Phosphorus Characterization of Alabama Soils and Evaluation of Extractants for Environmental Phosphorus Loss Risk Assessment

by

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Abstract

Poultry industry is the second largest agricultural industry in Alabama and generates 1.5 million tons of poultry litter, which contains approximately 19,000 tons of phosphorus (P). Long term application of fertilizers and manure in excess of crop requirement leads to buildup of P in soil. Increase in soil test P level increases risk of P loss from soil to surface water which can accelerate eutrophication and reduce water quality. As the P transport from agricultural land to water systems and looming concerns on water quality increases, it becomes necessary to evaluate the risk of P loss from an environmental standpoint. Soil test phosphorus (STP) originally developed for agronomic purposes is also used as the first line of defense to evaluate P loss risk. For example, STP methods have been integrated into P index (PI) which is considered the most comprehensive tool for environmental P loss risk assessment. Underestimation or overestimation in STP may misguide nutrient recommendations for fertilizer or manure management. Therefore, it is important to choose an appropriate soil test method that adequately captures environmental P loss risk as well as agronomic P needs. The objectives of this study were (i) to survey the soil P levels of major soils areas of Alabama at different soil depths and land use management (ii) to evaluate and compare the extraction potential of Mehlich-1/Lancaster and Mehlich-3 for P, aluminum (Al), iron (Fe), calcium (Ca), and magnesium (Mg) for major Alabama soil areas (iii) determine the conversion equations between Mehlich-1/Lancaster and M3 STP values and (iv) to determine if observable distinct relationship exist between WSP and STP for different soil types and depths in Alabama soils. Soil samples were collected form five major soil areas of Alabama, namely, Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valleys (LV), Piedmont Plateau (PP) and Blackland Prairie (BP). Based on calcium content, Alabama soils from AP, CP, LV, and

PP are typically categorized as non-calcareous soils whereas soils from BP are categorized as calcareous soil. The soil samples were collected from four soil depths (0-5, 5-15, 15-30 and 30-45 cm). Non-calcareous soil samples were extracted for water-soluble phosphorus (WSP), M1, and M3 extractable P, Fe, Al, Ca, and Mg using the standard protocols and relationship were studied. Lancaster was used for calcareous soils from Blackland Prairie instead of M1 and the above stated relationship for calcareous were studied. The critical soil test level for Alabama soils are 25 mg kg^{-1} M1-P (non-calcareous) and 36 mg kg^{-1} Lancaster-P (La-P) (Blackland Prairie) soil. The mean M1-P for AP, CP, LV, and PP soils at 0-5 cm depth were 143 mg kg^{-1}, 76 mg kg^{-1}, 73 mg kg^{-1}, and 91 mg kg^{-1} soil, respectively. The mean La-P for BP soil at 0-5 cm depth was 60 mg kg^{-1} soil. This indicated that STP at surface 0-5 cm are above the critical soil test level for Alabama soils. The percent distribution of major soil areas above soil test critical level for AP, LV, PP, BP, and CP for 0-5 cm depth were 93%, 84%, 77%, 67%, and 54%, respectively. Similarly, the distribution of soils from major soil areas above soil test critical level for 5-15 cm depth followed the order: AP (87%) > CP (69%) > LV (42%) > PP (41%) > BP (10%). Mehlich-3 showed better extraction efficiency than M1 for P, Al, Fe, Ca, and Mg for wide range of Alabama soils. A linear and strong correlation was observed between M1-P and M3-P for all soil from Alabama. For Blackland Prairie soils, Lancaster method showed better extraction for P, Ca and Mg compared to M3. However, correlation between La-P and M3-P showed, both the extractant have similar P extraction potential in BP soils. For the combined depths, the relationship between WSP and M3-P was better correlated (based on r^2 value) than the relationship between WSP and M1-P for all soil types except CP. The correlation between WSP and M3-P was better than WSP and La-P which suggested the suitability of use of M3 for BP soils. This research provides solid background for introducing M3 as an alternative soil test method for environmental purpose for wide range of Alabama soils.

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List of Abbreviations

AP	Appalachian Plateau
BP	Blackland Prairie
CP	Coastal Plain
LS	Limestone Valley
M1	Mehlich-1
M3	Mehlich-3
NRCS	Natural Resources Conservation Service
P	Phosphorus
PL	Poultry Litter
PP	Piedmont Plateau
STP	Soil Test Phosphorus
TP	Total Phosphorus
WSP	Water-Soluble Phosphorus

CHAPTER 1

Literature Review

1.1 Poultry Industry in Alabama

During the 1950s, approximately 90% of the poultry production in United States (US) was vertically integrated, which contributed to rapid expansion of broiler industry in Alabama (AL) and other parts of the nation. Alabama ranks second in the poultry production among the US states and the poultry industry contributes 65.6% of Alabama's agricultural income offering 86,000 jobs opportunities directly or indirectly (AP&EA, 2019). The industry generates more than 1.25 million tons of poultry litter (PL) annually (Lamba et al., 2013) which contains approximately 19,000 tons of phosphorus (P). Land application of PL is the most common practice adopted by farmers as PL supplies plant nutrients and helps to improve soil properties such as soil tilth, organic matter content, pH, cation exchange capacity, water holding capacity, and problems associated with soil compaction (Bolan et al., 2010a; Ghaly and Alhattab, 2013). Besides these advantages, it is important to understand the risks associated with PL application because excessive inputs of PL results in buildup of soil P level and can cause groundwater contamination (Almeida et al., 2019).

Feeding the growing population without degrading natural resources is a challenge. The Food and Agriculture Organization (FAO) reported a projected 76% increase in global meat consumption by 2050, including a doubling in poultry demand (Alexandratos and Bruinsma, 2012). The trend of accelerated consumption of poultry products have led to industrialization, geographic concentration and intensification in poultry production (Gerber et al., 2007). Although poultry industry has significant impact on US economy (Sims and Wolf, 1994), the industry has challenges in waste management. Large scale accumulation of PL every year generated by intensive production pose disposal and contamination issues (Moore et al., 1995; Sharpley et al., 2007; Bolan et al., 2010b). The challenge will be never solved unless economically and environmentally sound measures for waste management are developed. Moreover, it is inconvenient to transport PL long distances because of its bulky nature, pathogenic hazards, and higher expenses. Therefore, major portion (greater than 90% of PL produced) of PL application is confined in limited farmlands which are close to the production sites (Moore et al., 1995). Long term application of PL beyond crop requirement leads to build up of soil P levels and P losses to water bodies cause water quality problems. Solubility of P and loss from soil to water bodies depends on several factors such as pH, concentration of P, presence of divalent (Ca^{2+}, Mg^{2+}, Fe^{2+}) and trivalent (Al^{3+}, Fe^{3+}) cations in soil (Arai and Sparks, 2007). Therefore, it is important to understand the P dynamics in soil.

1.2 Phosphorus in Soil

Phosphorus is a non-renewable and ubiquitous element and is essential to all living forms (Maciá, 2005; Dhillon et al., 2017). In agriculture, P plays a significant role in vegetative growth and development to achieve an optimum yield (Tening and Foba-Tendo, 2013). Adequate amounts of P are required for plant root growth, development of reproductive parts and to improve quality of fruit, vegetables, and grain crops. In the lithosphere, P is primarily conserved in soil and sediments as phosphate minerals rocks (such as fluorapatite, chlorapatite and hydroxyl-apatite), usually found in small concentration 0.15 - 2% by weight of rock. About 90% of the total P in soil-plant-animal system is stored in soil and remaining 10% is in biotic system (Arai and Sparks, 2007). During soil formation, weathering of primary minerals (apatite) and its transformation to secondary minerals and to organic forms occurs over time (Börling, 2003). The transformation into secondary mineral occurs through precipitation and to organic forms through microbial activity and plant uptake. Phosphorus transfer from soil minerals to plant system and back to soil is a complex biogeochemical process (Sims and Pierzynski, 2005). The biogeochemical processes associated with P cycling are dissolution- precipitation (mineral equilibrium), sorption- desorption (interaction of P between solution and soil solid phases) and mineralization-immobilization (microbial mediated transformation of P between organic and inorganic forms)(Sims and Pierzynski, 2005). The extent and rate of these reactions depends on soil properties and microbial community present in soil.

Phosphorus in soil exist in two major forms, organic and inorganic. Generally, the range of total P concentration in soil is between 200 to 5000 mg kg^{-1} with an average of 600 mg kg^{-1} soil (Lindsay, 1979). Organic forms of P are complex forms mainly comprising inositol phosphates, phospholipids, and nucleic acids compounds (Sims and Pierzynski, 2005). These forms of P in soil can range from 20-80% of total P concentration (Arai and Sparks, 2007). The poultry manure, applied to replenish P in soil are reported to contain 53% P in organic forms (Toor et al., 2006). The inositol phosphates (phytic acid) are mainly sugar molecules with one or more phosphate group and are the most dominant forms (more than 50%) of organic phosphorus in soil (Arai and Sparks, 2007). Phosphorus when combined with fatty acids compounds form phospholipids and accounts 0.5-7% of organic phosphorus in soil. Nucleic acids, which is formed by the decomposition of plants, animals, and microbes accounts less than 3% of the total organic P in soil. Enzymes play a significant role in hydrolyzing the organic forms of phosphate into inorganic forms that could be available to plants (Toor et al., 2006). The presence of phosphatase enzyme plays an active role in mineralization of organic forms of phosphates.

Inorganic P exist in ionic forms ($H_2PO_4^-$, HPO_4^{2-}) in soil solution and these ions can be adsorbed on surface of clay minerals or precipitate as complex compounds depending on soil pH (Sims and Pierzynski, 2005). Clay contains greater amount of amorphous oxides and hydroxides of Fe and Al in acidic (non-calcareous) soils (Bowden et al., 1977; Tsado et al., 2012). The phosphate ions react with Al and Fe oxides in soil and form low solubility secondary minerals Al and Fe phosphates (variscite, $AlPO_4.2H_2O$, and strengite, $FePO_4.2H_2O$). In calcareous soils, P availability is limited due to precipitation of phosphate ions with calcium forming insoluble hydroxyapatite [$Ca_5(PO_4)_3OH$], octa-calcium phosphate [$Ca_8H(PO_4)_6.5H_2O$] and dicalcium phosphates (Lindsay, 1979). The major proportion of soil P that is reserved as primary minerals, secondary minerals, occluded P and organically stabilized P are the non-labile forms in the soil (Börling, 2003). These forms of P are available to crops in a small fraction through dissolution and mineralization. Weathering of minerals over time under different environmental conditions ultimately mineralizes the complex forms of phosphates to plant absorbable forms. Although P concentration in soil is abundant, the amount of readily plant available P is generally low (Dhillon et al., 2017). Therefore, P sources are added to maintain adequate P level in soil for crop production. However, a small fraction of applied P solubilizes and is readily available to plants as labile forms and the remaining fraction get fixed into the soil. Typically less than 5% of the total P present in soil is considered as labile fraction and remaining larger fractions get associated with primary, secondary minerals and organic complexes (Hou et al., 2018).

Phosphorus in soil solution is either found primarily as monobasic ($H_2PO_4^-$) or in smaller amount as dibasic (HPO_4^{2-}) and phosphate ion (PO_4^{3-}) which are readily absorbed by plants (Tamungang et al., 2016). Soil P availability to plants is influenced by several factors such as soil clay content, organic matter content, soil pH and concentration of exchangeable Al, Fe and Ca in the soil solution (Arai and Sparks, 2007). Soils with high clay content have a larger P retention capacity because clay particles have large surface for P adsorption (McGechan and Lewis, 2002). Soil organic matter contains organic acids (humic acid and fluvic acid) which contains functional group such as $R\text{-}COO^-$, R-COH, R-SH and others. The functional groups have capacity to adsorb metal cations thus increasing P sorption in soil (Bianchi et al., 2008). Soil pH affects biological and chemical phenomenon like solubility, mobility, and availability of P to plants (Dhillon et al., 2017). Phosphorus is most available to plant at pH range of 6.5 to 7.0. However, several contradictory results had been reported on the effect of soil pH on P solubility. Phosphorus sorption has been found to be increased, decreased, or not affected by increasing pH depending on soil types and experimental conditions (Whitelaw, 1999). Thus, it becomes important to study soil P behaviors regarding differences in soil type and mineralogy. Several physical, chemical, and biological factors influence the concentration of P in soil solution, its availability for crop and microbial uptake and its transport from soil to surface water bodies.

1.3 Phosphorus Transport Mechanism

Phosphorus from soil can potentially be lost to water system through several transport pathways and in different forms. The main pathways of P transport from agricultural fields to freshwater system are surface runoff or erosion, and leaching (Haygarth et al., 1998). Phosphorus transported from soil-plant systems are primarily found in two forms, dissolved (reactive and unreactive) and particulate (reactive and unreactive). Distinction between dissolved and particulate forms is made based on filtration through 0.45 µm filter paper (Toor et al., 2005). Dissolved reactive P are the ortho-phosphates that are soluble in water and some fractions of readily hydrolysable organic P forms such as labile monoesters and diesters (Toor et al., 2005; Reid et al., 2018). Particulate reactive P are water insoluble forms which are adsorbed on surfaces of clay, Fe, Al, or Ca and hydroxides. These eroded sediments provide a long-term source of P to aquatic biota. Dissolved unreactive P are the organic P compounds and some inorganic compounds such as polyphosphate. These forms are not detected by molybdate method. Particulate unreactive P are those forms whose nature are unpredicted and they may contain mineral hemic acid complexes (Toor et al., 2006).

Traditionally it was thought that P applied in soil was fixed and the amount of P transported through leaching and runoff represented a very small fraction of total P concentration. However, from water quality perspective, a very small fraction (10 parts per billion) might accelerate eutrophication and this transported P may be significant over time (Haygarth et al., 1998). Therefore, it is important to understand P transport processes from soil to water bodies. Excess application of manures and fertilizers results in higher soil test P levels which possess higher risk of P transport to water system. The rate of P transport from agricultural lands is affected by several agronomic practices and transport factors (Allen et al., 2006). Agronomic factors include initial soil P level, applied P source, application rate, method, and time whereas transport factors include soil erosion, surface runoff, subsurface drainage, field slope and proximity of field to surface water system.

1.3.1 Surface and Subsurface Phosphorus Loss

Surface runoff occurs when rate of precipitation or snowmelt exceed soil’s maximum saturation capacity. The P loss in runoff is dependent on several components such as rainfall intensity, land slope, land use, manure and fertilizer application rate, application method, application time and in overall, on soil P level. Higher the soil P level in soil, greater the potential risk of P loss in surface runoff, either as dissolved P or particulate P (Lamba et al., 2013). Manure application methods plays a significant role in P transport. For example, broadcasting is a common method for application of manure in pastures and hayfield. Vegetative cover in pasture land prevents manure to come in contact with soil which reduces the chances of P adsorption by soil particles (Schroeder et al., 2004). This may enhance P loss through surface runoff. However, erosion rates in hay and pasture fields is minimum and the runoff carries little sediments. This indicates that P loss through sediments (particulate P) would be minimal and dissolved P would be the dominant P form in runoff water in pasture and hayfields (Sharpley, 1997). Furthermore, studies have showed a strong positive linear relationship between rate of manure application and concentration of phosphorus in runoff (Schroeder et al., 2004). Most of the P loss occurred in the first runoff event from the fields where manure was surface applied (Sharpley, 1997; Sauer et al., 1999).

Different management practices have been formulated through experiments for reducing nutrient loss in surface runoff. For example, injecting dairy manure into subsurface horizon reduced N and P losses greatly in runoff (Ross et al., 1979). Likewise, incorporation of manure or fertilizer into the soil can reduce the potential P losses through surface runoff by more than 95% (Pote et al., 1996). In addition, Lamba et al., (2013) reported that concentration of PO_4-P and total P in leachate were reduced by 96% and 37% respectively in subsurface banded applied PL compared with surface applied PL.

Previously it was thought that subsurface loss of P from soil profile was not significant as compared to surface pathway. However, research during recent years has evident that P transport through subsoil can be substantial and can potentially emerge as a serious issue in future (Maguire and Sims, 2002b; Liao et al., 2019). Also, subsurface flow through tile drain is considered a dominating factor for P export (Buczko and Kuchenbuch, 2007). Several factors, such as P sorption capacity, redox condition and preferential flow could affect P transport in subsurface soil. In sandy soils, P leaching and subsurface drainage is greater due to its low P adsorption capacity and high hydraulic conductivity (Buczko and Kuchenbuch, 2007). The soils with loam and clay as dominant texture have abundant macropores in soil profile which induces P transport through preferential flow (Djodjic et al., 1999). Leaching through soil profile also depends on land use and tillage system. Generally the density and connectivity of macropores is found to be greater in soils under conservation tillage system (no-till or reduced till) compared to conventional tillage (Buczko et al., 2006; Buczko and Kuchenbuch, 2007). Hence, conventional practices may increase P loss by erosion and surface runoff, but conservational tillage practices may increase P losses by preferential flow.

1.4 Phosphorus in Agriculture and Environmental Issues

Over past few decades, trend of increasing P concentration in freshwater system has been noticed in large scale both in USA and Europe (Buczko and Kuchenbuch, 2007). In 1996, more than 50% of the surveyed waters in the USA were nutrient impaired (Sharpley and Wang, 2014). Sincere attention was drawn among the environmentalists because of more frequent instances of P contamination in water resources; including Chesapeake Bay, Baltic Sea, Gulf of Mexico (Sharpley and Wang, 2014), Lake Erie (Smith et al., 2015), Gippsland lake in Victoria, Alpine lakes of Italy and Switzerland (Toor et al., 2006), Poyang Lake in China (Liao et al., 2017) and many more throughout the world. Land utilized for agricultural purposes which receives excessive amount of manure and fertilizers have been identified as a major non-point source of P pollution (Maguire and Sims, 2002b; Kleinman et al., 2015). Agriculture contribution to total P loads in EU water system ranges from 25 to 75% (Withers and Haygarth, 2007). Similarly, in US, agricultural P loss contribute significantly to total P loads in freshwater system. For example, The Chesapeake Bay Foundation estimated about 50% of the non-point P pollution to the Chesapeake Bay comes from agricultural runoff (Chesapeake Bay Foundation, 2012).

In fresh water system P is the limiting nutrient for biotic system (Liao et al., 2019). Phosphorus concentration in surface water through anthropogenic activities has been on rise and number one cause of eutrophication in fresh water system (Cordell et al., 2009; Némery and Garnier, 2016).

The eutrophication results imbalance in aquatic ecosystem contributing to fish kills, harmful and nuisance algal bloom, impairment of drinking water quality and recreational activities (Toor et al., 2005). The accelerated evidence of eutrophic water resources has enforced environmental authorities in many countries to implement new rules and regulations to combat against these water quality challenges. The United States Environmental Protection Agency (USEPA) in 2002 established water quality criteria for nutrients in freshwater system. The permissible maximum total P concentration for lakes and reservoirs is 8-38 µg L^{-1} and 10-128 µg L^{-1} for rivers and streams (USEPA, 2002).

As agricultural land is one of the major contributors to P contamination in surface water system, it becomes necessary to evaluate the potential risk of P loss from agricultural lands. Many studies have showed that managing soil P loss and P fertility status are crucial to control eutrophication from agricultural source (Withers et al., 2019). Maintaining soil test phosphorus (STP) level below the recommended threshold (for fertilizers application) was identified as a more reliable practice to reduce eutrophication in water bodies. Precision soil sampling, analysis and interpretation of STP and innovative approach to lower agronomic optimum STP level on farm scale are needed for sustainable management of soil P fertility and potential environmental risks (Withers et al., 2019).

1.5 Soil Test Phosphorus (STP) Methods

Soil test P identifies the soils on which P addition can help obtain optimum crop growth and yield (Gartley and Sims, 1994). Soil test also identifies soils, which are non-responsive to P addition. The addition of P to non-responsive soils is not sustainable from agronomic as well as environmental viewpoint. Based on the regional differences in soil chemical properties, many STP methods such as Olsen, M1, M3 and Bray-1 were established to determine the soil P concentration and make recommendations for nutrient application to crops for optimum production (Sims et al., 2000). Unnecessary application of P would be waste of resources and reduces profitability of the producers and pose P loss risk to the environment. The different STP methods used in USA and several parts of the world are discussed below.

The Bray and Kurtz P-1 (0.025 M HCl + 0.03 M NH_4F) soil test P method was developed at the Illinois Agricultural Experiment Station in 1945 and is now widely used throughout the Midwestern and North Central United States (Bray and Kurtz, 1945). This method is particularly more applicable to acid and neutral soils. The fluoride activity in Bray and Kurtz extractant release P from the aluminum phosphate complexes by reducing Al activity in solution forming Al-F complex. Moreover, the more acidic nature of extractant (pH = 2.6) dissolves P from Al, Ca and Fe bound forms in soils. Although, powerful in P extraction from soil, it has some limitations. This extractant is not suitable for clay soils with moderately high degree of base saturation, silty clay loam or finer-textured soils that are calcareous and have a high pH value (pH > 6.8) or have a high degree of base saturation, soils with a calcium carbonate equivalent > 7% of the base saturation, or a soils with large amounts of lime applied to them (> 2% $CaCO_3$) (Pierzynski, 2000).

The M1 soil P test, also called dilute double acid or North Carolina extractant, was developed in the early 1950s by Mehlich and his co-workers. Mehlich-1 constitutes 0.05 M HCl and 0.0125 M H_2SO_4 solution (Mehlich, 1953). This method is primarily used in southeastern and mid- Atlantic states in US and identified as a multi-element extractant for P, K, Ca, Mg, Cu, Fe, Mn, and Zn. This is best suited for acid soils (pH < 6.5) with low cation exchange capacity (CEC < 10 cmol kg^{-1}) and low organic matter content (< 5%). Mehlich-1 had shown poor correlation to crops grown on calcareous soils, soil containing phosphate rocks, and high organic matter (> 6%). In such soils, the acidity of the extraction solution is neutralized by bases, reducing the capability of dilute acid to extract P from soil. In general, M1-P concentration of 20 to 25 mg P kg^{-1} soil is adequate for plants grown in sandy soils (Kamprath and Watson, 1980) but only 10 mg P kg^{-1} soil is required for fine-textured soils (Pierzynski, 2000).

Mehlich-3 was an improvement over M1 and was introduced by Mehlich in 1984 for P, K, Ca, Mn, Cu, Fe, Mn, and Zn (Mehlich, 1984). This is widely accepted STP methods in US and Canada as it has potential to extract P from both acidic and alkaline soils. Mehlich-3 is quite similar in principle to the Bray and Kurtz P-1 test as both are strongly acidic solution that contains ammonium fluoride. Fluoride forms a complex with aluminum in solution and prevent refixation of dissolved P. Fluoride can also precipitate as calcium fluorite (CaF_2) in soils that has high affinity for resorbing dissolved P at higher pH. To prevent P resorption, acetic acid acts as a buffer to maintain pH at 2.5 (Mehlich, 1978). Ammonium nitrate serves to extract exchangeable cation nutrients such as Ca, Mg, and K. Ethylenediaminetetraacetic acid (EDTA), in the solution improves the extraction of micronutrients (copper, manganese, and zinc) from the soil (Mehlich, 1984). Studies from past showed that the M3 soil test is highly correlated with P extracted from soils by the Bray and Kurtz P-1, M1, and Olsen-P methods (Sims, 1989). Mehlich-3 value of 45-50 mg P kg^{-1} soil is generally considered optimum for plant growth, and is higher than the critical values used for other standard soil P tests, such as the Bray and Kurtz P-1, M1, and Olsen-P (Pierzynski., 2000).

Olsen-P or sodium bicarbonate STP method was introduced by Sterling R. Olsen and co-workers in 1954 preferably for calcareous soils (Olsen et al., 1954) found in North Central and Western United States (with > 2% calcium carbonate). The solution is 0.5 M $NaHCO_3$ and contains

HCO^{3-}, CO_3^{-3} and OH^- (pH = 8.5). These ions are responsible for reducing the concentrations of soluble Ca^{2+} by forming precipitates of $CaCO_3$ and soluble Al^{3+} and Fe^{+3} by formation of Al and Fe oxyhydroxides, increasing P solubility. An Olsen-P value of 10 mg P kg^{-1} soil is generally considered to be optimum for plant growth (Pierzynski, 2000). This is lower than the critical values used for the Bray and Kurtz P-1, M1, and M3 because the Olsen extract lower amount of P from soils than the other acidic extractants.

J. D. Lancaster in 1970s introduced an extractant designed to estimate P from calcareous soils from Mississippi with wider pH ranges. Lancaster method uses a two-stage process. In first stage, 5 g soil is treated with a weak acidic solution (0.05 M HCl) without agitation. This step dissolves the readily plant available calcium associated P from the soil. In the second step, 20 ml of extraction solution (1.58 M glacial acetic acid + 0.125 M malonic acid + 0.187 M malic acid + 0.037 M ammonium fluoride + 0.03 M aluminum chloride hexahydrate) is added and shaken for 10 minutes (Cox, 2001). Fluoride in the solution extracts Al-P where organic acids formed soluble complexes with aluminum and promotes Al-P dissolution. Role of aluminum in the extraction solution is to ensure formation of soluble aluminum-fluoride complexes which minimizes Ca and Mg fluoride precipitations. Organic acids also act as a buffer to maintain pH at 4.0. In Alabama, Lancaster method is used as a routine soil test method for calcareous soils from Blackbelt.

Acid ammonium oxalate solution is not a conventional P extraction procedure but used for soil Fe analysis. This extractant contains (0.1 M oxalic acid + 0.175 M ammonium oxalate) and dissolves most of the iron and aluminum from amorphous minerals (ferrihydrite and Fe associated with organic matter) but very little from crystalline oxides (McKeague and Day, 1966).

Citrate bicarbonate dithionate (0.2 M sodium bicarbonate + 0.24 M sodium citrate) is not a routine soil test extractant and introduced by Mehra and Jackson, 1960. Compared to oxalate, dithionate dissolved a large fraction of crystalline Fe oxides and much of the amorphous materials as well (McKeague and Day, 1966). The dithionate functions as a reducing agent and citrate acts as a chelating agent.

Several environmental soil test P or bio-available soil P test are used with the purpose of estimating soluble and easily desorbable P from soil (Schindler et al., 2009). The commonly used extractants are deionized water (WSP), unbuffered salt solutions like $CaCl_2$, Fe-oxide impregnated filter paper, an anion-exchange resin membrane, and isotopic exchange method. These extractants can extract very small fraction of plant available P from the soil and are not usually used as agronomic soil P test.

Water soluble phosphorus (WSP) is used for environmental evaluation of reactive P that can cause water quality issues. Water soluble P is a measure of soluble and easily desorbable P present in soil (Sharpley et al., 1996; Sims et al., 2000) and was identified as a precise indicator to predict P concentration in runoff from agricultural soils compared to other STP methods (Pote et al., 1996). Highly significant and strong correlation was observed ($r^2 = 0.82$) between dissolved reactive P in runoff water (DRP runoff) and WSP (Pote et al., 1996; Torbert et al., 2002; Schroeder et al., 2004). Although, WSP showed more precision in estimating environmental P loss through surface runoff, the method only extracts a small fraction of labile P from the soil which limits the use of WSP as a routine soil test.

Some of the STP methods (M1, M3, Olsen) are used as routine soil test extractant in state extension laboratories. Extensive researches have been conducted on those routine STP methods and have shown good correlation with soluble and bioavailable P in soil (Maguire and Sims, 2002a). Based on STP values and its interpretations, decisions are made for further addition of P into the soil for optimum production. Furthermore, agronomic soil P tests are used for determining cut off point or threshold value used for both agronomic and environmental purposes.

1.6 Soil Test Phosphorus Fertility Ratings

United States and several European countries have been using STP methods for P based fertilizer recommendation as well as for environmental P loss risk assessment (Buczko and Kuchenbuch, 2007). The existing soil test phosphorus (STP) methods were primarily developed for P recommendation for optimum crop production, therefore same guidelines, and interpretations on STP would not be suitable to address environmental issues. Different guidelines and interpretations for STP values would be required so that STP could be used to address the environmental P loss and potential water quality issues (Sharpley et al., 2003). Generally, the soil P status is categorized into classes based on STP values which differ between soil types and extractant. For example, in Germany, a five categorical fertility classes (A-E) was derived using STP values determined with calcium-acetate-lactate as extraction solution (Buczko and Kuchenbuch, 2007). They provide STP value of 220 mg P kg^{-1} soil as an environmental threshold, above which greater P loss risk is assumed and no further P addition is recommended. Similarly, in USA, every state has adopted specific STP methods and categorized STP values into different P levels/ratings, a critical soil test level was assigned based on STP method used, soil types and specific crops. Critical soil test level is that concentration at which 95% of maximum relative yield is achieved and additional application of nutrient is not expected to increase yield (Mitchell and Huluka, 2012). For Alabama, group 1 soils (sandy soils) and group 2 (loams) soils the critical STP value (M1-P) is 25 mg P kg^{-1} soil. For group 3 (clay soils of Limestone Valley) critical STP values (M1-P) is 15 mg P kg^{-1} soil. Similarly, for group 4 (Blackbelt) soils the critical STP (La-P) is 36 mg P kg^{-1} soil. The different P fertility ratings and their respective range for STP values used by few Southeastern US states is provided in Table 1. The agronomic critical level lies at the junction of medium and high soil test P ratings. However, P status of soil is logically not sufficient to assess the risk of P loss because several hydrological and geomorphological factors influences the P export from agricultural land to water bodies.

Soil test P is one of the parameters used in P index. Southern Region Water Quality Planning Committee Working group considered STP as one of the important factor which most likely affect the P-index ratings (Osmond et al., 2006). Underestimation or overestimation in STP may misguide nutrient recommendations to the farmers and ultimately pose greater risk to environment. Therefore, it is important to choose an appropriate soil test method to determine P level in soil precisely. Understanding the extraction efficiency of STP methods for P extraction would be helpful in selecting a reliable and efficient extraction method. Currently, Alabama uses M1 (for non-calcareous soils) and La (for calcareous soils found in Blackbelt) as a routine soil test extractant. Both the STP methods have been used for environmental purposes such as P-index calculation in Alabama.

1.7 Extraction Efficiency

Some of the STP methods discussed above, such as oxalate and CBD are not routine soil test procedure adopted by soil testing laboratories. Therefore, the use of these STP procedure would not be convenient for laboratories which have to perform soil test analysis in large scale. Mehlich-3 has gained popularity in many of the states in US (Alaska, Florida, Arkansas, North Carolina, Oklahoma, Louisiana, Kentucky) and other parts of the world. Because of its applicability in wide range of soils and efficiency of nutrients extraction than other STP methods, many state laboratories in Southeastern US have shifted from M1 to M3 (Mylavarapu et al., 2017).

Extraction efficiency of M3, ammonium acetate (AA), ammonium chloride (NH_4Cl), ammonium bicarbonate-DTPA (AB-DTPA) for P, Ca, Mg, and K was studied in Florida (Alva, 1993). Results showed that P and K extracted by M3 was greater than other extractant. However, M1 extraction efficiency was greater for Ca and Mg than M3. Sims, (1989) also reported similar results showing M3 extractable P, Mn, Cu and Zn was greater compared to M1. The greater extractability of P was due to fluoride present in M3 solution which dissolves P boned with Al and Fe releasing P to soil solution. In addition, presence of ammonium ions in M3 favors dissolution of cations and EDTA enhance extraction of micronutrients (Mehlich, 1984). Extraction efficiency of M1 and M3 was calculated based on total elemental concentration in soils from South Carolina (Franklin et al., 2006). They reported M3 recovered larger amount of all the elements than M1 but neither of the extractant (M1 or M3) were capable in extracting substantial amount compared to total elemental concentration. A study for highly weathered Alabama soils showed P extraction efficiency for different extractant followed the order; M3 < Bray 1 < M1 < Olsen < H_2O < $CaCl_2$ (Senwo et al., 2003). Mehlich-3 extractable P, Fe, and Al was found closely related to oxalate extractable P, Fe, and Al (Maguire and Sims, 2002b) although M3 extracted small fraction of Fe compared to oxalate. This indicated the applicability of M3 solution in soils dominated by Fe and Al. Therefore, many researchers have documented M3 as an efficient extractant for non-calcareous soils from different regions.

Huge variation in extraction efficiency was reported between La and M3 solution by Cox, (2001) for calcareous soils found in Mississippi. Cox found that La method consistently extracted higher amount of nutrients than M3. The extraction efficiency was dependent on Ca content of soil. At low Ca level, La method extracted more Ca than M3 method, up to Ca level of 2030 mg kg^{-1}. Above that concentration of Ca, M3 extracted more amount of Ca than La. This may be due to neutralization of acidity of La solution at higher Ca concentration. Similarly, La extracted greater Mg up to 390 mg kg^{-1} than M3 and above that level M3 started extraction more Mg than La. Moreover, Cox, (2001) reported that P extraction was greater for La than M3 up to 57 mg kg^{-1} and above that level M3 extraction efficiency was greater than La.

1.8 Conversion Equation for Mehlich-3

Extensive field calibration and verification are needed to introduce a new extraction procedure in any state (Mylavarapu et al., 2002). It is difficult to obtain sufficient data for calibration and validation of a new STP method in short period of time. Therefore, conversion equations can be developed for nutrients concentration to convert from current STP method to a new method for interim period (Sims, 1989; Buondonno et al., 1992; Mylavarapu et al., 2002). Separate equation to convert between current STP equivalent to new method have been developed for nutrients through linear regression model.

Mylavarapu et al., (2002) studied correlation between M1 and M3 extractable P, Ca, Mg, K, and other micro-nutrients and developed the conversion equations for those elements. Highly significant linear relationships between M3 and M1 were obtained for all nutrients. The regression equations developed in the study showed M3 extracted 47%, 94%, 101% and 111% more P, Cu, Mn, and B, respectively than M1. They come to conclusion that the conversion equations developed could reasonably be used to estimate M1 values based on M3 values for acid mineral soils of Florida. Similar results were reported in different states for different soil types (Wolf and Baker, 1985; Sims, 1989). Sims, (1989) also reported highly significant relationship between M3 and M1 extractable P, K, Ca, Mg, and Zn ($r = 0.92$ to 0.97) for coarse-textured soil from Atlantic Coastal Plain. He reported that the conversion equation developed were acceptable for interim use for any laboratory that adopts M3 as routine soil test.

Furthermore, conversion equation between Olsen-P and M3-P was evaluated in a study in South Italy (Buondonno et al., 1992). The result showed that Olsen-P could be converted into M3-P by simple linear regression. However, the conversions were more significant when multiple regression equations were developed separately for calcareous and non-calcareous ($CaCO_3$-free soils) soils.

Cox, (2001) studied the relationship between La and M3 extractable Ca, Mg, K and P for soils found in Mississippi. He reported a strong correlation between La and M3 extractable Ca, Mg and K and stated the possibility of using the conversion equation for these three elements. However, his results showed weak correlation between La-P and M3-P and suggested no applicability of conversion equation for P in calcareous soils from Mississippi.

1.9 Relationship between Environmental Soil Test and Agronomic Soil Test

Soil test phosphorus values have been integrated into water quality assessment. Therefore, it becomes crucial to understand whether the adopted STP methods are technically adequate and effective for environmental P loss risk assessment. The relationship between agronomic STP methods and WSP was studied to validate agronomic soil P tests before using them as a tool in estimating environmental P loss (Wang et al., 2015). Maguire and Sims, (2002a) proposed M3 as a single soil test extractant that can potentially be useful for both agronomic (M3 STP value) and environmental purposes (M3-PSR) to assess environmental P loss risk. Relationship between agronomic soil P test (M1, M3) and environmental soil P test (WSP, $CaCl_2$, FeO-P) was studied in soils from Delmarva Peninsula USA (Maguire and Sims, 2002a). They evaluated the ability of agronomic and environmental STP methods to predict P loss in leachate from agricultural soils. They reported that all methods were significantly correlated ($P < 0.001$) with each other but environmental soil tests were slightly better than agronomic soil tests in estimating leachate.

Moreover, they also reported that degree of phosphorus saturation calculated using M3 (DPS_{M3}) was as better as the environmental soil tests in estimating environmental P loss in leachate. Similarly, M1-P was found to be significantly correlated with total P, oxalate extractable P, FeO-P and dilute $CaCl_2$-P in a study from Delaware (Pautler and Sims, 2000). Likewise, M3 was found to be linearly correlated with WSP ($r^2 = 0.68$***), FeO-P ($r^2 = 0.84$***), and oxalate degree of phosphorus saturation (DPS_{OX}) ($r^2 = 0.72$***) for 465 soils from Delaware (Sims et al., 2002).

Additionally, several researches have reported that FeO-P, M1-P, M3-P, WSP, M3 degree of phosphorus saturation (DPS_{M3}) and oxalate degree of phosphorus saturation (DPS_{OX}) could be good predictors of dissolved reactive P in runoff and can be used in environmental P loss risk assessment (Pote et al., 1996; Torbert et al., 2002; Penn et al., 2006).

1.10 Need for Phosphorus Loss Risk Assessment in Alabama Soils

Alabama soils have a long cultivation history that goes back more than 150 years. Many farms have been fertilized continuously over years. Farmers started applying PL at the advent of poultry production in the state. The rate of manure application is generally based on crop N requirement that leads to excess application of P (Sharpley et al., 1996; Hoover et al., 2015). The N/P ratio of PL is approximately 1:1 and crops usually require lower amount of P than N. Generally, most crops will require five times as much N as P. Therefore, quantity of manure applied to meet the crop N requirement results in over application of P to soil than crop P requirement. Application of P in soils beyond the crop need is a waste of resource and pose environmental risk. For example, an experiment was conducted in Southeastern Oklahoma to study the impact of PL application on soil STP (Sharpley et al., 2007). They found that PL applied at 11 Mg ha^{-1} for three consecutive years increased the STP value (M3-P) from 10 to 258 mg kg^{-1} in surface soils. The mean dissolved P concentration in surface runoff increased from 0.08 mg L^{-1} to

4.08 mg L^{-1} in three years. When they stopped application of PL after 3 years, mean STP and dissolved P decreased significantly in following years. Similar results were reported by Kingery et al., (1994) in a field experiment at Sand Mountain, Alabama. They reported that P concentration (M1-P) was six times higher in long term litter applied plots than in non-littered plots. This situation prevailed in other agricultural lands where poultry litter was applied annually. Many countries throughout the world are facing P accumulation in soils through disposal of animal manure where intensive confined livestock industries are in operation.

Unfortunately, once a soil reaches excessively higher STP level, the management becomes challenging, and possess a greater environmental risk. Gartley and Sims, (1994) proposed two practices for managing soils with high STP levels: (i) completely stop addition of any types of P source; (ii) introduce high P demanding crops such as alfalfa to maximize crop P removal. However, P reduction by crop removal may take many years. For example, 16 to 18 years of corn or soybean production would be needed to lower STP from 100 to 20 mg P kg^{-1} soil without further addition of P (McCollum, 1991).

Alabama is the second largest broiler producing state in US and processes 21 million birds per week (AP&EA, 2019) and this production will continue to increase as the meat demand increases. With further expansion of poultry industry, more litter is expected to be generated and used in Alabama farms. The evidence of P enrichment in water bodies have been prominent in Alabama and is of great concern, since one sixth of total surface area is occupied by water bodies. According to Integrated Water Quality Monitoring and Assessment Report, (2020) about 151,021 acres of lakes, reservoirs, and ponds and 510 miles of rivers and streams were impaired by P. Thus, it is important to evaluate the status of P in Alabama soils so that BMPs can be targeted to reduce the P loss risk. The two primary land use management in Alabama where litter is applied are row crop and pastures. Additionally, there are five major soil types in Alabama with varying texture, soil OM, and mineralogical characteristics. It is important to understand how the soil P level varies between land use management, soil types, and depths so that BMPs can be targeted to reduce the risk of P loss in the environment. Additionally, precise P loss risk assessment tools would be needed to identify P hotspots which can be instrumental in minimizing P loss risks to the environment.

1.11 Objectives

The objectives of this research were to identify the soil phosphorus status of Alabama soils and evaluate potential of M3 as an alternative soil test method for environmental purpose for wide range of Alabama soils. The specific objectives of the study were:

1. To survey the soil P levels of major five soil areas of Alabama at four soil depths (0-5, 5-15, 15-30 and 30-45 cm) and two land use management (row crop and grazing systems).
2. To evaluate and compare the extraction potential of Mehlich-1/Lancaster and Mehlich-3 for P, Al, Fe, Ca, and Mg for five major Alabama soils.
3. To determine the conversion equations between Mehlich-1/Lancaster and Mehlich-3 STP values.
4. To determine if observable distinct relationship exist between WSP and STP for different soil types and depths in Alabama soils.

1.12 Tables

Table 1: Soil Test Phosphorus Ratings (STP) calibrated for Mehlich-1, Mehlich-3, and Lancaster/Mississippi extractant used by few of the Southeastern states in the United States.

State	Method	Soil	Crop	Phosphorus, mg kg^{-1}					
				VL	L	M	H	VH	EH
Alabama	Mehlich-1	All soil CEC < 9	All except peanuts	< 7	7-12	13-25	26-50	50-100	> 100
		All soils CEC 9+	All except peanuts	< 4	4-7	8-15	16-30	31-60	> 60
		All	Peanuts	< 3	3-5	6-9	10-25	26-100	> 100
	Lancaster	All Blackland Prairie soils	All	< 10	10-18	19-36	37-72	73-144	> 144
Florida	Mehlich-3	All	All		< 26	26-40	> 40		
Georgia	Mehlich-1	Coastal Plain	All except peanut, lawns, and ornamentals		< 16	16-30	31-50	> 50	
			Peanuts		< 8	8-15	16-30	> 30	
		Piedmont	All except peanut, lawns, and ornamentals		< 11	11-20	21-37	> 37	
			Peanuts		< 6	6-10	12-17	> 17	
		All	Golf greens, trees, and ornamentals		< 26	26-50	51-100	> 100	
Mississippi	Lancaster	All	All except rice	< 10	10-18	19-36	37-72	> 72	
			Rice	< 5	5-9	10-18	19-22	> 22	
South Carolina	Mehlich-1	Coastal Plain	All except peanut		< 16	16-30	31-60	> 60	
		Piedmont	All except peanut		<11	11-20	21-40	> 40	
		All	Peanut		< 6	6-9	10-25	> 25	
Tennessee	Mehlich-1	All	All except cotton		< 10	10-15	16-60	> 60	
	Mehlich-3	All	All except cotton		<20	20-30	31-105	>105	
Virginia	Mehlich-1	All	All	< 2	2-5	6-17	18-55	>55	

VL, L, M, H, VH, and EH denotes very low, low, medium, high, very high and extremely high categories of soil test P level. Adopted from Sikora and Moore, 2014 and Mitchell, 2012 . The values are converted from lb acre^{-1} to mg kg^{-1} using a conversion factor 0.5 .

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CHAPTER 2

Phosphorus Status of Alabama Soils under Different Land Use Practices for Environmental Loss Risk Assessment

2.1 Abstract

Poultry industry is the second largest agricultural industry in Alabama and the industry contributes 65.6% of the total Alabama's agricultural income. This industry generates 1.5 million tons of poultry litter (PL) annually which contains approximately 19,000 tons of phosphorus (P). Long term application of manure in excess of crop requirement results in accumulation of P in soils and P levels may build beyond soil P retention capacity. Loss of excess P from soil to water bodies through surface runoff, leaching and soil erosion, accelerates eutrophication in freshwater system. The objective of this study was to identify the soil P status of major soil areas of Alabama at different soil depths (0-5, 5-15, 15-30 and 30-45 cm) under different land use system (row crop and grazing systems) based on soil test phosphorus (STP) ratings. Soil samples were collected from several farms in Alabama representing five major soil areas namely Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) that had a history of PL application. Each sample was separated into 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depths. Non-calcareous soils (AP, CP, LV, PP) were extracted with M1 and calcareous soils found in BP were extracted with La. Agronomic critical soil test level is defined as the concentration at which 95% of maximum relative yield is achieved and additional application of nutrient is not expected to increase yield. The mean M1-P for AP, CP, LV, and PP soils at 0-5 cm depth were 143 mg kg^{-1}, 76 mg kg^{-1}, 73 mg kg^{-1}, and 91 mg kg^{-1} soil, respectively. The mean La-P for BP soil at 0-5 cm depth was 60 mg kg^{-1} soil. This indicated that STP at surface 0-5 cm are above the critical soil test level for Alabama soils. The percent distribution soils from

major soil areas above soil test critical level for 0-5 cm depth are 93%, 84%, 77%, 67%, and 54% for AP, LV, PP, BP, and CP soils, respectively. Similarly, the distribution of soils from major soil areas above soil test critical level for 5-15 cm depth followed the order: AP (87%) > CP (69%) > LV (42%) > PP (41%) > BP (10%). Results showed that majority of soil P status in PL- impacted surface soils from five major soil types of Alabama was above the environmental STP level and indicate the necessity for implementation of management practices based on precise and accurate soil test P values.

2.2 Introduction

Initiation of vertical integration in poultry production during 1950s in the United States (US) became a major milestone that installed rapid expansion of broiler industry. Alabama is one of the 2nd largest poultry producing state in the US. The poultry industry contributes 65.6% of the total Alabama's agricultural income through 86,000 jobs opportunities (AP&EA, 2019). Besides, the poultry industry also generates more than 1.25 million tons of poultry litter (PL) annually (Lamba et al., 2013) which contains approximately 19,000 tons of Phosphorus (P). Present trend of accelerated consumption of poultry products have led to industrialization, geographic concentration, and intensification in poultry production (Gerber et al., 2007). Although poultry industries have significant contributions to state's economy, the industry is facing challenges with disposal of PL in an economically and environmentally sound manner (Moore et al., 1995; Sharpley et al., 2007; Bolan et al., 2010). Additionally, transportation of PL to longer distances is challenging due to its bulky nature and the risk of mobilizing pathogens across country borders. This is why, majority of PL (> 90% of PL produced) ends up being applied to agricultural fields in close vicinity from the production site (Moore et al., 1995).

At present times, most of the issues related to water quality originate in areas with high density animal operations. Agricultural lands that receive excessive amount of manure and fertilizers have been identified as a non-point source of P pollution (Maguire and Sims, 2002b; Kleinman et al., 2015). Long-term application of manure in excess of plant requirement results in accumulation of P in soils and the P levels may build beyond the soil P adsorption capacity. Unfortunately, if a soil reaches excessively higher STP level, the P management becomes challenging, and it possess a greater environmental risk. Gartley and Sims, (1994) proposed two practices for managing soils with high STP levels: (i) completely stop addition of any types of P source; (ii) introduce high P demand crops such as alfalfa to maximize crop P removal. However, P reduction by crop removal may take many years. For example, 16 to 18 years of corn or soybean production would be needed to lower STP from 100 to 20 mg P kg^{-1} soil without further addition of P (McCollum, 1991). Loss of excess P from soil to water bodies occurs primarily through surface flow, leaching (dissolved form) and soil sediment transport (particulate form), which accelerates eutrophication in fresh water system (Hart et al., 2004; Cordell et al., 2009; Chakraborty et al., 2011; Liao et al., 2019).

United States and several European countries have been using STP methods for P based fertilizer recommendation as well for potential P loss risk assessment (Buczko and Kuchenbuch, 2007). Generally, the soil P status is categorized into classes based on STP values which differ between soil types and extractants. For example, in Germany, five categorical fertility classes (A-E) were derived using STP values determined with calcium-acetate-lactate as extraction solution (Buczko and Kuchenbuch, 2007). A STP value of 220 mg kg^{-1} was assigned as cut off point or threshold level above which P loss risk increased and no P application was recommended. Similarly, in the US, several states have categorized STP values into different P fertility levels and assigned a critical STP beyond which the P loss risk to environment increases. Critical soil test level for agronomic purpose is defined as the concentration at which 95% of maximum relative yield is achieved and additional application of nutrient is not expected to increase yield (Mitchell and Huluka, 2012a). Soil test beyond the critical level is assumed to increase with environmental P loss risk (Mitchell and Huluka, 2012a). Some states have chosen a critical P level for environmental purposes which is usually greater than critical P level for agronomic purposes. Different states in US use different STP methods which makes it different to use a universal threshold for environmental P loss risk assessments.

For example, Alabama uses M1 for non-calcareous soils and La for calcareous soils as standard STP methods for agronomic purpose and in Phosphorus Site Index (PI), which is the most commonly used tool for environmental P loss risk assessment at field scale. Based on calcium content, Alabama soils from AP, CP, LV, and PP are typically categorized as non-calcareous soils whereas soils from BP are categorized as calcareous soil. The critical soil test level lies at the junction of medium and high STP rating. Although several tools for environmental P loss risk assessment have been established, STP methods are still widely used because of its flexibility, cost effectiveness and correlation with soluble and bioavailable P forms (Maguire and Sims, 2002a). STP values would vary depending on soil types and soil characteristics so different soils have different critical STP values above which the release of P from soil increases significantly (Hesketh and Brookes, 2000). Moreover, soil from different geographic regions differ with respect to extraction efficiencies of soil test extractant (Wang et al., 2004). Alabama has five major soil areas and the cropping systems vary greatly depending on the geographic region.

The state has approximately 8,580 thousand acres of farmlands, of which 32.8% is occupied under croplands which comprises row crops, hay land and pastureland (USDA-NASS, 2017). Alabama soils have a long cultivation history, dating 150 years. Some of the farms have been fertilized continuously for many years. Farmers started applying PL at the advent of poultry production in the state. As the P transport from agricultural land to water system are prominent issue affecting water quality, it becomes necessary to investigate the P status of Alabama soils so that Best Management Practices (BMPs) can be developed and promoted for adoption by farmers. Thus, the specific objective of this study was to survey the soil P levels affected by a) major soil types b) land use system (row crops vs grazing) and c) soil depths on agricultural lands that have history of PL application.

2.3 Materials and Methods

2.3.1 Soil Sampling

Alabama soil are categorized into four soil groups based on effective cation exchange capacity (ECEC) of soils (Mitchell and Huluka, 2012b). The group 1 soils are sandy soils with Dothan, Orangeburg, Alaga, Ruston, and Troup soil as the most common series in this group. Group 2 soils are loamy and clayey soils with Madison, Lucedale, Allen, Hartsells, Cecil, Pacolet, and Savannah as the most common series in this group. Group 3 soils are clayey soils (other than Blackbelt) with Colbert, Decatur, Dewey, Talbott, Boswell, and Iredell as the most common soil series in this group. Group 4 soils are calcareous soils from Blackbelt with Houston, Sumter, Oktibbeha, Leeper, and Vaiden as the most common series in this group. Soil samples were collected from sixty-eight agricultural fields representing five major soil areas of Alabama (Fig. 1 and Table 2) located within 18 counties. The sampled farms were either in row crops or grazing system and most farms had poultry litter application history. A total of two hundred nine sampling points were visited where multiple soil cores were taken using a gas powered soil corer (Part 360.01, AMS Equipment, American Falls, ID) at individual depths of 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm (Fig. 2). Soil samples from similar depth were composited from each sampling points to get a most representative sample. A total of 814 samples (including four soil depths at each sampling point) were individually analyzed for parameters listed below. Information on soil order, soil series, and sand, silt, clay fractions and textural class information's were obtained from SUURGO database (Soil Survey Staff, 2020). The information on farm management such as fertilizer application, PL application rate and history, cropping system was collected from the farm owners. The Blackland Prairie soils was studied separately, as their characteristics were different from other non-calcareous soils and therefore, a different extraction method, La was used instead of M1.

2.3.2 Chemical Analysis

The soils samples were air-dried, ground (using mortar and pestle), and passed through a 2-mm sieve before analysis. Soil pH was measured with deionized water at 1:1 (w/v) soil to water ratio. Water-soluble phosphorus (WSP) was determined by extraction 2 g of soil with 20 ml of DI water (1:10 (w/v) soil to water ratio) shaken for an hour (Graetz and Nair, 1995; Chakraborty et al., 2011). Phosphorus in the solution was analyzed by ammonium molybdate-ascorbic acid method by Murphy and Riley, (1962). All soil samples were extracted with M1 extraction solution (0.0125 M H_2SO4 + 0.05 M HCl) using 1:4 (w/v) soil to solution ratio shaken for five minutes (Mehlich, 1953). The Blackbelt soil samples were extracted with La method using two-step process. In first step, 5 g soil was treated with 5 ml of 0.05 M HCl solution for 10 minutes and in second step 20 ml of extraction solution (1.58 M glacial acetic acid + 0.125 M malonic acid + 0.187 M malic acid + 0.037 M ammonium fluoride + 0.03 M aluminum chloride hexahydrate) was added and shaken for 10 minutes (Cox, 2001). Mehlich-1 (M1) and Lancaster (La) extractable P were analyzed using inductively coupled plasma spectroscopy (ICP) (Spectro Ciros, Spectro

Analytical Instruments Inc., Mahwah, NH). Total P in soil samples was determined following ignition method (Andersen, 1976) and analyzed for P and metals (Al, Fe, Ca, and Mg) using ICP.

Phosphorus concentration from M1 soil test (mg kg^{-1}) was used to rate the P fertility level of soils from Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV) and Piedmont Plateau (PP) whereas P concentration obtained from La soil test was used to rate fertility levels of soils from Blackland Prairie (BP). Based on soil series information obtained from SUURGO database, we categorize soil samples into respective soil group as information provided by Mitchell and Huluka, (2012b). For example, if soil sample belonged to Orangeburg soil series, then we categorized that soil as a group 1 soil. All the soils under study were categorized into four soil groups and five major soil types. This way we have a soil group for each of the major soil areas and classified Coastal Plain soils as group 1, Appalachian and Piedmont as group 2, Limestone Valley as group 3 and Blackland Prairie as group 4 soils. The categories and range of STP values used to categorize soils in the study into different P fertility ratings are provided in Table 3.

2.3.3 Statistical Analysis

Data arrangement and computation was performed using R version 3.6.2. (RStudio Team, 2018). Analysis of variance was conducted using the Proc GLIMMIX in SAS® 9.4 (SAS institute, 2013). Locations were considered random effect and soil depth and land use management was considered as fixed effect in the model. The means differences were determined using Tukey-Kramer adjusted P values at $\alpha = 0.05$ significance level. Difference among means and coefficient of determination were considered significant at the $P < 0.05$ level, unless stated. To study the differences between soil depths and among the soil types, logarithmic transformation was applied to all the variable under study. The Shapiro-Wilk W test was performed to test normality assumptions of the residuals.

2.4 Results and Discussion

2.4.1 Soil Properties

The general information on sample distribution and selected properties of soils are provided in Table 4. We found a significant effect of soil types ($P < 0.0001$) and depth ($P = 0.0007$) on soil pH but the interaction effect was not significant ($P = 0.4417$). The mean pH for AP soil (all depths combined) was 5.8 and ranged from acidic to neutral (3.9 -7.2). We found no significant difference in pH between 0-5, 5-15 and 15-30 cm, however pH at 30-45 cm depth was significantly different ($P = 0.0076$) from the 0-5 and 5-15 cm depth for AP soils. Similarly, the mean pH for CP soils was 5.7 and ranged from 3.8 to 7.4. However, the pH in CP soils did not vary significantly ($P= 0.5810$) between depths. The mean pH for LV soil was 6.2 and ranged from acidic to alkaline (4.6 -8.1). The pH of LV soils did not vary significantly up to 30 cm depth, however pH at 30-45 cm depth was significantly different ($P = 0.0117$) than the upper depths. The mean pH for PP soil was 5.4 and ranged from 4.0 to 7.8. We found no significant difference ($P = 0.1172$) in pH between soil depths for PP soils. The mean pH for BP soil was 7.5 and ranged from acidic to alkaline (4.8-9.1). We found no significant difference ($P = 0.8986$) in pH between soil depths for BP soils.

We also compared the soil pH for individual soil depths across soil types. Among the five major soil types, pH in BP, LV, and PP soils were significantly different ($P < 0 .0001$) at 0-5 cm and 15-30 cm depth. Similarly, the pH at 5-15 cm was significantly different ($P < 0 .0001$) among BP, CP, LV, and PP soils. Mean pH in 30-45 cm soil depth in AP, CP, and PP were found statistically similar. The mean pH was greatest in BP soil followed by LV, AP, CP, and PP. The wider range in pH observed within a same soil type might be due to influence of management practices such as PL and fertilizer application, type of crop grown, irrigation, and soil minerology.

Soils pH ranged from acidic to alkaline in Blackland Prairie soils because some of the soils in this area were assumed to be developed from acid residual clay soils.

A significant effect of soil types ($P < 0.0001$) and depth ($P < 0.001$) was observed on WSP but the interaction effect was not significant ($P = 0.3462$). The mean WSP in AP soil was 4.1 mg P kg^{-1} and ranged from 0 to 24 mg P kg^{-1} soil. Water-soluble phosphorus (WSP) in AP soil was significantly different ($P < 0.001$) up to 30 cm depth, however WSP at 30-45 cm depth did not vary significantly from 15-30 cm depth. The mean WSP for CP soil was 4.8 mg kg^{-1} and ranged from 0 to 60 mg P kg^{-1} soil. We found no significant difference in WSP between 0-5 and 5-15 cm, however WSP vary significantly ($P < 0.0001$) between 0-5, 15-30, and 30-45 cm depths. Similarly, the mean WSP for LV soils was 2.2 mg kg^{-1} and ranged from 0 to 32 mg P kg^{-1} soil. Water-soluble phosphorus in LV soils vary significantly ($P < 0.0001$) up to 30 cm depth, however WSP at 30-45 cm did not vary significantly with 15-30 cm depth. The mean WSP for PP soil was 3.2 mg kg^{-1} and ranged from 0 to 36 mg P kg^{-1} soil. We found significant difference ($P < 0.0001$) in WSP between 0-5, 5-15 and 15-30 cm depth, however WSP did not vary significantly at lower depth. The mean WSP for BP soil was 1 mg kg^{-1} and ranged from 0 to 13 mg P kg^{-1} soil. In BP soils, there was significant difference ($P < 0.0001$) in WSP between 0-5 and 5-15 cm depth but no significant difference in WSP observed between 5-15, 15-30, and 30-45 cm depths. Among the five major soil types, the WSP at 0-5 cm did not vary significantly among AP, CP, LV, and PP soils whereas mean WSP in BP soil was significantly lower ($P = 0.0105$) compared to other soil types at similar depth. Mean WSP at 0-5 cm depth was greater for AP (10 mg kg^{-1}) followed by CP (8.2 mg kg^{-1}), PP (7.8 mg kg^{-1}), LV (5 mg kg^{-1}), and BP (3 mg kg^{-1}) soils. The WSP at 5-15 cm was significantly different ($P < 0.0001$) among CP, LV, and BP soils whereas WSP did not vary significantly among AP, CP, and PP soils. Similarly, WSP at 15-30 cm did not vary significantly among AP, LV, PP and BP soils, however WSP from CP soil was significantly different ($P < 0.0001$) than other soil types. At 30-45 cm depth, WSP vary significantly ($P = 0.0141$) among CP and BP soils however, WSP was not significantly different among AP, LV, and PP soils at similar depth. For all depths combined, the mean WSP was greatest for CP followed by AP, PP, LV, and BP soils. The sampling depth affected the WSP concentration which decreased down the soil profile. This could be due to fixation of P in non-labile pools at lower depth (Schroeder et al., 2004). There was wider range in WSP concentration in some soil samples which might be due to higher amount of readily soluble P in surface soil through PL application.

We found a significant effect of soil types ($P < 0.0001$) and depth ($P < 0.0001$) on soil total phosphorus concentration (TP) but the interaction effect was not significant ($P = 0.1516$). For all depths combined, the mean TP was greatest in BP (487 mg kg^{-1}) soils followed by PP (445 mg kg^{-1}), AP (377 mg kg^{-1}), LV (348 mg kg^{-1}), and CP (223 mg kg^{-1}) soils and ranged between 84 to 1917 mg kg^{-1} for BP, 20 to 1695 mg kg^{-1} for PP, 47 to 1,299 mg kg^{-1} for AP, 64 to 2072 mg kg^{-1} for LV and 22 to 830 mg kg^{-1} for CP soils. In general, TP decreased down the soil profile for all the soil types, however the values differed between soil types. We found no significant difference in TP between 0-5 and 5-15 cm, however TP at 15-30 and 30-45 cm were significantly different ($P < 0.0001$) from the 0-5 cm and 5-15 cm depth for AP soils. In CP soils, TP did not vary significantly ($P = 0.3066$) between soil depths. The mean TP at 0-5 cm and 5-15 cm in LV soils were significantly different ($P < 0.0001$) from 15-30 cm and 30-45 cm depth. The TP in PP soils vary significantly ($P < 0.0001$) up to 30 cm depth, however TP at 15-30 and 30-45 cm depth were not significantly different. Similarly, the mean TP in BP soil was significantly different ($P < 0.0001$) up to 30 cm depth, however TP at 30-45 cm was not significantly different from 15-30 cm depth.

We also compared the soil TP for individual depths across soil types. Among five major soil types, TP in CP soils at 0-5, 5-15, and 30-45 cm depths were significantly different ($P < 0.01$) than other soil types at similar depth. Furthermore, there was no significant difference in TP at 0-5, 5-15, and 30-45 cm depths among AP, LV, PP, and BP soils which are significantly different ($P < 0.01$) from CP soils at similar depth. We found no significant difference ($P = 0.1020$) in TP among the five major soil types at 15-30 cm depth. The mean TP value observed in our study was in agreement with the result from a fractionation experiment by Senwo et al., (2003) where they observed the mean TP value of 338 mg kg-1 soil for five major soil series: Hartselle, Decatur, Cowart, Dothan, and Troup. These soils series were also the predominant in our sampling sites. However, M1-P was far greater (41.5 mg kg-1) in our study than Senwo et al., (2003) who reported a mean value of 9.01 mg P kg-1 soil.

There was significant effect of soil types ($P < 0.0001$) but no significant effect of depth ($P = 0.2080$) and interaction ($P = 0.4411$) on TFe concentration. For all depths combined, the mean TFe was greatest in PP (35,408 mg kg^{-1}) soils followed by BP (26,021 mg kg^{-1}), LV (21,787 mg kg^{-1}), AP (13,788 mg kg^{-1}), and CP (11,082 mg kg^{-1}) soils and ranged between 8,659 to 100,941 mg kg^{-1} for PP, 8,523 to 50,939 mg kg^{-1} for BP, 8,145 to 67,239 mg kg^{-1} for LV, 4,962 to 32,573 mg kg^{-1} for AP, and 643 to 49,189 mg kg^{-1} for CP soils. In general, TFe increased down the soil profile for all the soil types, however the values differed between soil types. The TFe in AP soils did not vary significantly up to 30 cm depth, however TP at 30-45 cm depth was significantly different ($P = 0.0297$) than the upper depth. We found non-significant difference in TFe between depths in CP ($P = 0.3671$), LV ($P = 0.5581$), and BP ($P = 0.21939$). In PP soils, TFe did not vary significantly between 5-15, 15-30, and 30-45 cm depth however, TP at 0-5 cm depth was significantly different ($P = 0.0028$) from 15-30 and 30-45 cm depths.

Among the five major soil types, TFe at 0-5 cm depth were significantly different ($P < 0.0001$) among AP, CP, and PP soils however, TFe did not vary significantly among BP, LV, and PP soils. Similarly, TFe at 5-15 cm in PP, LV, and BP did not vary significantly, however these soils were significantly different ($P < 0.0001$) with AP and CP soils at similar depth. There was not significantly different in TFe at 15-30 cm depth among AP, CP, LV, and BP soils however, TFe for PP soils was statistically different ($P < 0.0001$) with all other soil types at similar depths. At 30-45 cm depth, soils from LV, PP, and BP was significantly different ($P < 0.0001$) with CP soils.

We found significant effect of soil types ($P < 0.0001$) but no significant effect of depth ($P = 0.0649$) and interaction ($P = 0.5714$) on TAl concentration. For all depths combined, the mean TAl was greatest in PP (33,811 mg kg^{-1}) soils followed by BP (28,741 mg kg^{-1}), LV (27,165 mg kg^{-1}), CP (20,060 mg kg^{-1}), and AP (16,582 mg kg^{-1}) soils and ranged between 6,732 to 103,037 mg kg^{-1} for PP, 9060 to 56,283 mg kg^{-1} for BP, 7,167 to 94,499 mg kg^{-1} for LV, 2,621 to 84,551 mg kg^{-1} for CP, and 4,067 to 47,395 mg kg^{-1} for AP soils. In general, the mean TAl concentration increased down the soil profile for all the soil types, however the values differed between soil types. The TAl up to 30 cm depth did not vary significantly in AP soils, however TAl at 30-45 cm depth was significantly different ($P = 0.0141$) from 0-5, and 5-15 cm depth. The TAl was not significantly different between depths in CP ($P = 0.3595$), LV ($P = 0.7101$), and BP ($P = 0.0884$) soils. There was no significant difference in TAl between 0-5, and 5-15 cm depth, however TAl at 0-5 cm was significantly different ($P < 0.0001$) from 15-30, and 30-45 cm depth in PP soils.

Among the major five soil types, TAl in CP soils at 0-5 cm depths was significantly different ($P < 0.0001$) with LV, PP, and BP soils. Similarly, TAl in CP soils at 5-15 cm depths were significantly different ($P < 0.0001$) with LV, PP, and BP soils. We found no significant difference in TAl at 15-30 cm depth among the soil types. The TAl at 30-45 cm did not vary significantly among AP, LV, and BP soils, however TAl was statistically different ($P = 0.0188$) in CP and PP soils.

We found significant effect of soil types ($P < 0.0001$) on TMg but there was no significant effect of depth ($P = 0.5102$) and interaction ($P = 0.8583$). For all depths combined, the mean TMg was greatest in BP (2173 mg kg^{-1}) soils followed by LV (1,173 mg kg^{-1}), PP (884 mg kg^{-1}), AP (832 mg kg^{-1}), and CP (401 mg kg^{-1}) soils and ranged between 613 to 3,770 mg kg^{-1} for BP, 503 to 3,263 mg kg^{-1} for LV, 371 to 1,987 mg kg^{-1} for PP, 288 to 1651 mg kg^{-1} for AP, and 1 to 2,808 mg kg^{-1} for CP soils. In general, the mean TMg concentration increased down the soil profile, however the values differed between soil types.The TMg was not significantly different ($P = 0.05$) between soil depth in AP, CP, LV, and BP soils. We found no significant difference in TMg at 0-5 and 5-15 cm depths, however TMg at 30-45 cm depth was significantly different ($P = 0.0005$) from 0-5, and 5-15 cm soil depth for PP soils. Among the five major soil types, TMg in AP and PP soils at 0-5 cm depth did not vary significantly, however these soils were significantly different ($P = 0.0001$) with BP and CP soils at similar depth. There was no statistical difference in TMg among AP, LV, and PP soils at 5-15 cm depths, however these soils were significantly different ($P = 0.0001$) with BP and CP soils at similar depth. At 15-30 cm depth, TMg was significantly different ($P = 0.0001$) among BP, LV, and CP soils whereas no significant difference in TMg among AP and PP soils at that depth. The mean TMg at 30-45 cm depth did not vary significantly among AP, LV, PP, and BP soils which were significantly different with CP soils at similar depth.

2.4.2 Phosphorus Status of Major Soil Areas with Depth

The phosphorus status of major soil areas according to their respective depth is presented in Table 5. There was significant effect of soil depth ($P < 0.0001$), soil type ($P < 0.0001$) and interaction ($P = 0.0236$) on M1 STP. The mean M1 values decreased down the soil depth. In AP soils, we found no significant difference in M1 STP between 0-5 and 5-15 cm depth, however M1-P at 15-30, and 30-45 cm depths were significantly different ($P < 0.0001$) from 0-5 and 5-15 cm depths. Similarly, in CP soils, M1 STP did not vary significantly between 0-5 and 5-15 cm depth, however M1 STP vary significantly ($P < 0.0001$) between 0-5, 15-30, and 30-45 cm depths. The M1 STP in LV soils was significantly different ($P < 0.0001$) between soil depths. Similarly, there was significant difference ($P < 0.0001$) in M1 STP between depths in PP soils.

We also compared the M1 STP at individual depth across the four major soil types. The greatest amount of P was observed in surface soil (0-5 cm depth) for AP soils (143 mg kg^{-1}) followed by PP (91 mg kg^{-1}), CP (76 mg kg^{-1}), and LV (73 mg kg^{-1}). We found no significant difference in M1 STP at 0-5 cm depth among AP and PP soils which were significantly different ($P = 0.0006$) from CP and LV soils at similar depth. Similarly, M1 STP at 5-15 cm and 15-30 cm did not vary significantly in AP and PP soils, however M1 STP in CP and LV soils were significantly different ($P < 0.0001$) from AP and PP soils at similar depth. At 30-45 cm depth, M1 STP did not vary significantly in AP, CP, and LV soils, however M1 STP in PP soils was significantly different ($P = 0.0058$) from CP and LV soils at similar depth. Higher amount of P accumulation in surface soil is reflection of PL application history. Most sites received PL at rate 4 to 5 tons per acre for several years based on information collected through personal communication with the farmers.

The critical soil test P level for most crops grown (except crops with a low P requirement such as peanuts, blueberries, centipede-grass and pine trees) in Alabama is 25 mg kg^{-1} M1-P and 36 mg kg^{-1} La-P (Blackland Prairie soils) (Mitchell and Huluka, 2012a). From environmental perspective, soil reflecting higher STP values are source of P and are considered significant contributor of P movement to water bodies (Pote et al., 1996; Schroeder et al., 2004). For all four major soils areas (AP, CP, LV, and PP), mean M1-P concentration for surface soil, 0-5 and 5-15 cm, were found above the critical soil test P level (25 mg kg^{-1} soil). For 15-30 cm depth, AP, and CP soils, STP were found above critical level.

In BP soils, the La STP vary significantly ($P < 0.0001$) up to 30 cm depth however, La STP at 15-30 and 30-45 cm depth did not vary significantly at similar depth (Table 5). The mean soil test P in BP soils were 60 mg kg^{-1}, 23 mg kg^{-1}, 11 mg kg^{-1}, and 10 mg kg^{-1} at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depth, respectively.

2.4.3 Phosphorus Status of Soils under Different Land Use System

Further, we categorized all the data into two management practices namely, row crops and grazing system. The comparison of M1-P/La-P values between row crops and grazing system at four soil depth are shown in Fig. 3. We found no significant difference in M1 STP values between row crops and grazing system at all depth for AP and PP soils. However, for CP soils, M1-P value was significantly greater ($P < 0.0001$) for grazing systems than row crops for all soil depths. In LV soils, M1-P values were significantly greater ($P < 0.0001$) for grazing systems in top 0-5 and 5-15 cm depths than row crops. There was no significance difference between M1-P values at 15-30 and 30-45 cm for either grazing or row crops systems in LV soils. The greater STP at 0-5 and 5-15 cm soils in CP and LV grazing system might be the long-term accumulation of P through surface broadcasted poultry litter. Similarly, in BP soils, La-P values were significantly greater (P

< 0.0001) for grazing systems in top 0-5 and 5-15 cm depths than row crops. There was no significant difference in La -P values between 15-30 and 30-45 cm for either grazing or row crops system in BP soils.

We combined the data sets for AP, CP, LV, and PP and categorized them as non-calcareous soils and referred BP soils as calcareous soils. We then compared the difference between mean STP values between grazing and row crop systems (Table 6). There was significant effect of soil depth (P < 0.0001), and practices (P = 0.0257) on STP and no significant effect of interaction on STP for non-calcareous soils. In non-calcareous soil, M1 STP vary significantly (P = 0.0256) between all depths in row crop system. Similarly, M1 STP vary significantly (P < 0.0001) between depths in grazing system for non-calcareous soils. Furthermore, mean STP in 0-5 cm soil was approximately two folds greater than 5-15 cm soil depth in both row crops and grazing system for non-calcareous soils. Surface broadcasting is the most common method of manure application in grazing system (Schroeder et al., 2004) which may be a reason for surface accumulation of P in the topsoil surface. In addition to this we studied the difference in STP at individual depth among the two management systems. We found no significant difference in M1 STP at all four individual depths among grazing and row crops system for non-calcareous soils.

We found significant effect of soil depth (P < 0.0001) on STP and no significant effect of practices and interaction on STP for calcareous soils from BP. In BP row crops system, there was no significant difference in La-P up to 30 cm depths however, La-P at 30-45 cm was significantly different (P = 0.0256) from 0-5 cm depth. In BP grazing system, La-P vary significantly (P < 0.0001) up to 30 cm depth, however La-P at 30-45 cm did not vary significant from 15-30 cm depth. Further, at 0-5 cm the La STP vary significantly (P = 0.0349) among row crops and grazing system. Similarly, at 5-15 cm the La STP vary significantly (P = 0.0017) among the row crops and grazing system in BP soils. We found no significant difference in La STP at 15-30 cm and 30-45 cm depth between row crops and grazing system. This result showed that mean STP values in row crops system of BP soils was greater than grazing system at surface 0-5 and 5-15 cm soil depths. The greater STP values in row crops system of BP soils reflect the nutrient management practices such as manure and fertilizer application in those soils because the soil samples under study were representative samples from commercial farms in Blackbelt areas of Alabama.

2.4.4 Percentage Distribution of Soils under Soil Test Fertility Ratings

From agronomical viewpoint, the soil test P rating slightly above medium is considered adequate (critical soil test P level) for optimum production and further addition of P is not recommended above critical soil test P level (Mitchell and Huluka, 2012b). Based on STP (M1 or La), we categorize all soil samples into soil test fertility rating developed for Alabama soils. We then calculated the percentage distribution of soil samples under each fertility ratings or individual depths. Total of 816 samples were categorized into six STP ratings of which 84 samples were from BP and were studied separately. The remaining 731 soil samples belonged to AP, CP, LV, and PP. The percentage distribution of soil samples in each of STP ratings category for non-calcareous and calcareous soils are presented in Table 7. In non-calcareous soils, 3%, 8%, 11%, 16%, 29%, and 33% of the soil samples from surface 0-5 cm were categorized as very low, low, medium, high, very high, and extremely high, respectively. Similarly, 12%, 16%, 21%, 19%, 22%, and 10% of the soil samples from surface 5-15 cm were categorized as very low, low, medium, high, very high, and extremely high, respectively in non-calcareous soils. In contrast to surface soils, 41%, 18%, 16%, 13%, 9%, and 3% of the soil samples from surface 15-30 cm were categorized as very low, low, medium, high, very high, and extremely high, respectively from non-calcareous soils. Similarly, at 30-45 cm depth, 64%, 16%, 14%, 4%, and 2% of the soil samples were categorized as very low, low, medium, high, and very high, respectively from non-calcareous soils. The percentage distribution of soil in very low, low, and medium ratings increases down the soil profile. In BP, soil samples from 0-5 cm depth were only categorized into medium (33%), high (38%) and very high (29%) ratings. There were no soil samples categorized into extremely high ratings from BP. The distribution of soils in each of the P ratings categories for all five major soil areas were studied separately to identify the soils which possess higher P loss risk. The percentage distribution of soils in each P ratings at individual depths for AP soils are presented in Fig. 4. Similarly, the percentage distribution of soils in each P ratings at individual depth for CP, LV, PP, and BP soils are shown in Figures 5-8, respectively. Overall, 78% of the surface 0-5 cm soil were above critical soil test P level (above medium rating) and 51%, 25%, and 5% of soils from 5-15, 15-30, and 30-45 cm depths were above critical soil test P level, respectively.

For surface 0-5 cm soil from AP, about 90% of the soils had extremely high and very high P rating. As we go down the soil profile, the percentage of soils that fall under very low, low, and medium rating increased and percentage of soils that fall under high, very high and extremely high decreased (Fig. 4). In case of CP soils (Fig. 5), the percentage of soils that fall above medium category are still higher for lower depths as well, which indicates movement of P from surface to lower soil profile. In case of BP soils (Fig. 8), about 67% soil samples were in high and very high P ratings from surface 0-5 cm depth and percentage of soil samples above medium ratings reduced to 10% at 5-15 cm depth. From the figures, the distribution of soils from major soil areas above soil test critical level for 0-5 cm depth followed the order: AP (93%) > LV (84%) > PP (77%) > BP (67%) > CP (54%). Similarly, the distribution of soils from major soil areas above soil test critical level for 5-15 cm depth followed the order: AP (87%) > CP (69%) > LV (42%) > PP (41%) > BP (10%).

This overall soil categorization and their percentage distribution revealed that all soils from surface 0-5 cm had P ratings above medium and those soils could act as a source of P which may pose P loss risk to the environment. For surface 0-5 cm soil, which is considered more vulnerable to P loss through surface runoff and erosion, among the five major soil areas AP soils were found to be at greater environmental risk as evident by higher mean WSP values. In Alabama, intensive poultry farms are in operation at Appalachian region which might have direct impact on amount of PL application into agricultural lands in that area. It has been identified that the intensive animal husbandry in particular area had increased the volume of manure that goes into the fields which subsequently builds P level in soils (Sims et al., 2000; Maguire and Sims, 2002b). An experiment was conducted in Southeastern Oklahoma to study the impact of PL application on soil STP (Sharpley et al., 2007). They found that PL applied at 11 Mg ha^{-1} for three consecutive years increased the STP value (M3-P) from 10 to 258 mg kg^{-1} in surface soils. The mean dissolved P concentration in surface runoff increased from 0.08 mg L^{-1} to 4.08 mg L^{-1} in three years. When they stopped application of PL after 3 years, mean STP and dissolved P decreased significantly in following years. Similarly, Kingery et al., (1994) reported that P concentration in PL plots had six times higher P concentration up to 60 cm than in non-littered plots at Sand Mountain, AL. In addition to this, texture plays important role in P release from the soil. Greater amount of P is required in order to raise STP values by same amount on clayey textured soil than in coarse textured soil (Johnson et al., 2005). Coastal Plain soils are most extensive soils of Alabama. The soil from CP are mainly coarse textured with small amount of clay and have lower P retention capacity (Nair et al., 2004), which indicates greater P loss risk from CP soils. The overall results in our study indicated that the P concentration in surface soils were above the environmental STP level and highlighted the necessity for environmental soil test and implementation of more precise quantitative measures in assessing P loss risk from Alabama soils.

2.5 Conclusions

Transport of P from agricultural sources to water bodies and degradation of water quality have been a growing concern throughout the world. Manure management have been challenging issue in areas with intensive animal farming, and the agricultural land in those sites were found to be heavily manure impacted which elevated the STP level beyond crop requirement. Poultry industry in Alabama flourished half century ago and farmers started applying PL at the advent of poultry production in the state. In this study we aimed to understand the P levels in soils collected from major soil areas of Alabama with diverse land use systems. We also aimed to estimate the percentage distribution of soils which are vulnerable to P loss risk to the environment. The mean M1-P for AP, CP, LV, and PP at 0-5 cm was 143 mg kg^{-1}, 76 mg kg^{-1}, 73 mg kg^{-1}, and 91 mg kg^{-1} soil, respectively which are above the critical soil test level for non-calcareous Alabama soils. The mean La-P for BP soil at 0-5 cm depth was 60 mg kg^{-1} and is above the critical soil test P level for calcareous soils. Greater percentage of soils samples from AP were above the soil test critical level. In AP 93% of the soil samples from 0-5 cm depths were above soil test critical level followed by LV (84%), PP (77%), BP (67%), and CP (54%). In surface 0-5 and 5-15 cm depths soils test ratings for most of the soils are high, very high and extremely high and these surface soils were found to be loaded with P resulting from PL application. However, as we go down the soil profile, the STP ratings for most of the soil are very low and low. This indicates the importance of management practices that need to be done for surface 0-15 cm soil in order to prevent P loss during runoff event and highlighted the necessity of implementations of more precise quantitative measures such as P index in assessing P loss risk from Alabama soils.

2.6 Tables

Table 2. Sample distribution in major soil areas, soil order, respective soil series and surface soil texture of Alabama soils under study.

Soil Areas	Soil Series	Soil Order	Surface Texture	No. of Samples
Appalachian Plateau (AP)	Albertville, Allen, Hartsells, Linker, Nauvo, Tilsit	Ultisols	Loam	116
Coastal Plain (CP)	Compass, Dothan, Lucy, Nankin, Orangeburg, Red Bay, Troup	Ultisols	Sand, Sandy Loam, Loam	142
Limestone Valley (LV)	Abernathy, Bodine, Cedarbluff, Colbert, Cookeville Cumberland, Decatur, Dickson, Holston, Monongahela, Mountview, McQueen	Alfisols, Ultisols	Loam, Silt Loam, Clay loam, Clay	222
Piedmont Plateau (PP)	Altavista, Augusta, Lousia, Madison, Ochlockonee, Vickham	Inceptisols, Entisols, Ultisols	Sandy loam, Silt loam, Loam,	251
Blackland Prairie (BP)	Faunsdale, Oktibbeha, Sucarnoochee, Tuscumbia, Sumter, Vaiden	Inceptisols, Vertisols	Silty Clay, Clay	84

Information on soil order, soil series, and textural class were obtained from SUURGO database.

Table 3: Soil test phosphorus ratings based on soil areas of Alabama and extractable phosphorus concentration (Adopted from Huluka and Mitchell, 2012).

Ratings	Soil areas of Alabama				
	Coastal Plain	Appalachian Plateau	Piedmont Plateau	Limestone Valley	Blackland Prairie
	………………………….. **mg kg^{-1}** ……………………………				
Very Low	< 7	< 7	< 7	< 4	< 10
Low	7-12	7-12	7-12	4-7	10-18
Medium	13-25	13-25	13-25	8-15	18-36
High	26-50	26-50	26-50	16-30	37-72
Very High	51-125	51-125	51-125	31-75	73-180
Extremely High	> 125	> 125	> 125	> 75	> 180

Blackland Prairie soils were extracted with Mississippi/Lancaster solution and all other soils were extracted with Mehlich-1

Table 4: Ancillary properties of five major soil areas of Alabama. The values are mean and standard error of means for respective soil depths.

Soil Areas	Depths (cm)	pH	WSP	TP	TFe	TAl	TMg	Sand	Silt	Clay	n
			……………………….mg kg^{-1} ………………………					………..%..........			
Appalachian Plateau (AP)	0-5	6.1 $^{a, bc}$ (0.7)	10 $^{a, a}$ (1.4)	617 $^{a, a}$ (51)	11885 $^{b, b}$ (1017)	13574 $^{b, ab}$ (1460)	794 $^{a, b}$ (65)	62$^{a, ab}$	24$^{c, b}$	14$^{b, c}$	29
	5-15	6.0 $^{a, bc}$ (0.7)	3.6 $^{b, abc}$ (1)	438 $^{a, a}$ (48)	12243 $^{ab, b}$ (1091)	13917 $^{b, bc}$ (1453)	731 $^{a, b}$ (55)	59$^{a, a}$	25$^{bc, b}$	15$^{b, c}$	29
	15-30	5.7 $^{ab, cd}$ (0.8)	1.8 $^{c, b}$ (0.8)	281 $^{b, a}$ (35)	14467 $^{ab, b}$ (1252)	18113 $^{ab, a}$ (1983)	868 $^{a, ab}$ (59)	47$^{b, b}$	31$^{ab, b}$	22$^{a, b}$	29
	30-45	5.4 $^{b, c}$ (0.8)	0.4 $^{c, ab}$ (0.3)	174 $^{c, ab}$ (21)	16546 $^{a, ab}$ (1347)	20723 $^{a, ab}$ (2070)	934 $^{a, a}$ (64)	43$^{b, b}$	31$^{a, b}$	26$^{a, b}$	29
Coastal Plain (CP)	0-5	5.8 $^{a, cd}$ (0.8)	8.2 $^{a, ab}$ (1.8)	325 $^{a, b}$ (29)	7671 $^{a, c}$ (1312)	13458 $^{a, b}$ (2054)	337 $^{a, c}$ (28)	74$^{a, a}$	15$^{a, c}$	10$^{c, d}$	34
	5-15	5.9 $^{a, c}$ (0.7)	5.1 $^{ab, a}$ (0.7)	228 $^{a, b}$ (17)	8119 $^{a, c}$ (1445)	14573 $^{a, c}$ (2268)	308 $^{a, c}$ (29)	74$^{a, a}$	16$^{a, d}$	10$^{c, d}$	35
	15-30	5.8 $^{a, bc}$ (0.6)	2.8 $^{b, a}$ (0.8)	203 $^{a, a}$ (17)	12213 $^{a, b}$ (2036)	22318 $^{a, a}$ (3417)	413 $^{a, c}$ (40)	70$^{a, a}$	15$^{a, c}$	15$^{b, c}$	36
	30-45	5.6 $^{a, bc}$ (0.6)	1.2 $^{c, a}$ (0.5)	143 $^{a, b}$ (18)	16159 $^{a, b}$ (2334)	29566 $^{a, b}$ (3798)	543 $^{a, b}$ (81)	64$^{b, a}$	15$^{a, c}$	21$^{a, c}$	36
Limestone Valley (LV)	0-5	6.3 $^{a, b}$ (0.6)	5 $^{a, ab}$ (1.0)	537 $^{a, a}$ (36)	19335 $^{a, ab}$ (1263)	22333 $^{a, a}$ (1637)	1035 $^{a, ab}$ (47)	29$^{a, c}$	48$^{a, a}$	23$^{b, b}$	57
	5-15	6.4 $^{a, b}$ (0.6)	1.7 $^{b, bc}$ (0.4)	353 $^{a, a}$ (19)	20430 $^{a, ab}$ (1164)	24917 $^{a, ab}$ (2015)	1055 $^{a, b}$ (42)	29$^{a, a}$	49$^{a, a}$	23$^{b, b}$	57
	15-30	6.2 $^{ab, b}$ (0.8)	0.5 $^{c, b}$ (0.1)	267 $^{b, a}$ (17)	22513 $^{a, b}$ (1316)	29738 $^{a, a}$ (2518)	1247 $^{a, b}$ (59)	21$^{a, c}$	50$^{a, a}$	29$^{a, b}$	56
	30-45	6.0 $^{b, b}$ (0.9)	0.3 $^{c, ab}$ (0.1)	214 $^{b, ab}$ (17)	25274 $^{a, a}$ (1316)	32352 $^{a, ab}$ (2425)	1380 $^{a, a}$ (73)	19$^{a, c}$	48$^{a, a}$	33$^{a, b}$	52
Piedmont Plateau (PP)	0-5	5.6 $^{a, d}$ (0.6)	7.8 $^{a, ab}$ (1)	714 $^{a, a}$ (46)	29453 $^{b, a}$ (1846)	26128 $^{c, a}$ (1637)	818 $^{b, b}$ (40)	55$^{ab, b}$	27$^{a, b}$	17$^{b, c}$	66

	5-15	5.3 $^{a, d}$ (0.6)	3.1 $^{b, ab}$ (0.5)	455 $^{b, a}$ (30)	34193 $^{ab, a}$ (2232)	30821 $^{bc, a}$ (2040)	806 $^{b, b}$ (34)	57$^{a, a}$	20$^{ab, c}$	22$^{b, c}$	66
	15-30	5.3 $^{a, d}$ (0.7)	1.2 $^{c, b}$ (0.3)	327 $^{c, a}$ (23)	39225 $^{a, a}$ (2636)	39192 $^{ab, a}$ (2718)	933 $^{ab, ab}$ (38)	49$^{bc, b}$	18$^{c, c}$	34$^{a, b}$	66
	30-45	5.4 $^{a, c}$ (0.7)	0.3 $^{c, ab}$ (0.1)	273 $^{c, a}$ (20)	39616 $^{a, a}$ (2655)	40284 $^{a, a}$ (2705)	999 $^{a, a}$ (41)	45$^{c, b}$	20$^{bc, c}$	36$^{a, b}$	53
Blackland Prairie (BP)	0-5	7.3 $^{a, a}$ (0.9)	3.0 $^{a, b}$ (3)	822 $^{a, a}$ (385)	23061 $^{a, ab}$ (8082)	25129 $^{a, a}$ (8813)	2149 $^{a, a}$ (737)	9$^{a, d}$	47$^{a, a}$	42$^{b, a}$	21
	5-15	7.6 $^{a, a}$ (1.1)	1.0 $^{b, c}$ (2)	499 $^{b, a}$ (271)	24929 $^{a, ab}$ (8601)	26596 $^{a, ab}$ (8896)	2092 $^{a, a}$ (611)	9$^{a, c}$	47$^{a, a}$	43$^{b, a}$	21
	15-30	7.5 $^{a, a}$ (1.3)	0.3 $^{b, b}$ (0.7)	334 $^{c, a}$ (237)	26693 $^{a, ab}$ (10013)	29733 $^{a, a}$ (10080)	2142 $^{a, a}$ (577)	11$^{a, d}$	39$^{b, ab}$	50$^{a, a}$	21
	30-45	7.4 $^{a, a}$ (1.4)	0.1 $^{b, b}$ (0.2)	293 $^{c, a}$ (176)	29400 $^{a, a}$ (10253)	33398 $^{a, ab}$ (11285)	2308 $^{a, a}$ (607)	13$^{a, c}$	36$^{b, ab}$	51$^{a, a}$	21

WSP is water soluble phosphorus; TP, TFe, TAl, and TMg total phosphorus, total iron, total aluminum, and total magnesium concentration in soil, respectively; n is no. of samples.

Information on sand, silt, clay fractions were obtained from SUURGO database.

Values within bracket are standard error of means.

The means differences were determined using Tukey-Kramer adjusted P values at $\alpha = 0.05$ significance level.

The initial alphabets separated by comma represents the means difference between the soil depths in a particular soil types and the second alphabets separated by comma represents the mean difference with-in a soil depth among the major soil types, the columns followed by the same letter are not significantly different ($P < 0.05$).

Table 5: Soil Test Phosphorus values for five soil areas of Alabama. Mehlich-1 was used for Appalachian Plateau, Coastal Plain, Limestone Valley and Piedmont Plateau whereas Lancaster was used for Blackland Prairie soils.

Soil Areas	Depths (cm)	Soil Test Phosphorus (mg kg^{-1})		n
		Mean (SE)	Range	
Appalachian Plateau	0-5	143 $^{a, a}$ (14.3)	10 - 289	29
	5-15	88 $^{a, a}$ (10)	4 - 188	29
	15-30	44 $^{b, a}$ (8.9)	3 - 171	29
	30-45	14 $^{c, ab}$ (2.8)	1 - 75	29
Coastal Plain	0-5	76 $^{a, b}$ (13)	3 - 290	34
	5-15	43 $^{ab, b}$ (6.7)	3 - 163	35
	15-30	31 $^{b, b}$ (5.5)	1 - 135	36
	30-45	11 $^{c, b}$ (4)	1 - 130	36
Limestone Valley	0-5	73 $^{a, b}$ (6.5)	7 - 214	57
	5-15	27 $^{b, b}$ (3.9)	3 - 143	57
	15-30	11 $^{c, b}$ (1.6)	2 - 83	56
	30-45	6 $^{d, b}$ (0.6)	2 - 29	52
Piedmont Plateau	0-5	91 $^{a, a}$ (11.3)	3 - 436	66
	5-15	35 $^{b, a}$ (5.2)	2 - 204	66
	15-30	12 $^{c, a}$ (2.2)	1 - 100	66
	30-45	5 $^{d, a}$ (0.9)	1 - 46	53
Blackland Prairie	0-5	60 ^{a} (7.6)	21 - 143	21
	5-15	23 ^{b} (5.9)	4 - 134	21
	15-30	11 ^{c} (3.2)	1 - 74	21
	30-45	10 ^{c} (4.5)	0 - 101	21

Values within bracket are standard error of means; n is no. of samples.

The means differences were determined using Tukey-Kramer adjusted P values at $\alpha = 0.05$ significance level.

The initial alphabets separated by comma represents the means difference between the soil depths in a particular soil types and the second alphabets separated by comma represents the mean difference with-in a soil depth among the major soil types, the columns followed by the same letter are not significantly different ($P < 0.05$). The mean comparison at individual depths was performed on all major soil types except BP.

Table 6: Soil phosphorus levels of non-calcareous (AP, CP, LV, and PP) and calcareous soils (BP) under two land use management systems and soil depths. Soil test phosphorus for Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV) and Piedmont Plateau (PP) is Mehlich-1 extractable phosphorus and Lancaster extractable phosphorus for Blackland Prairie.

Land Use System	Depths (cm)	Soil Test Phosphorus (mg kg^{-1})		N
		Mean (SE)	Range	
		AP, CP, LV, and PP combined		
Row crops	0-5	$74^{a, a}$ (6.0)	7 - 289	109
	5-15	$35^{b, a}$ (3.7)	2 - 188	109
	15-30	$18^{c, a}$ (2.8)	1 - 171	109
	30-45	$6^{d, a}$ (0.9)	1 - 75	102
Grazing	0-5	$100^{a, a}$ (10.5)	3 - 318	73
	5-15	$45^{b, a}$ (475.5)	2 - 204	73
	15-30	$19^{c, a}$ (3.2)	1 - 135	73
	30-45	$9^{d, a}$ (2.4)	1 - 130	65
Blackland Prairie (BP)				
Row crops	0-5	$85^{a, a}$ (15.9)	34 - 143	6
	5-15	$46^{ab, a}$ (18.4)	11 - 134	6
	15-30	$18^{ab, a}$ (11.4)	1 - 74	6
	30-45	$21^{b, a}$ (16.3)	0 - 101	6
Grazing	0-5	$49^{a, b}$ (7.5)	21 - 119	15
	5-15	$13^{b, b}$ (1.3)	4 - 23	15
	15-30	$7^{c,}$ a (0.8)	3 - 14	15
	30-45	$7^{c, a}$ (1.5)	1 - 27	15

Values within bracket are standard error of means; n is no. of samples.

The means differences were determined using Tukey-Kramer adjusted P values at $\alpha = 0.05$ significance level.

The initial alphabets separated by comma represents the means difference between the soil depths in a particular soil types and the second alphabets separated by comma represents the mean difference with-in a soil depth among the major soil types, the columns followed by the same letter are not significantly different ($P < 0.05$).

Table 7: Percentage distribution of soil samples in each of soil test P ratings category for combined four soil areas (AP, CP, LS, PP) and Blackland Prairie soils by depths. P fertility ratings, for combined soil (AP, CP, LV, and PP) rating is based on M1 extractable phosphorus and La extractable phosphorus for BP.

Soil Areas	Depths (cm)	Soil test P (mg kg^{-1})		Phosphorus Fertility Ratings						n
				% distribution						
		Mean (SE)	Range	V Low	Low	Medium	High	V High	E High	
Combined (AP, CP, LS, PP)	0-5	93^a (6.6)	3-436	3	8	11	16	29	33	187
	5-15	42^b (3.7)	2-444	12	16	21	19	22	10	187
	15-30	20^c (0.4)	1-171	41	18	16	13	9	3	187
	30-45	8^d (1.2)	1-130	64	16	14	4	2	0	170
Blackland Prairie (BP)	0-5	60^a (7.6)	21 - 143	0	0	33	38	29	0	21
	5-15	23^b (5.9)	4 - 134	14	52	24	5	5	0	21
	15-30	11^c (3.2)	1 - 74	76	19	0	0	5	0	21
	30-45	10^c (4.5)	0 - 101	80	5	10	0	5	0	21

n is no of samples, SE, Standard error of means,
The means differences were determined using Tukey-Kramer adjusted P values at $\alpha = 0.05$ significance level.
The alphabets in columns followed by the same letter are not significantly different ($P < 0.05$).

2.7 Figures

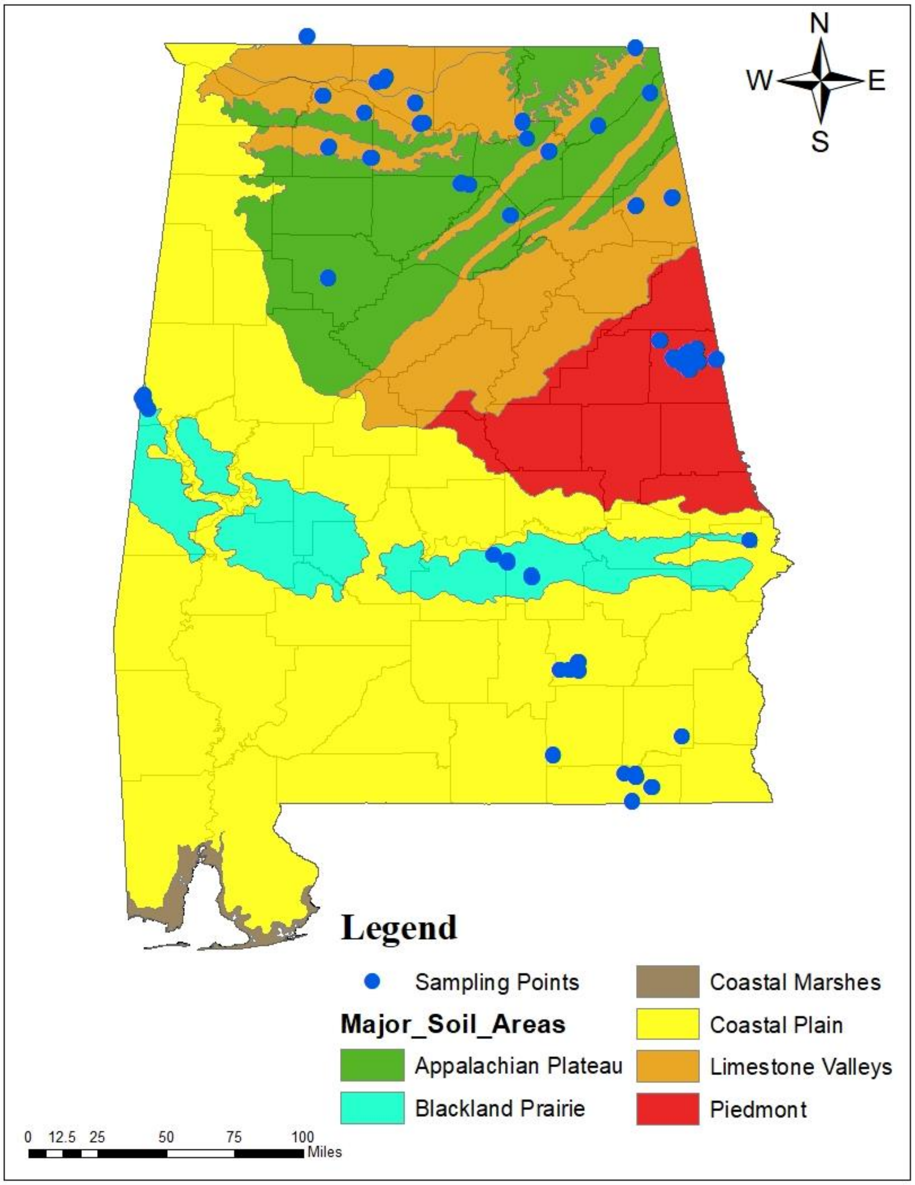

Fig. 1: Location of sampling points and major soil areas of Alabama, USA.

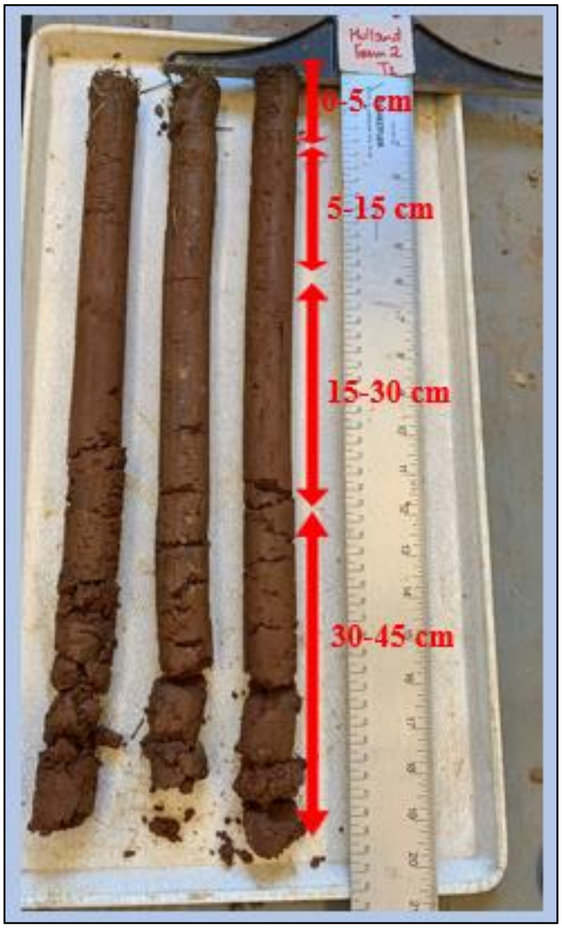

Fig. 2: Soil cores collected from the field showing different depths.

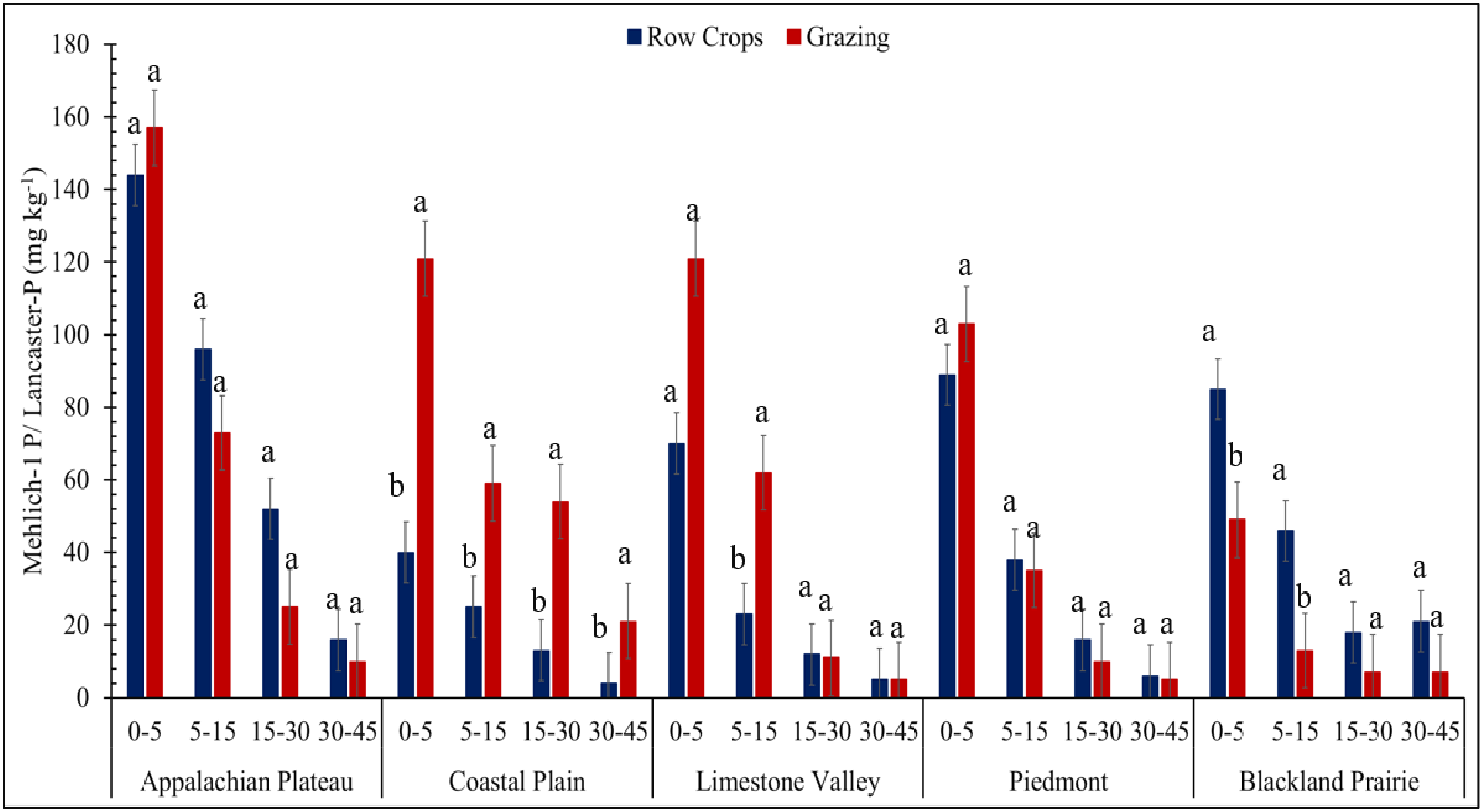

Fig. 3: Bar plot showing mean Mehlich-1 P (Appalachian Plateau, Coastal Plain, Limestone Valley, Piedmont) and Lancaster-P (Blackland Prairie) level of five soil areas of Alabama under row crops and grazing system with their respective depths. Bars represent standard error of the sample means. Means with same letter within each depth are not significantly different at $P < 0.05$.

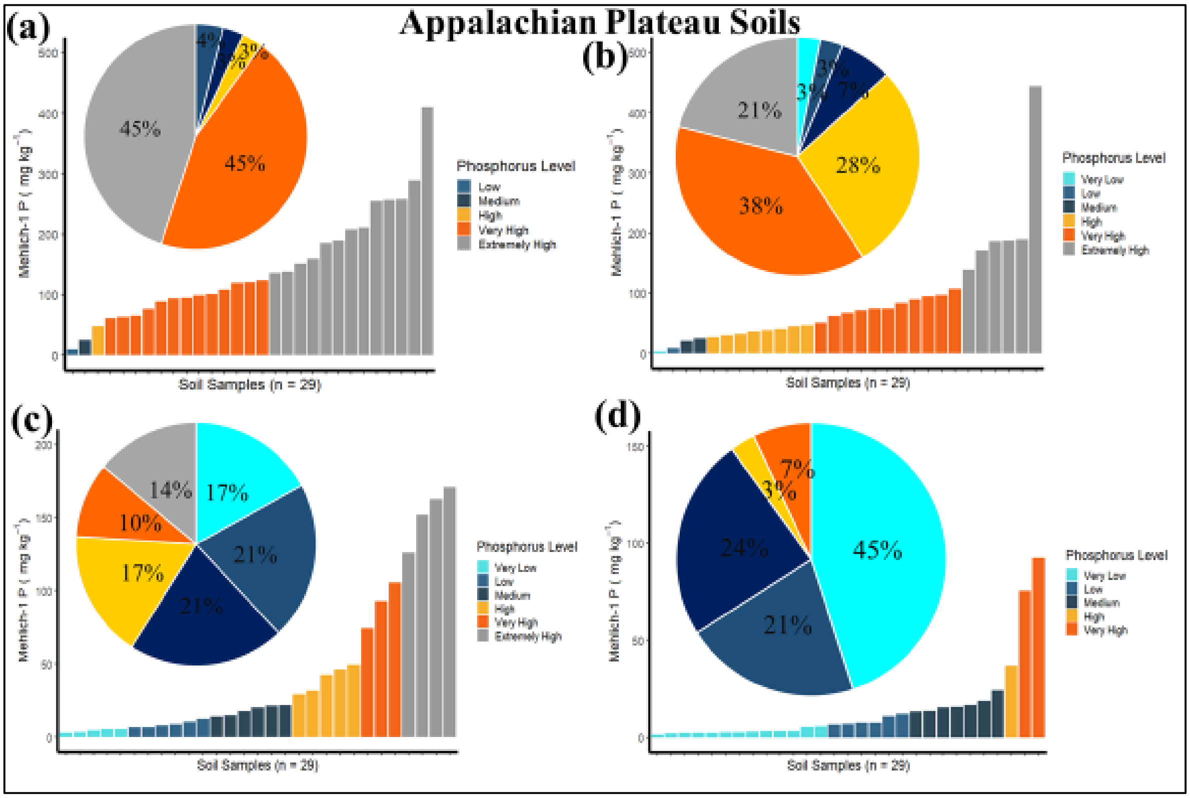

Fig. 4: Percent distribution of soil samples from Appalachian Plateau categorized into soil test phosphorus fertility ratings (very low, low, medium, high, very high, and extremely high) with depths a) 0-5 cm b) 5-15 cm c) 15-30 cm and d) 30-45 cm. The P fertility ratings was based on Mehlich-1 extractable P concentration.

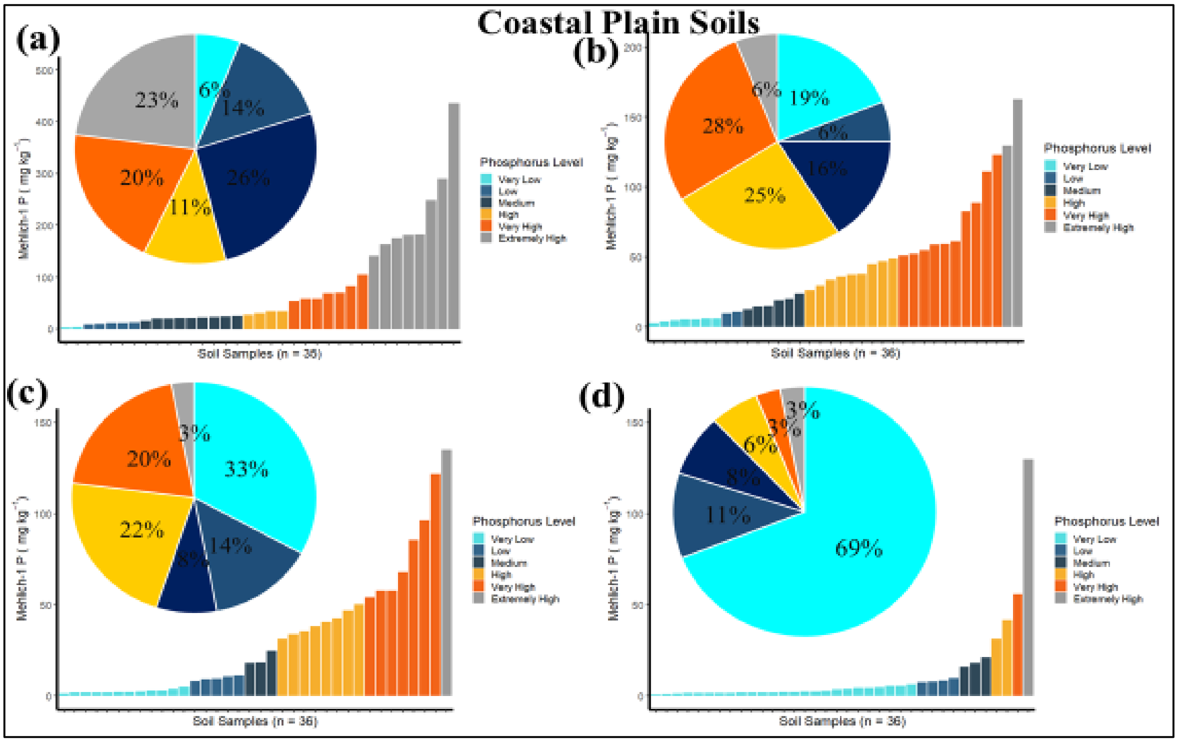

Fig. 5: Percent distribution of soil samples from Coastal Plain categorized into soil test phosphorus fertility ratings (very low, low, medium, high, very high, and extremely high) with depths a) 0-5 cm b) 5-15 cm c) 15-30 cm and d) 30-45 cm. The P fertility ratings was based on Mehlich-1 extractable P concentration.

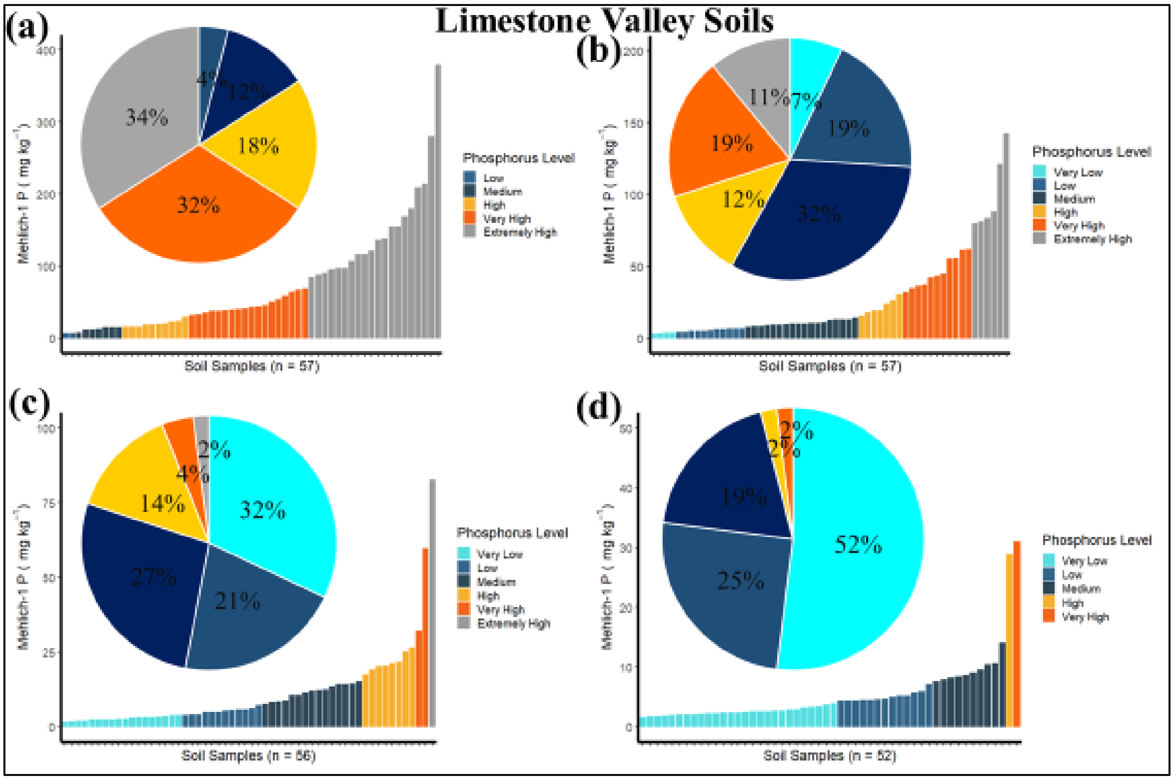

Fig. 6: Percent distribution of soil samples from Limestone Valley categorized into soil test phosphorus fertility ratings (very low, low, medium, high, very high, and extremely high) with depths a) 0-5 cm b) 5-15 cm c) 15-30 cm and d) 30-45 cm. The P fertility ratings was based on Mehlich-1 extractable P concentration.

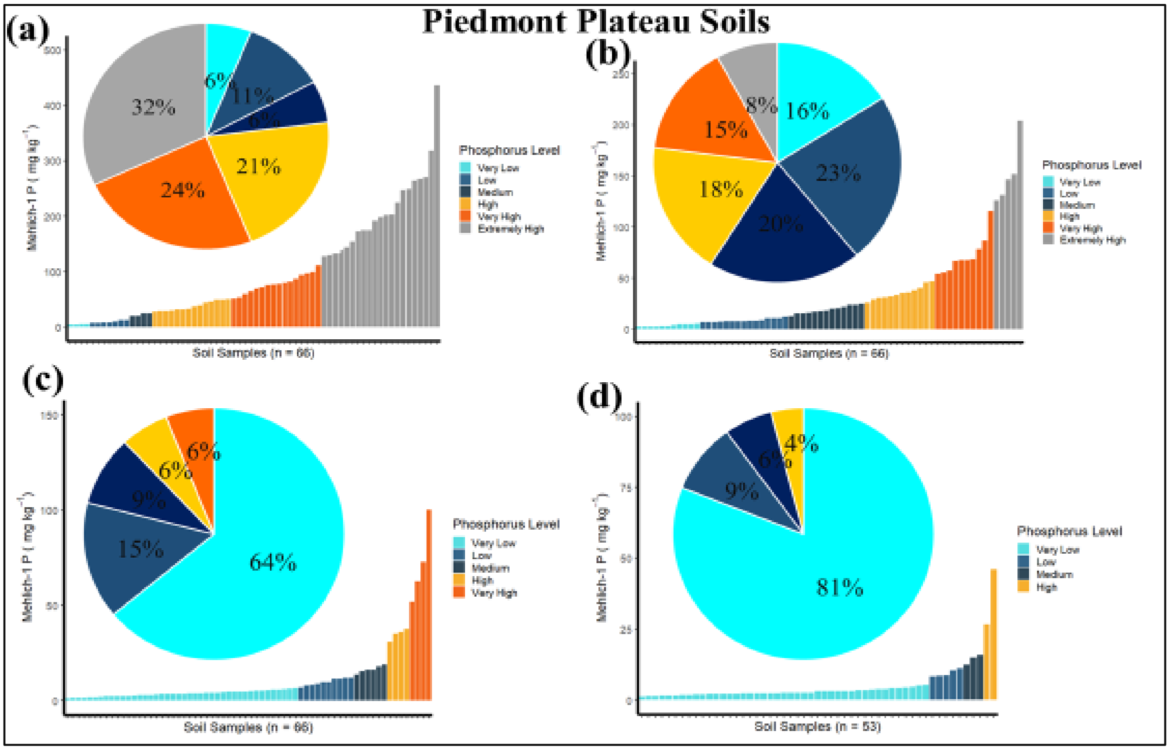

Fig. 7: Percent distribution of soil samples from Appalachian Plateau categorized into soil test phosphorus fertility ratings (very low, low, medium, high, very high, and extremely high) with depths a) 0-5 cm b) 5-15 cm c) 15-30 cm and d) 30-45 cm. The P fertility ratings was based on Mehlich-1 extractable P concentration.

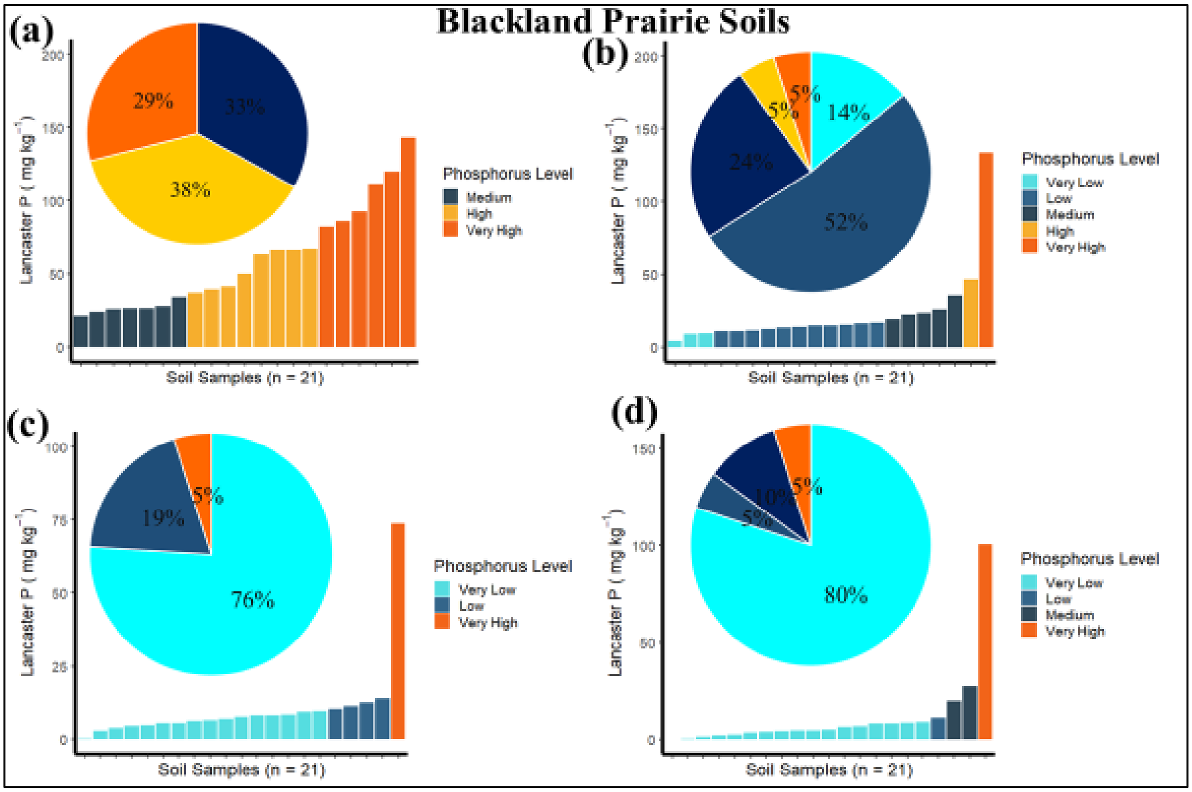

Fig. 8: Percent distribution of soil samples from Blackland Prairie categorized into soil test phosphorus fertility ratings (very low, low, medium, high, very high, and extremely high) with depths a) 0-5 cm b) 5-15 cm c) 15-30 cm and d) 30-45 cm. The P fertility ratings was based on Lancaster extractable P concentration.

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CHAPTER 3

Efficiency of Commonly Used Extractants for Assessment of Environmental Risk of Phosphorus Loss for Alabama Soils

3.1 Abstract

Individual states in the United States (U.S.) have chosen soil test phosphorus (STP) methods for agronomic purposes. However, the STP methods are also used in phosphorus index (P-index) for environmental P loss risk assessment. Laboratories in Alabama uses M1 and La as routine soil test for non-calcareous and calcareous soils, respectively. Majority of soils in Alabama are dominated by aluminum (Al) and iron (Fe) oxides. There have been questions raised about the extraction efficiency of currently adopted STP methods and use of those STP values in environmental P loss risk assessment. Mehlich-3 extractant has been identified as a more efficient and universal extractant applicable for wide range of soils. The objective of this study was to evaluate the extraction efficiency of common STP methods namely M1, M3 and La for P, Fe, Al, Ca, and Mg on five major soils of Alabama namely Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valleys (LS), Piedmont Plateau (PP) and Blackland Prairie (BP). Soil samples were collected from several farms in Alabama at four soil depths (0-5, 5-15, 15-30 and 30-45 cm). Soils were extracted with deionized water, M1, La and M3 following standard protocols. For AP, CP, LV, and PP soils, M3 extracted 1.3 times greater P from all soil depths and showed greater extraction efficiency for Fe and Al as compared to M1. Significant and strong correlation were observed between M1-P and M3-P for non-calcareous Alabama soils. However, the slope of the regression line between La-P and M3-P showed the extraction potential of both the extractants was similar. The conversion equations can be developed between M1/La and M3 for P, Ca, and Mg for

all soil types however, a single conversion equation developed for combined data can be used for conversions up to 30 cm depths. For the combined depths, the relationship between WSP and M3-P was better correlated than the relationship between WSP and M1-P/La-P for all soil types except CP. The high correlation obtained in this research indicated suitability of M3 as an alternative soil test method for environmental purpose for Alabama soils.

3.2 Introduction

Lands utilized for agricultural purposes which receives excessive amount of manure and fertilizers have been identified as a major non-point source of phosphorus (P) pollution in water bodies (Maguire and Sims, 2002b; Kleinman et al., 2015). Additionally, long term application of manure beyond crop P requirement results in accumulation of P in soils and cause soil test P (STP) levels to increase beyond the soil P retention capacity. Soil reflecting higher STP values are source of P and indicates greater risk of P loss to water bodies during storm events (Pote et al., 1996; Schroeder et al., 2004; Johnson et al., 2005). Therefore, the need to accurately measure soil P level using reliable STP method has become primary focus in P management and reducing environmental consequences.

In the U.S., many chemical extractants are widely used for soil phosphorus (P) test and making P fertilizer recommendations. For example, Bray and Kurtz P-1 is used in Midwestern and North Central states (Bray and Kurtz, 1945), M1 in Southeastern and Mid-Atlantic States (Mehlich, 1953), M3 in several states throughout the U.S (Alaska, Florida, Arkansas, North Carolina, Oklahoma, Louisiana, Kentucky) (Mehlich, 1984), Olsen in North Central and Western States (Olsen et al., 1954), and La in Mississippi soils (Cox, 2001). Although, STP methods were primarily designed for fertility recommendation, they are also used in environmental P loss risk assessment stewardship programs (Sims, 1993a; Sharpley et al., 1996; Sims et al., 2000, 2002).

For example, the Natural Resources Conservation Service (NRCS) Nutrient Management Conservation Practice standard (code 590) has integrated STP method into P site index (PI) (Sims, 1993b; Sharpley et al., 2003). The P-index is considered the most comprehensive tool for environmental P loss risk assessment for agricultural fields and uses an integrated approach that consider soil (source factor) and landscape features (transport factors) (Osmond et al., 2006). Source factors includes soil test P level, P application rate and method. Transport factors evaluate the potential for runoff to occur and includes soil erosion rate, underground outlet systems, soil hydrological group, field slope, P application distance to water and vegetative buffer width. Within the P-index calculation STP is an important determinant variable that affects PI ratings (Osmond et al., 2006). According to Osmond et al., (2006), when others input values in P index calculation were kept constant and STP was increased from 75 mg kg^{-1} to 150 mg kg^{-1}, the P index rating shifted from low to very high in Alabama P index tool. Similarly, for Florida, the P index rating shifted from medium to high as STP increased from 75 mg kg^{-1} to 150 mg kg^{-1} (Osmond et al., 2006). This underestimation or overestimation in STP may misguide nutrient recommendations for fertilizer or manure management. Therefore, it is important to choose an appropriate soil test method that adequately captures environmental P loss risk while meeting the agronomic P needs.

Mehlich-1 was found preferably useful for acidic soils with low CEC and was not capable of extracting P efficiently from soils with neutral to alkaline pH, high CEC soils, soils with high Al and Fe minerals, and high organic matter (> 5%) content (Mehlich, 1978; Mylavarapu et al., 2017). Lancaster was designed to estimate the P concentration from calcareous soils found in Blackland Prairies of Mississippi and Alabama (Cox, 2001; Mitchell and Huluka, 2012). Lancaster was found to extract P reasonably well from aluminum-P (Al-P) pool, however; the extractability varies for calcium-P (Ca-P) pool which is pH and calcium carbonate ($CaCO_3$) dependent.

Aluminum associated P (Al-P) is NH_4F- soluble P fraction in soil and the inclusion of malic and malonic acids with aluminum chloride ($AlCl_3$) facilitates uniform extraction of Al-P fractions at low Ca levels whereas in soils with higher Ca level, the acidity of extracting solution get neutralized with increase in Ca concentration and may reduce P extraction (Cox, 2001).

To overcome the limitations of M1 and make it useful for application to a wider range of soils, M3 was developed. Additionally, M3 was also useful in extracting micronutrients (Mehlich, 1984). Since its introduction, M3 has been extensively used in Southern, Mid-Atlantic, and Mid-Western United States (Wang et al., 2004; Penn et al., 2006; Dari et al., 2019). Interest in M3 as a "universal extractant" is based on the desire to improve uniformity in the soil test analysis and recommendations as well due to its broader applicability and its usefulness as agri-environmental soil test method (Sims et al., 2002; Zhang et al., 2009). Mehlich suggested that M3 extractant solution be buffered to a pH of 2.5 to prevent reaction of Ca and fluoride (F^-) to form calcium fluoride (CaF_2) precipitate, therefore, acetic acid was added as a buffering agent. Also, the cation dissociation is governed by NH_4^+ from ammonium nitrate which minimizes the influence of soil pH on extraction. Some of the state extension laboratories in the Southeastern US (e.g. Florida) have since moved to M3 extraction procedure because of its improved efficiency, particularly micronutrients and its broad range applicability, up to near neutral pH. However, for calcareous soils in which soil P pool shifts from Al and Fe associated pool to Ca dominated pool, the acidity of M3 solution will be partially neutralized and fixation of fluoride by Ca from non Ca-P minerals may reduce the extraction of soil P. In such soils the efficiency of M3 may be compromised (Penn et al., 2018).

Currently, Alabama uses M1 for non-calcareous soils and Lancaster/Mississippi, for calcareous soils (Mitchell and Huluka, 2012). It would be of great interest to compare the efficiency of P extraction and associated metals between current STP methods (M1 and La) and M3 for different soil types of Alabama under different management practices. This study will provide foundation for adopting M3 as soil test P for environmental P loss risk assessment program. No extensive datasets have been published till date with regards to extraction efficiencies of P and associated metals for major soil types and land uses of Alabama. Furthermore, the suitability of STP (M1, M3, La) methods for estimating environmental P loss have been evaluated through correlation of STP methods with environmental soil tests such as water-soluble phosphorus, iron-stripe phosphorus, $CaCl_2$-P (Maguire and Sims, 2002a; b; Beck et al., 2004) but no data or evaluation has been carried for Alabama soils.

Water soluble phosphorus was identified as a precise indicator and consistent tool in estimating P concentration in runoff from agricultural soils (Pote et al., 1996; Sharpley et al., 1996; Torbert et al., 2002; Maguire and Sims, 2002a; Schroeder et al., 2004; Penn et al., 2006; Wang et al., 2015). Water soluble phosphorus estimates soluble and easily desorbable P fraction in soil (Sharpley et al., 1996; Sims et al., 2000) and is not well correlated to crop response to added fertilizer unlike soil tests utilizing extractants such as M1. Due to the fact that, WSP is correlated well with dissolved reactive phosphorus (DRP) in runoff water, evaluating the relationship between STP method and WSP would be scientifically sound and economic approach that could be performed in large sample population for environmental P loss risk assessment purposes (Wang et al., 2015). This study will provide the background for future P works to develop promising tools that could be potentially used for environmental purposes.

The main objective of this study was to evaluate M3 as a possible alternative soil test method for evaluating environmental P loss risk for Alabama soils. Although our main interest was P, however, we also studied other elements such as Fe, Al, Ca, and Mg since they are directly associated with P retention chemistry. In acidic soils, P availability is mostly governed by oxides and hydroxides of Fe and Al (Tsado et al., 2012) whereas in calcareous soil, P is mostly associated with Ca compounds (Lindsay, 1979). The specific objectives of this study were (i) to evaluate and compare the extraction efficiency of M1/La and M3 for P, Al, Fe, Ca, and Mg in soils from major soil areas of Alabama; (ii) determine the conversion equations from M1/La to M3 procedure; (iii) determine if observable distinct relationship exist between WSP and STP for different soil types, depths, and land use management systems (primarily row crops and grazing systems) in Alabama soils.

3.3 Materials and Methods

3.3.1 Soil Sampling

Sixty-eight agricultural fields in 18 Alabama counties representing five major soil types (Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP) and Blackland Prairie (BP)) were sampled. The selected farms had been historically managed either under row crops or grazing system and most of the farms had a poultry litter application history. Two hundred nine soil samplings points were marked where multiple soil cores were collected using a gas-powered core sampling kit (AMS INC/Part number 360.01, American Falls, ID). Soil core (38.1 mm diameter) at individual sampling point was separated into individual depths of 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm. Soil samples from similar depth were composited from each sampling points to get a representative sample. The soil samples were air-dried, ground, and passed through a 2-mm sieve for analysis. A total of eight hundred fifteen samples (includes four soil depths at each sampling point) were individually analyzed. Soil order and soil series information was obtained from the SUURGO soil database (USDA-NRCS).

3.3.2 Chemical Analysis

Soil pH was measured with a suspension of soil in deionized water at 1:1 (w/v) soil to water ratio. Water-soluble phosphorus (WSP) was determined by extracting 2 g of soil with 20 ml of water, 1:10 (w/v) (Chakraborty et al., 2011). Phosphorus in the solution was analyzed by ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962). All soil samples were extracted with M1 extractant (0.0125 M H_2SO_4 + 0.05 M HCl) to determine P (M1-P), Fe (M1-Fe), Al (M1-Al), Ca (M1-Ca), and Mg (M1-Mg) using 1:4 (w/v) soil to solution ratio shaken for five minutes (Mehlich, 1953). Mehlich-3 (M3) solution (0.2 M CH_3COOH + 0.25 M NH_4NO_3 + NH_4F + 0.13 M HNO_3 + 0.001 M EDTA) was used to determine extractable P (M3-P), Fe (M3-Fe), Al (M3-Al), Ca (M3-Ca), and Mg (M3-Mg) using 1:10 (w/v) soil to solution ratio shaken for 5 minutes (Mehlich, 1984). Lancaster method was used for Blackbelt soils only. The method was a two-step process where, in first step, 5 g soil was treated with 5 ml of 0.05 M HCl solution for 10 minutes and in second step, 20 ml of extracting solution (1.58 M glacial acetic acid + 0.125 M malonic acid + 0.187 M malic acid + 0.037 M ammonium fluoride + 0.03 M aluminum chloride hexahydrate) was added and shaken for 10 minutes (Cox, 2001). Total P in soil samples was determined following ignition method (Andersen, 1976). Phosphorus and the metals in extracting solution (M1, M3, La, and ignition method) were analyzed using inductively coupled argon plasma spectroscopy (Varian Vista-MPX, ICP-OES, Ltd, Victoria, Australia). Aluminum was not determined by La method because of the presence of Al in extracting solution.

3.3.3 Statistical Analysis

Data arrangement and computation was performed using R version 3.6.2. (RStudio Team, 2018). Analysis of variance was conducted using the Proc GLIMMIX in SAS® 9.4 (SAS institute, 2013). Locations were considered random effect and soil depth and land use management was considered as fixed effect in the model. The means differences were determined using Tukey-Kramer adjusted P values at P = 0.05 significance level. Relationship between M1/La and M3 extractable P, Al, Fe, Ca, and Mg was determined using Pearson correlation test. Similarly, relationship between WSP and P extracted with different STP methods were determined using Pearson correlation test. Difference among means and coefficient of determination were considered significant at the $P < 0.05$ level, unless stated. To study the differences between soil depths and among the soil types, logarithmic transformation was applied to all the variable under study. The Shapiro-Wilk W test was performed to test normality assumptions of the residuals.

3.4 Results

3.4.1 Extraction Efficiency

3.4.1.1 Comparison of Extraction Efficiency of Mehlich-1 and Mehlich-3

Mean values and extraction efficiency of M1 and M3 for P, Fe, Al, and Mg for four non-calcareous soils of Alabama are presented in Table 8. Results showed that extraction efficiency (ratio of extractable to total concentration, where total metal concentration was determined using ignition method) of P, Fe, Al, and Mg decreased by depth (Table 8). The data for total Ca were not presented because the total Ca recovered by ignition method were not considered reliable similar to Franklin et al., (2003). The results are presented individually for each soil type.

Appalachian Plateau

For combined depth, the mean M1 extractable P, Fe, Al, and Mg for AP soils was 72 mg kg^{-1} (ranged from 2- 444 mg kg^{-1}), 42 mg kg^{-1} (ranged from 0-376 mg kg^{-1}), 262 mg kg^{-1} (ranged from 69-634 mg kg^{-1}), and 94 mg kg^{-1} (ranged from 11- 433 mg kg^{-1}), respectively. In general, the mean M1-P decreased down the soil profile in AP soils. Similarly, for combined depth, the mean M3 extractable P, Fe, Al, and Mg for AP soils was 134 mg kg^{-1} (ranged from 2-537 mg kg^{-1}), 188 mg kg^{-1} (ranged from 22-949 mg kg^{-1}), 920 mg kg^{-1} (ranged from 448-1684 mg kg^{-1}), and 107 mg kg^{-1} (ranged from 15-302 mg kg^{-1}), respectively. In general, the mean M3-P decreased down the soil profile in AP soils.

The mean M1-P in AP soils significantly decreased ($P < 0.0001$) down the soil profile. The P extraction efficiency of M1 at 0-5, 5-15 cm, 15-30 cm, and 30-45 cm were 21%, 16%, 13%, and 6%, respectively whereas P extraction efficiency of M3 at respective depths were 41%, 38%, 31%, and 18%. The M1-Fe from 0-5 cm and 5-15 cm was significantly different ($P = 0.0287$) than 30-45 cm depth. There was also a significant difference ($P = 0.05$) in M3-Fe between 0-5 cm, 15-30 cm, and 30-45 cm soil depth. The Fe extraction efficiency of M1 was < 1% at all four depths but the M3 extracted 2.2%, 1.9%, 1% and 0.6% of total Fe concentration at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depths, respectively. Mehlich-1 extractable Al at 0-5 cm depth was significantly greater ($P < 0.0001$) than 30-45 cm soil depth. In contrast, M3-Al at 15-30 cm and 30-45 cm depths were significantly greater ($P = 0.0001$) than 0-5 cm soil depth. The extraction efficiency of M1-Al was 2.2%, 1.9%, 1.3%, and 0.9% at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depths, respectively whereas M3-Al was 5.6%, 6.4%, 5.6%, 4.8% at respective depths. The mean M1-Mg at 0-5 cm was significantly greater ($P < 0.0001$) than other soil depths. Similarly, mean M3-Mg at 0-5 cm was significantly greater ($P = 0.0038$) than other soil depths. The Mg extraction efficiency for M1 and M3 were approximately similar in AP soils. In summary, for all depths, M1 extracted 19% of total P, 0.3% of TFe, 1.6% of TAl, and 11% of TMg from the soil whereas M3 extracted 36% of total P, 1.4% of TFe, 5.5% of TAl, and 12.8% of TMg from AP soils.

Coastal Plain

For combined depth, the mean M1 extractable P, Fe, Al, and Mg for CP soils was 40 mg kg^{-1} (ranged from 1-436 mg kg^{-1}), 16 mg kg^{-1} (ranged from 0-185 mg kg^{-1}), 166 mg kg^{-1} (ranged from 56-521 mg kg^{-1}), and 61 mg kg^{-1} (ranged from 2- 290 mg kg^{-1}), respectively. In general, the mean M1-P in CP soils decreased down the soil profile. Similarly, for combined depth, the mean M3 extractable P, Fe, Al, and Mg for CP soils was 87 mg kg^{-1} (ranged from 3-440 mg kg^{-1}), 79 mg kg^{-1} (ranged from 0-418 mg kg^{-1}), 799 mg kg^{-1} (ranged from 82-2752 mg kg^{-1}), and 70 mg kg^{-1} (ranged from 0-412 mg kg^{-1}), respectively. In general, the mean M3-P decreased down the soil profile in CP soils.

We found a significant difference ($P < 0.0001$) in M1-P between 0-5 cm, 15-30 cm, and 30-45 cm depths however, M1-P did not vary significantly between 0-5 and 5-15 cm depth. The P extraction efficiency of M1 at 0-5, 5-15, 15-30, and 30-45 cm depths were 20%, 19%, 14%, and 8%, respectively. In contrast, P extraction efficiency of M3 at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depths were 44%, 47%, 37%, and 18%, respectively. In CP soils, there was no significant difference ($P = 0.1648$) in M1-Fe between depths. However, there was significantly different ($P < 0.0001$) in M3-Fe between 0-5 cm, 15-30 cm, and 30-45 cm soil depth. The Fe extraction efficiency of M1 was < 0.5% at all four depths but the M3 extracted 1.6%, 1.1%, 0.6% and 0.2% of total Fe concentration at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depths, respectively. The M1-Al at 5-15, and 15-30 cm did not vary significantly, however M1-Al at 30-60 cm depth was significantly different ($P = 0.0032$) from 5-15, and 15-30 cm depth. The M3-Al at 0-5 cm depths was significantly lower ($P = 0.0031$) than other soil depths. The extraction efficiency of Al by M1 was 1.2%, 1.2%, 0.8%, and 0.5% at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depths, respectively whereas M3 extraction efficiency for Al at respective depths were 4.7%, 5.2%, 3.8%, and 3.2%. The mean M1-Mg at 0-5 cm was significantly greater ($P = 0.0221$) than other soil depth. There was no significant difference ($P = 0.1608$) in M3-Mg from 0-5 cm compared to other soil depth. The Mg extraction efficiency for M1 and M3 was approximately similar in CP soils. In summary, for all depths, M1 extracted 17% of total P, 0.1% of TFe, 0.8% of TAl, and 15% of TMg from the soil whereas M3 extracted 39% of total P, 0.7% of TFe, 4% of TAl, and 17% of TMg from CP soils.

Limestone Valley

For combined depth, the mean M1 extractable P, Fe, Al, and Mg for LV soils was 29 mg kg^{-1} (ranged from 2-379 mg kg^{-1}), 17 mg kg^{-1} (ranged from 0-136 mg kg^{-1}), 240 mg kg^{-1} (ranged from 74-789 mg kg^{-1}), and 70 mg kg^{-1} (ranged from 12-495 mg kg^{-1}), respectively. In general, the mean M1-P in LV soils decreased down the soil profile. Similarly, for combined depth, the mean M3 extractable P, Fe, Al, and Mg for LV soils was 44 mg kg^{-1} (ranged from 1-346 mg kg^{-1}), 91 mg kg^{-1} (ranged from 0-421 mg kg^{-1}), 922 mg kg^{-1} (ranged from 370-2028 mg kg^{-1}), and 81 mg kg^{-1} (ranged from 14-536 mg kg^{-1}), respectively. In general, the mean M3-P decreased down the soil profile in LV soils.

The M1-P in LV soil was significantly different ($P < 0.0001$) between depths. Similarly, there was significant difference ($P < 0.0001$) in M3-P between soil depths. The P extraction efficiency of M1 and M3 at 0-5 cm, 5-15 cm, 15-30 cm and 30-45 cm depths were 10%, 7%, 4%, and 2%, respectively whereas P extraction efficiency of M3 at 0-5 cm, 5-15 cm, 15-30 cm and 30-45 cm depths were 20%, 12%, 6%, and 4%, respectively. There was no significant difference ($P = 0.2350$) in mean M1-Fe between depths whereas M3-Fe at 0-5 cm was significantly different ($P < 0.0001$) from 15-30 cm, and 30-45 cm depth. The Fe extraction efficiency of M1 was < 0.1% at all four depths in LV soils whereas M3 extracted 0.7%, 0.5%, 0.3% and 0.2% of total Fe at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depths, respectively. There was no significant difference ($P = 0.0839$) in M1-Al between soil depth whereas M3-Al at 5-15 and 15-30 cm were significantly greater ($P < 0.0001$) than 0-5 cm soil depth. The extraction efficiency of Al by M1 was ≤ 1% at all four depths but M3 extraction efficiency for Al was > 3% at all four depths. The mean M1-Mg at 0-5 cm was significantly greater ($P < 0.0001$) than at other depths and similar results found for M3-Mg in LV soils. The Mg extraction efficiency for M1 and M3 was approximately similar at corresponding depths in LV soils. In summary, for all depth combined, M1 extracted 8% of total P, 0.1% of TFe, 0.9% of TAl, and 6% of TMg whereas M3 extracted 12% of total P, 0.4% of TFe, 3% of TAl, and 7% of TMg from LV soils.

Piedmont Plateau

For combined depth, the mean M1 extractable P, Fe, Al, and Mg for PP soils was 37 mg kg^{-1} (ranged from 1-318 mg kg^{-1}), 38 mg kg^{-1} (ranged from 0-240 mg kg^{-1}), 216 mg kg^{-1} (ranged from 60-826 mg kg^{-1}), and 71 mg kg^{-1} (ranged from 5-499 mg kg^{-1}), respectively. In general, the mean M1-P in PP soils decreased down the soil profile. Similarly, for combined depth, the mean M3 extractable P, Fe, Al, and Mg for PP soils was 81 mg kg^{-1} (ranged from 1-635 mg kg^{-1}), 172 mg kg^{-1} (ranged from 19-906 mg kg^{-1}), 880 mg kg^{-1} (ranged from 371-1573 mg kg^{-1}), and 92 mg kg^{-1} (ranged from 7-824 mg kg^{-1}), respectively. In general, the mean M3-P decreased down the soil profile in PP soils.

The M1-P in PP soil was significantly different ($P < 0.0001$) between depths. There was also significant difference ($P < 0.0001$) in M3-P between 0-5 cm, 5-15 cm, and 15-30 cm depths. The P extraction efficiency of M1 at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depths were 14%, 8%, 4%, and 2%, respectively. In contrast, P extraction efficiency of M3 at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depths were 24%, 19%, 11%, and 6.2%, respectively. The M1-Fe at 0-5 cm and 5-15 cm depths did not vary significantly, however M1-Fe at 15-30 cm and 30-45 cm depths were significant difference ($P < 0.0001$) from 0-5 and 5-15 cm depth. Similarly, there was significantly different ($P < 0.0001$) in M3-Fe between depths. The Fe extraction efficiency of M1 was ≤ 0.2% at all four depths in LV soils whereas M3 extracted 1.1%, 0.5%, 0.3% and 0.2% of total Fe at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depth, respectively. There was significant difference ($P = 0.0053$) in M1-Al between 0-5 cm and 30-45 cm depths whereas M3-Al at 15-30 cm was significantly greater ($P < 0.0001$) than 0-5 cm soil depth. The extraction efficiency of Al by M1 was < 1% at all four depths but M3 extraction efficiency for Al was > 2% at all four depths. The M1-Mg at 0-5 cm was significantly greater ($P < 0.0001$) than other soil depths and similar results found for M3-Mg in PP soils. The Mg extraction efficiency for M1 and M3 was approximately similar at corresponding depths in PP soils. For combined depth, M1 extracted 8% of total P, 0.1% of TFe, 0.6% of TAl, and 8% of TMg from the PP soil whereas M3 extracted 18% of total P, 0.5% of TFe, 3% of TAl, and 10% of TMg from PP soils.

We also explored the relationship between M1/La and M3 extractable P, Fe, Al, Ca, and Mg at individual depths for all non-calcareous (Table 10 and 11) and calcareous soils (Table 13). For all depths combined, there was a significant ($P < 0.001$) linear relationship between M1-P and M3-P for all individual non-calcareous soils (Fig. 9). Furthermore, to understand the effect of sampling depth, the data for each soil types was segregated by depth and the correlation analysis was performed (Table 12).

We also explored the data based on land management practices to understand whether cropping system might influence the extraction efficiency. Significant ($P < 0.001$) and strong correlation were observed for both row crops and grazing system with $r^2 = 0.69$ and $r^2 = 0.78$, respectively (Fig. 10). There was strong and significant correlation between M1-P and M3-P for the combined data with $r^2 = 0.7$. The slope for the relationship was steeper (slope = 1.5) for row crops than grazing systems (slope = 1.2). The 95% confidence interval ($CI_{95\%}$) for slope of row crops was 1.44 to 1.63 and $CI_{95\%}$ for grazing was 1.1 to 1.25. This indicated that the relationship between M1-P and M3-P for row crops and grazing system are not similar. One possible reason for difference in slope between row crops and grazing systems might be due to presence of large amount of recalcitrant P due to accumulation of plant residue mainly root biomass in grazing systems.

3.4.1.2 Comparison of Extraction Efficiency of Mehlich-1, Lancaster, and Mehlich-3 Blackland Prairie

Comparison of extraction efficiency between M1, La and M3 for different elements are presented in Table 9. For combined depth, the mean M1 extractable P, Fe, Al and Mg for BP soils was 11 mg kg^{-1} (ranged from 1-85 mg kg^{-1}), 6 mg kg^{-1} (ranged from 0-143 mg kg^{-1}), 53 mg kg^{-1} (ranged from 0-231 mg kg^{-1}), and 76 mg kg^{-1} (ranged from 5-326 mg kg^{-1}), respectively. In general, the mean M1-P in BP soils decreased down the soil profile. For combined depth, the mean La extractable P, Fe, and Mg for BP soils was 26 mg kg^{-1} (ranged from 0-143 mg kg^{-1}), 93 mg kg^{-1} (ranged from 21-677 mg kg^{-1}), and 128 mg kg^{-1} (ranged from 7-444 mg kg^{-1}), respectively. In general, the mean La-P in BP soils decreased down the soil profile. Similarly, for combined depth, the mean M3 extractable P, Fe, Al, and Mg for BP soils was 21 mg kg^{-1} (ranged from 1-189 mg kg^{-1}), 78 mg kg^{-1} (ranged from 17-502 mg kg^{-1}), 331 mg kg^{-1} (ranged from 1-1354 mg kg^{-1}), and 7 mg kg^{-1} (ranged from 1-23 mg kg^{-1}), respectively. In general, the mean M3-P decreased down the soil profile in BP soils.

The mean M1-P at 0-5 cm was significantly different ($P < 0.0001$) from other soil depths. The La-P was significantly greater ($P < 0.0001$) at 0-5 cm soil depth than other soil depths. Similarly, M3-P was significantly greater ($P < 0.0001$) at 0-5 cm than other soil depths. The P extraction efficiency of M1 at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm were 3%, 2%, 1%, and 1%, respectively. Phosphorus extraction efficiency of La were 7%, 5%, 3%, and 3% at 0-5 cm, 5-

15 cm, 15-30 cm, and 30-45 cm depth, respectively. Similarly, M3 extracted 6%, 4%, 3%, and 3%, of total P from soil at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm depth, respectively. The mean M1-Fe was non -significant ($P = 0.9890$) between soil depths in BP soils. Lancaster extractable Fe was significantly greater ($P < 0.0001$) at 0-5 cm depth than other soil depths. Similarly, the M3-Fe was significantly greater ($P = 0.0005$) at 0-5 cm depth than in 15-30 cm and 30-45 cm depth. The extraction efficiency of M1-Fe was < 0.1% at all depths. The extraction efficiency of La-Fe at 0-5cm, 5-15 cm, 15-30 cm and 30-45 cm were 0.7%, 0.4%, 0.3%, and 0.2%, respectively whereas M3 extraction efficiency for Fe at respective depths were 0.5%, 0.3%, 0.2%, and 0.2%. There was no statistical difference in Al concentration between depths for both M1 and M3 extractant. The M1 extracted ≤ 0.2% of total Al from BP soils whereas M3 extracted between 1% to 1.5% of total Al concentration in the soils. The mean M1-Mg at 0-5 cm was significantly different ($P < 0.0001$) with 15-30 cm and 30-45 cm soil depth. The mean La-Mg at 0-5 cm was significantly different ($P < 0.0001$) with 15-30 cm and 30-45 cm soil depths. Similarly, the mean M3-Mg at 0-5 cm was significantly different ($P < 0.0002$) with 15-30 cm and 30-45 cm depth. The extraction efficiency of M1-Mg was 6%, 4%, 3%, and 2% at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm soil depths, respectively. Lancaster extracted 10%, 7%, 4%, and 3% of total Mg in soil from 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm soil depths, respectively. Mehlich-3 extracted < 1% of total Mg from the soil at all depths. In summary, for all depths, M1 extracted 2.2% of total P, 0.02% of total Fe, 0.2 % of total Al, 12% of total Ca, and 3% of total Mg. Similarly, La extracted 5% of total P, 0.04% of total Fe, and 6% of total Mg whereas M3 extracted 4% of total P, 0.3% of total Fe, 1.1 % of total Al, and 0.3% of total Mg from BP soils.

Additionally, the relationship between La-P and M3-P for BP soils based on management system were explored (Fig. 12). The relationship between the two extractant La-P and M3-P for both row crops and grazing system were highly significant ($P < 0.001$) with $r^2 = 0.94$ for row crops and $r^2 = 0.93$ for grazing system. Although the coefficient of determination was similar, the slope of the regression line was steeper for row crops (slope = 1.2) than grazing system (slope = 0.83). The 95% confidence interval ($CI_{95\%}$) for slope of row crops was 1.03 to 1.29 whereas $CI_{95\%}$ for grazing was 0.76 to 0.88. This indicated that the relationship between La-P and M3-P for row crops and grazing system are not similar.

3.4.2 Conversion Equation for Mehlich-3 Extractant

Extensive field calibration data for specific crops and soil types are needed to introduce a new extraction method into routine laboratory soil tests for agronomic purposes. However, conversion equations can be developed for nutrients to convert from current STP method to a new method. Separate equation to convert the value from M1/La equivalent to M3 can be developed for P, Fe, Al, Ca, and Mg through regression analysis. The correlation analysis between M1 and M3 extractable Al, Fe, Ca, and Mg was also performed for individual depth for AP, CP, LV, and PP soils (Table 10 and 11).

Appalachian Plateau

The relationship between M1-P and M3-P at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm were significantly correlated ($P < 0.001$) with r^2 of 0.62, 0.82, 0.91, and 0.96, respectively (Table 10) Similarly, the relationship between M1-P and M3-P for the combined data (all depths) was strongly correlated (Fig. 9). The 95% confidence interval ($CI_{95\%}$) of slope for combined data for P overlapped with the $CI_{95\%}$ of slope for 0-5 and 5-15 cm depths. This suggested that the regression equation for P from combined data can be used for conversion up to 15 cm depth and is not applicable in lower depths. There was significant correlation between M1-Al and M3-Al at 0-5 cm, 5-15 cm, and 15-30 cm soil depths, however the relationship was not strongly correlated at all depths. The relationship between M1-Fe and M3-Fe at each of four depths were highly significant ($P < 0.001$) and strongly correlated. The slope of regression line for individual soil depth and the slope of combined data were not statistically different which indicated that the combined regression equation derived for Fe can potentially be used for conversions instead of different regression equations for separate depths. There was highly significant ($P < 0.001$) and strong correlation between M1-Ca and M3-Ca at all depths. The $CI_{95\%}$ of slope for combined data overlapped with $CI_{95\%}$ of slope for all individual depths. Similarly, the relationship between M1-Mg and M3-Mg for AP soils were highly significant ($P < 0.001$) and strongly correlated in each soil depth. The slope of regression line for combined data and the slope for all individual depths were not statistically different at all depths. For Ca and Mg, a separate regression equation derived for combined data can be used for conversions between M-1 and M-3 Ca and Mg up to 45 cm depth.

Coastal Plain

The relationship between M1-P and M3-P at all depth were highly significant ($p < 0.001$) and strongly correlated except 5-15 cm depth (r^2 of 0.39) (Table 10). Similarly, the relationship between M1-P and M3-P for the combined data was strongly correlated (Fig. 9). The $CI_{95\%}$ of slope for combined data overlapped with the $CI_{95\%}$ of slope for 0-5, 5-15, and 15-30 cm. This suggested that the regression equation for P derived from combined data can be used for conversion up to 30 cm depth. There was no linear relationship observed between M1-Al and M3-Al at all depths. The relationship between M1-Fe and M3-Fe at each four depths were significant however the relationships were not strongly correlated. There was significant ($P < 0.01$) and strong correlation between M1-Ca and M3-Ca at each individual depth. The slope of the regression line for combined dataset and the slope for 0-5, 5-15, and 15-30 cm were not statistically different.

This suggests that a separate single regression equation derived for combined data can be used for conversions between M1-Ca and M3-Ca up to 30 cm depths. Similarly, the relationship between M1-Mg and M3-Mg for CP soils were highly significant ($P < 0.001$) and strongly correlated for all soil depths. The $CI_{95\%}$ of slope for combined data overlapped with the $CI_{95\%}$ for slope of all individual depths except 30-45 cm. For Mg, a regression equation derived for combined data can be used for conversion for all three soil depths except 30-45 cm.

Limestone Valley

The relationship between M1-P and M3-P was highly significant ($P < 0.001$) and strongly correlated at all four depths. (Table 10). Similarly, the relationship between M1-P and M3-P for the combined data from LV was strongly correlated (Fig. 9). The $CI_{95\%}$ of slope for combined data overlapped with the $CI_{95\%}$ of slope for all individual depths. This suggested that the regression equation for P from combined data can be applicable for conversion at all four depths. The relationship between M1-Al and M3-Al was non-significant at all depths. Similarly, there was no relationship between M1-Fe and M3-Fe at all depths in LV soils. However, there was significant ($P < 0.001$) and strong correlation between M1-Ca and M3-Ca at all individual depth. The $CI_{95\%}$ of slope for combined dataset overlapped with $CI_{95\%}$ of slope for all individual depths except 30-45 cm. This indicated that the regression equation derived for combined data for Ca can be used up to 30 cm depth instead of separate regression equations derived for individual depths. Similarly, the relationship between M1-Mg and M3-Mg for LV soils were highly significant ($P < 0.001$) and strongly correlated at all depths. The slope of regression line for combined data and the slope for individual depths were not statistically different. A single regression equation derived from combined data can be used for conversions between M1-Mg and M3-Mg at all depths.

Piedmont Plateau

The relationship between M1-P and M3-P were highly significant ($P < 0.001$) and strongly correlated at all four depths (Table 10). Similarly, the relationship M1-P and M3-P for the combined data was strongly correlated (Fig. 9). The $CI_{95\%}$ of slope for combined data overlapped with the $CI_{95\%}$ of slope up to 30 cm depth. This suggested that the regression equation for P from combined data can be applicable for conversion up to 30 cm soil depth. There was no significant relationship between M1-Al and M3-Al at all depths. The relationship between M1-Fe and M3-Fe were significantly correlated ($P < 0.001$) at all depths except 30-45 cm. However, the relationship was not strongly correlated at 15-30 cm depth (r^2 of 0.39). The $CI_{95\%}$ of slope for combined depth overlapped with the $CI_{95\%}$ of slope for all individual depths except 30-45 cm. This indicated that combined regression equation can be applicable for conversions between M1-Fe and M3-Fe up to 15 cm depth only. There was significant ($p < 0.001$) and strong correlation between M1-Ca and M3-Ca at all individual depth. The slope of regression line for combined dataset and the slope for all individual depths were not statistically different which indicated that combined regression equation can be used instead of individual regression equations derived for each depth. Similarly, the relationship between M1-Mg and M3-Mg for PP soils were highly significant ($p < 0.001$) and strongly correlated at all soil depths. The $CI_{95\%}$ of slope for combined data overlapped with the $CI_{95\%}$ of slope for all individual depths. This suggested that a single regression equation for Mg derived from combined dataset can be used for conversions between M1 and M3 for all depths.

We further explored how the slope of the regression line changes among the soil types for combined data. The slope of regression line between M1-P and M3-P for AP (combined depths) was not statistically different from the PP soils. This indicated that a common regression equation can be derived for both AP and PP soils for conversions. Similarly, the slope of regression line between M1-P and M3-P for CP (combined depths) was not statistically different from the LV soils and this also indicated that a common regression equation can be used for conversions for CP and LV soils. We found that the slope of the regression line between M1-Ca and M3-Ca for combined data were not statistically different among all four soil types. This indicated that a single regression equation can be used for conversion between M1-Ca and M3-Ca for all non-calcareous soils. The slope of the regression line between M1-Mg and M3-Mg for combined data were not statistically different among CP, LV, and PP soils which indicated that a single regression equation can be used for conversions for three soil types (CP, LV, and PP).

3.4.3 Conversion between Lancaster and Mehlich-3

Blackland Prairie

Regression equations between La and M3 extractable elements for BP soils are presented in Table 13. The relationship between La-P and M3-P were highly significant ($P < 0.001$) and strongly correlated at all four depths. The $CI_{95\%}$ of slope for combined data overlapped with the $CI_{95\%}$ of slope for all individual depths except 5-15 cm depth. This suggested that the regression equation for P from combined data can be applicable for conversion on all soil depth except 5-15 cm. The relationship between La-Fe and M3-Fe were significantly correlated ($P < 0.001$) at all depths and was strongly correlated for the combined datasets as well. The $CI_{95\%}$ of slope for combined depth overlapped with the $CI_{95\%}$ of slope for all individual depths. This indicated that combined regression equation for Fe can be applicable for conversions between La and M3 for all depths. There was significant ($P < 0.001$) and strong correlation between La-Ca and M3-Ca at all individual depth. The slope of the regression line between La-ca and M3-Ca for combined dataset and the slope of all individual depth were not statistically different which indicated that combined regression equation can be used instead of regression equations derived for individual depth. The relationship between La-Mg and M3-Mg for BP soils were also highly significant ($p < 0.001$) and strongly correlated for each soil depth. The $CI_{95\%}$ of slope for combined data overlapped with the $CI_{95\%}$ of slope for all individual depths which suggested that a single regression equation derived for combined data can be used for conversions between La-Mg and M3-Mg for all depths.

3.4.4 Relationship between Agronomic and Environmental STP

3.4.4.1 Relationship between water-soluble P, Mehlich-1 P and Mehlich-3 -P

Because STP values have been integrated into water quality assessment, it is crucial to understand whether the adopted STP methods are technically adequate and effective for environmental P loss risk assessment. Several researchers have identified WSP as an indicator of readily desorbed fraction of P in soil (Pote et al., 1996; Sharpley et al., 1996; Maguire and Sims, 2002a), and used it to develop P retention relationship such as threshold phosphorus saturation ratio (Nair et al., 2004; Chakraborty et al., 2012). Our objective was to evaluate the relationship between WSP and M1-P/M3-P/La-P for various Alabama soils that would allow us to select a better STP for environmental P loss risk assessment. Regression analysis between WSP and M1-P/La and WSP/M3-P for all five major soils was carried for the individual soil depths and is presented in Table 14.

Appalachian Plateau

The relationship between WSP and M1-P at 0-5cm, 5-15 cm, 15-30 cm and 30-45 cm were significantly correlated ($P < 0.05$) with r^2 of 0.37, 0.59, 0.53, and 0.51, respectively (Table 14). Similarly, significant, and strong correlation (r^2 of 0.6) between WSP and M1-P was observed for combined data. The relationship between WSP and M3-P at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm were significantly correlated ($P < 0.05$) with r^2: 0.5, 0.65, 0.58, and 0.44, respectively. The relationship between WSP and M3-P for combined data was significant and strongly correlated (r^2

of 0.66). The relationships between WSP and M3-P were better correlated at 0-5 cm, 5-15 cm, and 15-30 cm depths as compared to relationships between WSP and M1-P. Similarly, for combined data the relationship between WSP and M3-P was better correlated than the relationship between WSP and M1-P in AP soils.

Coastal Plain

The relationship between WSP and M1-P at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm were significantly correlated ($P < 0.01$) with r^2 of 0.81, 0.46, 0.48, and 0.89, respectively (Table 14). Similarly, significant, and strong correlation (r^2 of 0.76) was found for the combined dataset. The relationship between WSP and M3-P at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm were significantly correlated ($P < 0.05$) with r^2 of 0.47, 0.56, 0.53, and 0.85, respectively. The relationship for the combined data was significant and strongly correlated with r^2 of 0.56. The relationships between WSP and M3-P were better correlated at 5-15 cm, and 15-30 cm depths as compared to relationships between WSP and M1-P. The relationship between WSP and M1-P were better correlated at 0-5 cm and 30-45 cm depths. Similarly, for combined data the relationship between WSP and M1-P was better correlated than the relationship between WSP and M3-P in CP soils.

Limestone Valley

The relationship between WSP and M1-P at 0-5 cm, 5-15 cm, and 15-30 cm were significantly correlated ($P < 0.01$) with r^2 of 0.56, 0.39, and 0.33, respectively, however the linear relationship was not significant at 30-45 cm soil depth (Table 14). Similarly, significant, and strong correlation (r^2 of 0.63) was found for the combined data set. The relationship between WSP and M3-P at 0-5 cm, 5-15 cm, and 15-30 cm were significantly correlated ($P < 0.05$) with r^2 of 0.65, 0.44, and 0.16, respectively. The relationship for combined data was significant and strongly correlated with r^2 of 0.69. The relationships between WSP and M3-P were better correlated at 0-5 cm, and 5-15 cm depths as compared to relationships between WSP and M1-P. Similarly, for combined data the relationship between WSP and M3-P was better correlated than the relationship between WSP and M1-P in LV soils.

Piedmont Plateau

The relationship between WSP and M1-P at 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm were significantly correlated ($P < 0.05$) with r^2 of 0.48, 0.7, 0.4, and 0.12, respectively. Similarly, significant, and strong correlation (r^2 of 0.64) between WSP and M1-P was found for combined data. The relationship between WSP and M3-P at 0-5 cm, 5-15 cm, 15-30 cm and 30-45 cm were significantly correlated ($P < 0.05$) with r^2 of 0.59, 0.62, 0.54, and 0.12, respectively. The relationship between WSP and M3-P for combined data was significant and strongly correlated with r^2 of 0.67. The relationships between WSP and M3-P were better correlated at 0-5 cm, and 15-30 cm depths as compared to relationships between WSP and M1-P. The relationship between WSP and M1P was better correlated at 5-15 cm soil depth. For combined data, the relationship between WSP and M3-P was better correlated than the relationship between WSP and M1-P in PP soils.

The relationship between WSP and M1-P for combined four major soil areas (AP, CP, LV, and PP) up to 30 cm depth was significantly correlated ($P < 0.001$) with r^2 of 0.57 (Fig. 13). Similarly, the relationship between WSP and M3-P for combined soils (AP, CP, LV, and PP) up to 30 cm depth was significantly correlated ($P < 0.001$) with r^2 of 0.64 (Fig. 13). This showed that, for combined data from non-calcareous soils, WSP better correlated with M3-P than M1-P. Further, the influence of soil depth on the relationship between WSP and M1-P/M3-P were studied at individual depth (Fig. 14). The relationship between WSP and M1-P were significantly correlated at depths: 0-5 cm ($r^2 = 0.51$), 5-15 cm ($r^2 = 0.47$), and 15-30 cm ($r^2 = 0.43$). This indicated that correlations between WSP and M1-P were affected with the sampling depth. The relationship between WSP and M3-P were significantly correlated at individual depths; 0-5 cm ($r^2 = 0.59$), 5-15 cm ($r^2 = 0.57$), and 15-30 cm ($r^2 = 0.57$). The correlations between WSP and M3-P were consistent up to 30 cm depth.

3.4.4.2 Relationship between water-soluble P, Lancaster-P and Mehlich-3 P

Blackland Prairie

The relationship between WSP and La-P/M3-P for BP soils for combined depths is presented in Fig. 15. and the relationship at individual depths is presented in Table 14. The relationships between WSP and La-P at 0-5 cm, 5-15 cm, 15-30 cm were significantly correlated ($P < 0.05$) with r^2 of 0.38, 0.48, and 0.58, respectively. There was no significant relationship between WSP and La-P in 30-45 cm soil depth. A significant correlation (r^2 of 0.54) between WSP and La-P was found for the combined dataset. The relationship between WSP and M3-P at 0-5 cm, and 5-15 cm were significantly correlated ($P < 0.05$) with r^2 of 0.48, and 0.42, respectively. There was no significant relationship between WSP and M3-P in 15-30 cm and 30-45 cm soil depth. The relationship between WSP and M3-P for combined data was significant and strongly correlated with r^2 of 0.6. The relationships between WSP and M3-P were better correlated at 0-5 cm depth as compared to relationships between WSP and La-P. The relationship between WSP and La-P was better correlated at 5-15 cm soil depth. For combined dataset up to 30 cm depth, the correlation between WSP and M3-P was better with greater coefficient of determination ($r^2 = 79$) than WSP and M1-P ($r^2 = 67$) for BP soils.

3.5 Discussions

3.5.1 Comparison of Extraction Efficiency of M1 and M3 for Non-Calcareous Soils

The results showed that M3 extracted larger fractions of P, Fe, Al, Ca, and Mg than M1 compared to total elemental concentration in non-calcareous soils. The percentage extraction efficiency of M1 for non-calcareous soils (all combined) was 11% P, 0.8% Al, and 0.12% Fe whereas M3 extracted 22% P, 3.3 % Al, and 0.6% Fe (Table 8). Senwo et al., (2003) reported that M3 extracted greater fractions of P for a subset of Alabama soils using different extractant such as M1, Bray 1, M3, Olsen, H_2O and $CaCl_2$. The strong acidic nature of M3 solution is capable of dissolving Ca and Al bound P, whereas other extractants fail to dissolve P associated with Ca and Al, resulting in their lower extraction efficiency (Senwo et al., 2003). Similarly, Mylavarapu et al., (2002) reported significantly greater extractable mean concentrations of M3-P along with other micronutrients in sandy soils compared to the corresponding M1 extractable amounts (Mylavarapu et al., 2002). Their study reported that M3 extracted 47%, 94%, 101% and 111% more P, Cu, Mn, and B, respectively than M1. Franklin et al., (2006) reported that P extraction efficiency of M1 and M3 were 5.9% and 8.7%, respectively for South Carolina soils which were lower than our study. The poor Fe extraction efficiency of M1 was also reported by Franklin et al., (2006). They reported that Fe recovery compared to total elemental concentration in soil was less than 1% for M1 whereas Fe extraction efficiency of M3 was 2.8%. Similarly, the Al extraction efficiency for South Carolina soils were 4% and 10% for M1 and M3, respectively which were greater than our study. The difference in extraction efficiency might be due to different soil types and land management practices.

Among the four major soil areas, relatively large fraction of P was recovered by M3 in CP soils at all depths followed by AP, PP, and LV soils. The efficiency of P extraction of M1 or M3 in soil is generally influenced by clay fractions associated with Al and Fe oxides and P linked with those fractions in the soil (Eriksson et al., 2015). Coastal Plain soils are mainly sandy with very small amount of clay resulting in lower amount of Fe and Al oxides associated with P. Therefore, a large fraction of P might have been associated with desorbed pool and hence extraction with M3 solution was efficient. Similarly, greater extraction efficiency of M3 was observed in AP soils. Appalachian Plateau soils were found to have greater amount of readily available P as evident by greater WSP, and M3-P than other soil types. The greater P content might be a reflection of management practices such as P application rate and source rather than native soil properties. Therefore, larger fractions of loosely sorbed P in soil might have influenced the extraction efficiency of both M1 and M3 for AP soils.

We found the extraction efficiency of M3 for Al was greater than Fe. The poor extraction efficiency of M3 for Fe compared to Al was also reported by other researchers (Maguire and Sims, 2002b; Penn et al., 2006). One reason for this poor extractability was because Fe oxides and oxyhydroxides are partly insoluble at pH 3.0 (minerals such as goethite and hematite) however minerals associated with Al such as gibbsite easily dissolve at pH 3.0 (Penn et al., 2018). Most Alabama soils are rich in Fe and Al oxides. Additionally, fluoride present in M3 solution have strong affinity for Al which forms complex with Al in solution (AlF3), and hence favors the dissolution of P associated with Al. On the other hand, fluoride does not readily complex with Fe (Penn et al., 2018).

3.5.2 Comparison of Extraction Efficiency of M1, M3 and La for Calcareous (BP) Soils

The Extraction Efficiency of M1 in BP soils for combined depths was 2.2% for P, 0.02% for Fe, 0.2% for Al, and 3% for Mg. Similarly, La extracted 5% P, 0.04% Fe, and 6% Mg compared to M3 which extracted 4% of total P, 0.3% of total Fe, 1.1% of total Al, and 0.3% of total Mg from

BP soils. The greater P extraction efficiency of La was also reported by Cox, (2001) in Mississippi soils. However, Cox reported greater P extraction efficiency of La up to 57 mg kg^{-1} beyond which M3 extracted greater amounts of P. Difference in extraction efficiency between La and M3 is potentially related to extraction mechanism driven by reagents in the solution. For example, fluoride in M3 extractant forms complex with Al, dissolving P associated with aluminum phosphates (Mehlich, 1978, 1984). At higher pH, precipitation of fluoride occurs as CaF_2 on $CaCO_3$ surface, which has greater affinity for resorbing dissolved P. To prevent resorption of P, acetic acid is added to maintain pH of 3. On the contrary, first step of La extraction involves dissolution of the plant available Ca-P by the weak acidic solution without dissolving apatitic calcium phosphate (Cox, 2001). In second step, fluoride in the solution forms complex with Al, dissolving Al-P. Aluminum present in extracting solution helps to accelerate the formation of aluminum-fluoride complexes which minimizes the risk of fluoride precipitation with Ca and Mg.

3.5.3 Conversion Equation for Mehlich-3 Extractant

We found highly significant and strong linear relationship between M1-P and M3-P; M1-Ca and M3-Ca; and M1-Mg and M3-Mg for four soil types. This indicated the possibility of using regression equation for interconversions between M1 and M3 nutrients. Although significant relationship was observed between M1-Al and M3-Al, poor correlation was found between the two extractants for Al except for AP soils. Sims, (1989) reported highly significant relationship between M3 and M1 extractable P, K, Ca, Mg, and Zn ($r = 0.92$ to 0.97) for coarse-textured soil from Delaware. He reported that the conversion equations developed were acceptable for interim use for any laboratory that adopts M3 as routine soil test.

Mylavarapu et al., (2002) studied correlation between M1 and M3 extractable P, Ca, Mg, K, and other micro-nutrients and developed the conversion equations for those elements. Highly significant linear relationships between M3 and M1 were found for all nutrients. The relationship between M1-P and M3-P was significant and highly correlated with r^2 of 0.64 for acid mineral soils of Florida. The coefficient of determination (r^2) reported by Mylavarapu (2003) was similar to CP soils in our study. In addition, Mylavarapu et al., (2002) developed regression equation between M1 and M3 for Ca and Mg. The correlations between M1 and M3 were similar with our results where they found r^2 of 0.74 for Ca and 0.83 for Mg, whereas our study showed r^2 of 0.76 for Ca and 0.70 for Mg in CP soils. Mylavarapu et al., (2002) come to the conclusion that conversion equations developed could reasonably be used to estimate M1 values based on M3 values for acid mineral soils of Florida. Similar results were reported in different states for different soil types (Wolf and Baker, 1985; Sims, 1989). Although the coefficient of determination for P, Ca and Mg were similar, the slope of the regression line was steeper in our study compared to Mylavarapu et al., (2002). Furthermore, when lower M3-P (less than 445 mg/kg) and M1-P values (less than 240 mg/kg) were considered in regression analysis, there was an improvement in r^2 value (0.72). The conversion equation derived from these lower levels indicated the M3-P values are approximately 1.5 times greater than M1-P values which was consistent with our results for the combined data set for non-calcareous soils (M3-P was 1.3 times greater than M1-P). Similarly, Franklin and Simmons, (2005) reported r^2 of 0.9 between M1-P and M3-P and slope of 1.45 between M3-P and M1-P for South Carolina soils.

Wolf and Baker, (1985) reported the relationships between M1-P and M3-P for 19 states in the southeast, north central, and northeastern regions. The result showed that the slope for the regression line was steeper (slope=1.85) compared to the slope obtained in our study. Wide variation in slope for the regression line between M1-P and M3-P was obtained in different studies. Gartley et al., (2002) reported slope of 2.04 for the regression between M1-P and M3-P for

Delaware soils. Similarly, M1 and M3 extractable Ca and Mg were better correlated (based on r^2 value) in their study than ours.

In case of BP soils, Cox, (2001) studied the relationship between La and M3 extractable Ca, Mg, K and P for soils found in Mississippi. He reported a strong correlation between La and M3 extractable Ca, Mg and K and stated the possibility of using the conversion equation for these three elements which are consistent with our study. However, Cox did not find good correlation between La-P and M3-P based on limited data sets in calcareous soils from Mississippi whereas we found significant and strong correlation (r^2 of 0.92) between La-P and M3-P for calcareous soils found in BP Alabama. The higher correlation coefficient indicated that the linear relationship between the two extraction methods were dependable and 92% of the variation between the two methods could be explained. Cox, (2001) justified that the poor relationship was most likely due to the two steps extraction in La method where the P fractions that was extracted by La from soil P pool was different from the P pool extracted by M3 and therefore were poorly related. Furthermore, La and M3 extractable Ca and Mg in our data set were significantly correlated which was consistent with the findings of Cox, (2001).

The good fit of the regression equations between M1 and M3 for both calcareous and non-calcareous soils up to 30 cm depths makes conversion equations suitable for P, Ca, and Mg in Alabama soils.

3.5.4 Relationship between Agronomic and Environmental STP

We found strong correlation between WSP and M1-P for combined depths for non-calcareous soils. However, the relationship was not consistent with the result obtained by Maguire and Sims, (2002a). They obtained the strongest relationship between WSP and M1-P with correlation coefficient of 0.93 for five soil series from Delmarva Peninsula USA. whereas in the present study, a relatively weaker correlation was found between WSP and M1-P. The disparity in results between Maguire and Sims, (2002a) and our study might be due to effect of sampling depth. Maguire and Sims, (2002a) used composited soil samples up to 20 cm depth. Another reason could be due to the difference in soil types. Schroeder et al., (2004) reported the disparity in relationship between STP and dissolved P in runoff were due to difference in soil types. Pautler and Sims, (2000) also reported correlation between STP (M1) and WSP for 127 soils from Delaware and Dutch with correlation coefficient of 0.71 which was more consistent with our findings.

The relationship between WSP and M3-P for combined depths for non-calcareous was significantly correlated. Similar results were obtained by Sims et al., (2002) between WSP and M3-P with coefficient of determination ($r^2 = 0.68$) which was consistent with our result ($r^2 = 0.64$). They also observed that correlation was lower for topsoil when the data was segregated by depth ($r^2 = 0.53$) and relationship improved for subsoils ($r^2 = 0.86$). However, the relationships between WSP and M3-P were consistent in our study up to 30 cm depths. Additionally, Schroeder et al., (2004) reported strong correlation between M3-P and WSP with r^2 of 0.87. Similarly, Maguire and Sims, (2002a) also obtained a better correlation coefficient of 0.82 for Delmarva Peninsula, USA, soils. We found lower r^2 values in our study compared to the previous findings from different areas. This could be due to difference in soil types, sampling depths and presence of higher amount of Al and Fe oxides in Alabama soils.

In BP soils, the relationship between WSP, La-P and M3-P were strongly correlated. However, the relationship between WSP and M3-P were stronger, which indicated M3 as a better estimator of WSP than La for BP soils.

3.6 Conclusion

The results showed that the extraction efficiency of M3 for non-calcareous Alabama soils, was better than M1 for P, Al, Fe, and Mg. The linear relationship between M1-P and M3-P were strongly correlated for all soils from Alabama and the slope of regression line showed that M3 extracted 1.3 times greater P than M1. For BP soils, La method showed better extraction efficiency than M3 for P and Mg. However, the regression between La-P and M3-P showed that both extractant have similar potential for P extraction. The conversion equations can be developed between M1/La and M3 for P, Ca, and Mg for all soil types however, a single conversion equation developed for combined data can be used for conversions up to 30 cm depths. The relationship between WSP and M3-P were better correlated than the relationship between WSP and M1-P for non-calcareous soils and similarly, the relationship between WSP and M3-P was better correlated than WSP and La-P for BP soils. The high correlation and greater coefficient of determination obtained in this research and in previous research in other states between M1-P/La-P and M3-P indicated suitable use of M3 as a better extractant for environmental P loss risk assessment for wide range of Alabama soils.

3.7 Tables

Table 8: Comparison of extraction efficiency between Mehlich-1 and Mehlich-3 for four major Alabama soils. The values outside the bracket are actual mean values while the ones inside bracket is percentage efficiency.

% efficiency											
Soil Areas	Depths (cm)	……………………………….mg kg^{-1}……….……………………………………									
		M1-P*	M3-P*	M1-Fe	M3-Fe	M1-Al	M3-Al	M1-Ca	M3-Ca	M1-Mg	M3-Mg
Appalachian Plateau (AP)	0-5	143 $^{a, a}$ (21.3)	251 $^{a, a}$ (40.7)	59 $^{a, a}$ (0.5)	277 $^{a, a}$ (2.2)	313 $^{a, a}$ (2.2)	766 $^{b, a}$ (5.6)	1233 $^{a, ab}$	1249 $^{a, ab}$	149 $^{a, a}$ (19)	148 $^{a, a}$ (18.6)
	5-15	88 $^{a, a}$ (15.8)	165 $^{a, a}$ (37.6)	52 $^{a, a}$ (0.4)	226 $^{ab, a}$ (1.9)	284 $^{a, a}$ (1.9)	887 $^{ab, a}$ (6.4)	776 $^{b, a}$	814 $^{b, a}$	87 $^{b, a}$ (11.3)	98 $^{b, a}$ (13.4)
	15-30	44 $^{b, a}$ (13.1)	87 $^{b, a}$ (30.9)	34 $^{ab, a}$ (0.2)	148 $^{b, a}$ (1)	257 $^{a, a}$ (1.3)	1029 $^{a, a}$ (5.6)	519 $^{c, b}$	642 $^{bc, b}$	79 $^{b, a}$ (8.8)	97 $^{b, a}$ (11.1)
	30-45	14 $^{c, ab}$ (6.3)	31 $^{c, a}$ (17.8)	21 $^{b, ab}$ (0.1)	99 $^{c, a}$ (0.6)	193 $^{b, a}$ (0.9)	997 $^{a, a}$ (4.8)	409 $^{c, b}$	534 $^{c, b}$	61 $^{b, a}$ (6.5)	86 $^{b, a}$ (9.2)
Combined	0-45	72 (19.1)	134 (35.5)	42 (0.3)	188 (1.4)	262 (1.6)	920 (5.5)	735	810	94 (11.3)	107 (12.8)
Coastal Plain (CP)	0-5	76 $^{a, b}$ (20)	142 $^{a, b}$ (43.7)	23 $^{a, b}$ (0.3)	123 $^{a, b}$ (1.6)	164 $^{ab, c}$ (1.2)	638 $^{b, b}$ (4.7)	650 $^{a, c}$	631 $^{a, c}$	92 $^{a, b}$ (24.9)	91 $^{a, b}$ (27)
	5-15	43 $^{ab, b}$ (18.8)	106 $^{a, ab}$ (46.5)	17 $^{a, b}$ (0.2)	95 $^{ab, b}$ (1.1)	182 $^{a, c}$ (1.2)	763 $^{ab, b}$ (5.2)	364 $^{ab, b}$	410 $^{ab, b}$	54 $^{b, b}$ (16.8)	55 $^{a, b}$ (17.8)
	15-30	31 $^{b, b}$ (13.7)	74 $^{b, a}$ (36.5)	17 $^{a, b}$ (0.1)	72 $^{b, c}$ (0.6)	182 $^{a, b}$ (0.8)	846 $^{a, b}$ (3.8)	289 $^{b, c}$	315 $^{b, c}$	51 $^{b, b}$ (12.3)	57 $^{a, b}$ (13.8)
	30-45	11 $^{c, b}$ (8.3)	26 $^{c, ab}$ (18.1)	8 $^{a, b}$ (0.1)	30 $^{c, c}$ (0.2)	135 $^{b, b}$ (0.5)	943 $^{a, a}$ (3.2)	243 $^{b, c}$	316 $^{b, c}$	50 $^{b, a}$ (10.1)	76 $^{a, b}$ (13.9)
Combined	0-45	40 (17.01)	86 (38.5)	16 (0.1)	79 (0.7)	166 (0.8)	804 (4)	372	412	61 (15.2)	69 (17.2)
Limestone Valley (LV)	0-5	73 $^{a, b}$ (10.2)	108 $^{a, b}$ (20.1)	21 $^{a, b}$ (0.1)	134 $^{a, b}$ (0.7)	262 $^{a, ab}$ (1.1)	802 $^{b, a}$ (4.5)	1573 $^{a, a}$	1612 $^{a, a}$	104 $^{a, ab}$ (8.9)	112 $^{a, a}$ (10.8)
	5-15	27 $^{b, b}$ (7.1)	41 $^{b, c}$ (11.6)	18 $^{a, b}$ (0.1)	98 $^{ab, b}$ (0.5)	246 $^{a, ab}$ (0.9)	959 $^{a, a}$ (3.8)	1105 $^{b, a}$	1242 $^{b, a}$	67 $^{b, ab}$ (6.4)	74 $^{b, a}$ (7)

	15-30	11 $^{c, b}$ (3.7)	17 $^{c, b}$ (6.4)	16 $^{a, b}$ (0.1)	74 $^{bc, bc}$ (0.3)	230 $^{a, ab}$ (0.7)	1016 $^{a, a}$ (3.1)	981 $^{bc, a}$	1167 $^{b, a}$	58 $^{b, ab}$ (4.7)	68 $^{b, b}$ (5.45)
	30-45	6 $^{d, b}$ (2.3)	8 $^{d, c}$ (3.7)	14 $^{a, b}$ (0.1)	55 $^{c, b}$ (0.2)	216 $^{a, a}$ (0.6)	912 $^{ab, a}$ (2.5)	841 $^{c, a}$	1091 $^{b, a}$	56 $^{b, a}$ (4.1)	68 $^{b, ab}$ (4.9)
Combined	0-45	29 (8.3)	43 (12.4)	17 (0.1)	90 (0.4)	240 (0.9)	924 (3.4)	1123	1274	70 (5.9)	79 (6.7)
Piedmont Plateau (PP)	0-5	91 $^{a, a}$ (13.5)	173 $^{a, b}$ (24.2)	55 $^{a, a}$ (0.2)	301 $^{a, a}$ (1.1)	240 $^{a, b}$ (0.9)	796 $^{b, a}$ (3.1)	1140 $^{a, b}$	1203 $^{a, b}$	129 $^{a, ab}$ (16.4)	151 $^{a, a}$ (18.5)
	5-15	35 $^{b, a}$ (7.7)	88 $^{b, b}$ (19.3)	42 $^{a, a}$ (0.1)	186 $^{b, a}$ (0.5)	228 $^{ab, bc}$ (0.7)	879 $^{ab, a}$ (2.9)	514 ^{b,}	609 b, b	61 $^{b, ab}$ (7.6)	82 $^{b, a}$ (10.2)
	15-30	12 $^{c, a}$ (3.7)	36 $^{c, b}$ (11)	27 $^{b, a}$ (0.1)	104 $^{c, ab}$ (0.3)	201 $^{ab, b}$ (0.5)	960 $^{a, a}$ (2.4)	336 $^{bc, c}$	405 bc, c	49 $^{b, b}$ (5.3)	62 $^{b, b}$ (6.6)
	30-45	5 $^{d, a}$ (1.8)	17 $^{c, bc}$ (6.2)	26 $^{b, a}$ (0.1)	80 $^{d, ab}$ (0.2)	188 $^{b, a}$ (0.5)	884 $^{ab, a}$ (2.2)	294 $^{c, c}$	356 c, b	49 $^{b, a}$ (4.9)	68 $^{b, ab}$ (6.8)
Combined	0-45	37 (8.3)	81 (18.2)	38 (0.1)	170 (0.5)	216 (0.6)	880 (2.6)	577	647	71 (8)	89 (10.1)

M1-P, Fe, Al, and Mg, Mehlich-1 extractable phosphorus, iron, aluminum, and magnesium, respectively; M3-P, Fe, Al, and Mg, Mehlich-3 extractable phosphorus, iron, aluminum, and magnesium, respectively.

Values within bracket are % extraction efficiency

*% efficiency = {(M1-extratable metal or M3-extractable metal)/Total metal*100}

The means differences were determined using Tukey-Kramer adjusted P values at $\alpha = 0.05$ significance level.

The initial alphabets separated by comma represents the means difference between the soil depths in a particular soil types and the second alphabets separated by comma represents the mean difference with in a soil depth among the major soil types, , the columns followed by the same letter are not significantly different ($P < 0.05$).

Table 9: Comparison of extraction efficiency between Mehlich-1, Lancaster, and Mehlich-3 for BP soils of Alabama soils. The values outside the bracket are actual mean values while the ones inside bracket is percentage efficiency.

% efficiency											
Depths (cm)	……………………………………………...mg kg^{-1}…………………………………………………										
	M1-P	La-P	M3-P	M1-Fe	La-Fe	M3-Fe	M1-Al	M3-Al	M1-Mg	La-Mg	M3-Mg
0-5	24 ^{a} (2.9)	60 ^{a} (7.3)	50 ^{a} (6.1)	10 ^{a} (0.04)	149 ^{a} (0.7)	124 ^{a} (0.5)	50 ^{a} (0.19)	229 ^{a} (0.9)	126 ^{a} (5.8)	205 ^{a} (9.5)	10 ^{a} (0.5)
5-15	11 ^{b} (2.2)	23 ^{b} (4.6)	19 ^{b} (3.8)	5 ^{a} (0.02)	91 ^{b} (0.4)	77 ^{ab} (0.3)	44 ^{ab} (0.16)	261 ^{a} (1)	83 ^{ab} (3.9)	140 ^{ab} (6.7)	8 ^{ab} (0.4)
15-30	4 ^{b} (1.19)	11 ^{c} (3.2)	10 ^{bc} (3)	4 ^{a} (0.01)	66 ^{b} (0.3)	55 ^{b} (0.2)	53 ^{b} (0.17)	376 ^{a} (1.3)	54 ^{b} (2.5)	95 ^{bc} (4.4)	5 ^{b} (0.2)
30-45	4 ^{b} (1.36)	10 ^{c} (3.4)	8 ^{c} (2.7)	4 ^{a} (0.01)	66 ^{b} (0.2)	54 ^{b} (0.2)	64 ^{b} (0.19)	457 ^{a} (1.4)	38 ^{b} (1.6)	73 ^{c} (3.2)	4 ^{b} (0.2)
0-45	11 (2.2)	26 (5.3)	21 (4.3)	6 (0.02)	93 (0.04)	78 (0.3)	53 (0.2)	331 (1.1)	76 (3.4)	128 (5.9)	7 (0.3)

M1-P, Fe, Al, and Mg, Mehlich-1 extractable phosphorus, iron, aluminum, and magnesium, respectively, La-P, Fe, and Mg, Lancaster extractable phosphorus, iron, and magnesium, respectively; M3-P, Fe, Al, and Mg, Mehlich-3 extractable phosphorus, iron, aluminum, and magnesium, respectively.

Values within bracket are % extraction efficiency

The means differences were determined using Tukey-Kramer adjusted P values at $\alpha = 0.05$ significance level, the columns followed by the same letter are not significantly different ($P < 0.05$).

The same letters represent the mean difference along the column

% efficiency = {(M1-extractable metal or La-extractable metal or M3-extractable metal)/Total metal*100}

Table 10: Regression analysis between Mehlich-1 and Mehlich-3 extractable P, Al, and Fe for four major soil areas of Alabama by depths.

Soil Areas	**Depths (cm)**	**M1-P and M3-P**				**M1-Al and M3-Al**				**M1-Fe and M3-Fe**				**n**
		m	**b**	**r^2**	$CI_{95\%}$	**m**	**b**	**r^2**	$CI_{95\%}$	**m**	**b**	**r^2**	$CI_{95\%}$	
Appalachian Plateau	Combined	1.4	32	0.83^{***}	1.3-1.5	0.73	730	0.094^{***}	0.03-1.1	2.2	96	0.72^{***}	1.9-2.4	116
	0-5	0.98	110	0.62^{***}	0.6-1.3	1.4	320	0.53^{***}	0.9-1.9	1.9	160	0.74^{***}	1.4-2.3	29
	5-15	1.3	51	0.82^{***}	1.1-1.6	1.6	440	0.35^{***}	0.7-2.4	2.1	120	0.76^{***}	1.6-2.5	29
	15-30	1.8	8.4	0.91^{***}	1.6-2	0.39	930	NS	-	2.6	59	0.72^{***}	1.9-3.3	29
	30-45	1.9	3.9	0.96^{***}	1.7-2.1	1.7	670	0.24^{***}	0.5-2.9	2.7	39	0.69^{***}	1.9-3.4	29
Coastal Plain	Combined	1.1	44	0.56^{***}	0.9-1.2	1.7	510	0.097^{***}	0.8-2.6	1.8	51	0.49^{***}	1.5-2	142
	0-5	0.76	83	0.52^{***}	0.5-1.	2.5	230	0.18^{***}	0.6-4.4	1.6	87	0.56^{***}	1.1-2.1	34
	5-15	1.3	54	0.39^{***}	0.6-1.7	1.8	430	NS	-	1.8	64	0.38^{***}	1-2.6	35
	15-30	1.6	23	0.66^{***}	1.2-2	1.6	550	0.16^{***}	0.3-2.9	1.6	45	0.57^{***}	1.1-2	36
	30-45	1.5	8.8	0.91^{***}	1.4-1.7	2.4	620	NS	-	1.8	15	0.45^{***}	1.1-2.5	36
Limestone Valley	Combined	1.1	12	0.78^{***}	1-1.2	0.24	860	NS	-	1	73	0.1^{**}	0.6-1.4	36
	0-5	0.87	44	0.66^{***}	0.7-1	0.27	730	NS	-	0.7	120	NS	-	222
	5-15	1.2	9.1	0.84^{***}	1-1.3	0.4	860	NS	-	1	80	0.14^{***}	0.3-1.7	57
	15-30	1.1	5.1	0.78^{***}	0.9-1.2	0.27	960	NS	-	0.98	58	NS	0.4-1.5	56
	30-45	1.1	2	0.73^{***}	0.9-1.3	0.25	860	NS	-	0.94	41	0.13^{***}	0.3-1.6	52
Piedmont Plateau	Combined	1.5	26	0.68^{***}	1.4-1.7	0.36	800	0.036^{**}	0.1-0.6	2.8	66	0.5	2.4-3.1	250
	0-5	1.2	67	0.59^{***}	0.9-1.5	0.5	670	NS	-	2.7	150	0.58^{***}	2.1-3.3	66
	5-15	1.9	21	0.65^{***}	1.5-2.2	0.39	790	NS	-	2.4	85	0.52^{***}	1.8-2.9	66
	15-30	2.1	2.8	0.85^{***}	1.7-2.5	0.42	880	NS	-	2.4	41	0.39^{***}	1.7-3.2	61
	30-45	3.7	-2.4	0.85^{***}	3.3-4.2	0.49	790	NS	-	1.1	50	NS	0.5-1.7	53

$^{*, **, ***}$ Indicate significance at 0.05, 0.01 and 0.001 levels of probability, respectively; NS, non-significant; M1-P, Fe, and Al, Mehlich-1 extractable phosphorus, iron, and aluminum, respectively; M3-P, Fe, and Al, Mehlich-3 extractable phosphorus, iron, and aluminum, respectively; n, no. of samples.

M3-extractable = m (M1-extractable) + b

m, slope; b, intercept; $CI_{95\%}$, 95% confidence interval for slope of regression line

Table 11: Regression analysis between Mehlich-1 and Mehlich-3 extractable Ca and Mg for four major Alabama soil by depths.

Soil Areas	**Depths (cm)**	**M1-Ca and M3-Ca**				**M1-Mg and M3-Mg**				**n**
		m	**b**	**r^2**	$CI_{95\%}$	**m**	**b**	**r^2**	$CI_{95\%}$	
Appalachian Plateau	Combined	0.81	220	0.73^{***}	0.7-0.9	0.76	35	0.81^{***}	0.7-0.8	116
	0-5	0.75	320	0.52^{***}	0.5-1	0.71	41	0.83^{***}	0.6-0.8	29
	5-15	0.73	250	0.76^{***}	0.5-0.9	0.84	25	0.86^{***}	0.7-1	29
	15-30	0.81	220	0.7^{***}	0.6-1	0.86	29	0.67^{***}	0.6-1.1	29
	30-45	1.1	74	0.8^{***}	0.9-1.3	1.1	15	0.66^{***}	0.8-1.5	29
Coastal Plain	Combined	0.8	110	0.76^{***}	0.7-0.9	0.96	10	0.7^{***}	0.8-1.1	142
	0-5	0.73	160	0.77^{***}	0.6-0.9	0.75	21	0.75^{***}	0.6-0.9	34
	5-15	1.1	34	0.71^{*}	0.7-1.2	0.99	5.7	0.66^{***}	0.8-1.2	35
	15-30	1	26	0.71^{***}	0.8-1.2	1.1	3.2	0.7^{***}	0.8-1.3	36
	30-45	1.3	-10	0.74^{***}	1.1-1.6	1.6	-9.9	0.87^{***}	1.4-1.8	36
Limestone Valley	Combined	0.88	290	0.62^{***}	0.8-0.9	1	6.4	0.87^{***}	1-1.1	36
	0-5	0.69	530	0.5^{***}	0.5-0.9	0.99	9.3	0.84^{***}	0.8-1.1	222
	5-15	0.86	300	0.59^{***}	0.7-1	1	7.3	0.85^{***}	0.9-1.1	57
	15-30	1.1	79	0.69^{***}	0.9-1.3	1.3	-4.4	0.9^{***}	1.1-1.4	56
	30-45	1.3	-29	0.71^{***}	1.1-1.6	1.1	6.1	0.89^{***}	1-1.2	52
Piedmont Plateau	Combined	0.91	120	0.85^{***}	0.8-1	0.98	19	0.82^{***}	1-1.2	
	0-5	0.8	290	0.78^{***}	0.7-0.9	1.1	9.5	0.79^{***}	0.9-1.2	66
	5-15	0.95	120	0.74^{***}	0.8-1.1	1.1	12	0.68^{***}	0.9-1.3	66
	15-30	1.1	37	0.77^{***}	0.9-1.2	1.2	5.4	0.82^{***}	1-1.3	61
	30-45	1.2	12	0.78^{***}	1-1.3	1.1	13	0.63^{***}	0.9-1.4	53

$^{*, **, ***}$ Indicate significance at 0.05, 0.01 and 0.001 levels of probability, respectively; NS, non-significant; M1-Ca, and Mg, Mehlich-1 extractable calcium, and magnesium, respectively; M3-Ca, and Mg, Mehlich-3 extractable calcium, and magnesium, respectively; n, no. of samples.

M3-extractable = m (M1-extractable) + b

m, slope; b, intercept; $CI_{95\%}$, 95% confidence interval for slope of regression line

Table 12: Regression analysis between Mehlich-1 and Mehlich-3 extractable nutrient for combined data (AP, CP, LV, PD) of Alabama by depths.

Depths (cm)	M1-P and M3-P				M1-Fe and M3-Fe				M1-Ca and M3-Ca				M1-Mg and M3-Mg				n
	m	b	r^2	CI$_{95\%}$	m	b	r^2	CI$_{95\%}$	m	b	r^2	CI$_{95\%}$	m	b	r^2	CI$_{95\%}$	
Combined	1.3	24	0.71	1.3-1.4	2.4	65	0.55	2.2-2.5	0.94	140	0.78	0.9-1	1	13	0.79	0.9-1.1	730
0-5	1.0	64	0.6	0.9-1.2	2.3	120	0.58	2-2.6	0.80	270	0.70	0.7-0.9	0.96	14	0.78	0.8-1	186
5-15	1.7	19	0.67	1.3-1.6	2.3	77	0.63	2-2.5	0.95	130	0.77	0.9-1	1.0	11	0.75	0.9-1.1	187
15-30	1.9	6.4	0.74	1.8-2.1	2.1	48	0.48	1.8-2.4	1.10	58	0.79	1.1-1.2	1.1	4.4	0.81	0.9-1.2	187
30-45	1.8	4.2	0.81	1.7-2	2.5	23	0.47	1.1-1.8	1.30	-11.3	0.81	1.2-1.4	1.27	1.17-1.37	0.8	1.1-1.4	170

r^2 significance at 0.01 levels of probability, respectively; NS, non-significant; M1-P, Fe, Ca, and Mg, Mehlich-1 extractable phosphorus, iron, calcium, and magnesium, respectively; M3-P, Fe, Al, Ca, and Mg, Mehlich-3 extractable phosphorus, iron, aluminum, calcium, and magnesium, respectively; n, no. of samples.

m, slope of regression line; b, intercept; r^2, coefficient of determination; CI$_{95\%,}$ 95% confidence interval for slope of regression line

M3-extractable = m (M1-extractable) + b

Table 13: Regression equation between Lancaster and Mehlich-3 extractable nutrients from Blackland Prairie soils of Alabama by depths.

Depths (cm)	La-P and M3-P				La-Fe and M3-Fe				La-Ca and M3-Ca				La-Mg and M3-Mg				n
	m	b	r^2	$CI_{95\%}$	m	b	r^2	$CI_{95\%}$	m	b	r^2	$CI_{95\%}$	m	b	r^2	$CI_{95\%}$	
Combined	1.06	-6.3	0.91	1-1.1	0.82	1.3	0.79	0.7-0.9	0.6	520	0.77	0.5-0.7	1.24	-27	0.93	1.2-1.3	82
0-5	1.13	-17.8	0.88	0.9-1.3	0.74	15	0.83	0.6-0.9	0.63	98	0.81	0.5-0.8	1.3	-56	0.87	1.1-1.5	20
5-15	1.34	-11.9	0.97	1.2-1.4	1.20	-33	0.71	0.8-1.6	0.63	-100	0.8	0.5-0.8	1.3	-39	0.97	1.2-1.4	20
15-30	1.22	-4.8	0.95	1.1-1.4	1.20	-24	0.70	0.8-1.5	0.52	1700	0.66	0.3-0.7	1.4	-34	0.96	1.3-1.5	21
30-45	1.18	-3.8	0.96	1.1-1.3	1.20	-21	0.72	0.8-1.5	0.64	200	0.81	0.5-0.8	1.3	-21	0.9	1.1-1.5	21

La-P, Fe, Ca, and Mg, Lancaster extractable phosphorus, iron, calcium, and magnesium, respectively; M3-P, Fe, Ca, and Mg, Mehlich-3 extractable phosphorus, iron, calcium, and magnesium, respectively; n, no. of samples

m, slope of regression line; b, intercept; r^2, coefficient of determination significant at 0.01 level of probability

M3-extractable = m (La-extractable) + b

Table 14: Regression equation between WSP and M1-P/M3-P for AP, CP, LV, and PP soils, and WSP and La-P/M3-P for BP soils.

		WSP Vs M1-P/La-P					WSP Vs M3-P					
Soil Areas	Depths (cm)	b	m	r^2	CI$_{95\%}$	P > F	b	m	r^2	CI$_{95\%}$	P > F	n
Appalachian Plateau	0-5	3.2	0.05	0.37	0.02-0.07	< 0.01	-1.7	0.05	0.5	0.029-0.067	< 0.05	29
	5-15	-0.26	0.05	0.59	0.03-0.06	< 0.01	-1.7	0.03	0.65	0.02-0.04	< 0.01	29
	15-30	-0.84	0.06	0.53	0.03-0.07	< 0.05	-1.1	0.03	0.58	0.02-0.04	< 0.01	29
	30-45	-0.21	0.05	0.51	0.03-0.07	< 0.01	-0.24	0.02	0.44	0.01-0.03	0.05	29
	combined	-0.17	0.05	0.6	0.05-0.06	<0.01	-1.29	0.04	0.66	0.03-0.04	<0.01	116
Coastal Plain	0-5	1.8	0.10	0.81	0.08-0.11	< 0.01	-0.72	0.07	0.47	0.04-0.09	< 0.05	34
	5-15	2.11	0.07	0.46	0.04-0.1	< 0.01	0.91	0.04	0.56	0.02-0.05	< 0.01	36
	15-30	0.48	0.09	0.48	0.05-0.12	< 0.01	-0.19	0.05	0.53	0.03-0.06	< 0.01	36
	30-45	-0.06	0.11	0.89	0.09-0.12	< 0.01	-0.58	0.07	0.85	0.058-0.078	< 0.01	36
	combined	0.72	0.1	0.76	0.09-0.11	<0.01	-0.5	0.06	0.56	0.05-0.07	<0.01	143
Limestone Valley	0-5	0.72	0.08	0.56	0.05-0.09	< 0.01	-2	0.08	0.65	0.06-0.09	< 0.01	57
	5-15	-0.03	0.06	0.39	0.04-0.08	< 0.01	-0.42	0.05	0.44	0.03-0.06	< 0.01	57
	15-30	0.18	0.03	0.33	0.01-0.03	< 0.05	0.23	0.02	0.16	0.006-0.02	< 0.01	56
	30-45	0.25	0.001	-	-	0.85	0.29	-0.01	-	-	0.91	52
	combined	-0.11	0.07	0.63	0.06-0.08	<0.01	-0.71	0.06	0.69	0.06-0.07	<0.01	222
Piedmont Plateau	0-5	1.9	0.07	0.48	0.04-0.08	< 0.01	0.13	0.04	0.59	0.03-0.05	< 0.01	66
	5-15	0.07	0.09	0.7	0.07-0.1	< 0.01	0.08	0.04	0.62	0.02-0.04	< 0.01	66
	15-30	0.19	0.09	0.4	0.06-0.11	< 0.01	0.27	0.03	0.54	0.02-0.03	< 0.01	66
	30-45	0.22	0.02	0.12	0.005-0.03	< 0.05	0.24	0.01	0.12	0.001-0.009	< 0.05	53
	combined	0.46	0.07	0.64	0.06-0.08	<0.01	-0.08	0.04	0.67	0.03-0.04	<0.01	250
Blackland Prairie	0-5	-0.56	0.05	0.38	0.02-0.08	< 0.01	0.17	0.05	0.48	0.02-0.07	< 0.01	21
	5-15	-0.91	0.08	0.48	0.07-0.09	< 0.01	-0.17	0.05	0.42	0.05-0.68	< 0.01	20
	15-30	-0.05	0.038	0.58	0.02-0.05	< 0.05	0.1	0.03	0.65	0.02-0.04	0.54	21

	30-45	0.12	-0.01	-	-	0.42	0.12	-0.001	0.02	-0.004-0.002	0.49	21
	combined	0.08	0.08	0.54	0.06-0.1	<0.01	0.007	0.04	0.6	0.03-0.05	< 0.01	84

AP, CP, LV, PP, BP; Appalachian Plateau, Coastal Plain, Limestone Valley, Piedmont Plateau, Blackland Prairie
n, no of samples; m, slope of regression line; b, intercept; r^2, coefficient of determination; $CI_{95\%}$, 95% confidence interval for slope of regression line

3.8 Figures

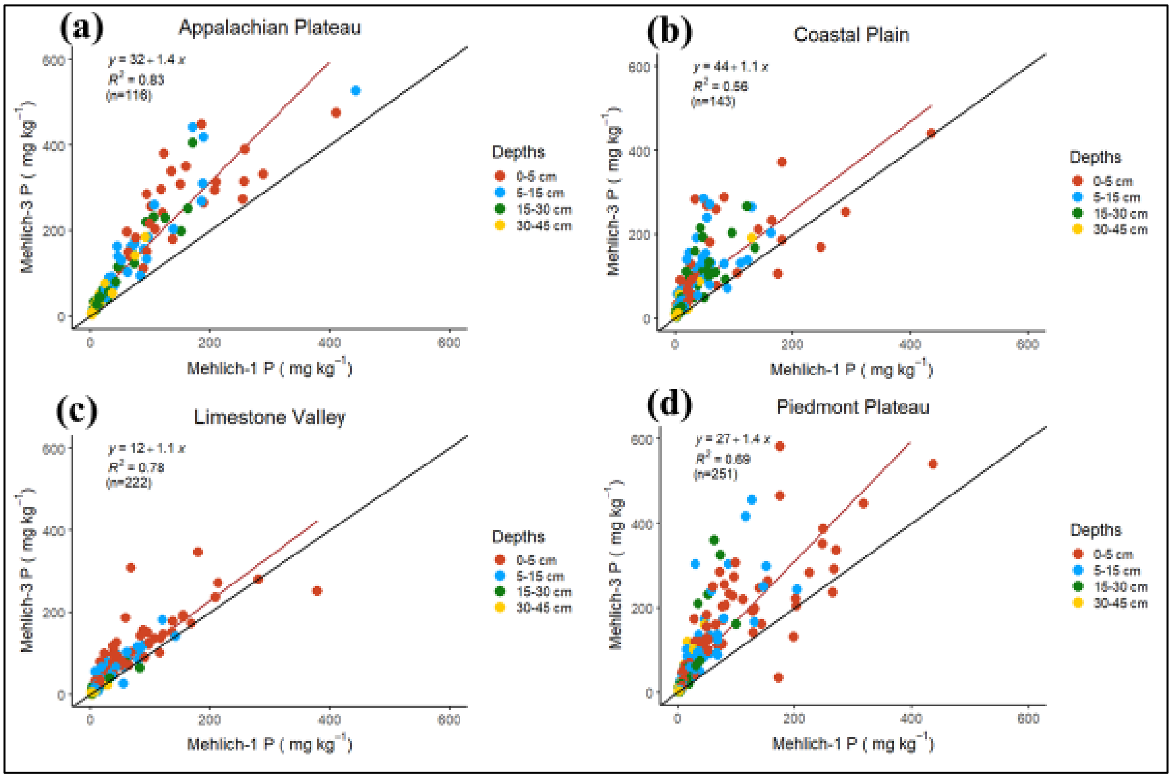

Fig. 9: Relationship between Mehlich-1 P and Mehlich-3 P for four major soil areas of Alabama (a) Appalachian Plateau (b) Coastal Plain (c) Limestone Valley (d) Piedmont Plateau. The solid black line is the 1:1 line.

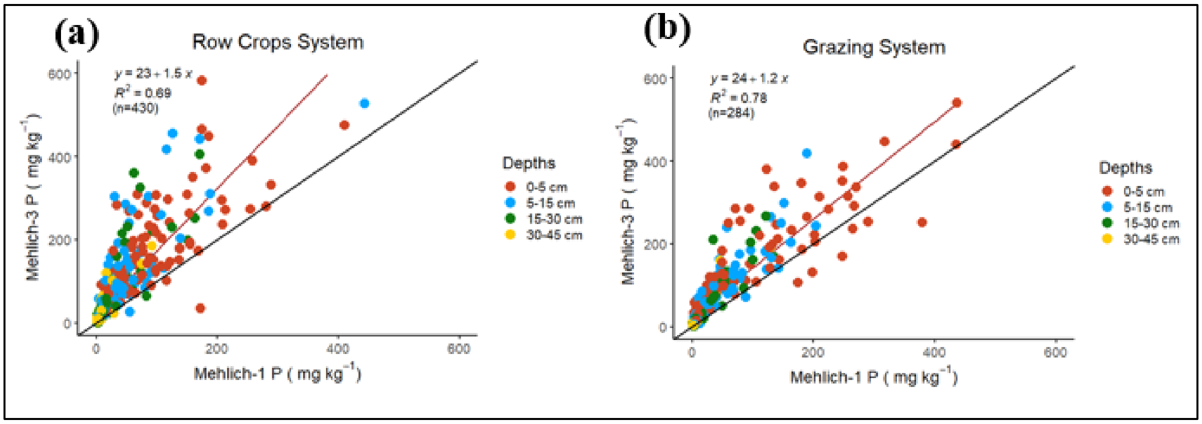

Fig. 10: Relationship between Mehlich-1 P and Mehlich-3 P for different management system (a) Row crops system (b) Grazing system. The solid black line is the 1:1 line.

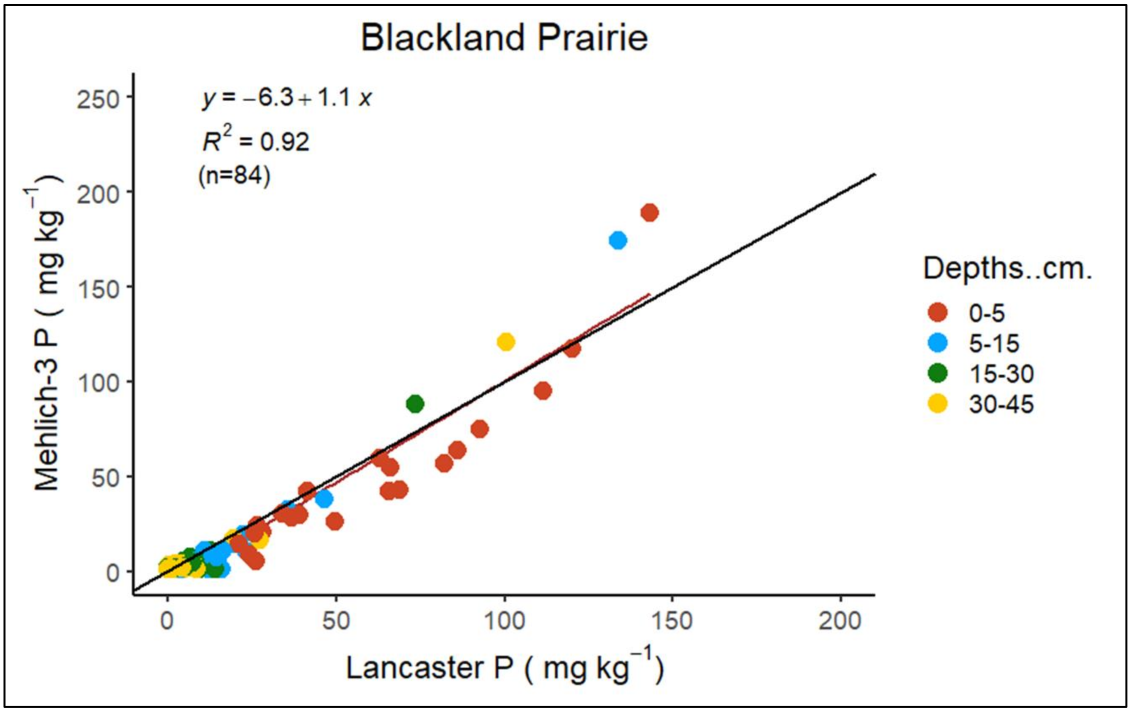

Fig. 11: Relationship between Lancaster- P and Mehlich-3 P for Blackland Prairie soils. The solid black line is the 1:1 line.

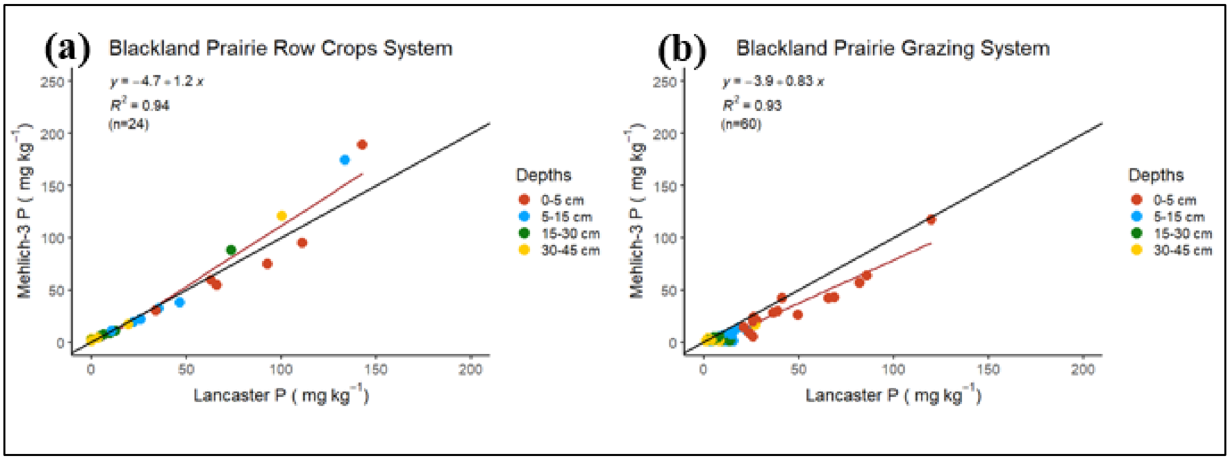

Fig. 12: Relationship between Lancaster- P and Mehlich-3 P for Blackland Prairie soils under different management system (a) Row crops system (b) Grazing system. The solid black line is the 1:1 line.

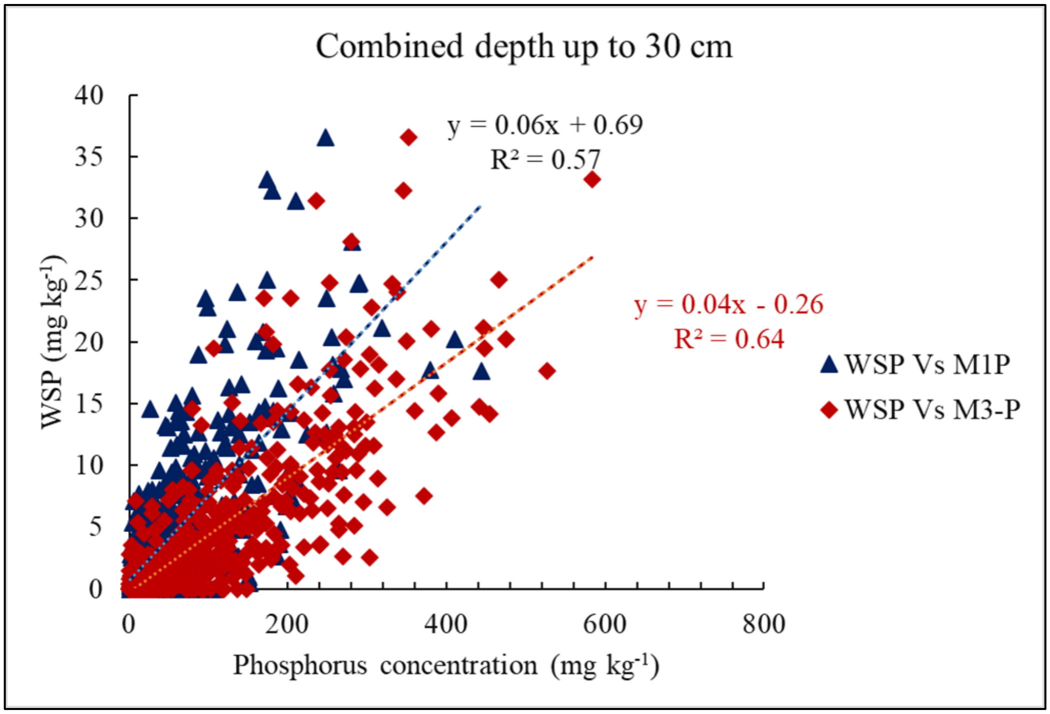

Fig. 13: Relationship between WSP and M1-P/M3-P for combined soils (AP, CP, LV, and PP) up to 30 cm depth.

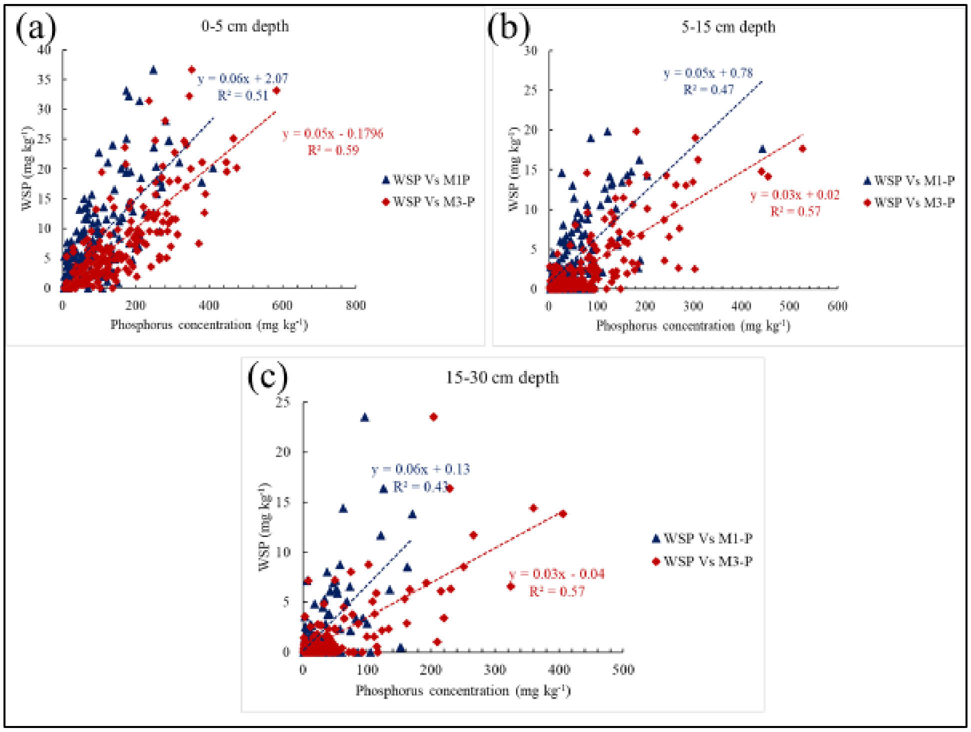

Fig. 14: Relationship between WSP and Mehlich-1 P/Mehlich-3 P for combined soils (AP, CP, LV, and PP) by depth (a) 0-5 cm (b) 5-15 cm (c) 15-30 cm.

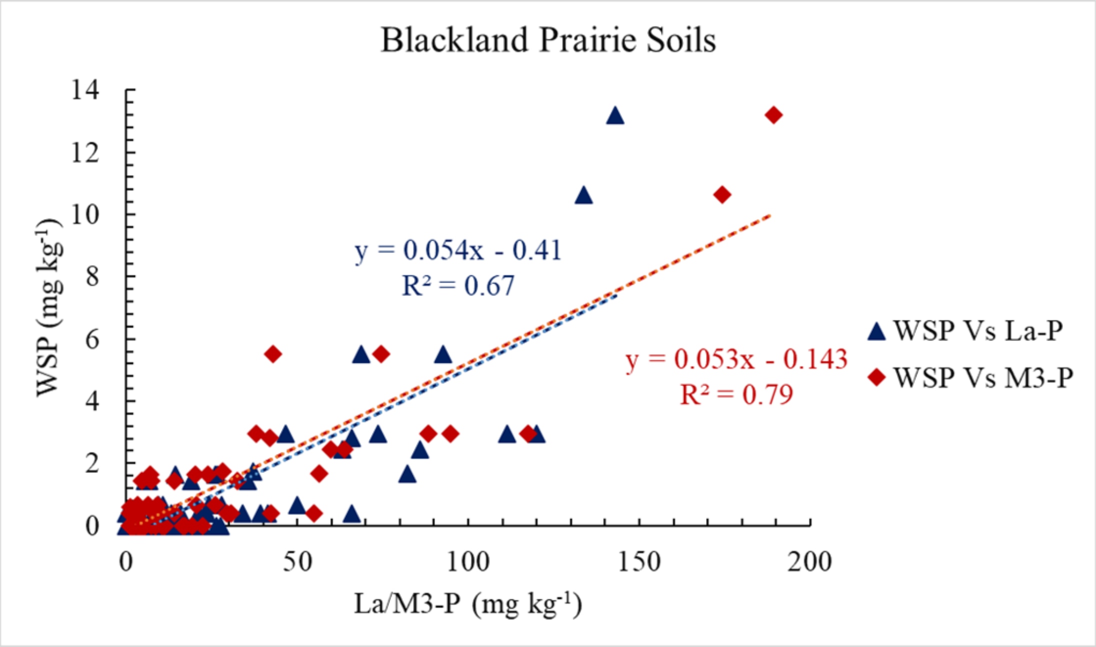

Fig. 15: Relationship between WSP and La-P/M3-P for BP soils up to 30 cm combined depth.

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