# UNDERSTANDING PHOSPHORUS DYNAMICS OF TWO ALLUVIAL SOILS GROWN WITH CORN AT DIFFERENT PHOSPHORUS RATES

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agriculture and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

in

The School of Plant, Environmental, and Soil Sciences

by Marilyn S. Dalen B.Sc. Agri., Visayas State University, Leyte, Philippines, 2000 December 2012

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# **Table of Contents**

Acknowledgements	ii
List of Tables	iv
List of Figures	v
Abstract	vii
Chapter 1. Introduction	1
Chapter 2. Influence of Phosphorus Rates on Growth and Yield of Corn Grown	on Two
Alluvial Soils of Louisiana.	7
2.1 Introduction	
2.2 Materials and Methods	
2.2.1 Site description, soil collection and preparation	
2.2.2 Greenhouse experiment.	
2.2.3 Laboratory analysis	
2.2.3.1 Soil analysis	
2.2.3.2 Plant analysis	
2.2.4 Data analysis	
2.3 Results and Discussion	
2.3.1 Soil physical and chemical properties of the two alluvial soils	
2.3.2 Corn response to P fertilization.	
2.3.3 Mehlich-3 and Bray-2 soil tests for extractable phosphorus	
2.4 Conclusions	
2.5 References	
215 100101005	
Chapter 3. Influence of Phosphorus Rate on Changes on Phosphorus Functional	Fractions
of Two Louisiana Alluvial Soils	
3.1 Introduction	
3.2 Materials and Methods	
3.2.1 Soil samples.	
3.2.2 Inorganic phosphorus fractionation	
3.2.3 Total phosphorus analysis	
3.2.4 Data analysis	
3.3 Results and Discussion	
3.3.1 Inorganic phosphorus	
3.3.2 Total P, residual P, and total inorganic P	
3.4 Conclusions	
3.5 References.	
Chapter 4. Conclusions	
Vita	51
Vita	J4

# List of Tables

Table 2.1. Soil classification, particle size distribution and chemical properties of   Commerce silt loam and Perry clay soils	.18
Table 2.2. Soil pH and Mehlich 3 extractable P, K, Ca, Mg of Commerce sl and Perry clay at 30 days after lime and P application (30 DAP), and at harvest of corn.	.19
Table 2.3. Effect of different phosphorus fertilizer rates on plant height (cm) atV8, V12, and tasseling stage of corn	21
Table 3.1. Distribution of inorganic phosphorus fractions of Commerce silt loam and   Perry clay at 30 days after P fertilizer   application	.42
Table 3.2. Distribution of inorganic phosphorus fractions of Commerce silt loam and   Perry clay after harvest of corn	.42

# List of Figures

Figure 2.1. Major land resource areas of Louisiana (Soil Survery Staff, 2008). Perry clay soil was obtained from a corn field near Monroe (a) while Commerce silt loam soil was collected from the LSU AgCenter Northeast Research
Station in St. Joseph (b)
Figure 2.2. Treatment establishment in potted Commerce sl and Perry clay soils: (A) pre-potted soil, (B, C) removal of the top 15 cm soil, (D) fertilizer application, (E) mixing and (F) re-potting treated soil
Figure 2.3. Total biomass of corn grown on Commerce silt loam and Perry clay soils in response to different phosphorus fertilizer rate
Figure 2.4. Grain yield of corn grown on Commerce silt loam and Perry clay soils in response to different phosphorus fertilizer rate
Figure 2.5. Grain P uptake of corn grown on Commerce silt loam and Perry clay soils in response to different phosphorus fertilizer rate
Figure 2.6. Relationship of P fertilizer rate and Mehlich-3 extractable P 30 days after P application (A) and at harvest (B) for both Perry clay and Commerce silt loam soils25
Figure 2.7. Relationship of P fertilizer rate and Bray-2 extractable P 30 days after P application (A) and at harvest (B) for both Perry clay and Commerce silt loam soils26
Figure 3.1. Sequential fractionation scheme for inorganic phosphorus (Zhang and Kovar, 2000)
Figure 3.2. Inorganic phosphorus pools of Commerce sl and Perry clay soils before P fertilizer application
Figure 3.3. Inorganic phosphorus pools of Commerce sl as influenced by different phosphorus rate at 30 days after application
Figure 3.4. Inorganic phosphorus pools of Commerce sl as influenced by different phosphorus application rate at harvest
Figure 3.5. Inorganic phosphorus pools of Perry clay as influenced by different phosphorus rate at 30 days after application
Figure 3.6. Inorganic phosphorus pools of Perry clay as influenced by different phosphorus application rate at harvest
Figure 3.7. Total inorganic phosphorus, residual P and total P of Commerce sl as influenced by different phosphorus application rate at 30 days after application

Figure 3.8. Total inorganic phosphorus, residual P and total P of Commerce sl as influenced by different phosphorus application rate at harvest	47
Figure 3.9. Total inorganic phosphorus, residual P and total P of Perry clay as influenc by different phosphorus application rate at 30 days after application	
Figure 3.10. Total inorganic phosphorus, residual P and total P of Perry clay as influenced by different phosphorus application rate at harvest	48

### Abstract

There is little information documented on the influence of soil properties on P availability of Louisiana alluvial soils thus this pot experiment was conducted in 2011 to: 1) evaluate the effect of P fertilizer rate on growth and development of corn grown on Perry clay and Commerce sl soils, 2) relate soil test P values using Mehlich-3 and Bray-2 procedures with yield, total biomass, and P uptake of corn, and 3) identify the soil properties that influence P partitioning into functional fractions of two alluvial soils. Different P fertilizer rates (0, 34, 67, 101 and 134 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) were applied, replicated four times and arranged in a randomized complete block design. After 30 days, corn was planted and grown until maturity. Mehlich-3 extractable-P, Bray-2, total-P and P<sub>i</sub> fractions (labile-P, Al-P, Fe-P, reductant-P, and Ca-P) of soil samples collected at 30 DAP and at harvest were quantified.

The Bray-2 P values were about six times higher than Mehlich-3 P values for Commerce sl while for Perry clay, the amounts of P extracted by these two procedures were very similar (1:1 ratio). Both Bray-2 and Mehlich-3 extractable-P of both soils increased with increasing P rate. Commerce sl and Perry clay soils tested low to medium for Mehlich-3 extractable-P but responded differently with the application of P fertilizer. Grain yield of corn grown on Perry clay significantly responded to P rate but not in Commerce sl which was testing very high for Bray-2 extractable-P. The applied P fertilizer was transformed into Ca-P for Commerce sl while Perry cl transformed into Feand reductant-P. Overall, the labile- and Al-P at 30 DAP increased with increasing P rate. With time across P rates, both soils showed build-up of less readily-available reductant-P. For total-P, residual-P and total-Pi components, Commerce sl and Perry clay

vii

differed significantly (P<0.05) at both 30 DAP and harvest; while total-P and residual-P of both soils were not affected. Refinement of soil test P prediction should be pursued such that P fertilizer recommendations will not be based solely on P soil test.

# **Chapter 1. Introduction**

Corn (*Zea mays* L.) is an important cereal crop which ranks the third after wheat and rice in the world (David and Adams, 1985). Corn is grown widely in many countries of the world; the US is, by far, the largest producer of corn in the world. According to the USDA World Agricultural Outlook Board and World Agricultural Supply and Demand Estimates (updated August 2012), corn is the primary US feed grain, accounting for more than 90 % of total feed grain production and use. Corn is also processed into a multitude of food and industrial products including starch, sweeteners, corn oil, beverage and industrial alcohol, and fuel ethanol (USDA-ERS, 2012). The average yield in the US for 2011 is estimated at 9.2 Mg ha<sup>-1</sup> and the area harvested for grain is estimated at 34 million ha (USDA-NASS, 2011). Corn is grown over 400,000 farms in the US including the state of Louisiana.

In Louisiana, corn is becoming an important crop commodity and currently cultivated in areas previously grown to cotton. According to USDA-NASS report in 2011, more than 200,000 ha were planted to corn in the state with an average yield of >8 Mg ha<sup>-1</sup>. The gross value of Louisiana corn production in 2011 was \$452.6 million. Corn is produced in twenty five (25) parishes in Louisiana where majority is located in northeast Louisiana. Soils in the Mississippi, Arkansas, and Ouachita River alluvial plains constitute one of the seven distinct soil areas in Louisiana commonly under crop production occupying approximately more than 2 million ha.

Alluvial soils contain a unique group of soils. Soil texture varies dramatically across the state of Louisiana. Sorting of the sediments during deposition, together with a diverse mineralogy, have resulted in a considerable differences in the deposits. The rich sediments deposited developed into soils that are fertile, productive and able to support

crops like soybeans, corn, cotton, wheat, sugarcane and many others. However, Louisiana is also a state of abundant rainfall. With most areas of the state receiving 125-150 centimeter (cm) of precipitation annually, nutrients can be readily leached out of the root zone, causing poor soil fertility (Weindorf, 2008). Furthermore, the use of cultural practices such as excessive tillage resulted in the depletion of organic matter in the soils and consequently in a loss of natural fertility. According to Mascagni et al. (2007), P deficiency symptoms on corn seedlings are commonly seen and are most pronounced on the sandy loam and silt loam Mississippi River alluvial soils with organic matter levels of 0.5 to 1.0 percent such as Commerce silt loam soil but rarely occur on the finer-textured silty clay and clay soils.

For the year 2012, farmers expected to plant corn on more than 230,000 ha to increase production. Corn production area is likely to continue to increase in the next years to sustain the needs for corn grain-based food, feeds, and ethanol production (USDA-ERS, 2012). With the increasing demand and continuous planting of corn in the same size area, farmers should maintain soil productivity to maximize yield potential. Some of the major causes of low corn yield are declining soil fertility and insufficient use of fertilizers resulting in severe nutrient depletion of soils (Buresh et al., 1997). Corn requires adequate supply of nutrients particularly nitrogen (N), phosphorus (P), and potassium (K) for good growth and high yield. Corn yields benefit from a robust soil fertility program, including optimization of macronutrients to provide a balanced nutrient supply for long term production.

Phosphorus is one of the most important life-supporting elements on Earth. It is one of the three crucial nutrients for plant growth (N, P, and K) which are fundamental

for modern farming and critical for global food security (Ulrich et al., 2009). It is an essential nutrient for all living organisms and indispensable because no other element can replace it in its vital role in many physiological and biochemical processes (Syers et al., 2008). Thus, P is essential for the general health and vigor of all plants.

Phosphorus fertilizers are frequently applied to ensure optimal P nutrition of crops. Plants require adequate P from the very early stages of growth for optimum crop production (Grant et al., 2001). Studies in Ontario have shown that corn grain yield was strongly affected by P supply and tissue P concentration in the leaf four (L4) to leaf five (L5) stage, rather than by P concentration later in growth (Barry and Miller, 1989; Lauzon and Miller, 1997). The importance of P as yield limiting factor in many Nigerian soils is also well established (Adepetu, 1993).

Many agricultural systems in which the application of P fertilizers to the soil is necessary to ensure plant productivity, but the recovery of applied P by crop plants in a growing season is very low (10-30%), because in the soil more than 80% of the P becomes immobile and unavailable for plant uptake because of adsorption, precipitation, or conversion to the organic form (Tisdale et al., 1993; Holford, 1997).

In most soils, inorganic phosphorus (Pi) occurs at fairly low concentrations in the soil solution a large proportion of it is more or less strongly held by soil minerals. Phosphate ions can indeed be adsorbed onto positively charged minerals such as Ca, Fe and Al oxides (Hinsinger, 2001; Tiessen, 1998). According to Karaman et al. (2001) soil physical and chemical characteristics greatly affect P nutrition of plants. Among these are: (1) type of parent material from which the soil is derived; (2) degree of weathering;

and (3) climatic conditions. In addition, soil P levels are affected by erosion, crop removal and P fertilization.

Due to low concentration and poor mobility of plant-available P in soils, proper applications and management of chemical P fertilizers are needed to improve crop growth and yield. Furthermore, the response of corn plant to application of P fertilizers varies by variety and growing conditions, and also depends on the availability of other essential nutrients (Onasanya et al., 2009). The reserves of P in the world are finite and are gradually being depleted (Tiessen, 1995) thus there is a need to develop agricultural systems based on meeting minimum P requirements for crops. Soil P must be managed at concentrations that allow for good crop production.

The soil P concentration that correlates with P bioavailability is the greatest determinant of the balance between adequate soil P fertility and offsite P escape. In this regard, soil testing is likely the best management tool available to determine the need for P fertilization and to ensure that soils has enough supply of P to optimize crop production. Among the methods that were developed to test soil for crop available P include Olsen, (Olsen et al., 1954), Bray (Bray and Kurtz, 1945) and Mehlich-3 (Mehlich, 1984). Each test method has unique characteristics and may have wide different interpretation index. For example, at a given soil test level the interpretation may be optimum for one test, but maybe interpreted as low or high for another test (Sawyer and Mallarino, 1999). Usually, three types of categories are used regarding soil test values: low, medium, and high. A low soil test value offers a high probability of response to added fertilizer. A medium soil test value offers a medium probability of

getting a response (Dahnke and Olson, 1990). According to Mallarino (2009), interpretations and recommendations vary among states of the region which can be attributed to differences in soil properties and cropping systems. The interpretations and recommendations differ even with similar soil-test calibration data because the philosophy and assumptions of those making the recommendations also differ across states.

With the adoption of Mehlich-3 procedure by LSU AgCenter's Soil Testing and Plant Analysis Laboratory (STPAL), it is essential to ensure the validity of this soil test based on correlations with Bray-2 procedure. In 2008, multiple P field calibration studies were established at different locations in Louisiana using corn and soybean as test crops. Based on yield response curve with soil test P value established from these calibration field trials, the critical P level for soils in Louisiana was set at 35 mg kg<sup>-1</sup> (Tubana et al., 2011). They further categorized Mehlich-3 soil test values into the following: <10 ppm is considered very low, 11-20 ppm is low, 21-35 ppm is medium and >36 ppm is high. However, there is a difference in soil test interpretation between Mehlich-3 and Bray-2 soil test values for some soils in this corn-growing region in Louisiana. A very good example is the soil test calibration data for Commerce sl in the upper Mississippi River alluvial plain. For this soil type, the Mehlich-3 soil test P value is currently interpreted as low while its Bray-2 P soil test P value is categorized as very high. There is no current explanation or documentation for such inconsistency in soil test interpretation thus this study was established to: 1) evaluate the effect of P fertilizer rate on growth and development of corn grown on Perry clay and Commerce sl soils, two alluvial soils of Louisiana which are acidic and testing low to medium for P using Mehlich-3 procedure,

and 2) relate soil test P values using Mehlich-3 and Bray-2 procedures with yield, total biomass, and P uptake of corn.

Furthermore, there is little information on the influence of soil properties on P availability for Louisiana alluvial soils particularly on P availability. Thus, this study was also conducted to identify the soil properties that influence P partitioning into functional fractions of two alluvial soils in Louisiana. To quantify Pi and Po (organic phosphorus) compounds, different sequential chemical P fractionation schemes have been developed (Chang and Jackson, 1957; Pratt and Garber, 1964; Williams et al., 1971; Hedley et al., 1982; Zhang and Kovar, 2000). Sequential extraction procedures utilize the ability of various chemical reagents to selectively solubilize the Al, Fe, or Ca phosphate phases contained in the soil. Although imperfect separation may exist, it has been shown that with careful design and interpretation chemical fractionation procedures can be very useful in revealing the controlling phases of soil P dynamics (Sui et al., 1999; Delgado and Torrent, 2000; Maguire et al., 2000). Also, the facts on P fractionation methodologies are also significant for accurate interpretation of P chemistry/fertility and for making nutrient management decisions.

# Chapter 2. Influence of Phosphorus Rates on Growth and Yield of Corn Grown on Two Alluvial Soils of Louisiana

#### **2.1 Introduction**

Corn (*Zea mays* L.) is an important cereal crop which ranks the third after wheat and rice in the world (David and Adams, 1985). Corn is grown widely in many countries of the world; the US is, by far, the largest producer of corn in the world. According to the USDA World Agricultural Outlook Board and World Agricultural Supply and Demand Estimates (updated August 2012), corn is the primary US feed grain, accounting for more than 90 % of total feed grain production and use. Corn is also processed into a multitude of food and industrial products including starch, sweeteners, corn oil, beverage and industrial alcohol, and fuel ethanol (USDA-ERS, 2012). Corn for grain production is estimated at 310 billion kg. The average yield in the US for 2011 is estimated at 9.2 Mg ha<sup>-1</sup> and the area harvested for grain is estimated at 34 million ha (USDA-NASS, 2011). Corn is grown over 400,000 farms in the US including the state of Louisiana.

In Louisiana, corn is becoming an important crop commodity and currently cultivated in areas previously grown to cotton. Corn is produced in twenty five (25) parishes in Louisiana where majority is located in northeast Louisiana. Soils in the Mississippi, Arkansas, and Ouachita River alluvial plains constitute one of the seven distinct soil areas in Louisiana commonly under crop production occupying approximately more than 2 million ha. According to USDA-NASS report in 2011, more than 200,000 ha were planted to corn in the state with an average yield of >8 Mg ha<sup>-1</sup>. The gross value of Louisiana corn production in 2011 was \$452.6 million; substantially higher than \$282.7 million attained in 2010 due to increased harvested areas, increased

yields and significantly higher grain prices (LSU AgCenter, 2011). For the year 2012, farmers expected to plant corn on more than 230,000 ha to increase production. Corn production area is likely to continue to increase in the next years to sustain the needs for corn grain-based food, feeds, and ethanol production (USDA-ERS, 2012). With the increasing demand and continuous planting of corn in the same size area, farmers should maintain soil productivity to maximize yield potential. Some of the major causes of low corn yield are declining soil fertility and insufficient use of fertilizers resulting in severe nutrient depletion of soils (Buresh et al., 1997). Corn requires adequate supply of nutrients particularly nitrogen (N), phosphorus (P), and potassium (K) for good growth and high yield. Corn yields benefit from a robust soil fertility program, including optimization of macronutrients to provide a balanced nutrient supply for long term production.

Phosphorus is one of the most important life-supporting elements on Earth. It is one of the three crucial nutrients for plant growth (N, P, and K) which are fundamental for modern farming and critical for global food security (Ulrich et al., 2009). It is an essential nutrient for all living organisms and is indispensable because no other element can replace it in its vital role in many physiological and biochemical processes (Syers et al., 2008). Thus, P is essential for the general health and vigor of all plants. Some specific growth factors that have been associated with P are: stimulated root development, increased stalk and stem strength, improved flower formation and seed production, more uniform and earlier crop maturity, increased N-fixing capacity of legumes, improvements in crop quality, and increased resistance to plant diseases (Griffith, 1999).

Phosphorus fertilizers are frequently applied to ensure optimal P nutrition of crops. Plants require adequate P from the very early stages of growth for optimum crop production (Grant et al., 2001). Studies in Ontario have shown that corn grain yield was strongly affected by P supply and tissue P concentration in the L4 to L5 stage, rather than by P concentration later in growth (Barry and Miller, 1989; Lauzon and Miller, 1997). The importance of P as yield limiting factor in many Nigerian soils is also well established (Adepetu, 1993).

Many agricultural systems in which the application of P fertilizers to the soil is necessary to ensure plant productivity, but the recovery of applied P by crop plants in a growing season is very low (10-30%), because in the soil more than 80% of the P becomes immobile and unavailable for plant uptake because of adsorption, precipitation, or conversion to the organic form (Tisdale et al., 1993; Holford, 1997). Due to low concentration and poor mobility of plant-available P in soils, proper applications and management of chemical P fertilizers are needed to improve crop growth and yield. Furthermore, the response of corn plant to application of P fertilizers varies by variety and growing condition in specific locations, and also depends on the availability of other essential nutrients (Onasanya et al., 2009).

The reserves of P in the world are finite and are gradually being depleted (Tiessen, 1995) thus there is a need to develop agricultural systems based on meeting minimum P requirements for crops. Soil P must be managed at concentrations that allow for good crop production. However, the soil P concentration that correlates with P bioavailability is the greatest determinant of the balance between adequate soil P fertility and offsite P escape. In this regard, soil testing is likely the best management tool

available to determine the need for P fertilization and to ensure that soils has enough supply of P to optimize crop production.

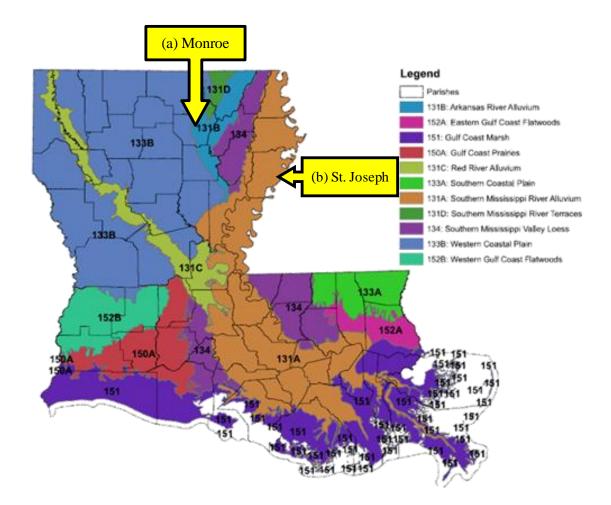
Among the methods that were developed to test soil for crop available P include Olsen, (Olsen et al., 1954), Bray (Bray and Kurtz, 1945) and Mehlich-3 (Mehlich, 1984). Each test method has unique characteristics and may have wide different interpretation index. For example, at a given soil test level the interpretation may be optimum for one test, but maybe interpreted as low or high for another test (Sawyer and Mallarino, 1999). Usually, three types of categories are used regarding soil test values: low, medium, and high. A low soil test value offers a high probability of response to added fertilizer. A medium soil test value offers a medium probability of response from added fertilizer and a high soil test value exhibits a low probability of getting a response (Dahnke and Olson, 1990). According to Mallarino (2009), interpretations and recommendations vary among states of the region which can be attributed to differences in soil properties and other production conditions across states. The interpretations and recommendations differ even with approximately similar crop response and soil-test calibration data because the philosophy and assumptions of those making the recommendations also differ across states.

With the adoption of Mehlich-3 procedure by LSU AgCenter's Soil Testing and Plant Analysis Laboratory (STPAL), it is essential to ensure the validity of this soil test based on correlations with Bray-2 procedure. In 2008, multiple P field calibration studies were established at different locations in Louisiana using corn and soybean as test crops. Based on yield response curve with soil test P value established from these calibration field trials, the critical P level for soils in Louisiana was set at 35 mg kg<sup>-1</sup> (Tubana et al.,

2011). They further categorized Mehlich-3 soil test values into the following: <10 ppm is considered very low, 11-20 ppm is low, 21-35 ppm is medium and >36 ppm is high. However, there is existing disagreement with soil test interpretation between Mehlich-3 and Bray-2 soil test values for some soils in this corn-growing region in Louisiana. A very good example of this soil type is the Commerce silt loam (sl) in the upper Mississippi River alluvial plain. For this soil type, the Mehlich-3 soil test P value is currently interpreted as low while its Bray-2 P soil test P value is categorized as very high. There is no current explanation or documentation for such inconsistency in soil test interpretation thus this study was establish to: 1) evaluate the effect of P fertilizer rate on growth and development of corn grown on Perry clay and Commerce sl soils, two alluvial soils of Louisiana which are acidic and testing low to medium for P using Mehlich-3 procedure, and 2) relate soil test P values using Mehlich-3 and Bray-2 procedures with yield, total biomass, and P uptake of corn.

### 2.2 Materials and Methods

**2.2.1 Site Description, Soil Collection and Preparation**. The soils used for the experiment were collected from two locations in northeast Louisiana: St. Joseph in Tensas and Monroe in Ouachita Parish (Figure 2.1). Soils from Tensas Parish consist of alluvium deposited by floodwaters from Mississippi River. The Commerce (sl) in St. Joseph (Latitude 31°, 56', 45" N; Longitude 91°, 13', 28") consists of deep, somewhat poorly drained, moderately slowly permeable soils that formed in loamy alluvial sediments. It is classified as thermic Fluvaquentic Endoaquepts.



**Figure 2.1** Major land resource areas of Louisiana (Soil Survery Staff, 2008). Perry clay soil was obtained from a corn field near Monroe (a) while Commerce silt loam soil was collected from the LSU AgCenter Northeast Research Station in St. Joseph (b).

On the other hand, the Perry clay soil collected from Monroe (Latitude 32°, 39', 7.83" N; Longitude 92°, 4', 7.81" W) consists of very deep, poorly drained, very slowly permeable soils that formed by clayey alluvium deposition by floodwaters from Arkansas River and classified as thermic Chromic Epiaquerts.

An area of about  $100 \text{ m}^2$  was selected from each location where bulk soil samples were collected from the top15 cm layer. The soils were mixed, air-dried, ground and

sieved to remove roots and plant debris before potting. Prior to potting, composite samples were taken from each soil.

**2.2.2 Greenhouse Experiment**. The greenhouse experiment was conducted at Louisiana State University campus in Baton Rouge from April 8, 2011 to July 26, 2011. For each soil, five rates of  $P_2O_5$  (0, 34, 67, 101 and 134 kg ha<sup>-1</sup>) were broadcast-applied. Treatments were replicated four times and arranged in a randomized complete block design.

Soil weight was adjusted based on moisture content of air-dried soils such that each plastic pot (diameter = 35.6 cm and height = 22.9 cm) was filled with 24 kg and 22 kg soils of Perry clay and Commerce sl soils, respectively. Phosphorus fertilizer was applied as Triple Superphosphate (TSP, 46% P<sub>2</sub>O<sub>5</sub>) at rates of 0, 0.78, 1.56, 2.34, and 3.11 g pot<sup>-1</sup> for Perry clay and 0, 0.72, 1.43, 2.15, 2.86 g pot<sup>-1</sup> for Commerce sl soils. These rates corresponded to 0, 34, 67, 101 and 134 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Based on soil test recommendation, K<sub>2</sub>O was applied at a rate of 34 kg ha<sup>-1</sup> (0.55 g pot<sup>-1</sup>) for Commerce sl and 67 kg K<sub>2</sub>O ha<sup>-1</sup> (1.19 g pot<sup>-1</sup>) for Perry clay as muriate of potash (KCl, 60% K<sub>2</sub>O). Lime application was also recommended at the rate of 1.12 Mt ha<sup>-1</sup> and applied as calcium carbonate (CaCO<sub>3</sub>). Lime, TSP and KCl were applied one month before planting.

Soil from each pot was transferred to a 20-L capacity plastic bin; P including the recommended rates of lime and KCl were thoroughly mixed with the soil. Soil was then placed back in the corresponding pot (Figure 2.2). Nitrogen fertilizer was applied at 200 kg ha<sup>-1</sup> in split in the form of urea ammonium nitrate (UAN, 32% N) at 7 and 45 days after planting to ensure adequate supply of N.



Figure 2.2. Treatment establishment in potted Commerce sl and Perry clay soils: (A) prepotted soil, (B, C) removal of the top 15 cm soil, (D) fertilizer application, (E) mixing and (F) re-potting treated soil.

The corn hybrid variety used was Pioneer 33R81. Three (3) seeds were sown per pot and at about V2 leaf growth stage, plants were thinned to one per pot. Water was maintained at field capacity until maturity. Plant height was measured at V8, V12, and tasseling growth stages. At harvest, whole plants were cut off at the soil surface. Each plant was partitioned into ear, husk, and stover, and dried in the oven for 48 hours at 65°C. Ear weight, grain weight, and number of grains per ear were determined for the yield and yield components. Total biomass and root were also weighed per pot.

Soils were sampled from initial, pre-planting and harvest for routine analysis and P sequential fractionation.

# 2.2.3 Laboratory Analysis

#### 2.2.3.1 Soil Analysis

Soil samples were dried, ground and passed through a 2 mm sieve for the following physical and chemical analysis.

2.2.3.1.a. Soil Particle Analysis (Gee and Bauder, 1986). Initial soil samples were determined for particle size distribution following pipette method using sodium hexametaphosphate (SHMP, 0.5% dispersing solution).

2.2.3.1.b. Soil pH (1:1 water). Ten (10) grams soil sample was weighed and added with 10 mL distilled water. Samples were shaken for 1 hour in a reciprocal shaker and sit undisturbed for 1 hour. The soil pH was measured using pH electrode meter.

2.2.3.1.c. Organic matter content (Walkley-Black, 1945). One (1) gram of soil was weighed and added with 10 ml 1 N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 20 ml concentrated sulfuric acid and

stand for 2 hours. Ninety (90) ml water was added and equilibrated for 16 hours and analyzed using colorimeter.

2.2.3.1.d. Extractable P, K and other elements. Soils were extracted by Mehlich-3 procedure (Mehlich, 1984). Two (2) grams of soil was weighed and 20 mL of extractant (dilute acid-fluoride-EDTA solution of pH 2.5) was added. The soil suspensions were shaken for 5 minutes using reciprocal shaker set at high speed. Solution was filtered using no. 42 Whatman filter paper and analyzed by Inductively-Coupled Plasma ICP-OES (SPECTRO CYRIOSCCD, Spectro Analytical Instruments, Inc., Fitchburg, MA). 2.2.3.1.e. Micronutrients by DTPA buffered at pH 7.3 (Lindsay and Norvell ,1978). Available metals were determined by using diethylene-triamine-pentacetic acid (DTPA) buffered at pH 7.3. Air-dried soil (10 g) was shaken with 20 mL of DTPA solution for 2 h. Extracts were collected by filtration through Whatman No.42 filter paper and analyzed for Zn, Cu, Mn, and Fe concentrations.

2.2.3.1. f. Extractable Phosphorus by Bray 2 Colorimetry method (Bray and Kurtz, 1945). Two (2) grams of soil was weighed and 20 mL of Bray-2 extractant (ammonium fluoride-HCl solution) was added. The soil suspensions were shaken for 5 minutes using reciprocal shaker set at high speed. Solution was filtered using no. 42 Whatman filter paper and analyzed for P by colorimetry ascorbic acid method using spectrophotometer with absorbance of 882 nm.

#### 2.2.3.2 Plant Analysis

Plant biomass was oven-dried and ground for total nutrient uptake determination. Biomass was digested by Nitric acid – Hydrogen peroxide method. A 0.5 gram plant tissue was weighed in kimwipes and placed into the digestion tube. Five (5 mL) of nitric

acid (HNO<sub>3</sub>, 67-70%) was added, ensuring that the acid washes any plant tissue on the side of the digestion tube down to the bottom and letting the samples stand undisturbed for 50 minutes. This time the digestion block was turned on to heat up to between 152 and  $155^{\circ}$ C. After 50 minutes, the sample was mixed using vortex mixer before placing the tube in the digestion block for 2-3 minutes to initiate vigorous boiling. Once the brown fumes appeared, tube was removed from the block and allowed to cool for 10 minutes. To each tube, 3 mL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added and placed back on the digestion block for 2.75 hours (2 hours and 45 minutes). After digestion, the samples were removed from the digestion block and were allowed to cool before transferring the digest to 15 mL centrifuge tubes making to a volume of 12.5 with deionized water. The digest solution was filtered using no. 42 Whatman for ICP multi-element analysis.

#### 2.2.4 Data Analysis

Analysis of variance was conducted using PROC MIXED in SAS (SAS, 2010) to determine the significance of differences among measured variables for each soil. The least square means test was performed to compare treatment means using the LSMEANS PDIFF option. The linear model between P extracted and P added was determined for both Bray-2 and Mehlich-3 procedure.

# 2. 3 Results and Discussion

#### 2.3.1 Soil physical and chemical properties of the two alluvial soils

The particle size distribution and chemical properties of the two alluvial soils are summarized in Table 2.1. Both soils had an initial pH value of <5.5 which is moderately acidic thus lime was applied at the rate of 1.12 Mt ha<sup>-1</sup> as CaCO<sub>3</sub> to raise the pH by at least 1 unit.

	Commerce silt loam	Perry clay		
Classification	Fine-silty, mixed, superactive,	Very-fine, clayey, smectitic,		
	nonacid, thermic Fluvaquentic	moderately acid, thermic		
	Endoaquepts	Chromic Epiaquerts		
Organic Matter, %	1.20	3.25		
Texture				
Sand, %	32	6		
Silt, %	52	36		
Clay, %	17	58		
pH (1:1 water)	5.40	5.25		
$P, mg kg^{-1}$	24	18		
K, mg kg <sup>-1</sup>	167	250		
Ca, mg kg <sup>-1</sup>	1324	1830		
Mg, mg kg <sup>-1</sup>	294	1526		
S, mg kg <sup>-1</sup>	15.4	28.0		
Fe, mg kg <sup>-1</sup>	70.50	150		
$Zn, mg kg^{-1}$	8.45	4.56		
Cu, mg kg <sup>-1</sup>	1.97	3.20		
Al, mg kg <sup>-1</sup>	0.52	2.60		
Na, mg kg <sup>-1</sup>	18.7	108		
$Mn mg kg^{-1}$	22.2	54.3		

Table 2.1. Soil classification, particle size distribution and chemical properties of Commerce silt loam and Perry clay soils.

Notes:

*Texture was analyzed using pipette method* 

P, K, Ca, Mg, S and Fe was analyzed using Mehlich-3

Zn and Cu was analyzed using DTPA

Table 2.2 summarizes soil pH and Mehlich-3 extractable P, K, Ca, and Mg of Commerce sl and Perry clay 30 days after lime and P application, and at harvest. The soil pH at 30 days after lime application increased to 6.9 for Commerce sl and 5.6 for Perry clay. Higher average pH values were recorded at harvest for both Commerce sl (pH 7.4) and Perry clay (6.3). Commerce sl has a course texture (32% sand and 52% silt) and lower OM (1.2%) compared with Perry cl which has a finer texture (58% clay) and higher OM content (3.25%). Soils which contain high clay and OM content are highly buffered thus, Perry cl has a higher buffering capacity and increase in pH was smaller than with Commerce sl. Correcting pH of cultivated soils is an essential program

Soil Type P2O5 Rate		рН		Р		K		Ca		Mg	
		30 DAP	Harvest	<b>30 DAP</b>	Harvest	30 DAP	Harvest	30 DAP	Harvest	30 DAP	Harvest
	kg ha⁻¹						mg k	g <sup>-1</sup>			
Commerce sl	0	6.9 a	7.3 a	30.1 d	22.2 d	172 a	135 a	1371 a	1492 a	229 a	214 a
	34	6.9 a	7.4 a	35.8 c	24.8 d	169 a	135 a	1379 a	1503 a	233 a	213 a
	67	7.0 a	7.5 a	39.8 b	30.8 bc	179 a	137 a	1412 a	1453 a	237 a	200 a
	101	7.0 a	7.5 a	42.1 b	35.0 b	159 a	134 a	1262 a	1400 a	213 a	204 a
	134	6.9 a	7.5 a	47.2 a	41.3 a	160 a	136 a	1340 a	1518 a	227 a	220 a
	Mean	6.9	7.4	39.0	30.8	168	135	1353	1473	228	210
Perry clay	0	5.6 a	6.3 a	18.6 d	11.8 a	255 a	194 a	1923 a	1893 a	1344 a	1428 a
	34	5.6 a	6.3 a	19.3 c	13.7 a	239 b	197 a	1792 b	1995 a	1259 b	1497 a
	67	5.7 a	6.3 a	25.3 b	15.4 a	254 a	192 a	1957 a	1888 a	1362 a	1404 a
	101	5.6 a	6.3 a	27.8 b	15.9 a	257 a	204 a	1950 a	2133 a	1369 a	1627 a
	134	5.7 a	6.4 a	33.1 a	16.0 a	260 a	188 a	1978 a	1890 a	1371 a	1423 a
	Mean	5.6	6.3	24.8	14.5	253	195	1920	1960	1341	1476

Table 2.2. Soil pH and Mehlich-3 extractable P, K, Ca, Mg of Commerce sl and Perry clay at 30 days after lime and P application (30 DAP), and at harvest of corn.

Note: Values within a column within soil type with the same letter are not significantly different at P < 0.05.

to ensure availability of essential plant nutrients such as P. Phosphorus in soil solution, soil pH and buffering capacity are determining factors of plant available P. Morel and Hinsinger (1999) reported that in highly P fertilized soils, the P concentration in soil solution is high (>1 ppm) and depletion is readily replenished however, replenishment is slow if soil solution P is low and soil's solid phase has a high buffering capacity. The availability of P in the soil depends largely on pH.

The greatest mobilization occurs at a pH value between 6 and 7. Phosphorus fixation is enhanced if soil pH becomes too low (acidic) or too high (alkaline). A study conducted by Gudu et al. (2005) showed that lime and P application stimulated the growth of corn grown on acid soils of Western Kenya. The combination of the low rate of lime (500 kg CaCO<sub>3</sub> ha<sup>-1</sup>) and P (10 kg P) was found to be optimum for plant growth (Oluwatoyinbo et al., 2005). Lime had significant positive effect on P concentration in plant and actually reduced the amount of fertilizer P required for optimum yield.

At both 30 DAP and harvest, Perry cl soil had higher Mehlich-3 extractable K, Ca, and Mg than Commerce sl (Table 2). On the other hand, Commerce sl soil had consistently higher Mehlich-3 extractable P than Perry clay (39 vs. 24.8 mg kg<sup>-1</sup>) for each sampling date. The amount of extractable P, K, and Mg of Commerce sl soil was reduced at harvest while slight increase in extractable Ca was noted which can be attributed to lime application. Due to nutrient removal by plant, the amount of extractable nutrients were expected to decline at the completion of crop cycle. For Perry cl, extractable P and K were both reduced but not Ca and Mg.

# 2.3.2 Corn response to P fertilization

Corn planted on Commerce sl was generally taller than corn planted on Perry cl. At tasselling, Commerce sl recorded an average corn height of 245 cm while Perry clay was only 198 cm. There was no clear and consistent effect of P rate on plant height measured at V8, V12, and tasseling stage of corn for either soil (Table 2.3). At tasselling on Commerce sl, height of corn with P tended to be shorter than corn without P. At the highest P rate of 134 kg  $P_2O_5$  ha<sup>-1</sup> corn height was significantly lower than that of the check (*P*<0.05). In contrast to earlier study conducted by Ayub et al. (2005), corn plants under similar level of applied N responded to P application. Corn plants with P rates of 80 kg  $P_2O_5$  ha<sup>-1</sup> were taller than those which received only 40 kg  $P_2O_5$  ha<sup>-1</sup>. Heckman et al. (2006) reported that based on combined analysis of variance over 51 experimental sites in Northeast USA showed than broadcast P application enhanced corn plant height at 35 days after planting and at silk emergence.

G - 11 T	P Rate	Plant Height (cm)				
Soil Type	$P_2O_5$ kg ha <sup>-1</sup>	<b>V8</b>	V12	Tasseling		
Commerce sl	0	159 a	206 a	254 a		
	34	156 ab	202 a	252 ab		
	67	156 ab	208 a	242 ab		
	101	146 b	180 a	233 ab		
	134	153 ab	194 a	243 b		
	Mean	154	198	244.6		
Perry clay	0	140 a	147 a	176.4 a		
	34	138 a	162 a	202.3 a		
	67	139 a	145 a	187.5 a		
	101	141 a	175 a	205.4 a		
	134	143 a	171 a	217.2 a		
	Mean	140	160	197.8		

Table 2.3. Effect of different phosphorus fertilizer rates on plant height (cm) at V8, V12, and tasseling stage of corn.

Note: Values with the same letter within column within soil type are not significantly different at P < 0.05.

Similar to plant height, total corn biomass was not affected by P rate for either soil (Fig. 2.3). Corn grown on Perry cl had higher total biomass than Commerce sl. Phosphorus rate significantly affected grain yield of corn grown on Perry clay (P<0.05; Fig. 2.4). Based on mean separation procedure, grain yields of the check pot and pots which received P were significantly different (P<0.05) where the highest P rate (134 P<sub>2</sub>O<sub>5</sub> kg ha<sup>-1</sup>) obtained the highest grain yield. However, the increase in grain yield was notably not proportionate with P rate. For example, pots which received 34 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> obtained higher grain yield than pots which received 67 and 101 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Grain P uptake for both soils showed similar pattern and response to P application as grain yield (Fig. 2.5).

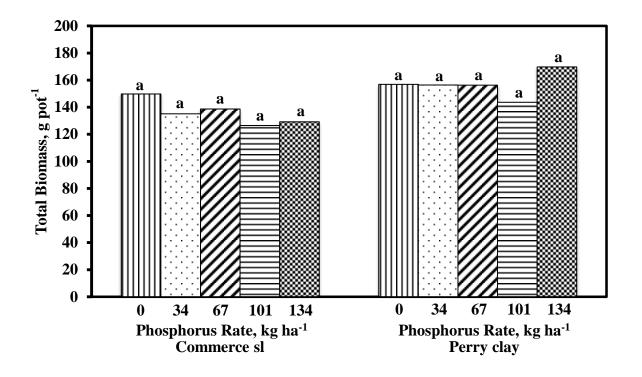


Figure 2.3. Total biomass of corn grown on Commerce silt loam and Perry clay soils in response to different phosphorus fertilizer rate.

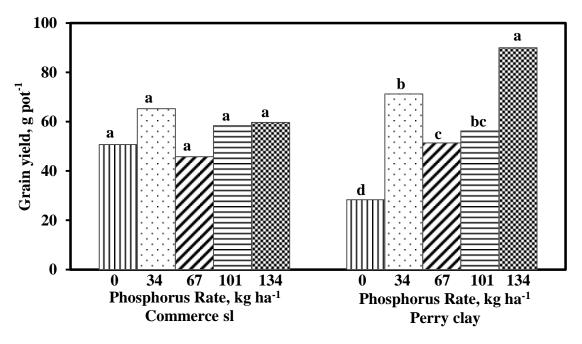


Figure 2.4. Grain yield of corn grown on Commerce silt loam and Perry clay soils in response to different phosphorus fertilizer rate.

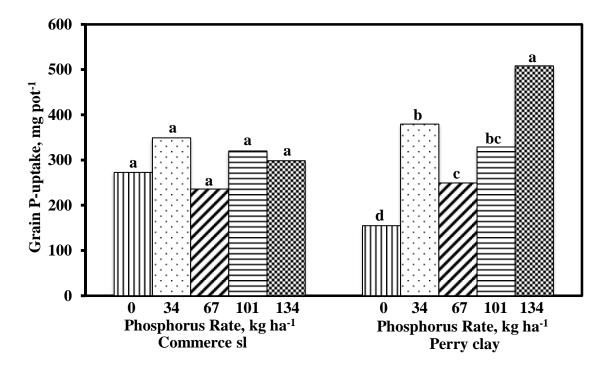


Figure 2.5. Grain P uptake of corn grown on Commerce silt loam and Perry clay soils in response to different phosphorus fertilizer rate.

The probability of crop to respond to fertilizer P is expected to be higher on soils testing low in P then declines with increasing soil test P levels. It is important to note that even if the soil test P levels are below the set critical P level in a specific region, crop response to P application is not always certain. Heckman et al. (2006) conducted soil test calibration studies during 1998 to 1999 at 51 experimental sites in northeastern states. Their results showed that only 17 to 47% of those sites with soil test levels below the currently used critical P level in the region exhibited positive grain yield response to P application. On the other hand, there were sites testing above the critical P level which responded as well to P application. For this study, the initial Mehlich-3 extractable P of Commerce sl and Perry clay are interpreted as medium and low-P testing soil, respectively (Tubana et al., 2011). This indicates that corn planted on Perry should likely to respond to P fertilization whereas response of corn grown on Commerce sl should be marginal. While the total biomass as a variable showed no response to P for both soils, the application of P raised corn grain yield on Perry clay by as much as 200 % with respect to grain yield level of the check pot.

## 2.3.3 Mehlich-3 and Bray 2 soil tests for extractable phosphorus

Figures 2.6 and 2.7 summarize the Mehlich-3 and Bray-2 extractable P of Commerce sl and Perry cl at different P rates at 30 DAP and harvest. There was a difference between Mehlich-3 and Bray-2 extractable P on Commerce sl as opposed to Perry cl soil. The initial soil test P values of Perry cl were categorized as soil testing very low and low for P based on soil test interpretation established using Bray-2 and Mehlich-3, respectively while Commerce sl was testing very high based on Bray-2 P but low to medium using Mehlich-3. On average, Bray-2 P values of Commerce at 30 DAP and harvest were 200 and 174 mg kg<sup>-1</sup> compared with its Mehlich-3 P values of 39 and 31 mg

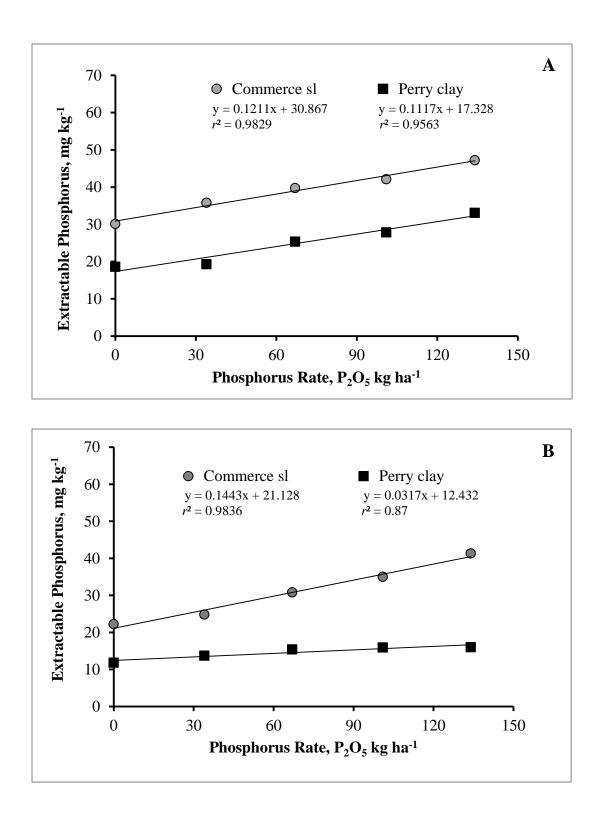


Figure 2.6. Relationship of P fertilizer rate and Mehlich-3 extractable P 30 days after P application (A) and at harvest (B) for both Perry clay and Commerce silt loam soils.

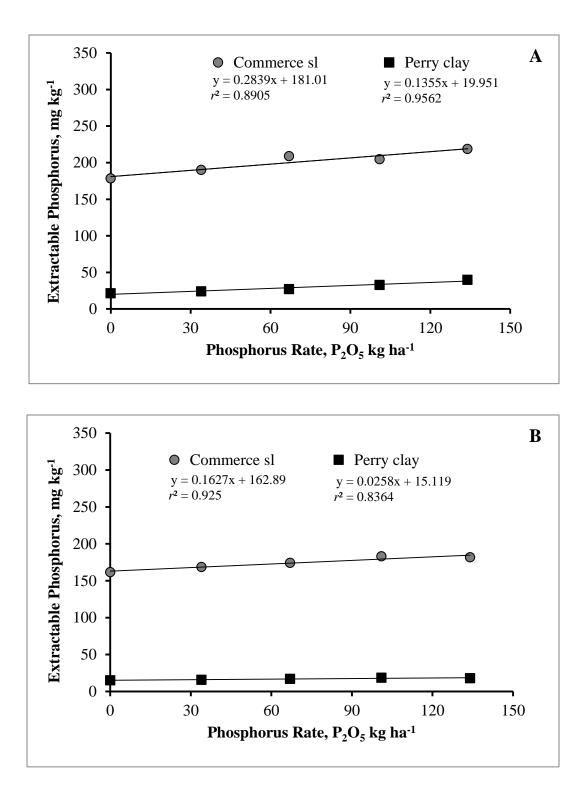


Figure 2.7. Relationship of P fertilizer rate and Bray-2 extractable P 30 days after P application (A) and at harvest (B) for both Perry clay and Commerce silt loam soils.

kg<sup>-1</sup>, respectively. The Bray-2 P values were about 6 times higher than Mehlich-3 P values for Commerce sl while for Perry cl, the amounts of P extracted by these two procedures were very similar (1:1 ratio). Sen-Tran (1990) evaluated six different soil test procedures to estimate the available P in 82 Quebec soils. Based on their findings, the amounts of P extracted by the Mehlich-3 method were closely related with those determined by the Bray-2 for about 80% of the soil population classified as Inceptisols. The Bray-2 extracted about 1.3 times the amount obtained by Mehlich-3. For the remaining 20% of soil samples (very acidic Spodosols), the Bray-2 extracted much more P than the Mehlich-3; the ratio varied from 1.6 to 6.6 times.

Both Bray-2 and Mehlich-3 extractable P of both soils increased with increasing P rate. With the highest P application rate of 134 kg P ha<sup>-1</sup>, Bray-2 extractable P of Commerce sl was raised by as much as 30 mg kg<sup>-1</sup> at 30 DAP compared with only 18 mg kg<sup>-1</sup> for Perry cl. Smaller increases in Mehlich-3 extractable P were determined for both soils having an increase of only 17 mg P kg<sup>-1</sup> for Commerce sl and 14 mg P kg<sup>-1</sup> for Perry cl. At harvest, there were significant differences among extractable P (P<0.05) for both procedures except for Mehlich-3 P of Perry cl soil. Figure 6 shows the linear relationship between Mehlich-3 extractable P and added P as P<sub>2</sub>O<sub>5</sub> kg ha<sup>-1</sup>. The linear models indicate that for every unit of P applied there was a corresponding increase of 0.12 and 0.11 mg P kg<sup>-1</sup> for Commerce sl and Perry clay soils, respectively. At harvest, there was a different pattern of Mehlich-3 for Commerce sl for every unit of P added (0.14 mg P kg<sup>-1</sup>) whereas for Perry cl, smaller amount of extractable P was recorded (0.03 mg P kg<sup>-1</sup>). These results were consistent based on the linear model between Bray-2 extractable P and

P added for Perry clay except that Commerce sl intercept value (soil test P level at 0 P rate) was very high. A significant reduction in extractable P was observed at harvest compared with what obtained at 30 DAP (0.026 vs. 0.136 mg P kg<sup>-1</sup>). This might be due to higher clay content of Perry cl, as the amount of clay increases in the soil, the P-sorption capacity increases as well. This is because clay particles have a tremendous amount of surface area for which phosphate sorption can take place (Penn et al., 2005). In addition, the reduction in both Bray-2 and Mehlich-3 extractable P at harvest is consistent with the removal of P by corn uptake.

# **2.4 Conclusions**

This study evaluated Bray-2 and Mehlich-3-based soil test P interpretations for corn on soils in the alluvial plains of Louisiana. Both Commerce sI and Perry cl soils were tested to having low to medium Mehlich-3 extractable P but responded differently with the application of P fertilizer. Grain yield of corn grown on Perry cl significantly responded to P rate. This was not the case for corn grown on Commerce sI. According to Bray-2 extraction method, Commerce sI was testing very high for P which was consistent to the lack of corn grain yield response to P application. Except for soil organic matter and particle size distribution, there were no outstanding differences in physical and chemical properties which could have resulted in differential response of corn grown on these two soils to P application. Also, there might be possible effect of other growth limiting condition or factors that could mask the effect of P application. Different P extraction procedures were established to obtain soil test P index suitable for specific groups of soil. The recent advancement in analytical procedure allowing analysis of

multiple elements using one extraction procedure resulted in the adoption of Mehlich-3 procedure by many soil testing laboratories. However, the disagreement between the soil test P level and probability of crop response documented in earlier field studies including the current pot experiment highlighted the potential limitation of Mehlich-3 procedure in gauging plant available P in specific soils. The findings in this study suggest that refinement of soil test P prediction should be pursued such that P fertilizer recommendations will not be based solely on P soil test.

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# **Chapter 3. Influence of Phosphorus Rate on Changes on Phosphorus Functional Fractions of Two Louisiana Alluvial Soils**

#### **3.1 Introduction**

The total phosphorus (P) content of most surface soils is low, averaging only 0.6%. This compares to an average soil content of 0.14% nitrogen and 0.83% potassium. The P content of soils is quite variable ranging from less than 0.04% as P<sub>2</sub>O<sub>5</sub> in the sandy soils of the Atlantic and Gulf coastal plain to over 0.3% in soils of the northwestern United States (Griffith, 1999). Soil P exists in inorganic and organic compounds that range from ions in solution to very stable inorganic and organic compounds. The inorganic P (Pi) compounds are mainly coupled with amorphous and crystalline forms of AI, Fe, and Ca. The organic (Po) compounds are associated with rapidly to slowly decomposable organic molecules such as nucleic acids, phospholipids, sugar phosphates, inositol phosphates, and recalcitrant humic substances (Hedley et al., 1982; Tiessen and Moir, 1993; Reddy et al., 1999).

The concentration of available soil Pi seldom exceeds 10  $\mu$ M (Bieleski, 1973), which is much lower than that in plant tissues where the concentration is approximately between 5 to 20 mM (Raghothama, 1999). According to Karaman et al. (2001) soil physical and chemical characteristics greatly affect P nutrition of plants. Among these are: (1) type of parent material from which the soil is derived; (2) degree of weathering; and (3) climatic conditions. In addition, soil P levels are affected by erosion, crop removal and P fertilization. The utilization of P fertilizers by crops is generally low (10-30%), and fixation of P significantly affect P accumulation in the soil (Tisdale et al., 1993). The low efficiency of plant uptake is the main problem associated with P

application. When P input from fertilizer exceeds P output in crop, P accumulation in the soil gradually increase over time (Kuo et al., 2005). Due to low concentration and poor mobility of plant-available P in soils, proper applications and management of chemical P fertilizers are needed to improve crop growth and yield.

Alluvial soils contain a unique group of soils. Soil texture varies dramatically across the state of Louisiana. Sorting of the sediments during deposition, together with a diverse mineralogy, have resulted in a considerable differences in the deposits. The rich sediments deposited resulted in soils that are fertile, productive and able to support crops like soybeans, corn, cotton, wheat, sugarcane and many others. However, Louisiana is also a state of abundant rainfall. With most areas of the state receiving 125-150 cm of precipitation annually, nutrients can be readily leached out of the root zone, causing poor soil fertility (Weindorf, 2008). Furthermore, the use cultural practices such as excessive tillage has resulted in the depletion of organic matter in the soils and consequently in a loss of natural fertility. According to Mascagni et al. (2007), P deficiency symptoms on corn seedlings are commonly seen and are most pronounced on the sandy loam and silt loam Mississippi River alluvial soils with organic matter levels of 0.5 to 1.0 percent such as Commerce silt loam soil but rarely occur on the finer-textured silty clay and clay soils. Mineralogical studies of the Mississippi alluvium indicate that smectite minerals are predominant in the clay-size fraction, and secondary amounts of micaceous clays are also present (Southern Research Publication, 1970). The sand and silt-size fractions consist predominantly of quartz and feldspar. Harrell and Wang (2006) also found out that the Mississippi River alluvial soil was generally dominated by smectites (78%) followed by kaolinite and clay mica (approximately 10% each).

In most soils, Pi occurs at fairly low concentrations in the soil solution a large proportion of it is more or less strongly held by diverse soil minerals. Phosphate ions can indeed be adsorbed onto positively charged minerals such as Ca, Fe and Al oxides (Hinsinger, 2001; Tiessen, 1998). In addition, many cropping systems in which the application of P to the soil is necessary to ensure plant productivity, the recovery of applied P by crop plants in a growing season is very low, because in the soil more than 80% of the P becomes immobile and unavailable for plant uptake because of adsorption, precipitation, or conversion to the organic form (Holford, 1997). Hence, there is little information on P availability on Louisiana alluvial soils differing in soil properties. Thus, this study aims to identify the soil properties that influence P partitioning into functional fractions of two alluvial soils in Louisiana.

To quantify Pi and Po compounds, different sequential chemical P fractionation schemes have been developed (Chang and Jackson, 1957; Pratt and Garber, 1964; Williams et al., 1971; Hedley et al., 1982; Zhang and Kovar, 2000). Sequential extraction procedures utilize the ability of various chemical reagents to selectively solubilize the Al, Fe, or Ca phosphate phases contained in the soil. Although imperfect separation may exist, it has been shown that with careful design and interpretation chemical fractionation procedures can be very useful in revealing the controlling phases of soil P dynamics (Sui et al., 1999; Delgado and Torrent, 2000; Maguire et al., 2000). Also, the facts on P fractionation methodologies are also significant for accurate interpretation of P chemistry/fertility and for making nutrient management decisions.

#### **3.2 Materials and Methods**

#### 3.2.1 Soil samples

Corn seeds were sown on potted Commerce sl and Perry clay soils treated with different P rates (0, 34, 67, 101, and 134 kg P ha<sup>-1</sup>) and grown until harvest. Composite soil samples were collected and analyzed for soil pH, organic matter content, Mehlich-3 extractable nutrients, and soil particle size distribution (Table 1, Chapter 1). Soil samples from individual pots were obtained 30 days after P (DAP) application but prior to sowing and after harvest. Five (5) sampling points from 0-9 inches were collected for each pot. These samples were then mixed, air-dried and processed to pass 2-mm sieve for soil pH, Mechlich-3 extractable nutrients, total P and sequential inorganic P fractionation.

## 3.2.2 Inorganic Phosphorus fractionation

Inorganic P sequential fractionation analysis was determined following the method by Zhang and Kovar (2000). The method is outlined in Figure 3.1 showing the different solutions (1*M* NH<sub>4</sub>Cl, 0.5*M* NH<sub>4</sub>F, 0.1*M* NaOH, 0.3*M* Na<sub>3</sub>C<sub>3</sub>H<sub>6</sub>O<sub>7</sub> + 1*M* NaHCO<sub>3</sub> + Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>, and 0.25*M* H<sub>2</sub>SO<sub>4</sub>) and procedures (shaking or washing requirements) to extract labile P (extract A), Al-P (extract B), Fe-P (extract C), reductant soluble P (extract D), and Ca-P (extract E) fractions.

Phosphorus concentration was determined by colorimetry using ascorbic acid method (Murphey and Riley, 1962) with some modifications on the determination of the reductant-soluble phosphate fraction because of potential interferences from dithionite, citrate, iron, and silicon (Weaver, 1974). Phosphorus standards series (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg P kg<sup>-1</sup>) was prepared containing the same volume of extracting solution as the sample extracts used for colorimetry. The absorbance of the sample solutions was

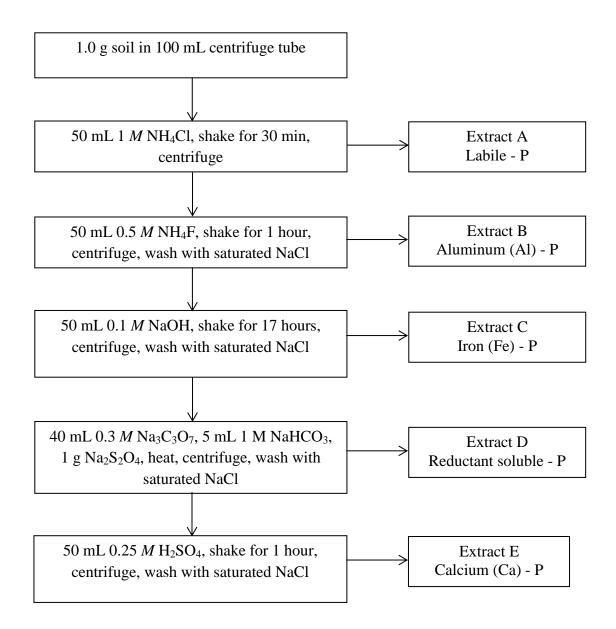


Figure 3.1. Sequential fractionation scheme for inorganic phosphorus (Zhang and Kovar, 2000).

measured at 882 nm. The concentration of P in the solution was derived from the slope and intercept between the P standard series and their absorbance readings such that:

Concentration 
$$(\frac{\text{ug P}}{\text{mL}}) = \frac{(Absorbance_{extract} - \text{int } ercept)}{\text{slope}} -$$

The amount of each of the P fractions is calculated using the following equation:

Concentration 
$$\left(\frac{\text{ug P}}{\text{g}}\right) = \left(\frac{\text{ug P}}{\text{mL}}\right) \times \left(\frac{\text{volume}_{\text{extractant}} (\text{mL})}{\text{weight}_{\text{soil}} (\text{g})}\right) \times \left(\text{dilution factor}: \frac{50 \text{ mL}}{2 \text{ ml aliquot}}\right)$$

3.2.3 Total Phosphorus Analysis (Microwave Acid Digestion, Inductive Coupled Plasma-Atomic Emission Spectroscopy, EPA Method 200.7)

Soil sample (0.5 g) was weighed and placed in a plastic digestion tube. Ten (10 mL) of nitric acid (HNO3) was added. Sample was placed in a microwave digester for 40 minutes at 200°C. Sample was allowed to cool down, transferred to a flask and made to a final volume of 250 mL with deionized water. Sample was shaken 3-4 times and allowed to stand overnight. About 100 mL solutions was decanted and total P concentration was quantified by ICP (Optical Emission Spectrometer, Optima 4300 DV).

### 3.2.4 Data Analysis

Analysis of variance was conducted using PROC MIXED in SAS (SAS, 2010) to determine the significance of differences among measured variables for each soil. The least square means test was performed to compare treatment means using the LSMEANS PDIFF option.

#### **3.3 Results and Discussion**

#### 3.3.1 Inorganic Phosphorus

The soil Pi fractions quantified in this study included labile P, Al-P, Fe-P, reductant-P, and Ca-P which correspond to sequentially extracted NH<sub>4</sub>Cl-P<sub>i</sub>, NH<sub>4</sub>F-P<sub>i</sub>, NaOH-P<sub>i</sub>, NaHCO<sub>3</sub>-P<sub>i</sub>, and H<sub>2</sub>SO<sub>4</sub>-P<sub>i</sub>. Kuo (1996) categorized total Pi into active and inactive P forms: Al-P, Fe-P and Ca-P consisting the active while occluded, reductant

soluble and residual P consisting the inactive P forms. Based on the initial Pi fractionation, Commerce sl contained higher amount of Ca-bound P (152 mg kg<sup>-1</sup>) compared with 11 mg kg<sup>-1</sup> of the Perry cl soil (Fig. 3.2). Perry cl has higher amount of Fe-P (values) and reductant-P (values) than with Commerce sl. Both soils have low amount of labile-P (<5 mg kg<sup>-1</sup>) and Al-P (<10 mg kg<sup>-1</sup>). The total Pi of Commerce sl was >200 mg kg<sup>-1</sup> whereas Perry cl recorded only about 80 mg kg<sup>-1</sup>. The largest contributing fraction on Commerce sl was Ca-P with 75% of the total Pi while reductant-P (40%) and Fe-P (40%) fractions were recorded for Perry cl. Calcium-P is a dominant form of inorganic P in high pH soils while Fe and Al hydroxyl phosphates dominate in low pH soils. However, Beauchemin et al. (2003) noted that Ca-P can also exist in soils of all pH values which agree with the result of this study where large contribution of Ca-P to the total Pi was observed despite of Commerce sl's low pH.

The changes in Pi fractions distribution following P application and after harvest of corn for Commerce sl are summarized in Figures 3.3 and 3. 4, respectively. There were significant (P<0.05) changes on labile- and Al-Pi pools in response to P rate at 30 days after P fertilizer application. An increasing trend in both labile- and Al- P was observed with increasing P rate; the highest P application rate (134 kg ha<sup>-1</sup>) obtained the highest labile- and Al-P fractions with mean value of 14 mg kg<sup>-1</sup>(Table 3.1). The check pot has lower mean values of 11 and 8 mg kg<sup>-1</sup> for labile-P and Al-P, respectively. On the other hand, the Fe-, reductant- and Ca-P fractions showed no significant difference between P application rates (P<0.05). At harvest, the labile-, Al- and Fe-P fractions decreased to about 60-75% (Figure 3.4). The decreased can be attributed to plant uptake and fixation or conversion of these Pi fractions to unavailable Pi forms. For example, the

reductant- and Ca-P fractions were increased by 5-10 mg kg<sup>-1</sup> (Table 3.2). It was also evident that the amount of Ca- and reductant-P at harvest was the highest at highest P application rate (134 mg kg<sup>-1</sup>):Ca-P was the highest concentration (>100 mg kg<sup>-1</sup>) of all inorganic P pools at pre-planting and harvest. Generally, the five inorganic P pools were influenced by the different P application rates.

For the Commerce sl soil the observed P pools trend at 30 DAP was in the order Ca-P>Fe-P>reductant-P>labile-P>Al-P which correspond to the following extracting solution:  $H_2SO_4-P_i>NaOH-P_i>NaHCO_3-P_i>NH_4Cl-P_i>NH_4F-P_i$ . At harvest, there was a slight change in trend such that the order was Ca-P>reductant-P>Fe-P>Al-P>labile-P ( $H_2SO_4-P_i>NaHCO_3-P_i>NH_4F-P_i>NH_4Cl-P_i$ ) which confirmed the Walker and Syers (1976) model of P transformation over time.

In general, the Ca bound P was higher in Commerce sl soil both at 30 DAP and harvest. This means that the unutilized fertilizer P did remain in the form of Ca-Pi. Contrary to the findings of Pierzynski et al. (1990) which indicate that on heavily fertilized soils Al, not Ca, was the predominant cation associated with P in P-rich particles regardless of soil pH. Harrell and Wang (2006) also studied the fractionation and sorption of Pi of calcareous soils of Louisiana; they reported that Commerce sl in Mississippi Red River alluvium contained large percentage of Fe phosphate suggesting that even these soils are classified as Commerce alluvium, they possess different characteristics.

For the Pi fractions of Perry cl soil, our results revealed that the most abundant Pi form was Fe-Pi (>30 mg kg<sup>-1</sup>) at 30 DAP (Figure 3.5; Table 3.1). All the Pi fractions except the reductant-P were significantly influenced by P application rate (P<0.05). It

was also observed that there were increasing levels of readily-available labile P and Al-P fractions with P application rates.

On the other hand, at harvest, the reductant-P had the highest concentration (>25 mg kg<sup>-1</sup>) as shown in Figure 3.6. Moreover, there was a significant difference of reductant-P with different P application rates particularly between the highest P application and without P application. There was also significant increase in mean concentration values around 15 mg kg<sup>-1</sup> of the reductant-P at harvest (*P*<0.05). The distribution trend of different Pi fractions of Perry clay soil was different at 30 DAP and harvest. The trend was Fe-P>Reductant-P>Ca-P>Labile-P>Al-P (NaOH-Pi> NaHCO<sub>3</sub>-P<sub>i</sub>>H<sub>2</sub>SO<sub>4</sub>-P<sub>i</sub>>NH<sub>4</sub>Cl-P<sub>i</sub>) and reductant-P>Fe-P>Ca-P>Al-P>Labile-P (NaHCO<sub>3</sub>-P<sub>i</sub>>NaOH-P<sub>i</sub>>H<sub>2</sub>SO<sub>4</sub>-P<sub>i</sub>>NH<sub>4</sub>F-P<sub>i</sub>>NH<sub>4</sub>F-P<sub>i</sub>>NH<sub>4</sub>Cl-P<sub>i</sub>) at 30 DAP and harvest, respectively. Results showed that the unutilized fertilizer P was transformed mainly to reductant-P and Fe-P.

Overall, the labile P and Al-P fractions of samples collected before planting increased with increasing P rate while the relationship of Fe-P with P rates became observable only at harvest for both soils. With time across P rates, both soils showed build-up of less readily-available reductant-P. Similar findings was observed by Osodeke and Ubah (2005) on P fraction in selected soils of southern Nigeria where Al-P significantly correlated with the available P indicating that the increase in Al-P increases the available P in the soil. Similar results were also reported by Osodeke and Kamalu (1992).

P Rate,						
kg $P_2O_5$ ha <sup>-1</sup>	Labile-P	Al-P	Fe-P	Reductant-P	Ca-P	Total
Commerce sil	t loam					
0	11c	8 d	22 a	13 a	112 a	167
34	12b	9 cd	23 a	13 a	122 a	180
67	12b	12 bc	23 a	14 a	120 a	181
101	14ab	13 ab	23 a	12 a	119 a	181
134	14a	14 a	25 a	12 a	103 a	169
Perry clay						
0c	9 d	4 bc	36 bc	19 a	20 a	89
34b	12 cd	7 c	34 c	22 a	15 b	89
67b	12 bc	7 bc	35 bc	21 a	16 b	91
101ab	13 ab	10 ab	39 ab	21 a	15 b	97
134a	12 a	12 a	42 a	21 a	15 b	101

Table 3.1. Distribution of inorganic phosphorus fractions of Commerce silt loam and Perry clay at 30 days after P fertilizer application.

Note: Values within a column within soil type with the same letter are not significantly different at P < 0.05.

Table 3.2. Distribution of inorganic phosphorus fractions of Commerce silt loam and Perry clay after harvest of corn.

P Rate,	Inorganic Phosphorus Fractions, mg kg <sup>-1</sup>						
kg $P_2O_5$ ha <sup>-1</sup>	Labile-P	Al-P	Fe-P	Reductant-P	Ca-P	Total	
Commerce sil	t loam						
0	3 c	4 c	14 b	18 b	124 b	162	
34	3 c	6 bc	15 ab	20 ab	128 b	172	
67	4 b	8 ab	17 ab	20 ab	129 b	178	
101	4 b	10 a	18 ab	21 a	128 b	181	
134	6 a	10 a	23 ab	23 a	156 a	218	
Perry clay							
0	2 b	1 c	23 b	26 b	6 b	58	
34	2 b	3 bc	29 ab	33 ab	8 b	75	
67	3 b	4 ab	31 ab	31 ab	8 b	76	
101	2 b	6 a	28 ab	35 a	8 b	80	
134	3 a	5 ab	32 a	37 a	12 a	89	

Note: Values within a column within soil type with the same letter are not significantly different at P < 0.05.

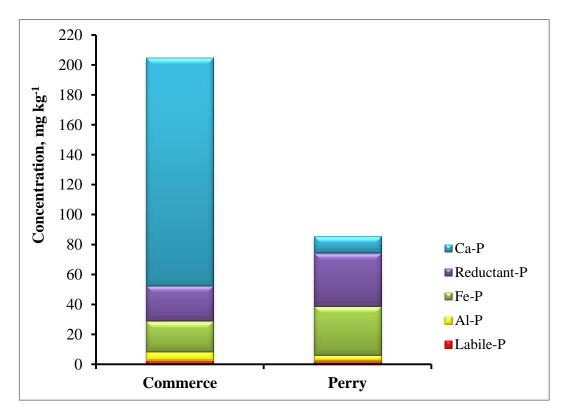


Figure 3.2. Inorganic phosphorus pools of Commerce sl and Perry clay soils before P fertilizer application.

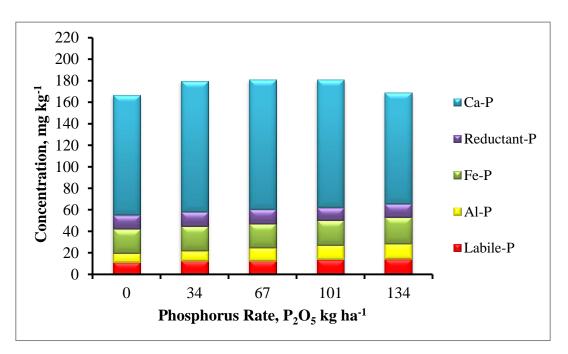


Figure 3.3. Inorganic phosphorus pools of Commerce sl as influenced by different phosphorus rate at 30 days after application.

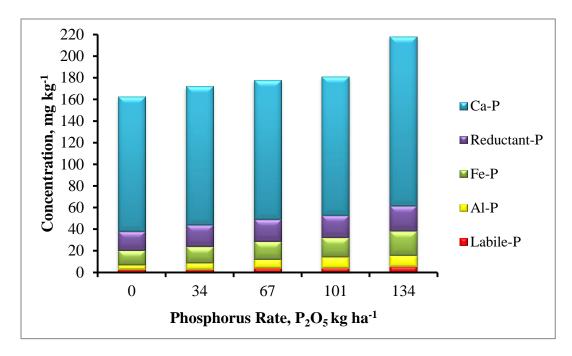


Figure 3.4. Inorganic phosphorus pools of Commerce sl as influenced by different phosphorus application rate at harvest.

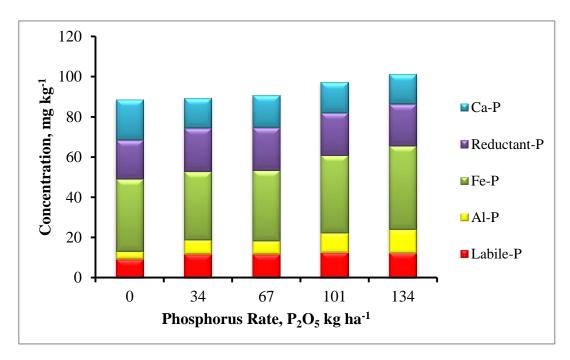


Figure 3.5. Inorganic phosphorus pools of Perry clay as influenced by different phosphorus rate at 30 days after application.

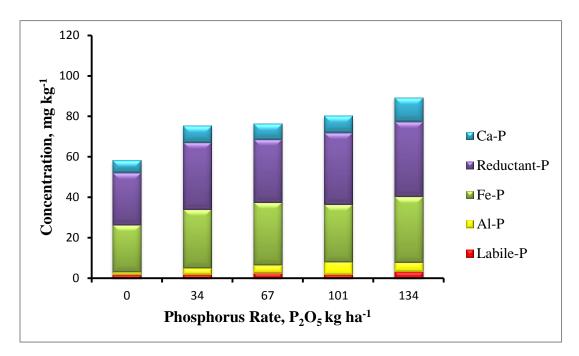


Figure 3.6. Inorganic phosphorus pools of Perry clay as influenced by different phosphorus application rate at harvest.

# 3.3.2 Total P, residual P and total inorganic P

The fractions of total P, residual P and total Pi between Commerce sl and Perry clay soils were significantly different at 30 DAP (P<0.05). Commerce sl has higher amount of total P, residual P and total Pi with a mean values of 458, 282 and 175 mg kg<sup>-1</sup>, respectively. Furthermore total Pi accounted 38% of the total P in the soil while 65% for residual P. Although Commerce sl showed higher concentration of the three components mentioned above, these components were not affected by P application rate (Figure 3.7).

On the other hand, Perry cl soil's total Pi differed among P rates at 30 DAP (Figure 3.9). The P application rate of 134 kg ha<sup>-1</sup> had the highest total Pi (P<0.05) with a concentration of 352 mg kg<sup>-1</sup> compared to the check pot, 34, and 67 kg ha<sup>-1</sup> with means values 316, 309, and 331 mg kg<sup>-1</sup>, respectively. It was also observed in Perry cl that the

concentration of the total Pi was only 28% of the total P which is 10% lower than the Commerce sl while the residual P was 10% higher on the Perry clay.

At harvest, the same pattern was also observed for total P and total Pi wherein there was a significant difference between the two soils (P<0.05). Commerce has a mean value of 419 and 182 mg kg<sup>-1</sup> for total P and total Pi, respectively while Perry cl has a mean value of 325 mg kg<sup>-1</sup> total P and 76 mg kg<sup>-1</sup> total Pi. There was a 5% increase in total Pi of Commerce sl and 10% reduction in residual P at harvest. However, with Perry cl soil there was a build-up of residual P with time and a reduction of total Pi.

Results also showed that there was a significant difference in total Pi concentration among P application rates for both soils at harvest (Figures 3.8 and 3.10). Soil that received fertilizer P showed higher total Pi concentration compared to the soil without P (check pot). Total Pi was the highest with P application rate of 134 kg ha<sup>-1</sup>; with mean values of 218 mg kg<sup>-1</sup> P and 89 mg kg<sup>-1</sup> for Commerce sl and Perry cl, respectively. This means that increasing the application rate also increased the amount of the inorganic functional fractions of Pi. Similar result was found by Takahashi and Anwar (2006) with their field experiment on P uptake and soil P fraction after 23 years of annual fertilizer application of wheat grown on Andosol, wherein they reported that total Pi increased in treatments with P application at 0-15 cm depths. In contrary, both soils showed no significant differences between P rates in terms of total P and residual P at 30 DAP and harvest. Results from this study had similar findings as Osodeke and Ubah (2005) wherein their results revealed that inactive Pi forms had the highest percent of the total P constituting 41.2% of the total P in all the soils they collected in 16 locations of Southern Nigeria.

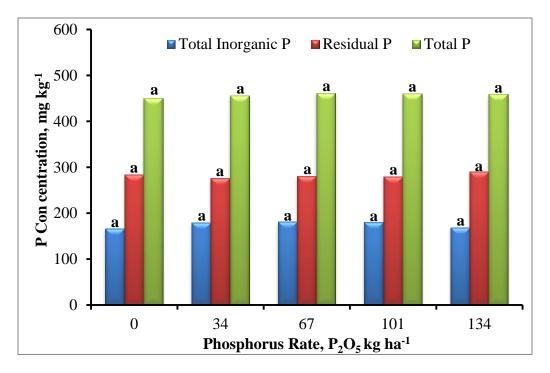
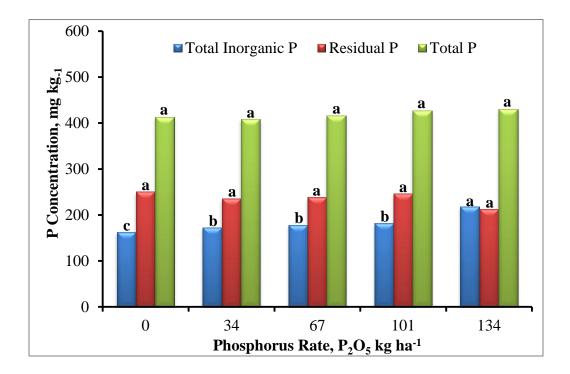
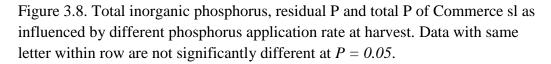


Figure 3.7. Total inorganic phosphorus, residual P and total P of Commerce sl as influenced by different phosphorus application rate at 30 days after application. Data with same letter within row are not significantly different at P = 0.05.





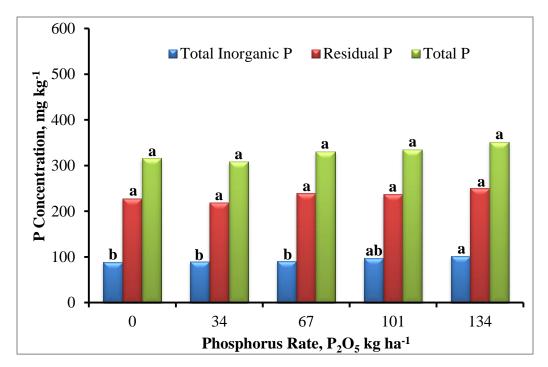


Figure 3.9. Total inorganic phosphorus, residual P and total P of Perry clay as influenced by different phosphorus application rate at 30 days after application. Data with same letter within row are not significantly different at P = 0.05.

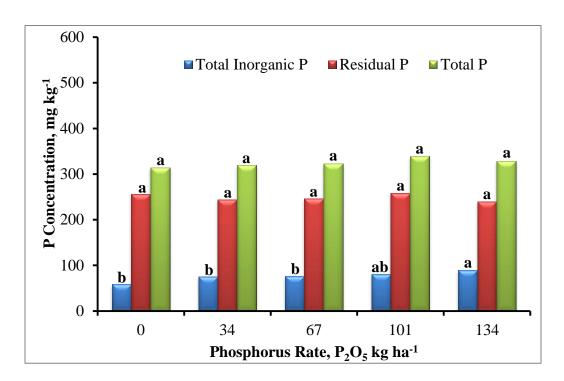


Figure 3.10. Total inorganic phosphorus, residual P and total P of Perry clay as influenced by different phosphorus application rate at harvest. Data with same letter within row are not significantly different at P = 0.05.

## **3.4 Conclusions**

The P fractionation conducted in this study revealed that the two alluvial soils (Commerce sl and Perry clay) differed considerably in terms of the distribution of different P fractions. The unutilized P fertilizer was transformed mainly into Ca-P for Commerce sl soil while for Perry clay it transformed into Fe- and reductant-P. Overall, the labile- and Al-P fractions before planting increased with increasing P rate while the relationship of Fe-P with P rates became observable only at harvest for both soils. With time across P rates, both soils showed build-up of less readily-available reductant-P. In terms of soil total P, residual P and total Pi components, our results revealed that Commerce sl and Perry cl soils differed significantly (P<0.05) at both DAP and harvest. On the other hand, the total P and residual P were not affected by P rates at both 30 DAP and harvest for both soils.

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#### **Chapter 4. Conclusions**

This study evaluated Bray-2 and Mehlich-3-based soil test P interpretations for corn on two Louisiana alluvial soils. Both Commerce sl and Perry cl soils were tested to having low to medium Mehlich-3 extractable P but responded differently with the application of P fertilizer. Grain yield of corn grown on Perry cl significantly responded to P rate. This was not the case for corn grown on Commerce sl. However, according to Bray-2 extraction method, Commerce sl was testing very high for P which was consistent to the lack of corn grain yield response to P application. Except for soil organic matter and particle size distribution, there were no outstanding differences on physical and chemical properties which could have resulted in differential response of corn grown on these two soils to P application. Also, there might be possible effect of other growth limiting condition or factors that could mask the effect of P application. Different P extraction procedures were established to obtain soil test P index suitable for specific groups of soil. The recent advancement in analytical procedure allowing analysis of multiple elements using one extraction procedure resulted in the adoption of Mehlich-3 procedure by many soil testing laboratories. However, the disagreement between the soil test P level and probability of crop response documented in earlier field studies including the current pot experiment highlighted the potential limitation of Mehlich-3 procedure in gauging plant available P in specific soils. The findings this study suggests that refinement of soil test P prediction should be pursued such that P fertilizer recommendations will not be based solely on P soil test.

The P fractionation conducted in this study revealed that the two alluvial soils (Commerce sl and Perry clay) differed considerably in terms of the distribution of

different P fractions. The unutilized P fertilizer was transformed mainly into Ca-P for Commerce sl soil while for Perry it transformed into Fe- and reductant-P. Overall, the labile- and Al-P fractions before planting increased with increasing P rate while the relationship of Fe-P with P rates became observable only at harvest for both soils. With time across P rates, both soils showed build-up of less readily-available reductant-P. In terms of soil total P, residual P and total Pi components, our results revealed that Commerce sl and Perry clay soils differed significantly (P<0.05) at both 30 DAP and harvest. On the other hand, the total P and residual P were not affected by P rates at both 30 DAP and harvest for both soils. Marilyn Sebial Dalen was born in September 11, 1979, in Leyte, Philippines. She finished her Bachelor of Science degree in Agriculture major in Soil Science in 2000 at the Visayas State University, Philippines. She also took some master's credits from the said university from 2008 to 2010. In January of 2011 she was admitted into the master's degree program in the School of Plant, Environmental, and Soil Science at Louisiana State University Agricultural and Mechanical College. She is under the guidance of Dr. Brenda Tubana working on P nutrition on corn grown on alluvial soils of Louisiana. The title of her thesis is "Understanding phosphorus dynamics of two alluvial soils grown with corn at different phosphorus rates".

## Vita