81	7.07	78.44
	168	0-15	7.53	0.00	5.72	8.78	10.38	15.20	15.79	159.55	19.95	4.69	99.10
		15-30	7.54	0.00	4.52	5.74	10.50	12.41	16.09	144.33	14.52	8.38	106.16
		30-45	7.57	0.00	3.12	4.94	9.28	11.09	14.85	139.64	28.27	0.00	93.26
		45-60	7.65	0.00	2.68	6.35	9.33	11.38	10.76	176.81	14.85	6.97	99.88
		60-120	7.70	0.00	1.96	7.67	4.13	9.02	3.56	149.20	21.76	0.00	68.34
	504	0-15	7.09	32.26	11.08	10.09	17.30	24.55	18.22	179.90	14.05	10.82	127.90
		15-30	7.19	6.61	0.13	6.74	8.18	14.10	31.17	156.51	14.51	10.83	101.22
		30-45	7.35	1.63	5.97	1.80	12.58	8.88	15.20	275.05	9.50	11.44	91.22
		45-60	7.37	6.80	2.87	7.33	3.57	11.51	20.79	142.10	27.88	0.00	83.52
		60-120	7.39	3.31	0.00	6.59	4.62	6.74	12.30	146.11	11.03	8.17	65.88

Table 13. Results of pH and phosphorus concentration after thirteen years application of N-fertilizer from a continuously cropped, conventionally tilled corn production system.

				H_2O		$NaHCO_3$ 0.5 M		NaOH 0.1 M		HCl 1.0 M	HCl 11.3 M		Residual-P
Source	Nrate	Depth	pH	Pi	Po	Pi	Po	Pi	Po	Pi	Pi	Po	
	kg ha^{-1}	cm		……………………………………………..………..mg kg^{-1}……………...………...…………...………………………..									
N fertilizer	0	0-15	7.58	1.68	0.00	9.19	9.54	12.56	37.22	152.19	17.93	9.86	93.60
		15-30	7.59	0.82	2.24	5.06	6.26	8.91	32.61	152.19	15.41	10.75	105.58
		30-45	7.67	0.76	3.31	6.87	5.57	15.44	19.56	180.79	15.27	6.49	109.46
		45-60	7.70	0.56	3.36	5.23	5.30	16.02	18.20	197.43	12.81	9.53	93.14
		60-120	7.73	0.00	2.60	12.28	3.98	12.41	6.78	155.13	9.53	6.48	103.38
	56	0-15	7.44	6.51	0.00	10.90	6.43	12.45	34.60	150.11	20.16	8.70	92.70
		15-30	7.50	2.09	2.41	4.25	5.86	8.32	33.76	138.10	22.57	3.33	99.88
		30-45	7.52	0.00	2.72	1.60	4.34	11.23	16.78	156.27	16.10	8.22	93.72
		45-60	7.56	0.00	3.20	3.02	2.56	11.60	13.16	211.85	17.63	7.27	90.60
		60-120	7.57	0.00	2.19	10.30	1.98	9.21	2.47	153.54	19.55	1.51	83.10
	168	0-15	7.29	2.84	5.94	14.85	14.44	20.21	20.84	155.30	15.19	11.12	110.98
		15-30	7.31	0.91	5.47	10.10	10.74	17.12	15.12	140.17	15.25	8.39	99.22
		30-45	7.37	0.13	4.13	6.86	7.30	14.69	14.45	154.15	15.02	7.98	113.68
		45-60	7.50	0.48	2.38	5.75	8.29	12.85	12.07	166.98	15.65	5.93	107.84
		60-120	7.57	0.00	2.56	6.86	5.28	9.76	4.60	128.40	12.42	7.42	78.26
	504	0-15	6.91	3.40	6.15	7.47	22.83	21.53	20.18	252.31	19.10	2.92	113.34
		15-30	5.98	30.89	0.00	19.84	13.48	19.13	34.29	132.48	14.78	9.82	113.48
		30-45	6.62	1.63	5.37	1.31	11.51	8.73	17.85	239.99	18.48	3.84	119.26
		45-60	7.05	1.67	0.71	3.74	6.39	5.25	20.93	187.45	21.19	6.99	130.86
		60-120	7.14	0.43	1.19	7.25	4.75	5.99	12.25	146.21	21.67	0.55	75.50

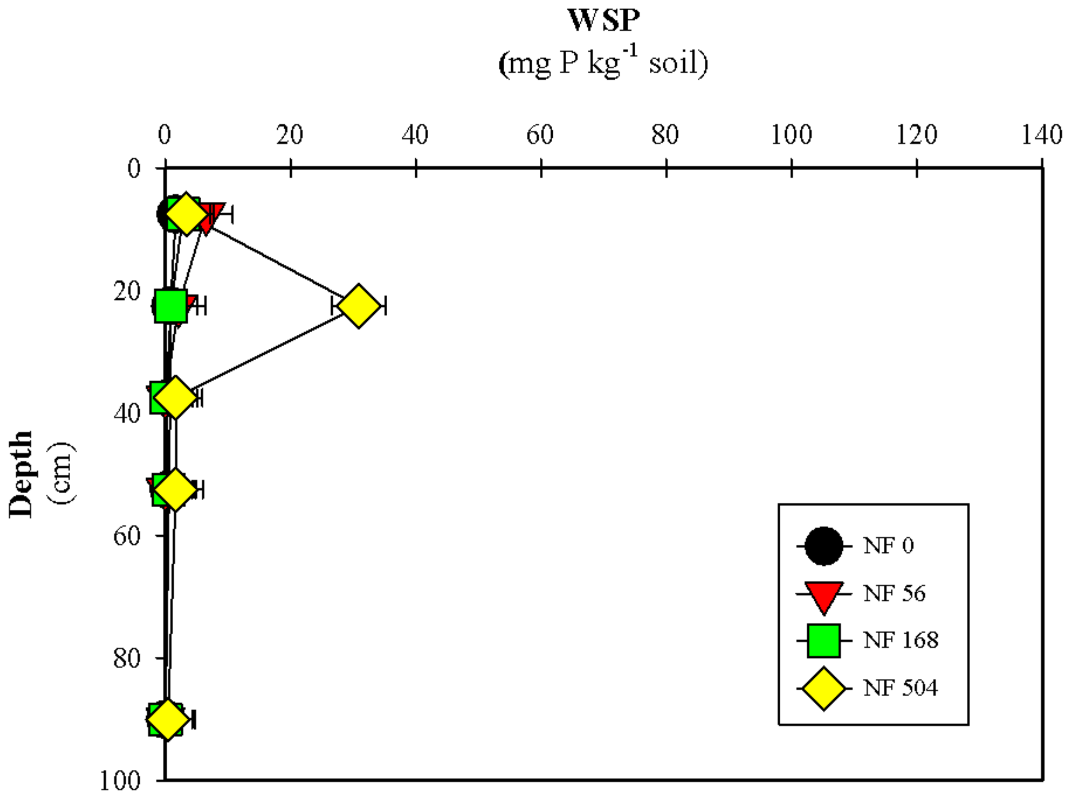

Figure 7. Distribution of water-soluble phosphorus (WSP)- Pi with depth in Gruver clay loam soil amended with N-fertilizer at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

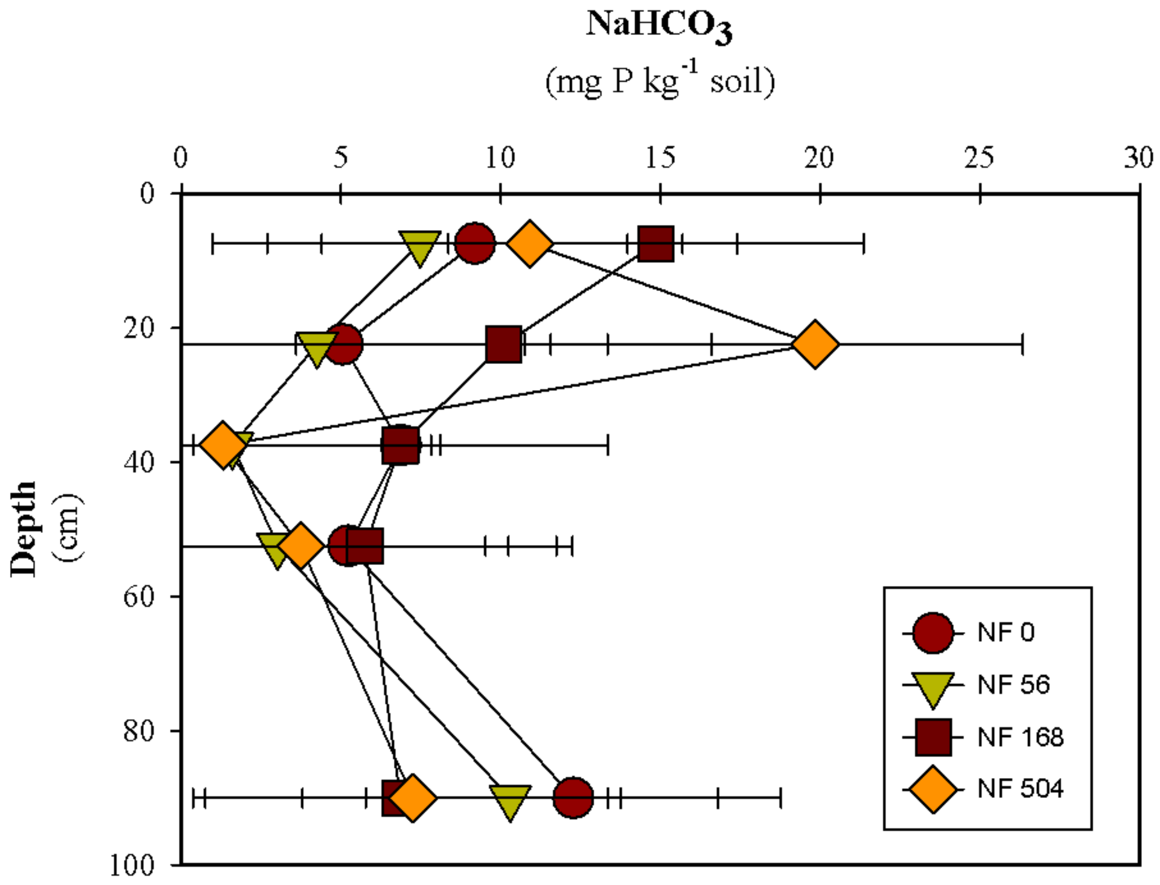

Figure 8. Distribution of sodium bicarbonate ($NaHCO_3$)-Pi with depth in Gruver clay loam soil amended with N-fertilizer at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

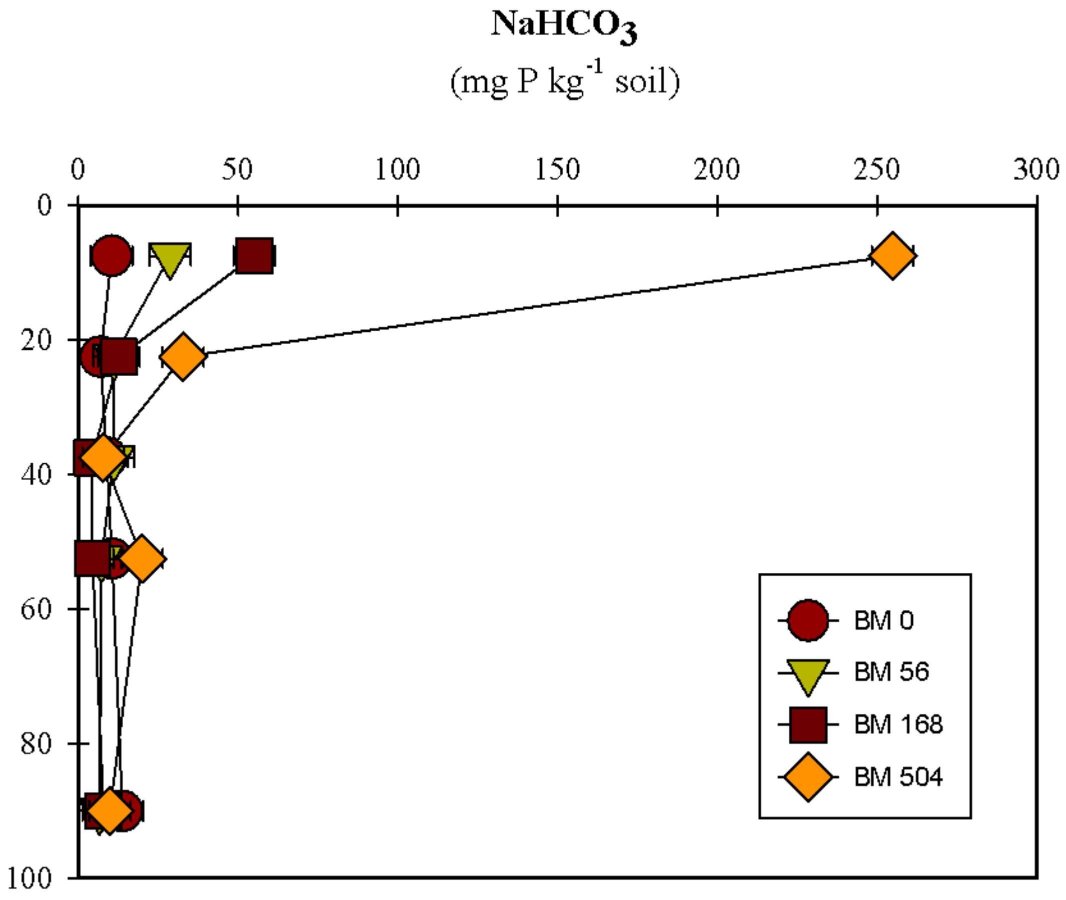

Figure 9. Distribution of sodium bicarbonate ($NaHCO_3$)-Pi with depth in Gruver clay loam soil amended with beef manure at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

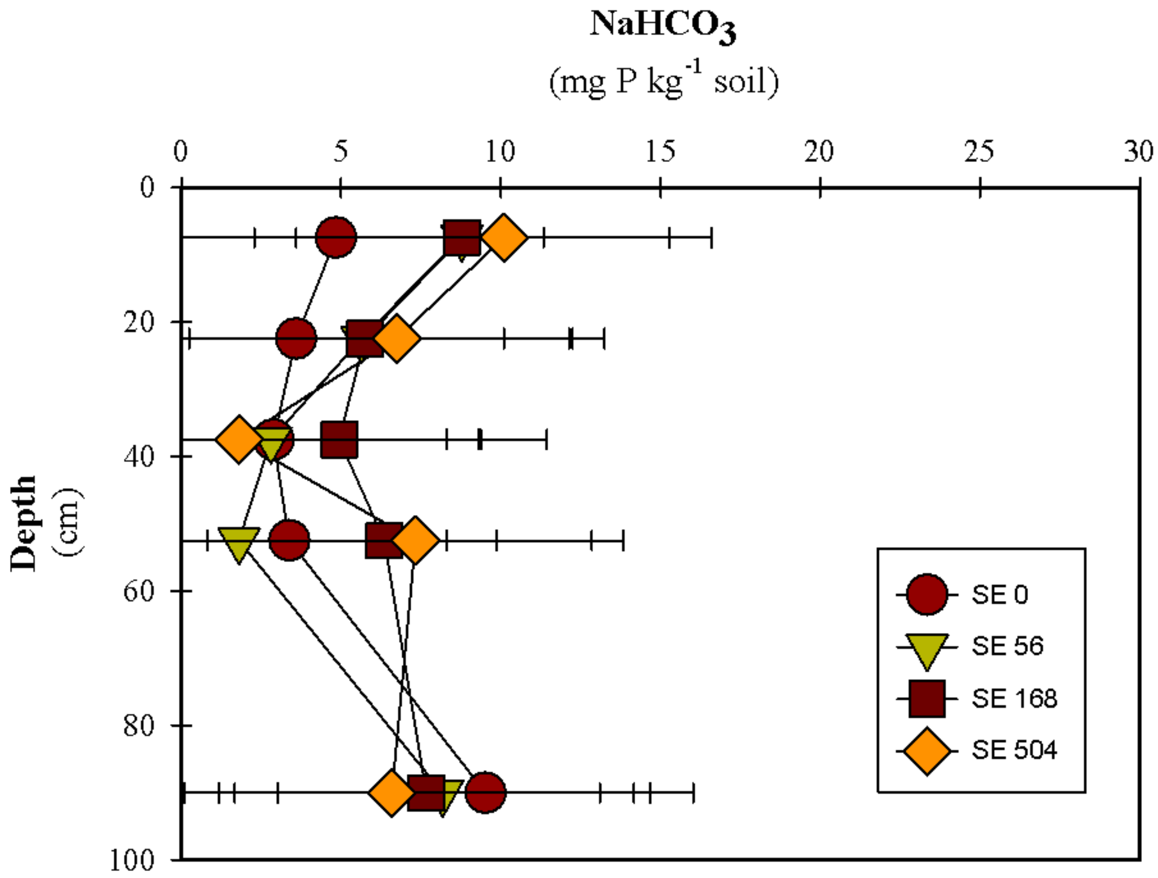

Figure 10. Distribution of sodium bicarbonate ($NaHCO_3$)-Pi with depth in Gruver clay loam soil amended with swine effluent at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

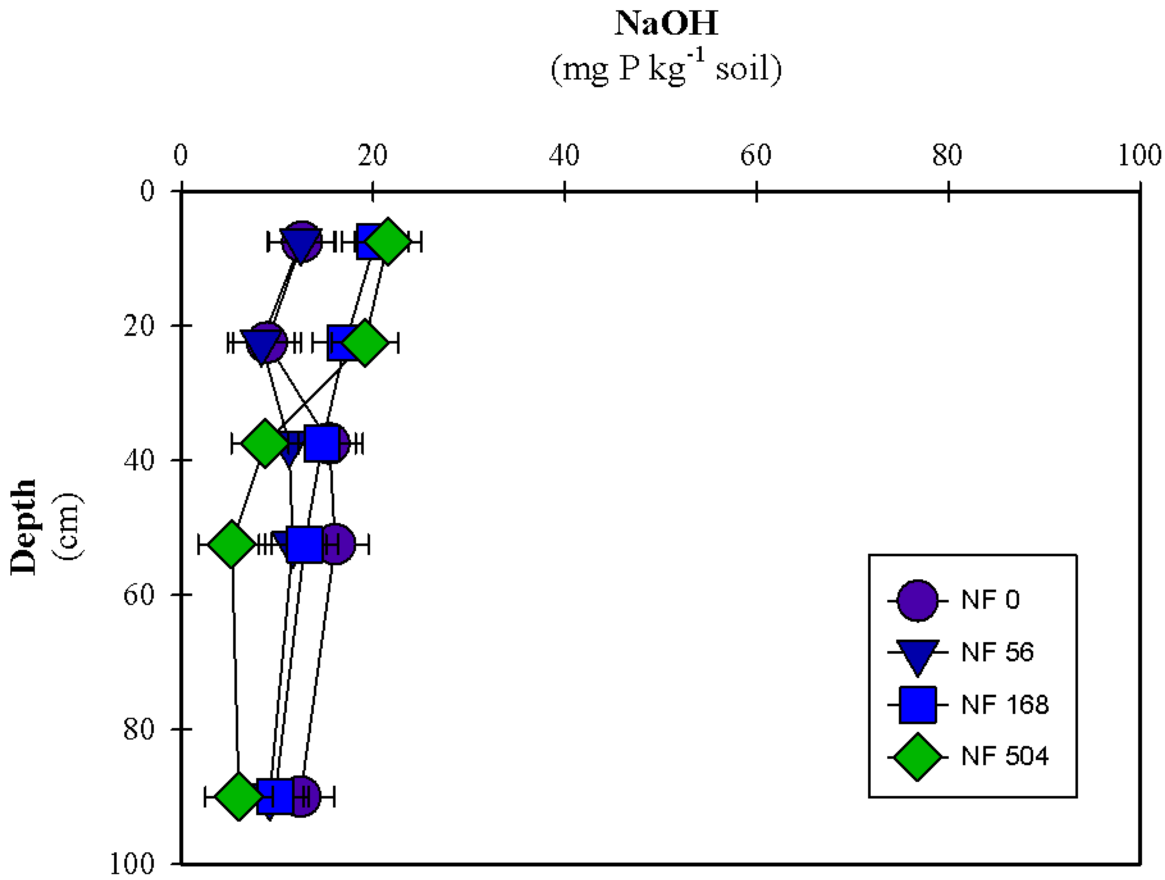

Figure 11. Distribution of sodium hydroxide (NaOH)-Pi with depth in Gruver clay loam soil amended with N-fertilizer at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

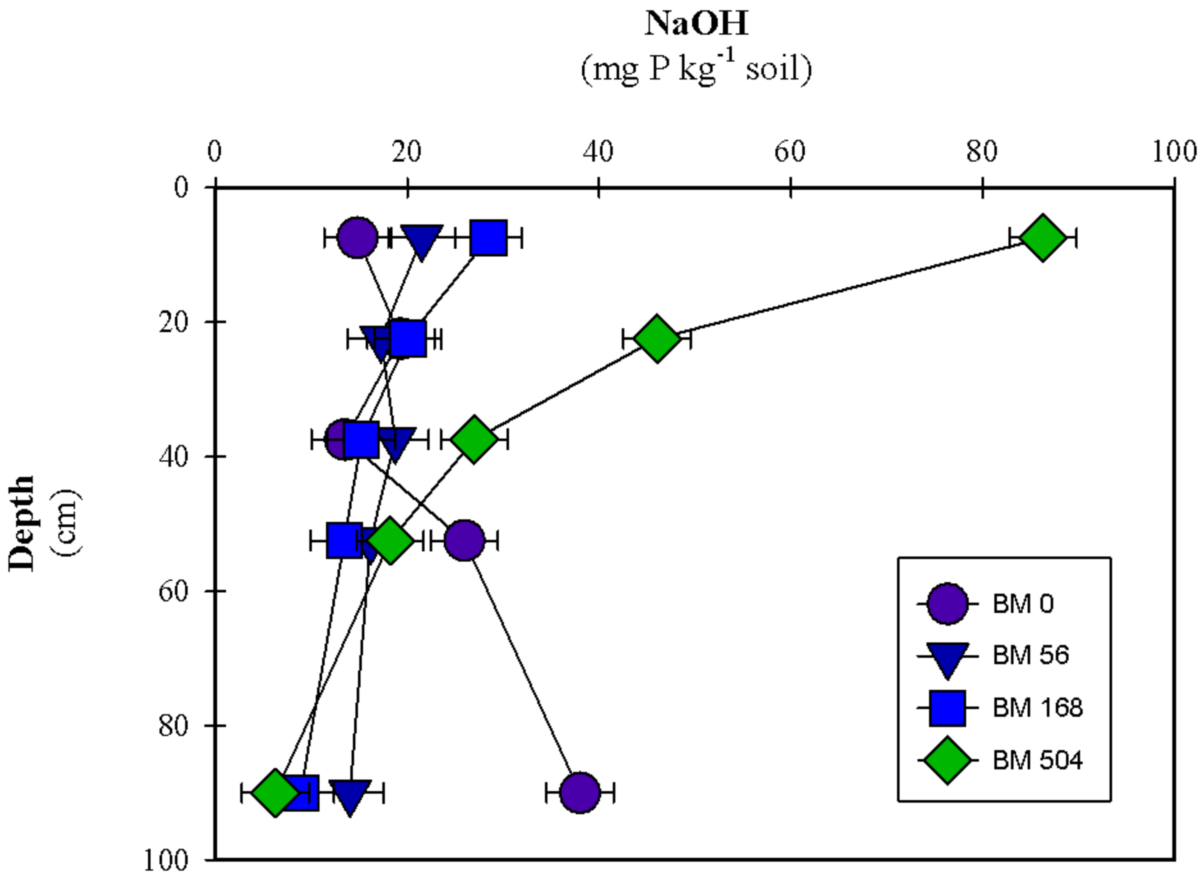

Figure 12. Distribution of sodium hydroxide (NaOH)-Pi with depth in Gruver clay loam soil amended with beef manure at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

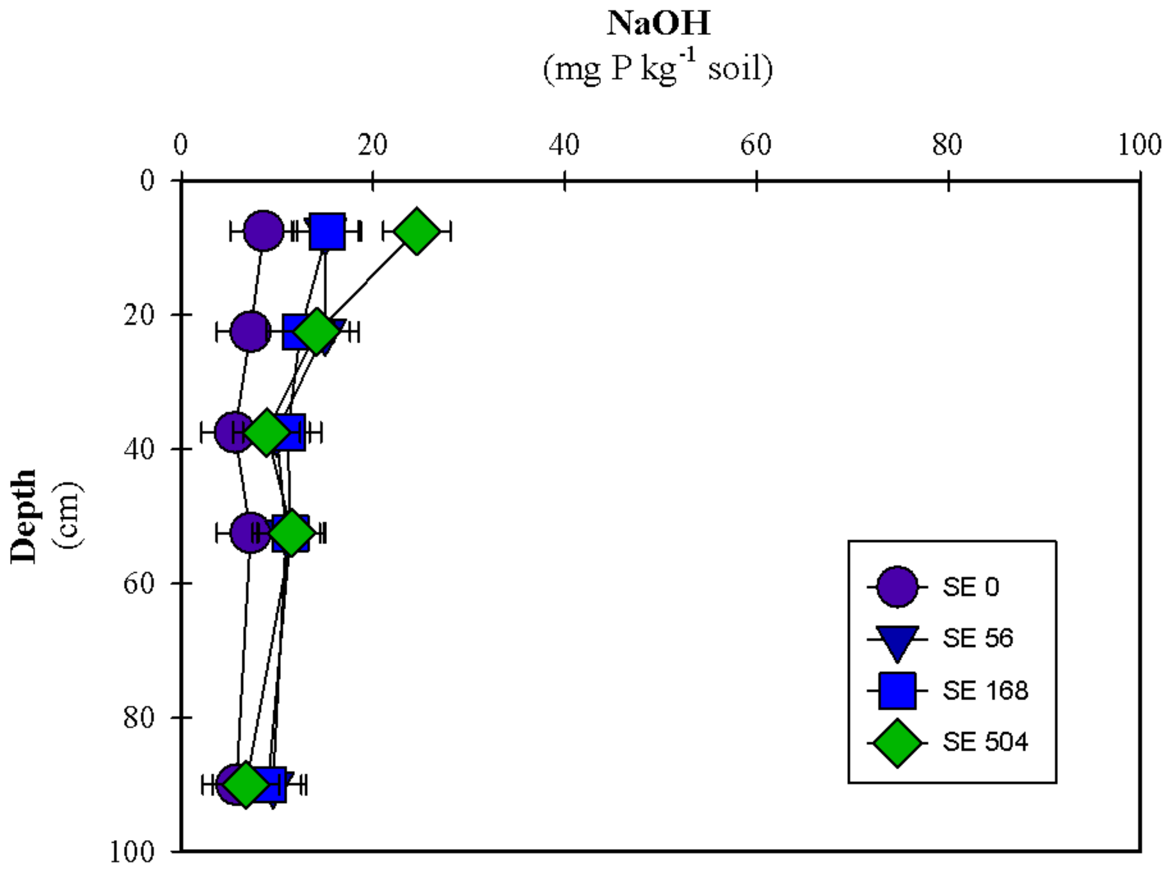

Figure 13. Distribution of sodium hydroxide (NaOH)-Pi with depth in Gruver clay loam soil amended with swine effluent at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

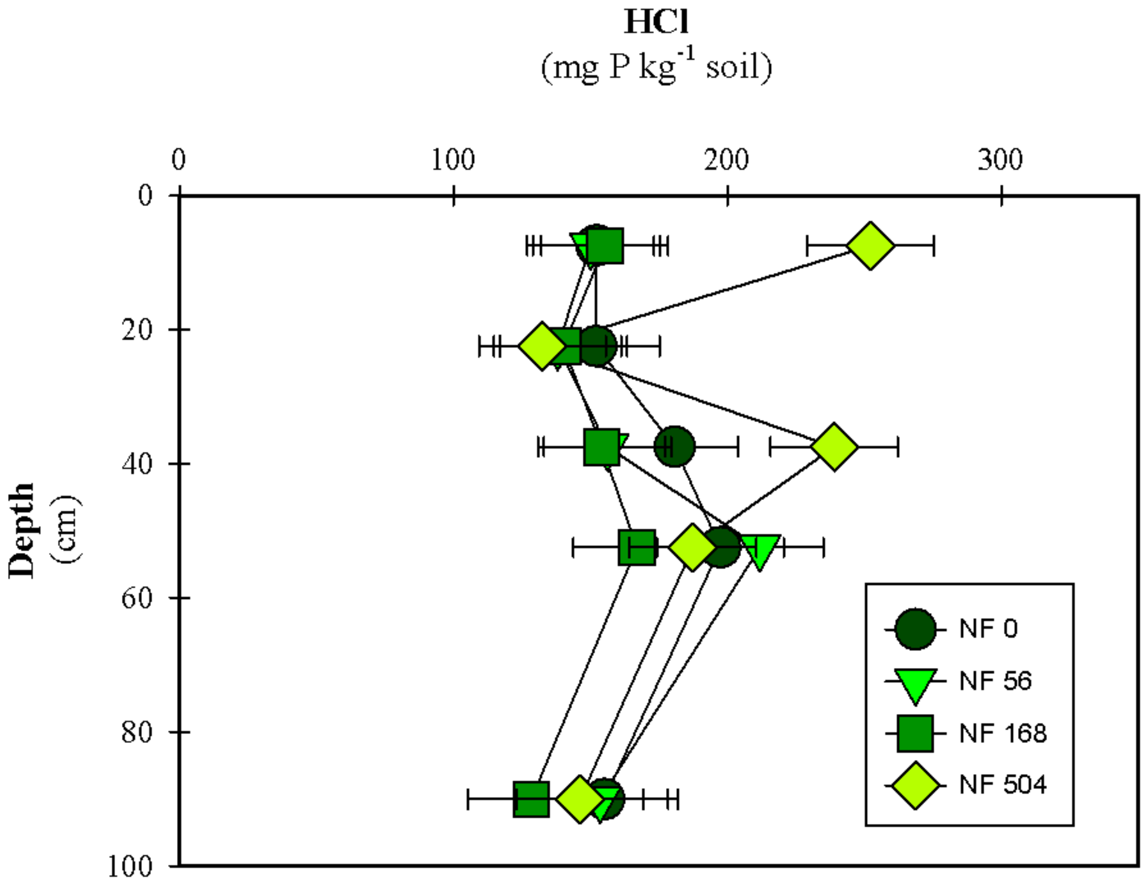

Figure 14. Distribution of 1M hydrochloric acid (HCl)-Pi with depth in Gruver clay loam soil amended with N-fertilizer at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

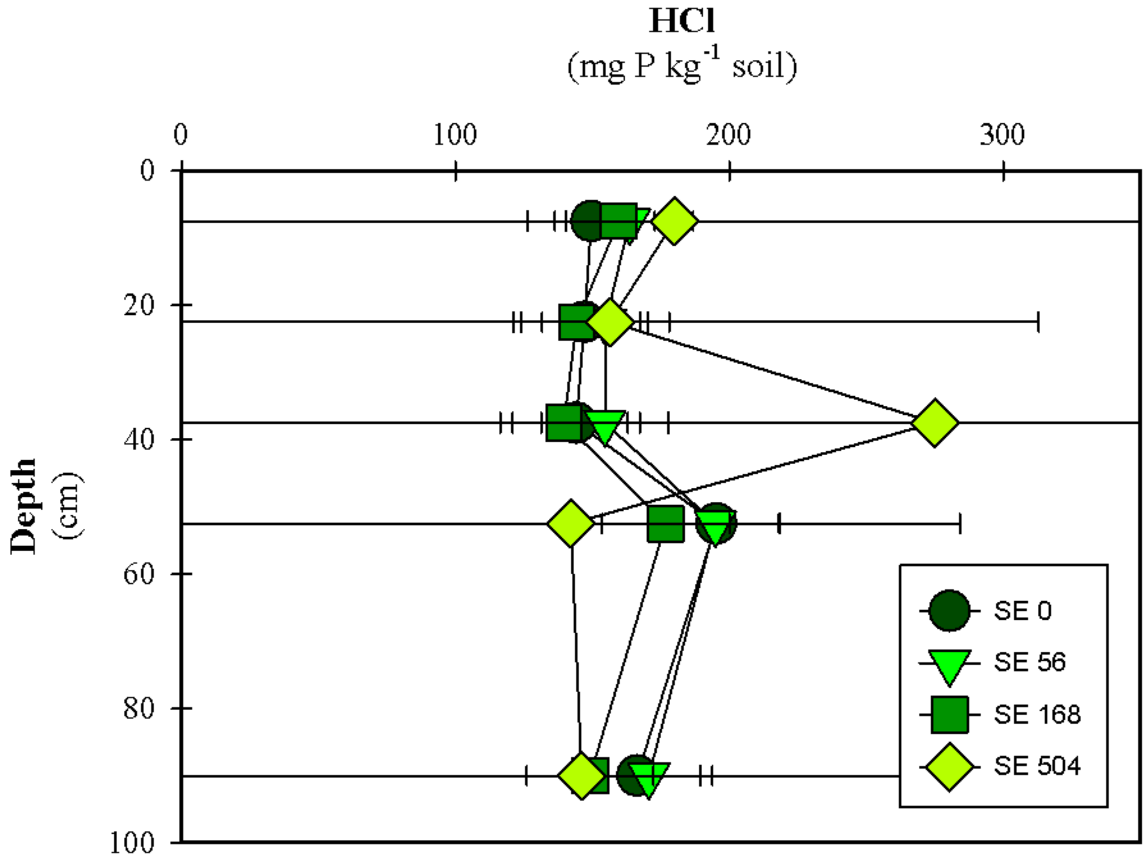

Figure 15. Distribution of 1M hydrochloric acid (HCl)-Pi with depth in Gruver clay loam soil amended with swine effluent at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

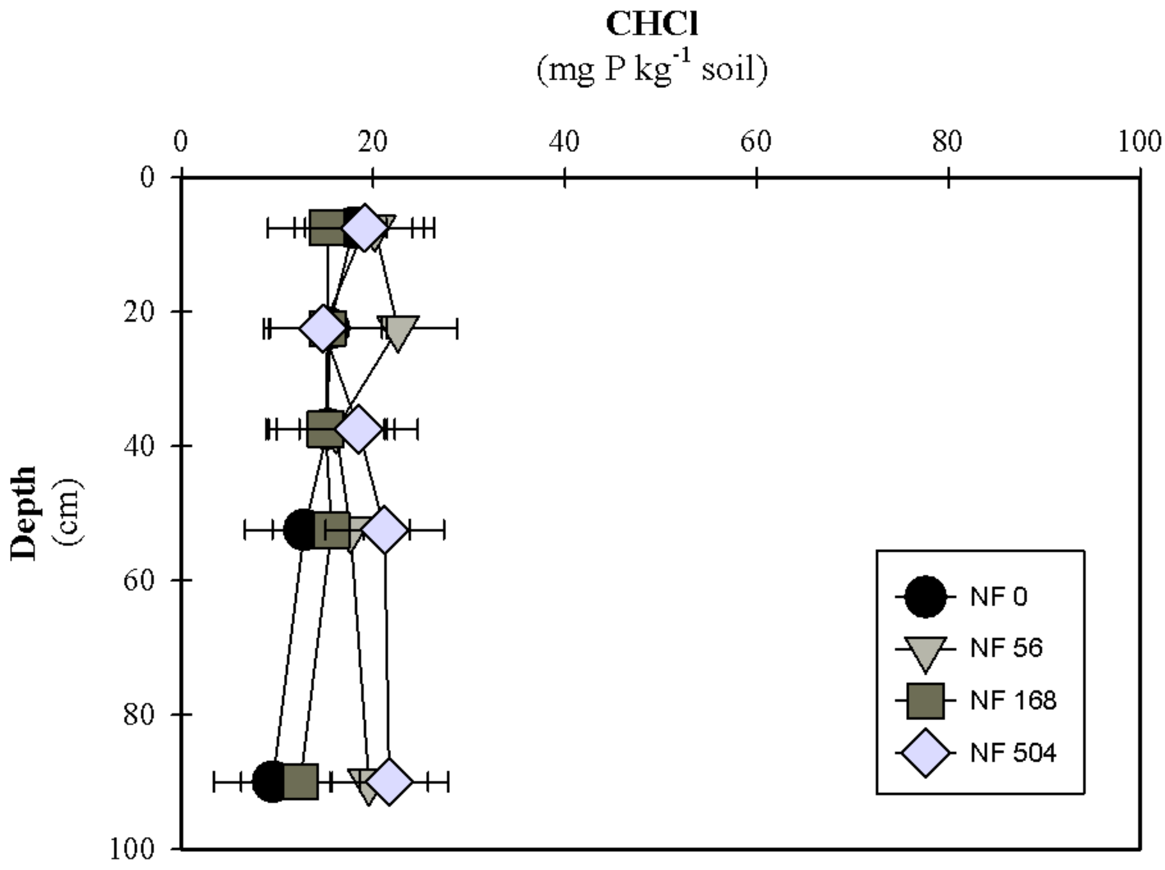

Figure 16. Distribution of 11.3M hydrochloric acid (CHCl)-Pi with depth in Gruver clay loam soil amended with N-fertilizer at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

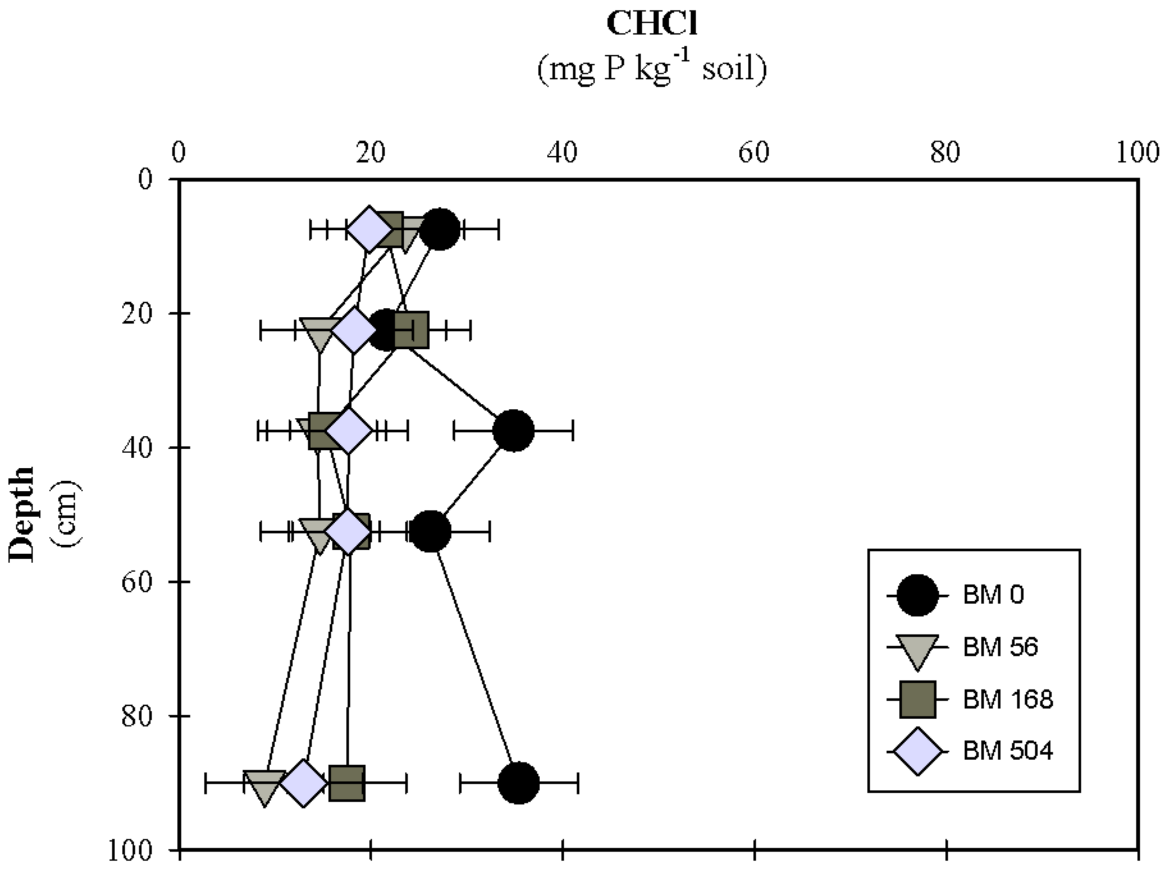

Figure 17. Distribution of 11.3M hydrochloric acid-Pi with depth in Gruver clay loam soil amended with beef manure at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

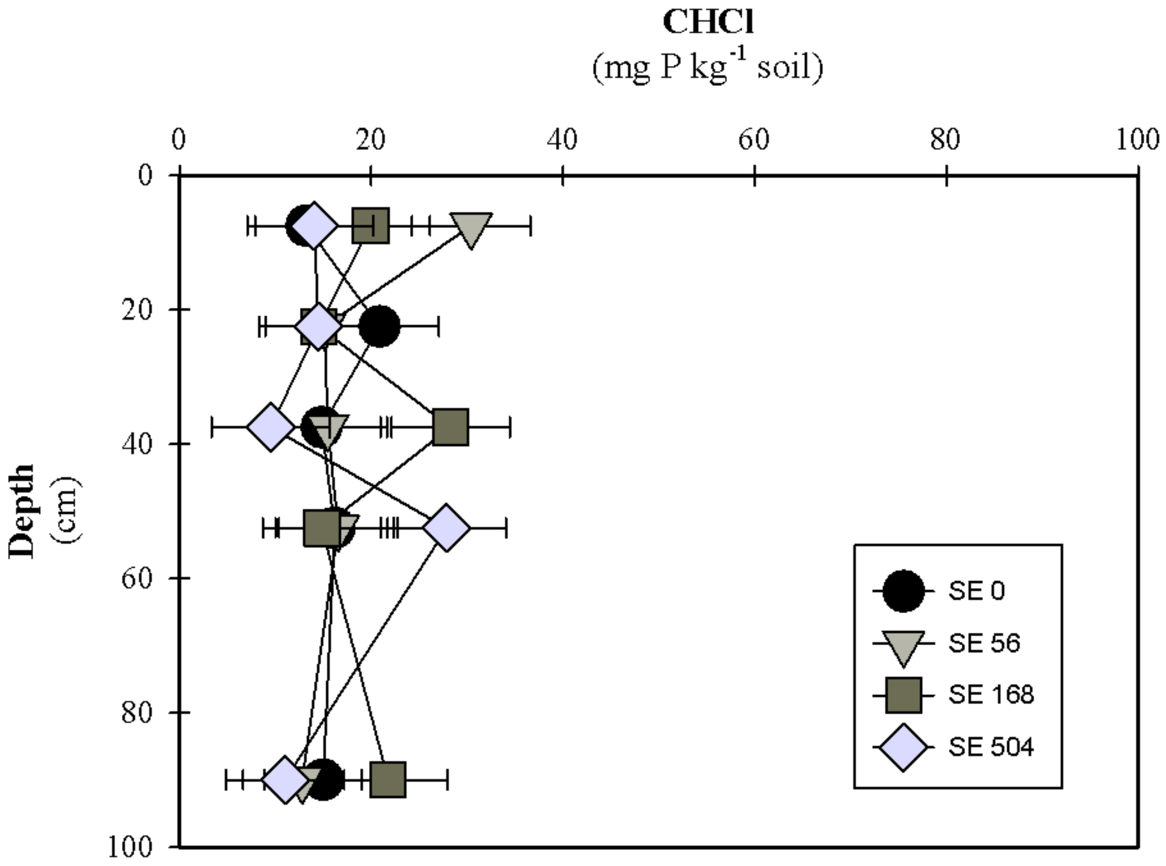

Figure 18. Distribution of 11.3M hydrochloric acid (CHCl)-Pi with depth in Gruver clay loam soil amended with swine effluent at 0, 56, 168 and 504 kg ha^{-1} rates over 13 years. Horizontal bars indicate standard error.

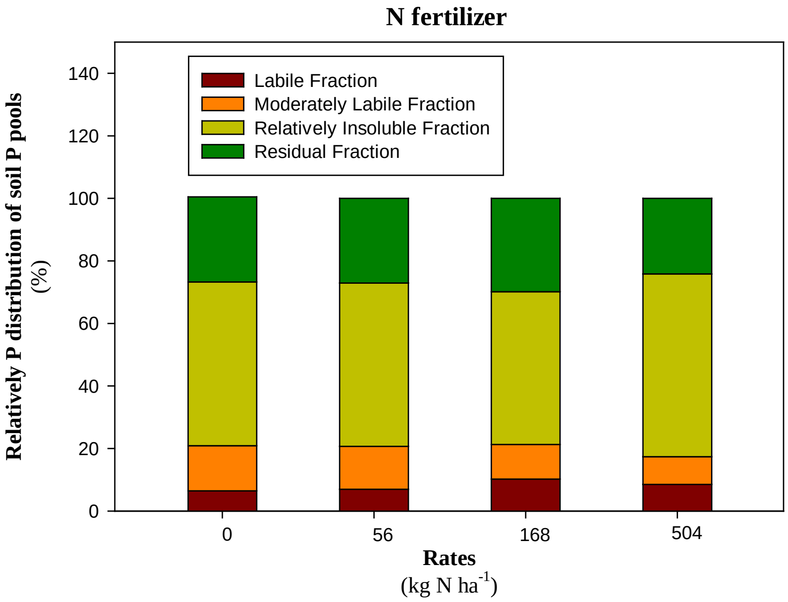

Figure 19. Relatively phosphorus distribution of soil phosphorus pools amended with N fertilizer at the surface for each rate.

VITA

Kaliana Moro Tanganelli

Candidate for the Degree of

Master of Science

Thesis: SEQUENTIAL FRACTIONATION AND WATER SOLUBLE PHOSPHORUS METHODS TO INVESTIGATE SOIL PHOSPHORUS IN A LONG-TERM MANURE APPLICATION

Major Field: Plant and Soil Science

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Education:

Completed the requirements for the Master of Science in Plant and Soil Science at Oklahoma State University, Stillwater, Oklahoma in May, 2011.

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ADVISER'S APPROVAL: Dr. Jeffory A. Hattey

Name: Kaliana Moro Tanganelli Date of Degree: May, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: SEQUENTIAL FRACTIONATION AND WATER SOLUBLE PHOSPHORUS METHODS TO INVESTIGATE SOIL PHOSPHORUS IN A LONG-TERM MANURE APPLICATION

Pages in Study: 87 Candidate for the Degree of Master of Science

Major Field: Plant and Soil Science

Scope and Method of Study: The objectives of this study were to investigate soil P using sequential fractionation, and to evaluate how long-term manure applications affect the amount and of bioavailable P and water soluble P (WSP) levels at multiple depths (0-120 cm) in the soil when different N sources (beef manure, swine effluent and anhydrous ammonia) are utilized as sources of plant nutrients.

Findings and Conclusions: The results showed that 13 years of animal manure application has increased soil P pools in the soil profile Gruver clay loam. The soil P fractionation results suggest that continuous application of beef manure on the basis of crop N requirements significantly increased all P fractions. A possible solution to this problem is applying animal manure based on crop P rather than N requirements. Thus, animal waste management practices are an important nutrient source that can be a viable alternative to a commercial fertilizer. However, soil and weather condition as well as animal manure composition should be considered for a sustainable model in the agricultural production.

ADVISER’S APPROVAL: Dr. Jeffory A. Hattey