EFFECTS OF EXTRACTION TIME AND PHOSPHORUS SPECIATION ON SOIL TEST PHOSPHORUS (STP) IN ILLINOIS AGRICULTURAL SOILS

BY

ANTHONY PAUL MILLER

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Natural Resources and Environmental Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2017

Urbana, Illinois

Adviser:

Associate Professor Yuji Arai

Abstract

Phosphorus (P) loss from agricultural soils is the leading cause of accelerated eutrophication of surface waters. As an essential plant nutrient, however, adequate soil P is required for plant growth. Soil test phosphorus (STP) methods have been developed to correlate P levels in soil to crop growth, so that producers may better estimate the supply of P. There are numerous limitations in STP procedures, however, including: inaccurate estimation of plant available P, (i.e., dissolved reactive P (DRP)), unknown speciation in STP extracts, and the unknown relationship between STP and temporal-scale P release. Assessing these limitations, the appropriate colorimetric methods to distinguish orthophosphate from organic phosphate (P_o) in Bray P-1, and Mehlich III extracting solutions were first evaluated, including the effects of acid hydrolysis reactions on P_o (phytic acid) and polyphosphate (tripolyphosphate and pyrophosphate) in the STP extracting solutions. Based on the assessment, the effects of extraction time and P speciation on the STP procedures were studied in P rich fertilizer-amended and manure-amended Central Illinois agricultural soils. Although the extent of P release is proportional to the total P extracted by STP methods, the results are highly influenced by longer extraction times and P species. A longer extraction time increases STP values, lowering fertilizer recommendations. Manure-amended soils released more inorganic P (P_i), P_o, and colloidal P than did fertilizer-amended soils. The fraction of P_o was substantial (10-50% of total P) in STP soil extracts. Overall, the results of this study suggest the complexity of interpreting current STP procedures for fertilizer recommendations.

Acknowledgements

Foremost, I would like to thank my advisor Dr. Yuji Arai for his guidance through this project. The research would not have been possible without his dependable counseling and leadership. His encouragement has led not only to greater knowledge in the field of soil chemistry, but to becoming a more professional and well-rounded person. I am deeply thankful for all of his efforts in helping me through this journey and I owe him the utmost gratitude for shaping me into a more confident individual.

I would also like to thank my committee members Dr. Richard Mulvaney and Dr. Chris Matocha for their time and effort in support of my research. Also, thanks to Brian Lenell for his guidance in laboratory and teaching techniques.

Finally, I would like to thank my family and friends for their support while working on this project. Their encouragement and reassurance has been paramount in overcoming the adversity I have faced along the way.

Table of Contents

Chapter 1: Introduction and literature review

1.1 General chemistry of phosphorus

Phosphorus (P) gains its name from the Greek word for light-bearing, *phosphoros*. Phosphorus is the 15th element and has an atomic weight of 31. The elemental form was discovered in 1669 by German alchemist Hennig Brandt in his search for the 'Philosopher's Stone,' that would supposedly turn base metals into gold (Ashley, Cordell, and Mavinic 2011). Phosphorus is commonly found in flora and fauna as phosphate. Phosphates are a critical component of nucleotides, which serve as energy storage units within cells (adenosine triphosphate (ATP)), or when linked together, form the nucleic acids DNA and RNA (Emsley 2002; Holtan, Kamp-Nielsen, and Stuanes 1988). Phosphorus is the second most abundant element in mammalian bones after calcium. 80% of P in human bodies are found in bones and teeth, with the remainder located in bodily fluids and soft tissue (Ashley, Cordell, and Mavinic 2011). Major metabolic processes for which P is a major factor include the development and maintenance of skeletal tissues, the maintenance of osmotic pressure and acid-base balance, energy utilization and transfer, protein synthesis, the transport of fatty acids, and amino acid exchange (Hanrahan et al. 2005; NAL 1993; Soetan, Olaiya, and Oyewole 2010). Even though plants are only about 0.2% P by dry weight, P is a critical component in their biochemical processes. Plants also contain DNA and RNA as their genetic "memory units," which contain the codes to build proteins and other compounds essential for plant structure, seed production, and genetic transfer. Phosphorus a vital component of adenosine triphosphate (ATP), which in plants forms in conjunction with CO_2 fixation through photosynthesis (Schachtman, Reid, and Ayling 1998). Adenosine triphosphate fuels plants from the beginning of seedling growth all the way through the formation of seeds and maturity. Adding P to a soil is known to promote root growth, winter hardiness, stimulate tillering, and hasten maturity, depending on the soil's native P availability (Macdonald, Bennett, and Taranu 2012; Ruttenberg 2013).

Many chemical species of P, both inorganic and organic, are found in the environment. Organic P, which is formed primarily by biological processes, consists of a phosphate molecule associated with a carbon-based molecule. Organic P forms include phospholipids, nucleic acids, glycerol phosphates, phosphotidyl choline, proteins, polysaccharides, nucleotide cofactors, and phosphonates (Hanrahan et al. 2005). Phosphate esters, such as the aforementioned phospholipids, glycerol phosphates, phosphatidyl choline, and nucleic acids, which are added to the soil through decomposing microbial, plant, and animal remains (Bower 1949; Dalal 1977), make up only 1-2% of total organic P. Inositol P in various combinations with humic and fulvic acids, or as metal complexes, may account for up to one third of the total organic P in soils (Baker 1977; Grindel and Zyrin 1965; Stewart 1987). The remainder of organic P is associated in chemical compounds with soil humus, which makes up a large TP portion of a soil. Organic P contained in soil biomass, can be converted to inorganic P by mineralization, the process of which is determined by the C:P ratio of the soil. A low C:P ratio dictates that there is sufficient P for both soil microbes and higher plants, resulting in greater gross mineralization than immobilization. A high C:P ratio, however, leaves both plants and microbes with insufficient P and microbes act to scavenge P from the soil, leading to higher gross immobilization than mineralization.

Besides insoluble organic P, the vast majority of P in soils is inorganic. Inorganic P (P_i) occurs in primary minerals, sorbed to hydrous oxides coating and in the interlayers of clays, as secondary precipitates, and in solution as ionic orthophosphate (also known as DRP – Dissolved Reactive Phosphate). As a component of primary minerals, the vast majority of P_i is contained in the apatite group of minerals, with fluoroapatite accounting for approximately 95% of mineral P. In acidic soils, Fe-P and Al-P minerals can be found such as strengite and variscite (Allen and Hajek 1989). Hydrous oxides on the surfaces and interlayers of clay particles possess a highly variable charge dependent on pH. In acidic soils, hydrous oxides tend to be protonated and have a positive charge, which DRP will readily fix to. Neutral and alkaline soils also contain positively charged hydrous oxides associated with clay, just to a lesser extent (Pierzynski et al. 2005). Phosphorus-containing secondary precipitates form when DRP ions in solution encounter metal ions. In acidic soils, Fe, Al, or Mn-P precipitates are likely to form. In alkaline soils, Ca-P secondary precipitates are likely to form. Solution DRP ions (PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$, H_3PO_4) comprise a very small percentage of P_i contained in soils. Species of DRP (conjugate bases of phosphoric acid) protonate and deprotonate depending on pH. The HPO_4^{2-} and $H_2PO_4^-$

species exist between pH ~4-10 and are the only P ions taken up by plants (Mullins 2009). Inorganic P solubility is highest between pH 5-7.

Condensed phosphates, also known as polyphosphates, such as $P_3O_{10}^{5-}$, are more complex inorganic P compounds composed of a chain of many hundreds of phosphate residues linked by high-energy phosphoanhydride bonds (Kornberg, Rao, and Ault-Riché 1999). Polyphosphates are common components of industrial products such as detergents. They are strongly complexed by metal ions and are unstable in water, eventually hydrolyzing to DRP if left uncomplexed. The rate of condensed P hydrolysis to DRP is principally influenced by enzymatic activity of microbes. Condensed phosphates have been utilized in chemical fertilizers because of their rapid conversion to DRP and because they are generally more mobile in soil solution (Olson 1972). The need for hydrolysis, however, can limit their effectiveness for P fertilization.

1.2 Anthropogenic and indigenous sources of phosphorus

1.2a Indigenous sources

Phosphorus is the eleventh most abundant element in the earth's crust. It has an average concentration of 0.1% by weight in the environment, and is thus classified as a trace element (Hansen et al. 2002). Only a fraction of total P (TP) in the environment is concentrated in deposits consisting of phosphate minerals.

Phosphate deposits can be separated into three broad groups, all of which are forms of apatite ($Ca_5(PO_4)_3(OH,F,Cl)$) (Holtan et al. 1988). The three apatite groups include apatite of igneous or metamorphic origin, sedimentary phosphorites, and guano deposits. About 95% of mineral P occurs as fluoroapatite of igneous or metamorphic origin, which is a common accessory mineral in most rock types (Sharpley et al. 2015). The largest and richest phosphate deposits in the world are found in marine marginal trough environments where upwelling of deep ocean waters rich in phosphates effected slow precipitation of sedimentary phosphorites (Liu and Wetzel 1983; Mckelvey 1967). The deposits are characterized by a typical sequence from bottom to top of black shale, phosphatic shale, phosphorite, dolomite, and sandstone. Phosphorite beds can be one or more meters thick, contain 13% or higher P, and can extend over hundreds of km^2. Sedimentary phosphorite deposits constitute over 80% of phosphate rock utilized commercially, compared with ~15% from igneous and metamorphic rocks, and ~5% from guano deposits (Emsley 2002; Smil 2000). Even when in low concentration, P will tend to form independent minerals, with more than 200 natural phosphates having been discovered (Holtan et al. 1988).

The weathering of P-rich rock produces P almost exclusively as DRP (PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$) (Beardsley 2011), which is readily utilized by plants. The mineral apatite, which is a family of phosphates containing calcium, iron, chlorine, and several other elements in varying quantities, contains the large majority of P contained in rocks of various origin (igneous, sedimentary, metamorphic) (Arai and Sparks 2007; Pope, Milligan, and Mau 2002). Behind only inorganic P contained in the Earth's core and mantle, apatite minerals represent the largest pool of P on Earth (Liu and Chen 2002). Large accumulations of guano, which are also considered P-rich rock, are formed on the land surface by the excrement of sea birds and served as the earliest reserves for P fertilizer production (Holtan et al. 1988). Phosphate is not an inexhaustible resource and much of the world's phosphate deposits have already been mined for production (Emsley 2002).

1.2b Anthropogenic sources

As anthropogenic sources, the two broad applications of phosphate rock are in fertilizers and industrial chemicals, with 22% of the phosphate rock used in the USA going into industrial chemicals and the remainder into fertilizer (Cordell, Drangert, and White 2009; Holtan et al. 1988). Industrial phosphates made from phosphate-containing compounds include detergents, toothpaste, pharmaceuticals, and food additives. The preparation of industrial phosphates requires highly pure phosphoric acid produced from elemental P (Cordell et al. 2009), which is obtained by treating a blend of prepared P ore, coke, and silica in large electric furnaces.

Phosphorus fertilizers are available in several forms, all of which are created using rock phosphate. Illinois fertilizer consumption is summarized in Table 1.1. Total P content in synthetic and animal based P fertilizers is varied depending on the mineralogy and or sources of manure. Phosphate fertilizers are most commonly produced in the solid or granular form, but are also produced in liquid form. Superphosphate (i.e., monocalcium phosphate (MCP) ($Ca(H_2PO_4)_2$), which is the most basic form of P fertilizer, is produced by treating rock phosphate with sulfuric acid, and contains ~9% of P by wt. (Ashley et al. 2011). Once applied to soils, it is often found in the soil solution in the form of $Ca(H_2PO_4)_2 \cdot H_2O$. Triple superphosphate ($3CaH_4(PO_4)_2$), which is produced by treating rock phosphate with phosphoric acid, or a mixture of sulfuric and phosphoric acid, has approximately 20% of TP by wt. The P content in monoammonium phosphate (MAP) ($NH_4H_2PO_4$), and diammonium phosphate (DAP), ($(NH_4)_2HPO_4$), is ~ 22% and 20%, respectively. Rock phosphates, which only contain ~15.5% of TP by wt., are insoluble, making them less marketable for agricultural uses.

Manures can be effective fertilizers, as they are high in organic P content, especially manures from grain-fed animals (Holtan et al. 1988). Total P content in manure is, however, highly variable with nearly 70% of the TP in labile form, which can be converted to soluble, biologically available P via mineralization by soil microbes (Shen et al. 2011). Phosphorus concentrations in dairy manure range from 4-4.7 mg/L as compared to 7-30 mg/L in poultry manure. Because of the variable nature of the P content of manure, it has been proven to be a less effective fertilizer than synthetic fertilizers like DAP. In a study by Kleinman et al. (1998), it was found that 100% of TP in DAP fertilizer was water-soluble, whereas 33, 26, and 27% of the TP in dairy manure, poultry manure, and swine slurry, respectively, were water soluble. Water-soluble and TP concentrations in DAP were overall 6 to 83 times greater than in the manure sources.

Manure amendment rates vary in the Midwest. In an experiment by Tomer and Liebman (Tomer and Liebman 2014) near Boone, Iowa, designed to model Midwestern cropping systems, an average of 19.5 kg P/ha in the form of cattle manure was applied to 2YS (two-year corn-soybean), 3YS (three-year corn-soybean-small grain/red clover), and 4YS (four-year corn-soybean-small grain/alfalfa-alfalfa) systems. Joosse and Baker (Joosse and Baker 2011) reported a manure amendment rate for the Lake Erie basin at 11 kg P/ha. An experiment conducted by Toor (Toor 2009), designed to resemble a typical agricultural system utilizing poultry manure, amended several soils with a typical application rate of 90-150 kg/ha. A similar study conducted by Kaiser et al. (2010) on poultry manure application in Iowa noted amendments of 53-104 kg P/ha, slightly lower than the values suggested by Toor (Toor 2009).

1.3 Phosphorus distribution in aquatic and terrestrial environments

1.3a Background phosphorus levels

Given the low solubility of indigenous minerals (e.g., apatite) and strong adsorption of P in soils (Arai and Sparks 2007), annual losses of P due to leaching and runoff in natural, non-agricultural areas of the Midwest, are only 0.01-0.6 kg P/ha (Clesceri, Curran, and Sedlak 1986). The natural background level of TP in surface fresh waters and rainfall in the Midwest is generally < 0.03 mg/L and 0.01-0.06 mg/L, respectively (Dunne and Leopold 1978; Smil 2000). In pristine rivers, TP concentrations are a median of 0.016 mg P/L (Litke 1999). Annual wet and dry deposition of P in the Midwest totals no more than 0.75 kg P/ha/yr (Smil 2000).

The majority of P found in groundwater of the Midwest is in the form of DRP and colloidal P form. Concentrations of P in groundwater of agriculturally affected areas are generally reported between 0.03-0.16 mg P/L (Burkart et al. 2004). In a national survey conducted by Mueller et al. (1995), groundwater in urban areas was found to average 0.02 mg P/L, while concentrations in aquifers used as drinking water sources, and thus assumed to be unaffected by pollution, were found to average 0.01 mg P/L (Nolan and Stoner 2000).

Because of long-term P fertilizer amendment and use of P-enriched detergents in urban systems, agricultural soils and urban water resources in Midwestern states generally contain a greater concentration of TP (i.e., sum of DRP and colloidal P) than remote non-agricultural systems (Holtan et al. 1988). The level of natural background P and anthropogenic P (i.e., systems impacted by agricultural practices and/or wastewater systems) in urban and agricultural systems is summarized in Table 1.2.

1.3b Phosphorus in Midwestern agricultural soils

Midwestern agricultural soils on average receive 34.1 kg P/ha yr in fields where corn is grown, 27.2 kg P/ha yr in fields where soybeans are grown and 27.9 kg P/ha yr in fields where wheat is grown for an average of 18.2 kg P/ha yr applied in the form of P fertilizer or manure (Sohngen et al. 2015). This is an equilibrant to annual application of 35,665 kg of P. The relationship between P fertilizer application and STP is proportional, regardless of geographic location (Shen et al. 2011; Wortman et al. 2005; Zhang et al. 2004).

While natural background TP in soils range from 245-350 mg/kg in the surface 8 in of soil (Pope et al. 2002; Sims, Simard, and Joern 1998), TP in agricultural soils of the Midwest generally ranges from 897 to 2,466 mg/kg in the surface 8 in (Guertal et al. 1991; McDowell and Wilcock 2015). Most of the P in soils is unavailable for plant growth because of 1) its strong adsorption to fine-grained loess and clay particles inherited from glacial debris and 2) the formation of insoluble calcium phosphates (Arai and Sparks 2007). However when the soil environment is high or saturated with P, P enrichment of surface waters occurs under specific environmental conditions (e.g., reduced condition, frequent runoff events, high pH) that favor transport of P to surface and ground waters (Arai and Sparks 2007). In general, P in the soil solution of most Midwestern agricultural soils is between <0.01 and 1 mg/L (Mullins 2009). That being the case, an entire acre generally contains less than 0.18 kg of P in solution throughout the growing season at any one time (Stewart 1987). When surface soils with high P loadings are eroded by surface runoff (Sharpley, Mcdowell, and Kleinman 2001) and/or subject to intense leaching through the soil profile via tile-drainage systems (Gentry et al. 2007; Sims et al. 1998), P is readily transported to natural and man-made water bodies (Ahiablame, Chaubey, and Smith 2010; Sharpley, Smith, and Naney 1987; Vidon and Cuadra 2011).

1.3c Surface runoff

The transport of P in surface runoff occurs in both dissolved and colloidal forms, accounting for 41-64% of P transported from cultivated soils (McDowell and Sharpley 2003b). Loss of P from the soil surface is controlled by multiple factors including how the soil is being used, crop management, and transport factors including rates of surface runoff and erosion and channel processes (McDowell et al., 2001). Small particles, which contain a greater percentage of P by mass, are selectively eroded during surface runoff (Sharpley and Smith, 1993; McDowell et al., 2001; Shigaki et al., 2006). In a rainfall simulation experiment conducted by Shigaki et al. (2006), average TP losses from soils of differing compositions was 0.48 mg/L, amounting to an average of 0.74-22.71 kg TP/ha lost.

1.3d Tile systems

Tile drainage is a commonly used practice in Midwestern agricultural soils. In fact, Illinois has more tile-drained acres than any other state. It has been estimated that tile drains account for 46-59% of the DRP export from agricultural soils with an average transport of 0.9 kg /ha yr (Kovacic et al. 2000). Dissolved reactive P accounts for 50-73% of the TP load exported from agricultural fields by tile drainage, leaving ~25% of P exported by tile drainage as colloidal P. Colloidal P is eroded from agricultural soils and is lost in runoff (Sims et al., 1998). Annual TP export from Midwest agricultural fields by tile drainage amounts for between 0.46-1.56 kg/ha (Gentry et al. 2007). There is relatively high variability from year to year in TP export from tile drains, which is related to precipitation frequency and intensity.

In the case of three Northwestern Indiana locations, it was reported that average colloidal P concentrations in drainage ditches were between 0.05 and 0.11 mg/L, with the highest concentrations of colloidal P measured above 6.0 mg/L (Sims et al. 1998). These colloids can release DRP into the water column, ranging from 0.03-0.58 mg/L (Ahiablame et al. 2010; Gentry et al. 2007; Sims et al. 1998). Colloidal P is also subject to leaching in agricultural subsoils, but is the main contributor of DRP in drainage ditches (King et al. 2014; Vidon and Cuadra 2011; Weld et al. 2001).

1.3e Phosphorus in urban systems

Total P concentrations in wastewater and septic systems can be found in even higher concentrations than in areas of heavy agricultural activity. Concentrations of TP average 1.2-12.1 mg/L in septic systems, but can be as high as 210 mg/L (Waschbusch, 1993). Human excrement and other contents of septic systems are the main contributions to elevated levels of P in urban wastewater. Based on an anticipated wastewater generation rate of 60 gallons per person per day in urban areas, and 0.75 kg P/capita annually, the resulting TP concentration in urban wastewater in the Midwest is between 4-15 mg/L (Holtan et al. 1988; USEPA 1996; Waschbusch 1993). This is substantially greater than the concentration of TP (0.1-0.4 of TP mg /L) in surface waters in urban areas associated with sources other than wastewater (Litke 1999; USEPA 1996; Waschbusch 1993).

Interestingly, average TP concentrations from all residential sources (e.g., lawn, streets, driveways, sidewalks, parking lots, roofs, parks, and woodlots) and for commercial areas are as high as ~3,007 mg/kg and ~3,580 mg/kg, respectively. Tree canopy, other vegetation, and lawn fertilizer (banned in Illinois and other states since 2010) are the major sources P in urban environments outside of human wastewaters (Waschbusch 1993).

1.4 Environmental consequences

Phosphorus is often the limiting nutrient to the growth of vegetation in surface freshwater bodies, meaning that there is often not enough P present for optimal growth. In these water bodies, increasing P concentrations, specifically DRP, will increase the growth of aquatic vegetation (McDowell et al. 2004; Sharpley et al. 1987). The case of P being a limiting nutrient is becoming increasingly rare, however, as human activities such as agriculture and the production of wastewater introduce massive amounts of anthropogenic P into surface waters, the levels of which would not occur in natural systems. When concentrations of P exceed what is required for optimal plant growth, excessive growth of aquatic vegetation, most notably algae, will occur (Viviani 1992). Negative consequences to the affected water body ensue such as the depletion of dissolved oxygen, reduction of light transmission and water clarity, and the production of algal toxins, which in concert act to deplete fish populations, reduce water quality for recreation, and impart undesirable odors and tastes that result in an increased cost of treating water for domestic use. The process of progressive increases in surface water P concentration that results in the deterioration of water quality through overstimulation of aquatic vegetation is known as eutrophication.

Eutrophication is a naturally occurring process, but is greatly accelerated with the intensification of agriculture or increased dumping of P-rich wastewaters into freshwater systems (Weld et al. 2001). Changes in water quality that may take centuries to occur naturally, can take just a few decades or less with the amounts of anthropogenic P in fresh water systems associated with intensive agriculture, industrial production, and dense human population (Volterra et al. 2002). Accelerated eutrophication is one of the most problematic and persistent surface freshwater quality problems in the United States. The

U.S. Environmental Protection Agency (USEPA) has identified excess P as the greatest impediment to achieving water quality goals stated in the Clean Water Act (David and Gentry 2000). Over 50% of streams and 40% of lakes and reservoirs in the Midwest have been rated as impaired in relation to an overabundance of P (USEPA 2002). Eutrophication, in fact, is the most widespread water quality problem in the US and many other nations (Carpenter et al. 1998).

Eutrophication stimulates an explosive growth of phytoplanktonic algal blooms, scums, and mats, which are composed primarily of cyanobacteria. Cyanobacteria deplete water of oxygen as they die and are oxidized (decomposed) by aerobic bacteria (Hecky and Kilham 1988). The consumption of oxygen by bacteria during the decomposition of algal blooms is attributed to their biochemical oxygen demand (BOD). The resulting oxygen depleted (hypoxic) water can no longer support fish and other aquatic organisms, which quickly die out. Anaerobic bacteria, which are able to thrive in anoxic conditions, establish large populations in bottom layers of the water body, subsequently producing gases such as hydrogen sulfide (H_2S) and methane (CH_4), which further reduce water quality (Correll 1998). If hypoxia occurs in the denser, cooler waters at the bottom of a water column, especially at the sediment/water interface, P release from sediments may occur, as ferric hydroxides, which are often bound to P compounds, are reduced to the ferrous form, thus weakening their bond with P. The released P in turn fertilizes algal blooms further and amplifies the effects of eutrophication (Vaquer-Sunyer and Duarte 2008).

Along with cyanobacteria, the algae-consuming heterotrophic dinoflagellate *pfiesteria* thrives in surface waters that have excess P (Dolah 2000; Glasgow H.B. and Burkholder 2000). While *pfiesteria* garners more attention in Mid-Atlantic regions, populations of *pfiesteria* have been detected in estuaries of the Gulf of Mexico as a result of eutrophic conditions produced by P influxes from Midwestern agriculture (Burkholder et al. 2001; Rublee et al. 2005). *Pfiesteria* display chemosensory “ambush-predator” behavior, in which their release from benthic dormant cysts is stimulated by the detection of their prey’s presence, followed by the rapid devouring of the prey’s live tissue (Burkholder and Glasgow 1997). Some strains of *pfiesteria* also produce hydrophilic toxins that can adversely affect mammalian cells as well as marine organisms. Among impacts on humans, fishermen during *pfiesteria* related fish kills, have reported multiple ailments including cognitive dysfunction manifested as short-term memory loss, nausea, migraine headaches, skin sores, and loss of hearing (Arai and Sparks 2007; Burkholder and Marshall 2012).

Pfiesteria's effect on fishermen illustrates that aquatic species are not the only organisms affected by poor water quality as a result of eutrophication. As cyanobacterial blooms die or are ingested, they release water-soluble neuro- and hepatotoxins (liver toxins), which can kill livestock and pose a serious health risk to humans and land animals (Arai and Sparks 2007; Carpenter et al. 1998; Shaw, Moore, and Garnett 2003). Toxins can be found either free in the water where the bloom occurs or bound to the algal or cyanobacterial cells from which they were produced. When cyanobacterial cells are young, during their growth phase, 70 to 90% of toxins are cell bound, whereas when the cells are aged, up to 70% of toxins can be found free in the surrounding water (Bláha, Babica, and Maršálek 2009). Toxins can be extremely difficult to remove by the normal processes used in treating water for drinking purposes. The resistance and persistence of toxic compounds produced by cyanobacteria depends on the nature of the compound. Certain toxins can withstand intense physico-chemical and biological constraints, especially high temperatures (Viviani 1992). In dark and cool freshwaters, toxins can persist for several months to years. To date, there are more than 50 identified species of cyanobacteria that are able to produce toxins. The most frequently observed in freshwaters of the Midwest include *microcystis, anabaena, aphanizomenon, oscillatoria, nodularia,* and *nostoc* (Vasconcelos 2006). People and land animals are exposed to these toxins through the consumption of contaminated drinking water, direct contact with fresh water, or the inhalation of aerosols. Once ingested, toxins induce damage on the molecular level by damaging individual cells, tissues, and organs (Skulberg, Codd, and Carmichael 1984). The nervous, digestive, respiratory, and cutaneous systems may be especially damaged with secondary effects being observed in numerous organs (Codd 2000).

The majority of freshwater eutrophication research has focused on lakes and reservoirs, but P enrichment of flowing waters, like rivers and streams, is also of great concern. As discussed previously, the mean TP concentration of stream water was found to be 0.13 mg/L (Smith, Alexander, and Wolman 1987), well beyond the mesotrophic-eutrophic TP boundary of 0.075 mg/L proposed by Dodds et al. (1998). The prevailing view for many years was that moving bodies of water are insensitive to nutrient inputs based on the assumption that other physical, chemical, and biotic factors restrict the effects of nutrient enrichment on algal growth in rivers and streams (Hoyer and Jones 1983). This, however, was found not to be the case in subsequent studies by Carpenter et al. (1998), who observed that concentrations of TP were highly elevated up to 4 km downstream of a point source and that periphyton growth on submerged artificial substrates increased threefold in response to the elevated concentrations. Similar results were found by Newbold et al. (1981), who enriched two sections of an oligotrophic stream for 95 days with inorganic P. Stream water P concentrations were increased by over an order of magnitude, and resulted in significantly increased benthic algal biomass, increased rates of detritus decomposition, and increased abundances of macroinvertebrate consumers. Multiple other studies of similar type also noted increases in downstream periphyton biomass following inorganic P additions, and the stimulation of primary productivity subsequently cascaded into reduced stream consumer populations (Hershey et al. 1988; Peterson, Hobbie, and Corliss 1983). Despite the residence time of P being significantly lower in flowing waters, increased P concentrations still have drastic negative effects on stream and river ecosystems.

1.5 The phosphorus cycle

Biological production is contingent on the availability of P. Phosphorus that is fixed in bedrock, soils, and sediments is not readily available to organisms, however, and must go through conversion to DRP in order to be utilized first by plants, and subsequently by animals higher in the food chain. This process occurs through multiple geochemical and biochemical processes that work in unison to cycle P through the terrestrial and marine environments. The global P cycle consists of four primary components; tectonic uplift and exposure of marine phosphorites, erosion and chemical weathering of P-rich rocks producing soils and providing DRP to rivers, riverine and subsurface transport of P from soils to lakes and oceans, and marine sedimentation of P associated with colloidal and organic P, which can then be uplifted and exposed, beginning the cycle anew (Ruttenberg 2013).

The P cycle begins with the weathering of P-rich minerals in parent rock, a slow process that prior to pronounced human influence, maintained P balance in soils along with atmospheric deposition (Follmi 1996; Stewart et al. 2002). The total reservoir of P contained in crustal rocks has been estimated to be approximately 1.3×10^{20} mole P (Ruttenberg 2013). Weathering of primary P-minerals releases P in DRP form, which is readily usable by plants. Anthropogenic deposition of P to soils is currently the dominant process of P deposition (Filippelli 2008). Modern P deposition is carried out by four mechanisms; applications of animal manures and biosolids, the degradation of plant residues, atmospheric deposition, and the application of mineral fertilizers (Gužys 2013; Liu et al. 2008). Animal manures and biosolids contain P associated in organic compounds, which become part of the organic P pool of a soil, and inorganic DRP. Plant residues also contribute organic P compounds to the organic P pool as they undergo decomposition near the soil surface (Bolan et al. 2005). The total reservoir size of P in soils (< 60 cm deep), has been estimated to be $3100\text{-}6450 \times 10^{12}$ mole P. Atmospheric deposition contributes a very small influx of P to soils, but what it does contribute is DRP that becomes part of the soil solution (Haygarth et al., 2013). By far the smallest reservoir of P, the atmospheric P pool is estimated to consist of 0.0009×10^{12} mole P (Ruttenberg 2013). Mineral fertilizers have been designed to contain DRP that readily becomes part of the soil solution and is usable by crops. Agricultural soils often receive P in great excess of what is actually required for optimal crop growth, however, leading to a great surplus of soil P and P loss in runoff and leaching (Carpenter et al. 1998). Excessive P is promoted by the practice of farmers using fertilizer as an insurance policy to insure that their yield goals are met.

Phosphorus can become part of the atmosphere as a constituent of soil particles after erosion by wind, in spores of fungi and pollen, on sea-salt particles derived from the ocean, or as emissions from industrial and agricultural activities (Graham and Deice 1979). The great majority of P that becomes part of the atmosphere is derived from erosion of crustal material. Based on different deposition rates, the residence time of P in the atmospheric-P cycle associated with terrestrial and marine environments differ, with estimated times of 3 days and 1 day respectively (Robinson et al. 1971). Roughly 25% of the crustal P that becomes part of the atmosphere is transported to the oceans, with Saharan dust accounting for about half of that total (Chen et al. 2006). Overall, the continental portion of the atmospheric P-cycle accounts for a much larger flux than does the oceanic with contributions of 105×10^{10} g P/yr being transported from land to ocean and only 3×10^{10} g P/yr being transported from oceans to land (Graham and Deice 1979). A study by Blanchard and Keith (1963) indicates that the ratio of dry to wet deposition from the atmosphere is roughly 2:1.

Dissolved reactive P ions in the soil solution are most subject to sorption reactions with soil particles that generate anion exchange capacity (AEC) such as Al and Fe-oxides, clays (e.g., kaolinite) under acidic conditions, calcareous minerals in alkaline conditions, and amorphous material (Holtan et al. 1988; Stuanes 1982). The mineral content of a soil has a defined effect on P-sorption ability, as a higher content of oxides or amorphous material will result in greater sorption of P while less weathered and organic soils have low P-sorption capacities (Liu and Wetzel 1983). As clay content increases in a soil, the P-sorption capacity will increase due to the large surface area onto which P-sorption can occur (Räty et al. 2009). As a general principal, P-sorption will also increase as temperature increases due to reactions with soil components becoming thermodynamically more favorable (Arai and Sparks 2007). Opposing conditions can develop where desorption of P from insoluble compounds is favored and DRP is released to the soil solution. Desorption will occur when anions such as silicates, carbonates, sulfates, arsenate, and molybdate compete with P for positions on anion exchange sites, which results in the displacement of P into solution (Holtan et al., 1988). Higher organic matter content increases P desorption by replacing P on anion exchange sites and forming humus coatings on Fe and Al-oxides (Stewart 1987). The oversaturation of a soil reduces P-sorption by increasing the solubility of P that is bound to Fe/Al-oxides and amorphous minerals (Heathwaite et al. 2005).

Soils in the Midwest are primarily Mollisols and Alfisols, which have differing capacities to adsorb P based on Al/Fe-oxide content, pH, clay content, type of clay present, and organic matter content. It is important to differentiate between P-fixation capacities of different soil orders to gauge the rate and amount specific soils effect P concentrations of surface waters. Fixation of P under acidic conditions ($\text{pH} < 5.5$) is primarily dependent on the pH-dependent charge of soil colloids. Exposed hydroxyl groups associated with clay minerals or Fe/Al-oxides are protonated in acidic conditions and generate an anion exchange capacity (AEC) (Stuanes 1982). Under alkaline conditions, P forms complexes with Ca^{2+} in soils with high calcareous content, a process which is maximized at pH ~ 8.0 (Arai and Sparks 2007). Functional groups associated with soil organic matter (SOM) have the ability to adsorb P, but only when protonated under highly acidic conditions, so SOM should not be considered a source of P fixation by itself. Midwestern soils are generally lightly weathered having formed from recently (on the pedogenic timescale) deposited glacial debris and loess. They contain high contents of 2:1 type clays including montmorillonite, vermiculite, illite, and limited chlorite and contain only small concentrations of 1:1 type clays; e.g. kaolinite, and hydrous oxides, which are products of more intense weathering. The slight degree of weathering also leads to limited AEC in neutral to basic pH conditions, so comparatively, Midwestern soils have low P-fixation capacities, but there is variation among soil orders.

Alfisols are a dominant soil order in the Midwest, occurring in most Midwestern states as Udalfs, and in some as Aqualfs. They are poorly weathered soils formed in forested environments with high contents of 2:1 clays (Allen and Hajek 1989; Quigley 1961), high SOM content, although not as high as Mollisols, acidic to neutral pH (~ 5-6), and low P-fixation capacity. Mollisols are also widespread, occurring as Udolls and Aquolls in the western Midwest, and as Aquolls in the eastern Midwest. They contain almost exclusively 2:1 clays, are high in SOM due to being formed under grasslands, have neutral pH (~6.5) (Buol et al. 2011), and are also low in P-fixation capacity. Entisols are dispersed throughout the Midwest as Aquents, Psamments, and Fluvents (Liu et al. 2012). Being the least weathered soil type, Entisols contain little clay, are low in SOM, are at neutral pH (~7.3), and have very little P-fixation capacity (Ciolkosz et al. 1989). Spodosols occur in northern Michigan and Wisconsin as Orthods. Formed in humid areas dominated by coniferous trees, Spodosols are characterized by acidic pH (~5), average SOM concentration, and primarily the 2:1 clay minerals illite and smectite (Ross and Kodama 1974). As the most acidic of Midwestern soils, Spodosols have the highest capacity for P-fixation. Inceptisols occur in northern Minnesota, southern Indiana, eastern Michigan, and central Wisconsin. Much like Entisols they are recently formed soils that contain low contents of clay, SOM, are at a near neutral pH, and have very low P-fixation capacities (Castro and Torrent 1995). Histosols also occur in the northern Midwest, primarily in the wet regions associated with recent deglaciation of Michigan, Wisconsin, and Minnesota.

Due to their formation in settings such as swamps and bogs, Histosols are primarily composed of SOM. Under completely saturated anaerobic conditions, which is typical of Histosol formation, pH levels are near neutral, but upon drainage and subsequent oxidation, pH can drop to as low as 3. P is not readily fixed in saturated Histosols due to low clay content (Bridgham et al. 2000).

Solubility products form when DRP in the soil solution reacts with metal cations to form solid minerals. In contrast, dissolution reactions occur when a P-containing mineral dissolves and releases P into solution (Liu and Wetzel 1983). Together, these reactions profoundly influence the P content of the soil solution. As plants take up P from the soil solution, dissolution reactions act to counterbalance and replenish the solution, a process which will tend to happen when the soil solution is undersaturated in P (Holtan et al. 1988). When the soil solution is oversaturated with P, as might be the case after the application of a fertilizer, P-minerals will form via precipitation reactions whereby P is removed from solution (Ruttenberg 2013). The solubility of P-minerals is dependent on soil pH. The optimum soil pH for P availability in soil solution is ~6.5. In alkaline conditions, P will tend to react with Ca^{2+} to form minerals such as apatite, while in acidic conditions the genesis of Fe and Al-P minerals such as strengite and varescite will dominate (Jackman and Black 1951).

Reactions between the organic-P reservoir of a soil and soil solution also play a critical role in the amount of P in the soil solution. Organic P compounds primarily reside in microbial biomass, plant residues, and humus, all of which release DRP into the soil solution via decomposition in a process termed mineralization (Bolan et al. 2005). While organic P inputs to soils from microbial and plant sources consist primarily of nucleic acids and phospholipids, inositol phosphates comprise the largest proportion of organic P compounds in soils (Turner et al. 2002). This disparity can be attributed to the fact that organic P compounds with more P groups are more readily sorbed to soil particles due to higher charge density. Sugar phosphates and diesters (e.g. nucleic acids and phospholipids) are only weakly adsorbed and are vulnerable to microbial degradation while inositol phosphate is strongly sorbed to clays or precipitated in insoluble Fe, Al, or Ca-oxides (Celi et al. 1999). Soil microorganisms may also act to remove DRP from soil solution and convert it to organic P for incorporation into their living cells in a process known as immobilization.

Net mineralization or immobilization is determined by the C:P ratio of a soil. When the C:P ratio is less than 200:1, net mineralization prevails, indicating that there is enough P in the soil to sustain both plants and microorganisms. At C:P ratios between 200:1 and 300:1, immobilization and mineralization rates are fairly equal. When C:P ratios exceed 300:1, conditions are such that there is not enough P to support both microorganisms and plants, so microorganisms will scavenge the soil and net immobilization will prevail (Brady and Weil 2002). Soil C:P ratios vary primarily with the amount of microbial biomass present in a soil, which is dependent on SOM content. Microbial immobilization of P is a more significant process than immobilization of other elements illustrated by the fact that microbes often contain 20-30% of the total organic P pool, considerably higher than the proportions of C (~1-2%) and N (~2-10%) contained in microbes (Haygarth, Bardgett, and Condron 2013). Other factors that affect mineralization and immobilization are temperature, moisture, and soil aeration.

Plants take up P from the soil solution in order to support vital cellular functions and act as a primary reservoir of P in the soil/biota system. The reservoir size of P in plant tissues has been estimated to be 83.9-96.8 $\times 10^{12}$ mole P (Ruttenberg 2013). Mycorrhizal fungi play an important role in P acquisition for approximately 80% of terrestrial plants. Mycorrhizae produce phosphatase enzymes that cleave ester bonds that bind P to organic matter. They also produce low molecular weight organic acids that enhance the availability of soil P by increasing the weathering rates of Ca, Fe, and Al-P minerals (Haygarth et al. 2013). When plants are removed from the soil via such actions as crop harvests or forest cutting, it results in a net loss of P from the soil, as the natural process of decomposition and return of P to the soil does not take place (Liu and Wetzel 1983). Phosphorus is also lost from the soil through leaching, which tends to occur in soils that are oversaturated with P. This is typical of agricultural soils that have received a vast surplus of P in the form of mineral or manure fertilizers and have reached their P fixation threshold (Gužys 2013). DRP in soil solution is transported downwards through the soil column and is incorporated into groundwater, which flows in directions influenced by local hydrology. The majority of P lost from soils, especially in agricultural systems, takes place via overland erosion and runoff (Holtan et al. 1988). Colloidal P is often in high concentration on the surface of agricultural soils due to the application of fertilizers. During precipitation events, large amounts of colloidal P and DRP will incorporate into overland flow and move towards the nearest aquatic sink (Compton et al. 2000).

Upon exiting the soil system, P becomes part of an aquatic cycle, first joining stream or river systems adjacent to the soil system. The majority of P in rivers and streams is in colloidal inorganic form, specifically P occurring in apatite and other minerals and P adsorbed to Fe/Mn oxides/hydroxides (Eckert and Nishri 2014). Upon reaching a coastal area, the colloidal load of rivers is quickly deposited in estuarine and coastal shelf environments. Clay particles contain Fe/Al oxyhydroxides on their surface, which gives them a high capacity to adsorb P from freshwater solutions (Paytan and McLaughlin 2007) and subsequently release P to the ocean upon an increase of salinity in the estuarine environment. It has been estimated that the total load of P that desorbs from clay particles is 2-5 times more than the DRP load that enters the oceans via rivers (Slomp and Van Cappellen 2006). Riverine organic P is subject to multiple fates upon reaching coastal environments including entrapment in estuaries via flocculation or the process of photohydrolysis. The intensity of the P flux of a river is dependent on climate, topography, and the rock and soil composition of the drainage area (Delaney 1998).

Most P in the open ocean will be found in DRP form with the majority located in deep waters (Slomp and Van Cappellen 2006). Dissolved reactive P resides in the ocean for an extended period, estimated to be between 20- and 100-thousand years. Cycling of P between shallow and deep waters within oceans occurs on much shorter time scales. Deep water P turnover is estimated to take approximately ~1500 years, while surface water P turnover is estimated to take only 1-3 years (Ruttenberg 2013). The ocean surface reservoir of P has been estimated to be 87.4×10^{12} mole P, while the deep ocean contains a reservoir of 2810×10^{12} mole P. Living matter in the marine environment contains P as a major biogenic element comprising a reservoir estimated to be $1.61\text{-}4.45 \times 10^{12}$ mole P (Ruttenberg 2013). Large accumulations of P-rich benthic sediments generated by the death of microorganisms such as phytoplankton and zooplankton, upon undergoing diagenesis, can form sedimentary P-rich rock known as phosphorite (Dyhrman, Ammerman, and Van Mooy 2007). Phosphorites are an important aspect of the global P cycle, as over geologic time they are uplifted and exposed, becoming part of the terrestrial environment. Phosphorite deposits are the main source of P minerals for the manufacture of agricultural fertilizer, with extensive mining operations taking place around the world (Follmi 1996).

1.6 Current management and regulations of P in aquatic and terrestrial environments

Current management and regulations for P in aquatic and terrestrial environments consist of a combination of efforts including agricultural P management, wastewater treatment, and the remediation or protection of aquatic systems that are prone to eutrophication. Agricultural soils have been recognized as the biggest source of P to surface waters, and as such require strict regulation and monitoring in order to ensure P is not applied in great excess of crop needs, however historical and modern P inputs are still often excessive (Beegle 2005; Kleinman et al. 2011; Shepard 2005).Wastewater generated in urban areas, consisting of a combination of excreta, detergents, and industrial byproducts, is also of great concern when it comes to the transport of P to aquatic systems. Wastewater treatment plants thus have strict guidelines for the concentration of P in effluent they release (Bowker and Stensel 1990; Morse et al. 1998; Rybicki 1998; Yeoman et al. 1988). Regulations and technologies to limit P transfer to aquatic environments are a relatively recent development. Numerous remediation techniques and management strategies have been developed to try and combat the effects of eutrophication, and when applied in unison, they can greatly hasten the time it takes for an aquatic system to recover (Benndorf et al. 2002; D'Arcy and Frost 2001; Jeppesen et al. 1990).

1.6a Agricultural management and related regulations

Agricultural P management is of paramount importance in regulating the amount of P entering aquatic systems, as diffuse P pollution from agricultural soils has been identified as the main contributor to anthropogenic eutrophication. Dealing with the issue of P pollution from agriculture begins with identifying and managing P sources at individual field scale (Kleinman et al. 2011). Major sources of P in agricultural fields include recently applied chemical fertilizers, manure, and legacy P in soils from previous fertilizer applications. Phosphorus transfer to aquatic environments is at a maximum during the first precipitation and runoff events following fertilizer or manure application (Austin, Prendergast, and Collins 1996). Soil erosion presents the greatest concern to most P mitigation programs. The concentration of P attached to particles (colloidal P) in runoff is several times greater than DRP. The magnitude and duration of P transfer during runoff events can be reduced by fertilizer and manure application methods that promote rapid or immediate incorporation of P into the soil (Sharpley 1985). Other agricultural methods that can aid in P loss prevention include precision agriculture, best management practices, and assessment of on-farm nutrient resources.

The use of precision agriculture techniques has the ability to drastically reduce the impact of agricultural P loss. Precision agriculture implements techniques that help farmers adjust P fertilization rates in response to soil test phosphorus (STP) concentration variability in individual fields (Iho and Laukkanen 2012). Instead of applying fertilizers and manures on predetermined estimates, precision techniques allow for accurate calculation of P-fertilizer requirements within different areas of agricultural fields, which corrects for over-application in some areas, and under-application in others (Goss 1998). Precision agriculture has yet to be widely adopted, however, in the US only 11% of corn areas, 7% of soybean areas, and 3% of wheat areas currently apply technologies that adjust application rates to local field conditions (Srinivasan 2007).

Best Management Practices (BMPs), otherwise known as nutrient management plans, are incentive-based conservation plans developed for individual farms by the USDA that aim to reduce nutrient input to aquatic environments (University of Wisconsin 1995). Plans are site-specific and target areas of high nutrient and sediment runoff. Many Midwestern states require crop and livestock producers to comply with agricultural performance standards for manure, chemical fertilizers, and other nutrient sources (Shepard 2005). A list of common source and transport measure BMPs are listed in Tables 1.3 & 1.4. Nutrient management plans help to optimize the use of on-farm sources of nutrients such as manure and residual nutrients from previous crops. Theoretically, this would allow for a reduction in commercial fertilizer use while maintaining soil productivity and crop yields (Beegle, Carton, and Bailey 2000). The first element of a nutrient management plan in relation to P is accurate and extensive testing of soil P levels, or STP. It is a requirement of active BMPs that all cropland fields are to be tested, or have been tested within the past four years (University of Wisconsin 1995).

Soils in the Midwest are primarily at or near a neutral pH, or slightly acidic (pH 5-7). With that fact in mind, it is no coincidence that the STPs most commonly used on Midwest farms are the Bray P-1 soil P test and the Mehlich III soil P test. Table 1.5 shows the soil mineral properties that affect the selection of an appropriate STP. Organic P is not targeted by STP extractants. The Bray P-1 method involves the extraction of soil P via application of dilute acid fluoride. This is most effective in neutral and acid soils (pH < 7.4). The fluoride ion promotes P desorption by decreasing aluminum activity via the formation of Al-F complexes (Ketterings and Flock 2005). It is less effective in alkaline soils due to acid neutralization by calcium carbonate and formation of insoluble CaF_2-P complexes. A Bray P-1 value of 25 to 30 mg P/kg is generally considered optimum for plant growth (Pierzynski 2000). The Mehlich III extractant method also employs dilute acid and fluoride, but includes acetic acid, which increases its buffering capacity (Ketterings and Flock 2005). It is a multi-element extractant, which is effective in acid soils and alkaline soils. It is generally more effective than the Bray P-1 test for soil extraction, as indicated by consistently higher P concentrations (Mallarino 2003). When using the Mehlich III test, the optimum concentration of P for crop growth is considered to be between 45-50 mg P/kg (Pierzynski 2000). The Olsen extractant method differs from both the Bray and Mehlich methods, as it employs a buffered alkaline solution in order to extract P. Sodium bicarbonate solution decreases the concentration and activity of Ca^{2+} and Al^{3+} thereby increasing P solubility (Iatrou et al. 2014). It is most effective in alkaline and calcareous soils. Farmers should keep optimum P values in mind when they plan for fertilizer amendments. The goal should be to reach, but not exceed, these values. Nonetheless, it is often the case that many Midwestern soils are far above optimum STP levels.

There are multiple regulations for agricultural BMPs in the Midwest based on the condition of surface water bodies in the geographical area. The National Nutrient Criteria Program, run by USEPA Regional Offices of Water, sets standards called Regional Nutrient Criteria (Sharpley et al. 2006). In order to set these criteria, the background levels of P in the local environment are measured from pristine streams, lakes, reservoirs, and other surface waters, which then are set as a benchmark against which similar watercourses in the area can be compared (Gibson, Carlson, and Simpson 2000). The difference between the reference condition for P concentration and measurements taken from other surface waters in the area reflects the extent of management required to protect or restore the nutrient quality of the water to its natural state. Regional Nutrient Criteria are significant in that they determine attainable targets for nonpoint P reduction for resource managers who are planning conservation farming practices (Havlin et al. 2005). With target conditions in mind, watersheds can be divided into constituent sub-watersheds and particular P goals can be set among tributary systems. Individual farmers can then target P load amounts as their share of water quality protection in their immediate area.

In order to meet Regional Nutrient Criteria, farmers must closely manage P application to their soil. Soil tests for P are a component to this management, as they identify current P levels in soils and allow farmers to calculate fertilizer amendment rates to meet optimum crop growth needs (Sims et al. 1998). It should be noted that soil P tests do not necessarily predict crop response to P fertilizer due to sampling depth and other limitations (Franzen and Peck 1995). Commercial fertilizer applications are commonly managed to minimize excessive accumulations of soil P because economic incentives are in place for farmers who do not over-apply (Pote et al. 1999). One popular program that offers incentives is the Environmental Quality Incentives Program (EQIP) conducted by the USDA-NRCS (National Resources Conservation Service). Farmers can choose to voluntarily participate in EQIP, which not only provides financial and technical assistance for fertilizer application, but also helps to plan and implement a wide range of other conservation practices (Pierson et al. 2001). For farmers who utilize manure applications to meet crop P needs, however, these same incentives are not in place (Sharpley et al. 1996) due to plant usable P levels being harder to determine in manure, and because manure application rates are often tailored to meet crop N needs, which results in elevated soil P (Pote et al. 1999).

The assessment of on-farm nutrient resources is an important aspect of a nutrient management plan. The amount of P applied to fields from on-farm sources such as manure should be determined and deducted from the base chemical fertilizer recommendation (Thomas et al. 2007). Nutrient assessments especially impact farms that focus on livestock production in the Midwest. The P-content of manure produced often far exceeds the farm's P-application needs (Wu, Satter, and Sojo 2000). The Federal Nutrient Management

Regulations for Animal Operations Act, passed by Congress in 2001, targets manure management and manure P-content as a key component to the protection of surface water quality (National Research Council 2001). A "P credit" can be developed in which the base fertilizer recommendation along with applied on-farm P sources is precisely tabulated. In a study by Powell et al. (2002), a P credit was developed on a Wisconsin dairy farm by calculating the average P return from alfalfa and corn residues and then adding it to all the animal-related P sources used for fertilization on the farm.

In order to properly calculate the amount of P being applied from manure, farmers must take into account application rates and the crop-available P content of the manure (Conley 1999). Manure inventories are developed as primary components of nutrient credits and involve estimating the amount of manure produced on a farm, followed by planning manure application rates for individual fields. With these data, manure spreading plans can be developed in order for manure spreaders to be calibrated for the number of spreader loads it will take to supply a given field (Ribaudo et al. 2003), though manure applications are imprecise by nature due to the variable P content they contain. Manure spreading plans are developed in accordance that manure applications should be made at rates that don't exceed crop nutrient needs identified by soils test P methods.

Manure application methods have been developed to improve manure use efficiency. Application of solid manure via subsurface applicators, and application of liquid manures via injectors have been demonstrated (Maguire et al. 2011). It is necessary to identify fields that will benefit the most from the application of manure while posing little threat to water quality (Feinerman, Bosch, and Pease 2004). Fields that may pose a threat to manure applications include fields adjacent to lakes and streams, sloping fields that have the threat of spring runoff, and fields that are in the vicinity of wells, sinkholes, or fractured bedrock, which are direct pathways to groundwater (Castellanos-Navarrete et al. 2014). Nutrient management plans are developed in accordance with farm conservation plans, which contain relevant information on planned crop rotations, identifications of slopes on all fields, and the conservation methods being followed to maintain low soil erosion rates (Manderson, Mackay, and Palmer 2007).

1.6b Wastewater treatment

In addition to agricultural systems as a primary P source, urban wastewaters are also a major contributor of P to aquatic systems. Phosphorus in wastewater results from the use of chemical detergents, human excreta, and industrial point sources. In a study by Rybicki (1998), TP concentration in wastewater was attributed to 30-50% human excreta, 50-70% detergents, and 2-20% industry based on the locality. Raw influent to wastewater treatment plants can be expected to contain approximately 5 mg TP/L (Litke 1999), based on the assumption that each day the average person discharges 2 g of P into wastewater. In light of elevated levels of P in such wastewaters, strict regulations have been developed for the treatment and release of wastewaters from urban systems. The general effluent TP limit for wastewater treatment in the US is 1-2 mg TP/L (Sedlak 1991). Limits vary greatly, however, based on local water quality and extent of eutrophication. For example, the Great Lakes Water Quality Agreement takes into account 400 municipal water treatment plants discharging into the Great Lakes. Effluent limits from these plants are set at a maximum of 1 mg TP/L in the upper Great Lakes region and 0.5 mg TP/L in the lower Great Lakes region (Yeoman et al. 1988). Other areas in the US have even stricter TP limits, as can be observed in the Potomac River Basin, where effluents are required to be lower than 0.2 mg TP/L (USEPA 1974). Lower TP limits generally require increased chemical dosage in the wastewater treatment process and also an increase in the amount of sludge produced.

A great variety of methods for P removal have been developed for wastewater facilities globally, but the two primary and longest tenured methods are 1) basic chemical precipitation and 2) biological removal (Morse et al. 1998). Chemical precipitation of P from wastewater is achieved via the addition of divalent or trivalent metal salts, which form insoluble metal phosphates. The addition of metal salts can occur during several stages of wastewater treatment, making application timing a differentiating factor among wastewater facilities. Primary precipitation occurs when raw sewage wastewater is dosed with reactant chemicals prior to primary sedimentation, after which P is removed in primary sludge (Szabó et al. 2008). Chemical application during the primary stage is the least effective method of chemical precipitation, as only 5-10% of P is removed. Secondary precipitation (also known as simultaneous precipitation) occurs when chemicals are applied directly to an aeration tank during the activated sludge process. Phosphorus is then removed as a component of secondary sludge, which results in the net removal of 10-20% of P (Sonune and Ghate 2004). Tertiary treatment involves the dosing of chemicals following secondary treatment. Tertiary treatment is the most effective chemical treatment method, removing as much as 99% of P, but is generally not favored due to high chemical costs and the creation of additional chemicals in tertiary sludge (Doka, Life, and Assessments 2007).

In addition to temporal variation of metal addition, a variety of divalent and trivalent metals can be used to form insoluble metal phosphates. Iron and aluminum added as chlorides or sulfates are the most suitable metals due to their high reactivity with P. Lime can also be used under certain conditions to precipitate Ca-phosphate (Sperling 2008). Iron is primarily added as $FeCl_3$ or $Fe_2(SO_4)_3$ to wastewater. Fe^{3+} forms strong complexes with pyrophosphate and tripolyphosphate, which are then adsorbed onto Fe-hydroxides (Olsson and Newell 1999). Aluminum is added as aluminum sulphate (alum), $Al_2(SO_4)_3$, and undergoes reactions to form $AlPO_4$. The use of aluminum comes with the condition that wastewater pH must be at a high enough alkalinity for alum to be buffered during the reaction (Morse et al. 1998). Lime can be used as an addition to alkaline wastewaters (pH 8-10). The most commonly applied forms are quicklime or CaO, which undergo reaction to form hydroxyapatite. Retention times of wastewater in treatment plants are often not sufficient for the formation of pure crystalline hydroxyapatite, but the presence of nucleating material allows for adequate reaction times (Merrill and Jorden 1975).

Biological removal of P is widely applied in wastewater treatment facilities. It is based on the principal of luxury consumption of P. Activated sludge composed of microorganisms takes in P far beyond what is needed for optimal biomass growth (Bowker and Stensel 1990). The advantage of using biological removal in comparison to chemical precipitation is avoiding the use of costly chemicals. Conditions for luxury consumption of P by microorganisms are achieved by introducing an anaerobic zone prior to an aerobic zone in a process known as Enhanced Biological Phosphorus Removal (EBPR) (Stratful et al. 1999). Enhanced Biological Phosphorus Removal takes advantage of bacteria known as Phosphorus Accumulating Organisms (PAOs), which are able to store intracellular P ranging from 5-30% of their total weight (Minnesota Pollution Control Agency 2006). In the anaerobic zone, PAOs consume volatile fatty acids and release polyphosphate compounds to generate energy, subsequently increasing the P content of the wastewater being treated. In the following aerobic phase, PAOs rapidly multiply and act to take up the P released during the anaerobic phase, as well as utilizing energy stored from consuming fatty acids to take up even more P (Seviour, Mino, and Onuki 2003). Enhanced Biological Phosphorus Removal can consume up to 99% of P in wastewater. Waste sludge, which contains biologically bound P, is removed and can be released back into solution if anaerobic conditions are introduced again. Waste sludge is also reported to be of interest to those in search of alternative P fertilizers (Yeoman et al. 1988).

Biological P removal is the most common form of wastewater treatment for large municipalities in the Midwest. Areas such as Manitowac, WI, Wisconsin Rapids, WI, Sheboygan, WI, Waukesha, WI, Schaumburg, IL, the North Shore Water District, IL, the County of Dupage, IL, Ottumwa, IA, Dubuque, IA, and Davenport, IA all utilize EBPR or modified EBPR to treat wastewater (USEPA 2010). Smaller municipalities and treatment operations in the Midwest often do not have sufficient funding for EBPR or chemical treatment and must rely on less efficient pond treatments. In a study by Wallace et al. (2005), it was found that of 739 treatment systems in Iowa, 537 (73%) utilized ponds. Treatment ponds store wastewater for up to six months at a time, allowing for a limited degree of natural biological P consumption to take place. Ponds will often become eutrophic and contain P-enriched algae (Sauer and Kimber 2003). Ideally, water is discharged from treatment ponds twice a year, during spring and fall coinciding with periods of high flow, but it is often the case that ponds will be discharged more than twice a year due to inadequate storage capacity (Wallace et al. 2005). These small, inefficient wastewater treatment operations are major sources of P to surface waters of the Midwest, and subsequently the Mississippi River and Gulf of Mexico.

While efforts to reduce P pollution have become increasingly effective, many aquatic systems still suffer from intense P loading. Remediation techniques for aquatic systems affected contain a combination of measures and guidelines, which in theory, act to return the system to its original state. One of these guidelines is that N:P ratios should be maintained at high values (Downing and Mccauley 1992). Any reductions in nitrogen loading should be accompanied by reduction in P loading as well in order to maintain healthy ratios. In the Experimental Lakes Area located in Ontario, Canada, experiments were conducted to measure phytoplankton growth response to nutrient loading (Schindler 1974). Rapid eutrophication occurred when N:P ratios in the lake measured below the average N:P ratio in phytoplankton (Schindler et al. 2008). It is of utmost importance to monitor the N:P ratio of aquatic systems to see that an abundance of N-fixing phytoplankton does not develop.

Aquatic systems will often show resilience to recovering from eutrophication even after P loading has been greatly decreased (Jeppesen et al. 1999; Søndergaard, Jensen, and Jeppesen 2003) due to P release from benthic sediments. Benthic sediments become enriched in P during periods when P loading is high. The resilience to recovering from eutrophication depends on the magnitude and duration of previous P loading, hydrological retention time, and Fe input (Cullen and Forsberg 1988; Sas et al. 1989). The period of resilience can be surprisingly long, as some shallow lakes retain so much P in benthic sediments compared to their volume that it takes 30 years to reach pre-loading P equilibrium (Søndergaard, Kristensen, and Jeppesen 1993). Methods have been introduced to combat internal P loading in order to hasten the recovery process of lakes following a reduction in external loading. Methods include sediment dredging and the oxygenation of the hypolimnion by pure O_2 or NO_3^- (Ripl 1976).

1.6c Biological remediation in aquatic systems

Planktivorous and benthic fish have been observed to form a biological resilience to recovery from eutrophication. Benthic dwellers and planktivorous fish physically stir benthic sediments as they feed, which increases the rate which sediments release P into the water (Sosnovsky and Quirós 2009). Benthic grazers consume submerged macrophytes, which are important for P uptake and retention (Scheffer 1990). Planktivorous fish often will consume an overabundance of zooplankton, which in turn limits population control of phytoplankton (Meijer et al. 1999). Both benthic and planktivorous fish species also contribute P-rich excreta directly into the water column. In order to combat biological resilience, fish manipulation practices have been developed, which enhance the top-down control of phytoplankton (Shapiro and Wright 1984). One way to accomplish fish manipulation is to introduce predatory fish such as pike or bass to the aquatic system, which will feed on the problem species and keep their numbers in check (Meijer et al. 1999). Removing all planktivorous fish from the aquatic system will result in negating effects, however, as zooplanktivore populations will increase, which will subsequently outcompete grazing herbivore fish, ultimately leading to the flourishing of phytoplankton and eutrophication (Prejs et al. 1994). A study by Shapiro and Wright (1984) was conducted at Round Lake, MN, which exhibited eutrophic conditions such as low transparency, high algal mass, and an abundance of planktivorous fish. The lake returned to its original condition in only two years after biological manipulation, suggesting that bioremediation is a viable option for such systems in the Midwest.

Reintroduction of wetlands to areas subject to previous land reclamation has shown to be of great benefit for P retention in aquatic systems (Jeppesen et al. 1999). Human influences on stream and river hydrology, such as culverting and channelizing, lowering stream beds and increasing stream and river widths has resulted in a significant reduction in hydraulic retention time. Temporary and permanent P holding capacity has likewise been greatly reduced (Iversen et al. 1993). Restoring streams and rivers to their original hydraulic condition reduces the influx of P to lakes. The net retentive P capacity of streams and rivers has a close correlation to the size of the riparian zone allowed to grow, along with the macrophyte coverage of the area (Svendsen and Kronvang 1993). In a study by Iversen et al. (1993) on the potential P retentive capacity of flooded riparian zones, it was shown that a 0.5-ha riparian zone in the lower part of a river catchment retained 94 kg P/ha, which corresponds to P loss of a 300-fold greater area of agricultural land. In the Midwest alone, during the past 200 years there has been a 50-90% reduction in wetland area due to drainage for agriculture (Hopple and Craft 2013). The Wetland Reserve Program (WRP) and Conservation Reserve Program (CRP) are NRCS programs that assist Midwestern landowners in creating, restoring, and enhancing wetlands by providing technical and financial support (USDA 2008). Many of the projects conducted under WRP and CRP consist of restoring wetland hydrology to an area by plugging drainage ditches or destroying tile drainage lines (Galatowitsch and van der Valk 1996).

1.7 Research justification

As reviewed in the previous sections, several integrated factors (e.g., policies, regulations, social science, and agricultural management) are contributing to an overabundance of P entering the Mississippi River Basin. The major reasons resulting in frequent eutrophication and outbreaks of hypoxia might lie in current agricultural management practices.

Soil Test Phosphorus was originally developed to assess the soil P status (Sims et al. 1998). In current agricultural management practices, it is used to recommend the application rate of P fertilizer to optimize crop yield. However, it has been a challenging task to predict labile (i.e., plant-available) P in agricultural soils. While STP is thought to be a reliable agronomic test, operationally defined extractable P in the STPs (e.g., Bray, Mehlich III) do not accurately predict plant-available P because 1) extractants do not accurately quantify the concentration of plant-available DRP, 2) extractants do not accurately speciate dissolved P species (e.g., colloidal- and organic-P) that are available to plants and 3) STP results do not predict the rate/extent of P release (i.e., desorption and dissolution) during the growing season.

1.7a Inaccurate estimate of dissolved reactive P (DRP) concentration

Phosphorus fertilizer recommendations are based on the results of STP. If STP predicts an inaccurate quantity of "plant-available P" in soils, it could result in an overestimation or underestimation of required P fertilizer application. A soil's STP consists of its operationally defined concentration of DRP, the variability of which is a major issue. There are recurring issues with extraction procedures that result in less accurately measured agronomic STPs. Current extractants being used are inadequate due to limited research relating knowledge of biogeochemistry to what would make a proper STP procedure. Dilute acid extracts such as Mehlich III and Bray P-1 have a common problem of re-adsorption of P dissolved in the extract reaction (Mehlich 1978). Modifications have been made to the extractant techniques, but it is still a great challenge to introduce anion species that will preferentially replace adsorbed P (Pierzynski 2000).

Phosphorus in soils is categorized into inorganic and organic P groups. The inorganic P pool has adsorbed DRP in soil components and P minerals while the organic P pool contains compounds such as inositol phosphate monoesters and diesters (e.g., phospholipids). Commonly used STP procedures alone are ineffective for analyzing DRP concentration from inorganic and organic P sources. The use of ICP instead of colorimetric P determination in some STP procedures could lead to an overestimation of DRP due to the inclusion of organic P fractions. Furthermore, the extractant being used may not be suited to predicting the solubility of P minerals (e.g., hydroxy apatite) in post-limed soils.

As previously discussed, the most common extractants used for STP in Illinois are the Bray P-1 method evaluated by colorimetric techniques, and the Mehlich III method with the option of either using colorimetric or ICP analysis. Among the labs that are certified by the Illinois Soil Testing Association, the majority offer colorimetric analysis of Bray P-1 extracts and Mehlich III ICP while a few labs offer Mehlich III colorimetric testing (Soiltesting.org 2015). One would expect that ICP should be more widely used, but calibration for plant uptake and response is far less developed for the ICP methodology than its predecessors, the Bray P-1 and Mehlich III-colorimetric analyses.

Several studies illustrate that extraction efficiency of DRP is highly dependent on soil properties (e.g., pH, carbonate content). A study by Mallarino (1995) tested 240 cultivated Mollisols and Alfisols from Iowa for comparison of Bray P-1, Olsen, and Mehlich III extractant use in calcareous soils and non-calcareous soils. In this case, pH values of the soils ranged from 5.3 to 8.2, and highly calcareous soils were included to represent soil series of western and north-central Iowa. A field-P response study was also conducted with data from numerous 48-year experimental plots. The trials involved varying P-fertilizer application rates and the experimental plots represented 14 different soil series. Mallarino (1995) found that the correlation between Bray P-1 and Mehlich III extractants along with STP extractability was highly dependent on soil pH. Ebeling et al. (2008) expanded on these findings with a study conducted to evaluate the Bray P-1 test on alkaline, calcareous soils. The study was conducted on soils from the ERS (eastern red soil) region of Wisconsin, which have pH values ranging from 7 to 8 and are often calcareous. It was concluded that while high $CaCO_3$ content (>5 g) neutralized Bray P-1 extracts and led to decreased correlation with Mehlich III, pH > 7.05 did not. From these results it seems that carbonate content and not pH, is the primary factor affecting the extractability of Bray P-1 and Mehlich III.

1.7b Unknown P speciation in STP

There are multiple steps that comprise a single STP procedure. A typical soil testing procedure starts with collecting field samples, the depth and thoroughness of which is often inadequate, followed by chemical extraction in which “plant-available P (i.e., labile DRP)” is targeted. It should be noted that “plant-available” forms of P are not easy to distinguish due to the low solubility of P and the multiple reaction pathways of release from organically and colloidal bound into soil solution (Fernández and Hoeft 2009). Depending on the region, animal-based fertilizers are also commonly used (Maguire, Sims, and Applegate 2005), and as such, labile organic P from manure amendments cannot be ignored. This leads to a main issue with STP in which it cannot speciate various forms of P (DRP VS. colloidal-P) that may play significant roles in supplying P to crops.

As previously discussed, the most common extractants used for STP in Illinois are the Bray P-1 method and the Mehlich III method with the option of either using colorimetric or ICP analysis for an Mehlich III extract. In most soil testing laboratories, Mehlich III extracts that are analyzed by ICP provide higher test values of P than Bray P-1 and Mehlich III colorimetric analysis because TP is measured, which takes into account organic P, colloidal P, and DRP. The ICP analytical technique is not suited to isolate the most important plant-available P species, DRP.

1.7c An unclear relationship between the extent of P release and STP

Soils have the variable capacity to release labile P into the soil solution. Equilibrium-based STP extractions using mild or strong chemical reagents do not account for temporal scale P availability in soils. Predicting the rate of P release becomes important, especially during the growing season. Unfortunately, the heterogeneity of soils affects the rate and extent of P release via dissolution and desorption. Equilibrium-based STP methods are not ideal to detect variable P release rates. Solubility of P in soils is dependent on the surface area and crystallinity of fertilizer and native P minerals (e.g., hydroxy apatite, variscite, strengite) in soils. Desorption of P is also often controlled by the reactivity of adsorbents including soil OM. Most importantly, the release of P is often kinetically controlled (Amer et al. 1955; Arai, Livi, and Sparks 2005; Kuo and Lotse 1973). A slow P release in agricultural soils has been documented in several studies (Elkhatib and Hern 1988; Fekri, Gorgin, and Sadegh 2011; McDowell and Sharpley 2003a).

In summary, research evidence from numerous studies suggests that there is a knowledge gap in assessing the quantity of labile P which is affected by variable P species and extraction time. In order to accurately predict labile P, one must address the knowledge gap between STP results, the concentration of DRP in the current STP extracts, and the extent of DRP release.

1.8 Research objective

The objective of this study is to investigate the effects of extraction time and P speciation on STP in Illinois agricultural soils. To achieve the goal, several phosphate colorimetric methods were first evaluated to accurately distinguish orthophosphate from organic P, including the acid hydrolysis reaction of polyphosphate and organic phosphate. Using the appropriate analytical techniques and data interpretation, the effects of extraction time and P speciation on STP were evaluated.

1.9 Tables

Table 1.1: Illinois consumption of phosphate fertilizers in fall 2013. (Illinois Department of Agriculture 2014)

Name	**Grade**	**Consumption (material short tons)**
Diammonium phosphate	18-46-0	398,186
Monoammonium phosphate	11-52-0	60,641
Bat guano	0-5-0	47,001
US1240D©†	12-40-0	31,121
"Autumn NPK"	5-15-20	16,014
Liqui-Grow SLX©‡	3-10-30	15,327
White Front© Starter	9-23-30	13,367
Triple superphosphate	0-46-0	11,245
Biogan§	3-3-0	6,570
Ammonium polyphosphate	10-34-0	6,004

† Used for grain crop applications

‡ Pre-plant P and K fluid suspensions for corn and soybean crops

§ Organic liquid fish fertilizer

Table 1.2: A summary of P concentrations in agricultural and urban systems.

Environmental medium	Concentration	Comments	References
Pristine Natural Water Systems	0.005-0.05 mg/L	Average from 63 unpolluted drainage basins in USA	(Dunne and Leopold 1978; Litke 1999)
Groundwater in Agricultural Areas	0.03-0.16 mg/L	Average from 103 observation wells in Western Iowa and from USGS national report	(Burkart et al. 2004; Mueller et al. 1995)
Groundwater in Urban Areas	0.02 mg/L	Average from national USGS survey	(Nolan and Stoner 2000)
Groundwater in Aquifers Used for Drinking Water	0.01 mg/L	Average from national USGS survey	(Nolan and Stoner 2000)
Surface Runoff (Non-Wastewater) in Urban Systems	0.1-0.4 mg/L	Estimated average for urban runoff for USA and from erosion study in Madison, WI	(Litke 1999; USEPA 1996; Waschbusch 1993)
Wastewater in Urban systems	4-15 mg/L	Estimated average concentration for USA and from erosion study in Madison, WI	(Holtan et al. 1988; USEPA 1996; Waschbusch 1993)
Septic Systems	1.2-12.1 mg/L	From studies of septic system effluent P concentration in Idaho and Canada	(Idaho Department of Environmental Quality 2012; Robertson, Schiff, and Ptacek 1998)
Polluted Surface Water in Agricultural System	0.03-0.58 mg/L	Observed in tile drainage system in IN and in general study of Midwestern agricultural soils	(Ahiablame et al. 2010; Gentry et al. 2007; Sims et al. 1998)
Background P in Unaffected Soils	7.28-13 mg/kg	Average for multiple virgin soils in Kansas and Texas	(Hedley, Kirk, and Santos 1994; Sims et al. 1998)
Colloidal P in Urban Systems	3,294 mg/kg	Observed in an erosion study in Madison, WI	(Waschbusch 1993)
Fertilized Agricultural Soils	897-2,466 mg/kg	Observed in field study in Ohio	(Guertal et al. 1991; McDowell and Wilcock 2015)

Table 1.3: Best management practices – source measures (University of Wisconsin 1995).

Practices	Description
Feed supplements	Match feed to animals' nutritional requirements
Feed additives	Increase nutrient utilization by animals
Crop hybrids	Low phytic-acid corn used for feed reduces P in manure
Manure management	Includes compost, lagoons, pond storage, barnyard runoff control
Rate of application	Specifically matching crop needs by evaluating soil P content and on farm nutrient sources
Timing of application	Avoid applications to frozen ground, apply during seasons of low runoff probability
Method of application	Applications such as incorporation, banding, or injecting to incorporate fertilizer more effectively
Source application	Due to differences in P-solubility based on fertilizer source
Manure amendment	Addition of alum to manure reduces P-solubility
Soil amendment	Amendments such as Fe-oxides or gypsum to reduce P solubility
Cover crops / residues	Once harvested, reduce residual soil P
Inversion of stratified soils	Redistributes surface P through profile by plowing

Table 1.4: Best management practices – transport measures (University of Wisconsin 1995).

Practices	Description
Cover crop	Covering soil during winter to reduce runoff events
Conservation tillage	Reduced tillage increases infiltration and reduces soil erosion
Grazing management	Keeping animals away from fresh water sources
Buffer, riparian, constructed wetlands, grassed waterways	Removes sediment-bound P (Colloidal P) and enhances denitrification
Soil drainage	Tile drainage and drainage ditches enhance water removal and reduce erosion
Strip cropping, contour tillage, terraces	Reduces transport of sediment-bound P (Colloidal P)
Sediment delivery structures	Structures to deliver runoff in order to protect and stabilize stream banks
Critical source area treatment	Specifically targeting P sources in a watershed

Table 1.5: Soil reaction in relation to selection of appropriate soil test-P (STP) (Elrashidi 2010).

Soil	pH range	Targeted minerals	STP Methods
Acidic	< 6.0	Al-P, Fe-P, Mn-P	Bray P-1, Mehlich III
~Neutral	6.0-7.2	Al-P, Fe-P, Mn-P, Mg-P, Ca-P	Bray P-1, Mehlich III
Alkaline	> 7.2	Ca-P, Mg-P	Olsen

1.10 References

Ahiablame, Laurent, Indrajeet Chaubey, and Douglas Smith. 2010. “Nutrient Content at the Sediment-Water Interface of Tile-Fed Agricultural Drainage Ditches.” *Water* 2(3):411–28.

Allen, B. L. and B. F. Hajek. 1989. “Mineral Occurrence in Soil Environments.” *Minerals in Soil Environments* 2:199–278.

Amer, F., D. R. Bouldin, C. A. Black, and F. R. Duke. 1955. “Characterization of Soil Phosphorus by Anion Exchange Resin Adsorption and P32-Equilibration.” *Plant and Soil* 6(4):391–408.

Arai, Yuji, K. J. T. Livi, and D. L. Sparks. 2005. “Phosphate Reactivity in Long-Term Poultry Litter-Amended Southern Delaware Sandy Soils.” *Soil Science Society of America Journal* 69(3):616–29.

Arai, Yuji and D. L. Sparks. 2007. “Phosphate Reaction Dynamics in Soils and Soil Components: A Multiscale Approach.” *Advances in Agronomy* 94(6):135–79. Retrieved (http://linkinghub.elsevier.com/retrieve/pii/S0065211306940036).

Ashley, K., D. Cordell, and D. Mavinic. 2011. “A Brief History of Phosphorus: From the Philosopher’s Stone to Nutrient Recovery and Reuse.” *Chemosphere* 84(6, SI):737–46.

Austin, N. R., J. B. Prendergast, and M. D. Collins. 1996. “Phosphorus Losses in Irrigation Runoff from Fertilized Pasture.” *Journal of Environmental Quality* 25(1):63–68.

Baker, R. T. 1977. “Humic Acid-Associated Organic Phosphate.” *New Zealand Journal of Science* 20(4):439–41.

Beardsley, Timothy M. 2011. “Peak Phosphorus.” *BioScience* 61(2):91–91.

Beegle, D. B., O. T. Carton, and J. S. Bailey. 2000. “Nutrient Management Planning: Justification, Theory, Practice.” *Journal of Environmental Quality* 29:72–79. Retrieved (http://dx.doi.org/10.2134/jeq2000.00472425002900010009x).

Beegle, Douglas. 2005. “Assessing Soil Phosphorus for Crop Production by Soil Testing.” *Agronomy* 46:123.

Benndorf, Jürgen, Wiebke Böing, Jochen Koop, and Ivonne Neubauer. 2002. “Top-Down Control of Phytoplankton: The Role of Time Scale, Lake Depth, and Trophic State.” *Freshwater Biology* 47(12):2282–95.

Bláha, Luděk, Pavel Babica, and Blahoslav Maršálek. 2009. “Toxins Produced in Cyanobacterial Water Blooms – Toxicity and Risks.” *Interdisciplinary Toxicology* 2(2):36–41.

Blanchard, Duncan C. and Charles Keith. 1963. “The Electrification of the Atmosphere by Particles from Bubbles in the Sea.” *Progress in Oceanography* 73(1)

Bolan, Nanthi S., Benjamin L. Turner, Emmanuel Frossard, and Darren S. Baldwin. 2005. “Organic Phosphorus in the Environment.” *Journal of Environment Quality* 34(5)

Bower, Charles Arthur. 1949. *Studies on the Forms and Availability of Soil Organic Phosphorus*. Agricultural Experiment Station, Iowa State College of Agriculture and Mechanic Arts.

Bowker, R. P. and H. D. Stensel. 1990. *Phosphorus Removal From Wastewater*. Noyes Data Corp. Retrieved (https://books.google.com/books?id=50dSAAAAMAAJ).

Brady, N. C. and R. R. Weil. 2002. *The Nature and Properties of Soils*. Macmillan Publishing Co. Retrieved (http://www.amazon.ca/exec/obidos/redirect?tag=citeulike09-20&path=ASIN/013227938X).

Bridgham, Scott D., Chein-Lu Ping, Jimmie Larry Richardson, and Karen Updegraff. 2000. “Soils of Northern Peatlands: Histosols and Gelisols.” Pp. 343–70 in *Wetland Soils: Genesis, Hydrology, Landscapes, and Classification*. Boca Raton, FL: CRC Press.

Buol, S. W., R. J. Southard, R. C. Graham, and P. A. McDaniel. 2011. “Mollisols: Grassland Soils of Steppes and Prairies.” Pp. 331–47 in *Soil Genesis and Classification*. Retrieved (http://dx.doi.org/10.1002/9780470960622.ch15).

Burkart, M. R., W. W. Simpkins, A. J. Morrow, and J. M. Gannon. 2004. “Occurrence of Total Dissolved Phosphorus in Unconsolidated Aquifers and Aquitards in Iowa.” *Journal of the American Water Resources Association* 40(3):827–34.

Burkholder, J. M., H. G. Marshall, H. B. Glasgow, D. W. Seaborn, and N. J. DeamerMelia. 2001. “The Standardized Fish Bioassay Procedure for Detecting and Culturing Actively Toxic Pfiesteria, Used by Two Reference Laboratories for Atlantic and Gulf Coast States.” *Environmental Health Perspectives* 109(October):745–56. Retrieved (http://193.144.41.80:8086/4733.pdf).

Burkholder, Joann M. and Howard B. Glasgow. 1997. “Pfiesteria Piscicida and Other Pfiesteria-Like Environmental Controls.” *Limnology & Oceanography* 42(5(2)):1052–75.

Burkholder and Marshall. 2012. “Toxigenic Pfiesteria Species-Updates on Biology, Ecology, Toxins, and Impacts.” *Harmful Algae* 14:196–230. Retrieved (http://dx.doi.org/10.1016/j.hal.2011.10.022).

Carpenter, Stephen R. et al. 1998. “Nonpoint Pollution of Surface Waters with Phosphorus and Nitrogen.” *Ecological Applications* 8(3):559–68.

Castellanos-Navarrete, A., P. Tittonell, M. C. Rufino, and K. E. Giller. 2014. “Feeding, Crop Residue and Manure Management for Integrated Soil Fertility Management – A Case Study from Kenya.” *Agricultural Systems* 134:1–12. Retrieved (http://linkinghub.elsevier.com/retrieve/pii/S0308521X14000316).

Castro, B. and J. Torrent. 1995. “Phosphate Availability in Calcareous Vertisols and Inceptisols in Relation to Fertilizer Type and Soil Properties.” *Fertilizer Research* 40(2):109–19.

Celi, Luisella, Sebastiano Lamacchia, Franco Ajmone Marsan, and Elisabetta Barberis. 1999. “Interaction of Inositol Hexaphosphate on Clays: Adsorption and Charging Phenomena.” *Soil Science* 164(8):574–85. Retrieved (http://www.scopus.com/inward/record.url?eid=2-s2.0-0032826509&partnerID=tZOtx3y1).

Chen, H. Y., T. H. Fang, M. R. Preston, and S. Lin. 2006. “Characterization of Phosphorus in the Aerosol of a Coastal Atmosphere: Using a Sequential Extraction Method.” *Atmospheric Environment* 40(2):279–89.

Ciolkosz, Edward J., William J. Waltman, Thomas W. Simpson, and Robert R. Dobos. 1989. “Distribution and Genesis of Soils of the Northeastern United States.” *Geomorphology* 2(1–3):285–302. Retrieved November 2, 2015 (http://www.sciencedirect.com/science/article/pii/0169555X89900160).

Clesceri, Nicholas L., Sidney J. Curran, and Richard I. Sedlak. 1986. “Nutrient Loads To Wisconsin Lakes: Part I. Nitrogen and Phosphorus Export Coefficients.” *Water Resources Bulletin* 22(6):983–90. Retrieved (http://www.scopus.com/inward/record.url?eid=2-s2.0-0022920648&partnerID=tZOtx3y1).

Codd, Geoffrey a. 2000. “Cyanobacterial Toxins, the Perception of Water Quality, and the Prioritisation of Eutrophication Control.” *Ecological Engineering* 16(1):51–60.

Compton, J. et al. 2000. “Variations in the Global Phosphorus Cycle.” *Marine Authigenesis: From Global to Microbial* 66(66):21–33. Retrieved (http://www.soest.hawaii.edu/Marine_Authigenesis/03compto.pdf).

Conley, Daniel J. 1999. “Biogeochemical Nutrient Cycles and Nutrient Management Strategies.” *Hydrobiologia* 410:87–96.

Cordell, Dana, Jan-Olof Drangert, and Stuart White. 2009. “The Story of Phosphorus: Global Food Security and Food for Thought.” *Global Environmental Change-Human and Policy Dimensions* 19(2):292–305.

Correll, David L. 1998. “The Role of Phosphorus in the Eutrophication of Receiving Waters: A Review.” *Journal of Environment Quality* 27(2):261.

Cullen, Peter and Curt Forsberg. 1988. “Experiences with Reducing Point Sources of Phosphorus to Lakes.” *Hydrobiologia* 170(1):321–36.

D’Arcy, B. and A. Frost. 2001. “The Role of Best Management Practices in Alleviating Water Quality Problems Associated with Diffuse Pollution.” *The Science of the Total Environment* 265(1–3):359–67.

Dalal, R. C. 1977. “Soil Organic Phosphorus.” *Advances in Agronomy* 29:83–117.

David, Mark B. and Lowell E. Gentry. 2000. “Anthropogenic Inputs of Nitrogen and Phosphorus and Riverine Export for Illinois, USA.” *Journal of Environment Quality* 29(2):494.

Delaney, M. L. 1998. “Phosphorus Accumulation in Marine Sediments and the Oceanic Phosphorus Cycle.” *Global Biogeochemical Cycles* 12(4):563.

Dodds, Walter K., John R. Jones, and Eugene B. Welch. 1998. “Suggested Classification of Stream Trophic State: Distributions of Temperate Stream Types by Chlorophyll, Total Nitrogen, and Phosphorus.” *Water Research* 32(5):1455–62. Retrieved October 9, 2015 (http://www.sciencedirect.com/science/article/pii/S0043135497003709).

Doka, Gabor, Doka Life, and Cycle Assessments. 2007. “Wastewater Treatment.” *Metal Finishing* 98(13):3–5. Retrieved (http://linkinghub.elsevier.com/retrieve/pii/S0026057600817626).

Dolah, Frances M. Van. 2000. “Marine Algal Toxins: Origins, Health Effects, and Their Increased Occurrence.” *Environmental Health Perspectives* 108(SUPPL. 1):133–41.

Downing, John a. and Edward Mccauley. 1992. “The Nitrogen:Phosphorus Relationship in Lakes.” *Limnology and Oceanography* 37(5):936–45.

Dunne, T. and L. B. Leopold. 1978. *Water in Environmental Planning*. illustrate. New York, NY: WH Freeman and Co.

Dyhrman, Sonya, James Ammerman, and Benjamin Van Mooy. 2007. “Microbes and the Marine Phosphorus Cycle.” *Oceanography* 20(2):110–16.

Ebeling, Angela M., Larry G. Bundy, Aaron W. Kittell, and Daniel D. Ebeling. 2008. “Evaluating the Bray P1 Test on Alkaline, Calcareous Soils.” *Soil Science Society of America Journal* 72(4):985. Retrieved (https://www.soils.org/publications/sssaj/abstracts/72/4/985).

Eckert, Werner and Ami Nishri. 2014. “The Phosphorus Cycle.” Pp. 347–63 in *Lake Kinneret SE - 20*, vol. 6, *Aquatic Ecology Series*, edited by T. Zohary, A. Sukenik, T. Berman, and A. Nishri. Springer Netherlands. Retrieved (http://dx.doi.org/10.1007/978-94-017-8944-8_20).

Elkhatib, E. A. and J. L. Hern. 1988. “Kinetics of Phosphorus Desorption from Appalachian Soils.” *Soil Science* 145(3):222–29.

Elrashidi, Ma. 2010. “Selection of an Appropriate Phosphorus Test for Soils.” *USDA NRCS, Lincoln, NE* 2–3. Retrieved (http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Selection+of+an+Appropriate+Phosphorus+Test+for+Soils#0).

Emsley, John. 2002. *The 13th Element: The Sordid Tale of Murder, Fire and Phosphorus*. 1st ed. Mississauga, ON, Canada: Wiley.

Feinerman, Eli, Darrell J. Bosch, and James W. Pease. 2004. “Manure Applications and Nutrient Standards.” *American Journal of Agricultural Economics* 86(1):14–25.

Fekri, M., N. Gorgin, and L. Sadegh. 2011. “Phosphorus Desorption Kinetics in Two Calcareous Soils Amended with P Fertilizer and Organic Matter.” *Environmental Earth Sciences* 64(3):721–29.

Fernández, Fg and Rg Hoeft. 2009. “Managing Soil pH and Crop Nutrients.” *… agronomy handbook. 24th ed. Univ. of …* 91–112. Retrieved (http://www.ebooksmagz.com/pdf/managing-soil-ph-and-crop-nutrients-129919.pdf).

Filippelli, Gabriel M. 2008. “The Global Phosphorus Cycle: Past, Present, and Future.” *Elements* 4(2):89–95.

Follmi, K. 1996. “The Phosphorus Cycle, Phosphogenesis and Marine Phosphate-Rich Deposits.” *Earth-Science Reviews* 40(1–2):55–124. Retrieved (http://www.sciencedirect.com/science/article/pii/0012825295000496).

Franzen, David W. and Ted R. Peck. 1995. “Field Soil Sampling Density for Variable Rate Fertilization.” *Journal of Production Agriculture* 8(4):568–74.

Galatowitsch, Susan M. and Arnold G. van der Valk. 1996. “The Vegetation of Restored and Natural Prairie Wetlands.” *Ecological Applications* 6(1):102–12.

Gentry, L. E., M. B. David, T. V Royer, C. A. Mitchell, and K. M. Starks. 2007. “Phosphorus Transport Pathways to Streams in Tile-Drained Agricultural Watersheds.” *Journal of environmental quality* 36(2):408–15. Retrieved October 5, 2015 (http://www.scopus.com/inward/record.url?eid=2-s2.0-33947410859&partnerID=tZOtx3y1).

Gibson, G. R., R. Carlson, and J. Simpson. 2000. “Nutrient Criteria Technical Guidance Manual: Lakes and Reservoirs.” (April):1–231.

Glasgow H.B., Jr and J. M. Burkholder. 2000. “Water Quality Trends and Management Implications from a Five-Year Study of a Eutrophic Estuary.” *Ecological Applications* 10(4):1024–46.

Goss, Michael J. 1998. “Precision Agriculture.” *Field Crops Research* 55(3):285–87.

Graham, William F. and Robert A. Deice. 1979. “Atmospheric Pathways of the Phosphorus Cycle.” *Geochimica et Cosmochimica Acta* 43:1195–1208.

Grindel, N. M. and N. G. Zyrin. 1965. “Method of Determination and Dynamics of Organic Phosphorus Compounds In Plow Horizon of Slightly Cultivated Sod-Podzolic Soils.” *Soviet Soil Science-USSR* (12):1393-.

Guertal, E. A., D. J. Eckert, S. J. Traina, and T. J. Logan. 1991. “Differential Phosphorus Retention in Soil Profiles Under No-Till Crop Production.” *Soil Science Society of America Journal* 55:410–13.

Gužys, Saulius. 2013. “The Cycles of Phosphorus in Crop Rotations Differing in

Fertilization." Pp. 175–81 in *Annual 19th International Scientific Conference Proceedings, Research for Rural Development*, vol. 2. Jelgava, Latvia: Latvia University of Agriculture.

Hanrahan, G., T. Salmassi, C. Khachician, and K. Foster. 2005. "Reduced Inorganic Phosphorus in the Natural Environment: Significance, Speciation and Determination." *Talanta* 66(2):435–44. Retrieved (http://linkinghub.elsevier.com/retrieve/pii/S0039914004006204).

Hansen, N. C., T. C. Daniel, A. N. Sharpley, and J. L. Lemunyon. 2002. "The Fate and Transport of Phosphorus in Agricultural Systems." *Journal of Soil and Water Conservation* 57(6):408–17. Retrieved (http://www.jswconline.org/content/57/6/408.full.pdf+html?sid=db0ad700-df25-46dd-a5a3-462e795e6862).

Havlin, John, James D. Beaton, Samuel L. Tisdale, and Werner L. Nelson. 2005. *Soil Fertility and Fertilizers: An Introduction to Nutrient Management*. Pearson Prentice Hall Upper Saddle River, New Jersey, USA.

Haygarth, Phil M., Richard D. Bardgett, and Leo M. Condron. 2013. "Nitrogen and Phosphorus Cycles and Their Management." *Soil Conditions and Plant Growth* (15):132–59.

Heathwaite, Louise, Phil Haygarth, Rachel Matthews, Neil Preedy, and Patricia Butler. 2005. "Evaluating Colloidal Phosphorus Delivery to Surface Waters from Diffuse Agricultural Sources." *Journal of environmental quality* 34(1):287–98.

Hecky, R. E. and P. Kilham. 1988. "Nutrient Limitation of Phytoplankton in Freshwater and Marine Environments: A Review of Recent Evidence on the Effects of Enrichment." *Limnology & Oceanography* 33(4):796–822. Retrieved (http://www.scopus.com/inward/record.url?eid=2-s2.0-0024200349&partnerID=tZOtx3y1).

Hedley, M. J., G. J. R. Kirk, and M. B. Santos. 1994. "Phosphorus Efficiency and the Forms of Soil Phosphorus Utilized by Upland Rice Cultivars." *Plant and Soil* 158(1):53–62.

Hershey, Anne E. et al. 1988. "Nutrient Incluence on a Stream Grazer: Orthocladius Microcommunities Respond to Nutrient Input." *Ecology* 69(5):1383–92. Retrieved (http://www.jstor.org/stable/1941635).

Holtan, H., L. Kamp-Nielsen, and a. O. Stuanes. 1988. "Phosphorus in Soil, Water and Sediment: An Overview." *Hydrobiologia* 170(1):19–34.

Hopple, Anya and Christopher Craft. 2013. "Managed Disturbance Enhances Biodiversity of Restored Wetlands in the Agricultural Midwest." *Ecological Engineering* 61:505–10. Retrieved (http://dx.doi.org/10.1016/j.ecoleng.2012.02.028).

Hoyer, Mark V and John R. Jones. 1983. "Factors Affecting the Relation Between Phosphorus and Chlorophyll in Midwestern Reservoirs." *Canadian Journal of Fisheries and Aquatic Sciences* 40(2):192–99. Retrieved (http://dx.doi.org/10.1139/f83-029).

Iatrou, M. et al. 2014. "Determination of Soil Available Phosphorus Using the Olsen and Mehlich 3 Methods for Greek Soils Having Variable Amounts of Calcium Carbonate." *Communications in Soil Science and Plant Analysis* 45(16):2207–14. Retrieved (http://www.tandfonline.com/doi/abs/10.1080/00103624.2014.911304).

Idaho Department of Environmental Quality. 2012. *Domestic Wastewater Phosphorus Concentration Report*. Boise, ID.

Iho, Antti and Marita Laukkanen. 2012. "Precision Phosphorus Management and

Agricultural Phosphorus Loading." *Ecological Economics* 77:91–102. Retrieved (http://dx.doi.org/10.1016/j.ecolecon.2012.02.010).

Illinois Department of Agriculture. 2014. "Tonnage Report Summary, Fall 2013." 1–32.

Iversen, Torben Moth, Brian Kronvang, Bent Lauge Madsen, Peter Markmann, and Mogens Bjørn Nielsen. 1993. "Re-Establishment of Danish Streams: Restoration and Maintenance Measures." *Aquatic Conservation: Marine and Freshwater Ecosystems* 3(2):73–92.

Jackman, R. H. and C. A. Black. 1951. "Hydrolysis of Iron, Aluminum, Calcium, and Magnesium Inositol Phosphates by Phytase at Different pH Values." *Soil Science* 72(4):261–66.

Jeppesen, E. et al. 1990. "Fish Manipulation as a Lake Restoration Tool in Shallow, Eutrophic, Temperate Lakes 2: Threshold Levels, Long-Term Stability and Conclusions." *Hydrobiologia* 200–201:219–27.

Jeppesen, Erik et al. 1999. "Lake and Catchment Management in Denmark." *Hydrobiologia* (395/396):419–32.

Joosse, P. J. and D. B. Baker. 2011. "Context for Re-Evaluating Agricultural Source Phosphorus Loadings to the Great Lakes." *Canadian Journal of Soil Science* 91(3):317–27. Retrieved (http://pubs.aic.ca/doi/abs/10.4141/cjss10005).

Kaiser, Daniel E., Antonio P. Mallarino, and J. E. Sawyer. 2010. "Utilization of Poultry Manure Phosphorus for Corn Production." *Soil Science Society of America Journal* 74(6):2211. Retrieved (https://www.soils.org/publications/sssaj/abstracts/74/6/2211).

Ketterings, Q. M. and M. Flock. 2005. "Comparison of Bray-1 and Mehlich-3 Tests in High Phosphorus Soils." *Soil Science* 170(3):212–19. Retrieved (http://www.scopus.com/inward/record.url?eid=2-s2.0-15244341148&partnerID=40&md5=3aaddac4f8fb31152c4a6081a997ceb4).

King, Kevin W. et al. 2014. "Phosphorus Transport in Agricultural Subsurface Drainage: A Review." *Journal of Environment Quality* 0. Retrieved (https://www.agronomy.org/publications/jeq/abstracts/0/0/jeq2014.04.0163).

Kleinman, Peter J. a et al. 2011. "Managing Agricultural Phosphorus for Water Quality Protection: Principles for Progress." *Plant and Soil* 349(1–2):169–82.

Kleinman, Peter J. a, Andrew N. Sharpley, Barton G. Moyer, and Gerald F. Elwinger. 1998. "Effect of Mineral and Manure Phosphorus Sources on Runoff Phosphorus." *Journal of environmental quality* 31(6):2026–33.

Kornberg, Arthur, Narayana N. Rao, and Dana Ault-Riché. 1999. "Inorganic Polyphosphate: A Molecule of Many Functions." *Annual Review of Biochemistry* 68(1):89–125. Retrieved (http://dx.doi.org/10.1146/annurev.biochem.68.1.89).

Kovacic, David A., Mark B. David, Lowell E. Gentry, Karen M. Starks, and Richard A. Cooke. 2000. "Effectiveness of Constructed Wetlands in Reducing Nitrogen and Phosphorus Export from Agricultural Tile Drainage." *Journal of Environment Quality* 29(4):1262.

Kuo, S. and E. G. Lotse. 1973. "Kinetics of Phosphate Adsorption and Desorption by Hematite and Gibbsite." *Soil Science* 116(6):400–406.

Litke, By David W. 1999. "Review of Phosphorus Control Measures in the United States and Their Effects on Water Quality." *Water-Resources Investigations Report 99-4007* 1–38. Retrieved (http://www.msue.msu.edu/waterqual/WQWEB/ReviewPUSGS.pdf).

Liu, Xiaobing et al. 2012. "Overview of Mollisols in the World: Distribution, Land-Use and

Management." *Canadian Journal of Soil Science* 92(3):383–402.

Liu, Y. and J. Chen. 2002. "Phosphorus Cycle." *Plant Physiology* 5(2003):322–34. Retrieved (http://www.sciencedirect.com/science/article/B7CTC-4FTGWNS-F/2/217ea26655fb632befb4a81bea973c8e).

Liu, Y. and Robert G. Wetzel. 1983. "The Phosphorus Cycle." *Plant Physiology* 48(2003):242–50. Retrieved (http://www.sciencedirect.com/science/article/B7CTC-4FTGWNS-F/2/217ea26655fb632befb4a81bea973c8e).

Liu, Yi, Gara Villalba, Robert U. Ayres, and Hans Schroder. 2008. "Global Phosphorus Flows and Environmental Impacts from a Consumption Perspective." *Journal of Industrial Ecology* 12(2):229–47.

Macdonald, Graham K., Elena M. Bennett, and Zofia E. Taranu. 2012. "The Influence of Time, Soil Characteristics, and Land-Use History on Soil Phosphorus Legacies: A Global Meta-Analysis." *Global Change Biology* 18(6):1904–17.

Maguire, R. O. et al. 2011. "Manure Application Technology in Reduced Tillage and Forage Systems: A Review." *Journal of Environmental Quality* 40(2):292–301.

Maguire, R. O., J. T. Sims, and T. J. Applegate. 2005. "Loss in Runoff Following Litter Application." *Journal of Environment Quality* 34(1):359–69.

Mallarino, Antonio P. 1995. "Comparison of Mehlich-3, Olsen, and Bray-P1 Procedures for Phosphorus in Calcareous Soils." *Proceedings of the twenty-fifth North Central Extension-Industry soil fertility conference* (11):96–101.

Mallarino, Antonio P. 2003. "Differentiating and Understanding the Mehlich 3, Bray, and Olsen Soil Phosphorus Tests." *Soil Sci Soc Am J* 67:1928–34. Retrieved (http://soil.scijournals.org/cgi/content/abstract/soilsci;67/6/1928%5Cnhttp://www.agronext.iastate.edu/soilfertility/info/mnconf11_22_99.pdf).

Manderson, Andrew K., Alec D. Mackay, and Alan P. Palmer. 2007. "Environmental Whole Farm Management Plans: Their Character, Diversity, and Use as Agri-Environmental Indicators in New Zealand." *Journal of environmental management* 82(3):319–31. Retrieved (http://www.ncbi.nlm.nih.gov/pubmed/17084958).

McDowell, R. W., B. J. F. Biggs, A. N. Sharpley, and L. Nguyen. 2004. *Connecting Phosphorus Loss from Agricultural Landscapes to Surface Water Quality*. Retrieved (http://www.tandfonline.com/doi/abs/10.1080/02757540310001626092#preview).

McDowell, R. W. and A. N. Sharpley. 2003a. "Phosphorus Solubility and Release Kinetics as a Function of Soil Test P Concentration." *Geoderma* 112(1):143–54.

McDowell, R. W. and A. N. Sharpley. 2003b. "Uptake and Release of Phosphorus from Overland Flow in a Stream Environment." *Journal of Environmental Quality* 32(3):937–48. Retrieved (http://www.ncbi.nlm.nih.gov/pubmed/12809294).

McDowell, R. W., A. N. Sharpley, L. M. Condron, P. M. Haygarth, and P. C. Brookes. 2001. "Processes Controlling Soil Phosphorus Release to Runoff and Implications for Agricultural Management." *Nutrient Cycling in Agroecosystems* 59(3):269–84.

McDowell, R. W. and R. J. Wilcock. 2015. "Sources of Sediment and Phosphorus in Stream Flow of a Highly Productive Dairy Farmed Catchment." *Journal of Environmental Quality* 36(2):540–48.

Mckelvey, V. E. 1967. "Phosphate Deposits." *U.S. Geological Survey Bulletin* (1252). Retrieved (http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Phosphate+Deposits#8).

Mehlich, A. 1978. “Influence of Fluoride, Sulfate and Acidity on Extractable Phosphorus, Calcium, Magnesium and Potassium.” *Communications in Soil Science & Plant Analysis* 9(6):455–76.

Meijer, Marie-louise, Ingeborg de Boois, Marten Scheffer, Rob Portielje, and Harry Hosper. 1999. “Biomanipulation in Shallow Lakes in the Netherlands: An Evaluation of 18 Case Studies.” *Hydrobiologia* 408–409:13–30.

Merrill, Douglas T. and Roger M. Jorden. 1975. “Lime-Induced Reactions in Municipal Wastewaters.” *Journal of Water Pollution Control Federation* 47(12):2783–2808. Retrieved (http://www.jstor.org/stable/25038445).

Minnesota Pollution Control Agency. 2006. *Phosphorus Treatment and Removal Technologies*. St. Paul, MN.

Morse, G., S. Brett, J. Guy, and J. Lester. 1998. “Review: Phosphorus Removal and Recovery Technologies.” *The Science of The Total Environment* 212(1):69–81. Retrieved May 31, 2015 (http://www.sciencedirect.com/science/article/pii/S004896979700332X).

Mueller, D. K., P. A. Hamilton, D. R. Helsel, K. J. Hitt, and B. C. Ruddy. 1995. “Nutrients in Ground Water and Surface Water of the United States An Analysis of Data Through 1992 Water-Resources Investigations Report 95-4031.” *USGS Report 95-4031*.

Mullins, Gregory. 2009. “Phosphorus, Agriculture & the Environment.” *Virginia Cooperative Extension* 1–11. Retrieved (https://pubs.ext.vt.edu/424/424-029/424-029_pdf.pdf).

NAL. 1993. “Phosphorus.” 44(1):65. Retrieved (http://linkinghub.elsevier.com/retrieve/pii/0042207X9390049G).

National Research Council. 2001. *Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001*.

Newbold, J.Denis, Jerry W. Elwood, Robert V O’Neill, and Webster Van Winkle. 1981. “Measuring Nutrient Spiralling in Streams.” *Canadian Journal of Fisheries and Aquatic Sciences* 38(7):860–63. Retrieved (http://dx.doi.org/10.1139/f81-114).

Nolan, Bt and Jd Stoner. 2000. “Nutrients in Groundwaters of the Conterminous United States.” *Environmental Science & Technology* 34(7):1156–65. Retrieved (http://pubs.acs.org/doi/abs/10.1021/es9907663).

Olson, R. A. 1972. “Effects of Intensive Fertilizer Use on the Human Environment: A Summary Review.” *Effects of Intensive Fertilizer Use on the Human Environment* 368.

Olsson, Gustaf and Bob Newell. 1999. “Modelling, Diagnosis and Control.” *Modelling, Diagnosis and*. Retrieved (http://test.iwaponline.com/wio/2005/04/pdf/wio200504RF1900222159.pdf).

Paytan, Adina and Karen McLaughlin. 2007. “The Oceanic Phosphorus Cycle.” *Chemical Reviews* 107(2):563–76.

Peterson, Bruce J., John E. Hobbie, and Teresa L. Corliss. 1983. “A Continuous-Flow Periphyton Bioassay: Tests of Nutrient Limitation in a Tundra Stream.” *Limnology and Oceanography* 28(3):583–91. Retrieved (http://www.jstor.org/stable/2835839).

Pierson, S. T. et al. 2001. “Phosphorus and Ammonium Concentrations in Surface Runoff from Grasslands Fertilized with Broiler Litter.” *Journal of Environmental Quality* 30(5):1784–89.

Pierzynski, Gary M. 2000. “Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters.” *Southern cooperative series bulletin* 110.

Pierzynski, Gary M., Richard W. McDowell, J. T. Sims, and A. N. Sharpley. 2005. “Chemistry, Cycling, and Potential Movement of Inorganic Phosphorus in Soils.” *Phosphorus:*

Agriculture and the Environment. 53–86. Madison, WI: ASA, CSSA, SSSA.

Pope, By Larry M., Chad R. Milligan, and David P. Mau. 2002. *Historical Contributions of Phosphorus From Natural and Agricultural Sources and Implications for Stream Water Quality , Cheney Reservoir Watershed , South-Central Kansas*. 1st ed. Lawrence, KS: U.S. Department of the Interior.

Pote, D. H. et al. 1999. "Relationship Between Phosphorus Levels in Three Ultisols and Phosphorus Concentrations in Runoff." *Journal of Environmental Quality* 28(1):170–75.

Powell, J.Mark, Douglas B. Jackson-Smith, Larry D. Satter, and Larry G. Bundy. 2002. "Whole-Farm Phosphorus Management on Dairy Farms." Pp. 13–24 in *Proceedings of the 2002 Wisconsin Fertilizer, Aglime, and Pest Management Conference, Madison, WI*.

Prejs, A., A. Martyniak, S. Borón, P. Hliwa, and P. Koperski. 1994. "Food Web Manipulation in a Small, Eutrophic Lake Wirbel, Poland: Effects of Stocking with Juvenile Pike on Planktivorous Fish." *Hydrobiologia* 275/276:65–70.

Quigley, R. M. 1961. "Chloritized Weathering Products of a New England Glacial Till." *Clays and Clay Minerals* 10(1):107–16.

Räty, Mari, Jaana Uusi-Kämppä, Markku Yli-Halla, Kimmo Rasa, and Liisa Pietola. 2009. "Phosphorus and Nitrogen Cycles in the Vegetation of Differently Managed Buffer Zones." *Nutrient Cycling in Agroecosystems* 86:121–32.

Ribaudo, Marc et al. 2003. *Manure Management for Water Quality : Costs to Animal Feeding Operations of Applying Manure. Agricultural Economic Report Number 824*. Retrieved (http://www.ers.usda.gov/media/522864/aer824_3_.pdf).

Ripl, Wilhelm. 1976. "Biochemical Oxidation of Polluted Lake Sediment with Nitrate: A New Lake Restoration Method." *Ambio* 5(3):132–35.

Robertson, W. D., S. L. Schiff, and C. J. Ptacek. 1998. "Review of Phosphate Mobility and Persistence in 10 Septic System Plumes." *Ground Water* 36(6):1000–1010. Retrieved (http://dx.doi.org/10.1111/j.1745-6584.1998.tb02107.x).

Robinson, E., R. C. Robbins, Stanford Research Institute, and American Petroleum Institute. 1971. *Emissions, Concentrations, and Fate of Particulate Atmospheric Pollutants*. Stanford Research Institute. Retrieved (https://books.google.com/books?id=azOvGwAACAAJ).

Ross, G. J. and H. Kodama. 1974. "Experimental Transformation Of A Chlorite into A Vermiculite." *Clays and Clay Minerals* 22(3):205–11.

Rublee, Remington, Schaefer, and Marshall. 2005. "Detection of the Dinozoans Pfiesteria Piscicida and P. Shumwayae: A Review of Detection Methods and Geographic Distribution." *Journal of Eukaryotic Microbiology* 52(2):83–89. Retrieved (http://dx.doi.org/10.1111/j.1550-7408.2005.05202007.x).

Ruttenberg, K. C. 2013. "The Global Phosphorus Cycle." Pp. 499–558 in *Treatise on Geochemistry: Second Edition*, vol. 10.

Rybicki, Stanislaw M. 1998. *New Technologies of Phosphorus Removal from Wastewater. Proc. of a Polish-Swedish Seminar, Joint Polish Swedish Reports, Report.* (No. 3) Krakow, Poland.

Sas, H., I. Ahlgren, H. Bernhardt, and B. Bostroem. 1989. *Lake Restoration by Reduction of Nutrient Loading: Expectations, Experiences, Extrapolations*. 2nd ed. Berlin, Germany: St. Augustin: Academia Verlag Richarz.

Sauer, P. and A. Kimber. 2003. "Energy Consumption and Costs to Treat Water and Waste Water in Iowa." *Iowa Association of Municipal Utilities, Ankeny, IA*.

Schachtman, Daniel P., Robert J. Reid, and Sarah M. Ayling. 1998. “Phosphorus Uptake by Plants: From Soil to Cell.” *Plant physiology* 116(2):447–53.

Scheffer, Marten. 1990. “Multiplicity of Stable States in Freshwater Systems.” *Hydrobiologia* 200–201(1):475–86.

Schindler, D. W. 1974. “Eutrophication and Recovery in Experimental Lakes: Implications for Lake Management.” *Science* 184(4139):897–99.

Schindler, D. W. et al. 2008. “Eutrophication of Lakes Cannot Be Controlled by Reducing Nitrogen Input: Results of a 37-Year Whole-Ecosystem Experiment.” *Proceedings of the National Academy of Sciences* 105(32):11254–58.

Sedlak, R. I. 1991. *Phosphorus and Nitrogen Removal from Municipal Wastewater: Principles and Practice, Second Edition*. Taylor & Francis. Retrieved (https://books.google.com/books?id=bBSRPv87Ms8C).

Seviour, Robert J., Takashi Mino, and Motoharu Onuki. 2003. “The Microbiology of Biological Phosphorus Removal in Activated Sludge Systems.” *FEMS Microbiology Reviews* 27(1):99–127.

Shapiro, Joseph and David I. Wright. 1984. “Lake Restoration by Biomanipulation: Round Lake, Minnesota, the First Two Years.” *Freshwater Biology* 14(4):371–83. Retrieved (http://onlinelibrary.wiley.com.proxy.lib.muohio.edu/doi/10.1111/j.1365-2427.1984.tb00161.x/abstract%5Cnhttp://onlinelibrary.wiley.com.proxy.lib.muohio.edu/store/10.1111/j.1365-2427.1984.tb00161.x/asset/j.1365-2427.1984.tb00161.x.pdf?v=1&t=hszcfmij&s=8e3c653).

Sharpley, A. et al. 2006. “Best Management Practices To Minimize Agricultural Phosphorus Impacts on Water Quality.” ARS-163(July). Retrieved (http://www.ars.usda.gov/is/np/BestMgmtPractices/Best Management Practices.pdf).

Sharpley, A., R. Mcdowell, and P. Kleinman. 2001. “Phosphorus Loss from Land to Water: Integrating Agricultural and Environmental Management.” Pp. 287–307 in *Plant and Soil*, vol. 237. Retrieved (http://www.scopus.com/inward/record.url?eid=2-s2.0-0035704574&partnerID=tZOtx3y1).

Sharpley, A. N. 1985. “Depth of Surface Soil-Runoff Interaction as Affected by Rainfall, Soil Slope, and Management.” *Soil Science Society of America Journal* 49(4):1010.

Sharpley, A., S. Smith, and J. Naney. 1987. “Environmental Impact of Agricultural Nitrogen and Phosphorus Use.” *Journal of Agricultural and Food Chemistry* 35(5):812–17.

Sharpley, Andrew, T. C. Daniel, J. T. Sims, and D. H. Pote. 1996. “Determining Environmentally Sound Soil Phosphorus Levels.” *Journal of Soil and Water Conservation* 51(2):160–66.

Sharpley, Andrew N. et al. 2015. “Future Agriculture with Minimized Phosphorus Losses to Waters: Research Needs and Direction.” *Ambio* 44(S2):163–79. Retrieved (http://link.springer.com/10.1007/s13280-014-0612-x).

Sharpley, Andrew N. and S. J. Smith. 1993. “Prediction of Bioavailable Phosphorus Loss in Agricultural Runoff.” *Journal of Environment Quality* 22(1):32.

Shaw, Glendon R., David P. Moore, and Corinne Garnett. 2003. “Eutrophication and Algal Blooms.” Pp. 1–21 in *Encyclopedia of Life Support Systems*, vol. II.

Shen, Jianbo et al. 2011. “Phosphorus Dynamics: From Soil to Plant.” *Plant physiology* 156(3):997–1005.

Shepard, R. 2005. “Nutrient Management Planning: Is It the Answer to Better

Management?" *Journal of Soil and Water Conservation* 60:171+. Retrieved (http://go.galegroup.com/ps/i.do?id=GALE%257CA135773515&v=2.1&u=uiuc_uc&it=r&p=AONE&asid=94de3ebe9d065abad662d5083957f155).

Shigaki, Francirose, Andrew Sharpley, and Luís Ignácio Prochnow. 2006. "Source-Related Transport of Phosphorus in Surface Runoff." *Journal of Environment Quality* 35(6):2229. Retrieved (https://www.agronomy.org/publications/jeq/abstracts/35/6/2229).

Sims, J. T., R. R. Simard, and B. C. Joern. 1998. "Phosphorus Loss in Agricultural Drainage: Historical Perspective and Current Research." *Journal of Environment Quality* 27(2):277.

Skulberg, O. M., G. A. Codd, and W. W. Carmichael. 1984. "Toxic Blue-Green Algal Blooms in Europe: A Growing Problem." *Ambio* 13(4):244–47. Retrieved (http://www.scopus.com/inward/record.url?eid=2-s2.0-0021640056&partnerID=tZOtx3y1).

Slomp, C. P. and P. Van Cappellen. 2006. "The Global Marine Phosphorus Cycle: Sensitivity to Oceanic Circulation." *Biogeosciences Discussions* 3:1587–1629.

Smil, Vaclav. 2000. "Phosphorus In the Environment: Natural Flows and Human Interferences." *Annual review of energy and the environment* 25(1):53–88.

Smith, Richard A., Richard B. Alexander, and M.Gordon Wolman. 1987. "Water-Quality Trends in the Nation's Rivers." *Science* 235:1607+. Retrieved (http://go.galegroup.com/ps/i.do?id=GALE%257CA4757702&v=2.1&u=uiuc_uc&it=r&p=AONE&sw=w&asid=dbb3443a8c6315fd683e51f74b2203be).

Soetan, K. O., C. O. Olaiya, and O. E. Oyewole. 2010. "The Importance of Mineral Elements for Humans, Domestic Animals and Plants: A Review." *African Journal of Food Science* 4(May):200–222.

Sohngen, Brent, Kevin W. King, Gregory Howard, John Newton, and D.Lynn Forster. 2015. "Nutrient Prices and Concentrations in Midwestern Agricultural Watersheds." *Ecological Economics* 112(October):141–49. Retrieved (http://linkinghub.elsevier.com/retrieve/pii/S0921800915000476).

Soiltesting.org. 2015. "Illinois Soil Testing Association." Retrieved January 1, 2015 (http://www.soiltesting.org/5certifiedlabs.html).

Søndergaard, Martin, Jens Peder Jensen, and Erik Jeppesen. 2003. "Role of Sediment and Internal Loading of Phosphorus in Shallow Lakes." *Hydrobiologia* 506–509:135–45.

Søndergaard, Martin, Peter Kristensen, and Erik Jeppesen. 1993. "Eight Years of Internal Phosphorus Loading and Changes in the Sediment Phosphorus Profile of Lake Søbygaard, Denmark." *Hydrobiologia* 253(1–3):345–56.

Sonune, Amit and Rupali Ghate. 2004. "Developments in Wastewater Treatment Methods." *Desalination* 167(1–3):55–63.

Sosnovsky, Alejandro and Rolando Quirós. 2009. "Effects of Fish Manipulation on the Plankton Community in Small Hypertrophic Lakes from the Pampa Plain (Argentina)." *Limnologica* 39:219–29.

Sperling, Marcos Von. 2008. *Wastewater Characteristics, Treatment and Disposal*. illustrate. London, UK: IWA publishing. Retrieved (http://www.cro3.org/cgi/doi/10.5860/CHOICE.45-2633).

Srinivasan, Ancha. 2007. "Handbook of Precision Agriculture: Principles and Applications." *Euphytica* 156(1–2):269–70.

Stewart, J. W. B. 1987. "Dynamics of Soil Organic Phosphorus." *Biogeochemistry* 4(1):41–60.

Stewart, John W. B., Robert B. McKercher, Y. Liu, and J. Chen. 2002. "Phosphorus Cycle." *Experimental Microbial Ecology* 5(2003):221–38. Retrieved (http://www.sciencedirect.com/science/article/B7CTC-4FTGWNS-F/2/217ea26655fb632befb4a81bea973c8e).

Stratful, I., S. Brett, M. B. Scrimshaw, and J. N. Lester. 1999. "Biological Phosphorus Removal, Its Role in Phosphorus Recycling." *Environmental Technology* 20(7):681–95.

Stuanes, A. 1982. *Phosphorus Sorption by Soil; A Review*. 1st ed. Rotterdam: Springer Netherlands.

Svendsen, Lars M. and Brian Kronvang. 1993. "Retention of Nitrogen and Phosphorus in a Danish Lowland River System: Implications for the Export from the Watershed." *Hydrobiologia* 251(1–3):123–35.

Szabó, A. et al. 2008. "Significance of Design and Operational Variables in Chemical Phosphorus Removal." *Water Environment Research: A Research Publication of the Water Environment Federation* 80(5):407–16.

Thomas, M. A. et al. 2007. "Evaluation of Nutrient Management Plans Using an Integrated Modeling Approach." *Applied Engineering in Agriculture* 23(6):747–55.

Tomer, Mark D. and Matt Liebman. 2014. "Nutrients in Soil Water under Three Rotational Cropping Systems, Iowa, USA." *Agriculture, Ecosystems and Environment* 186(3):105–14. Retrieved (http://dx.doi.org/10.1016/j.agee.2014.01.025).

Toor, Gurpal S. 2009. "Enhancing Phosphorus Availability in Low-Phosphorus Soils by Using Poultry Manure and Commercial Fertilizer." *Soil Science* 174(6):358–64.

Turner, B. L., M. J. Paphazy, P. M. Haygarth, and I. D. Mckelvie. 2002. "Inositol Phosphates in the Environment." *Philosophical Transactions of the Royal Society: Biological Sciences* 357(1420):449–69. Retrieved (http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2001.0837).

University of Wisconsin. 1995. "What Is A Farm Nutrient Management Plan?" 4. Retrieved (http://ipcm.wisc.edu/download/pubsNM/what_is_NMP.pdf).

USDA. 2008. "At A Glance: Wetlands Reserve Program." (May):1. Retrieved (papers2://publication/uuid/8CEF930D-88EE-4995-B37B-3354C3A40F2C).

USEPA. 1974. *Potomac Estuary Case Study*. Washington D.C.: United States Environmental Protection Agency.

USEPA. 1996. *4.0 Environmental Assessment*. Washington D.C.: United States Environmental Protection Agency.

USEPA. 2002. "National Water Quality Inventory: 2002 Reporting Cycle Section 305 of the Clean Water Act." *October* 35(October). Retrieved (http://www.epa.gov/305b/2002report/factsheet2002305b.pdf).

USEPA. 2010. "U.S. Wastewater Treatment Plants - Partial List March 2010." (March).

Vaquer-Sunyer, Raquel and Carlos M. Duarte. 2008. "Thresholds of Hypoxia for Marine Biodiversity." *Proceedings of the National Academy Of Sciences of the United States of America* 105(40):15452–57.

Vasconcelos, Vitor. 2006. "Eutrophication, Toxic Cyanobacteria and Cyanotoxins: When Ecosystems Cry for Help." *Limnetica* 25(1–2):425–32.

Vidon, P. and P. E. Cuadra. 2011. "Phosphorus Dynamics in Tile-Drain Flow During Storms in the US Midwest." *Agricultural Water Management* 98(4):532–40. Retrieved September 15, 2015 (http://www.sciencedirect.com/science/article/pii/S037837741000315X).

Viviani, Romano. 1992. *Marine Coastal Eutrophication*. Elsevier. Retrieved October 8, 2015 (http://www.sciencedirect.com/science/article/pii/B9780444899903500560).

Volterra, L., Boualam, M., Ménesguen, A., Duguet, J. P., Duchemin, J., & Bonnefoy, X. (2002). Eutrophication and health. *World Health Organization and European commission*.

Wallace, Scott, Gene Parkin, and Brett Ballavance. 2005. *Ecological Wastewater Management in Iowa Hope for Iowa's Small Communities*. Mount Vernon, IA.

Waschbusch, R. J., Selbig, W. R., & Bannerman, R. T. (1993). Sources of phosphorus in stormwater and street dirt from two urban residential basins in Madison, Wisconsin, 1994-95. In *National Conference on Tools for Urban Water Resource Management and Protection proceedings, February 710, 2000, Chicago, IL.* (p. 9). Diane Publishing.

Weld, J. L., A. N. Sharpley, D. B. Beegle, and W. J. Gburek. 2001. "Identifying Critical Sources of Phosphorus Export from Agricultural Watersheds." *Nutrient Cycling in Agroecosystems* 59(1):29–38.

Wortman, C. et al. 2005. *Agricultural Phosphorus Management and Water Quality Protection in the Midwest*. Book 161. Ames, IA. Retrieved (http://extension.missouri.edu/p/NCR187).

Wu, Z., L. D. Satter, and R. Sojo. 2000. "Milk Production, Reproductive Performance, and Fecal Excretion of Phosphorus by Dairy Cows Fed Three Amounts of Phosphorus." *Journal of dairy science* 83(5):1028–41.

Yeoman, S., T. Stephenson, J. N. Lester, and R. Perry. 1988. "The Removal of Phosphorus During Wastewater Treatment: A Review." *Environmental Pollution* 49:183–233.

Zhang, T. Q., A. F. MacKenzie, B. C. Liang, and C. F. Drury. 2004. "Soil Test Phosphorus and Phosphorus Fractions with Long-Term Phosphorus Addition and Depletion." *Soil Science Society of America Journal* 68(2):519–28.

Chapter 2: Comparative evaluation of phosphate spectrophotometric methods in soil test phosphorus extractants

2.1 Abstract

Various methods utilizing ammonium molybdate and ascorbic acid have been developed for the detection of orthophosphate in aqueous solution. However, the optimal wavelengths (nm) for UV-Vis spectrophotometry are often reported having only used ultrapure water as a background medium. The methods developed by Murphy and Riley (1962), He et al. (1998), Dick and Tabatabai (1977), and Asher (1980) were compared based on the absorbance spectra they produced using 0-25 mg/L phosphate standards with ultrapure water, Bray P-1 extractant, and Mehlich III extractant as background mediums. Via linear regression, suggested wavelengths (nm) in the original publications were compared with optimal wavelengths suggested by the absorbance spectra in each medium. It was found that the suggested wavelength of 882 nm was optimal for the Murphy and Riley (1962) and He et al. (1998) methods, regardless of background medium. Using the Dick and Tabatabai (1977) method, this study suggests using wavelengths of 886 and 888 nm with Bray P-1 and Mehlich III extractants as a background medium respectively, and 888 nm using the Asher (1980) method, regardless of background medium.

2.2 Introduction

Orthophosphate detection in solution is critical for many environmental and agronomic soil and water tests for phosphorus. Known to be the primary biologically available species of phosphorus, orthophosphate abundances in natural water systems generated by agricultural runoff and urban pollution can cause adverse hypoxic conditions (Correll 1998; Dolah 2000; Kleinman et al. 2011). Concentrations as low as 0.025 mg/L can result in harmful algal blooms (Burkart et al. 2004; Nolan and Stoner 2000), and as such, accurate and precise determination of orthophosphate is of utmost importance in soil and water samples. Colorimetric techniques utilizing ascorbic acid-ammonium molybdate reagents are used widely by research facilities alike for rapid determination of orthophosphate concentration. The techniques are advantageous over Inductively Couple Plasma (ICP) atomic emission spectroscopy (AES) and optical emission spectrometry

(OES), since the spectrophotometric method is specific to dissolved orthophosphate via the formation of a heteropoly molybdophosphate complex, and does not overestimate the orthophosphate concentration when colloidal P, organic-P, and polyphosphate are present in samples.

Murphy and Riley (1962) introduced the ascorbic acid method that is still widely in use in soil and water science research today, utilizing antimony in the form of potassium antimonyl tartrate as a catalyst for rapid color development (Pierzynski 2000). Advantages of the Murphy and Riley (1962) method over the previous stannous chloride methods (Berenblum and Chain 1938) include a longer stability of the molybdenum blue color at 882 nm, tolerance to high salt and Fe^{3+} ion concentrations, and a detection limit of 10 μg/L (Kuo 1996). Though sensitive and rapid, interference with the determination of orthophosphate was noted in solutions with competing ligands such as arsenate, polyphosphates, fluoride, oxalate, citrate, and tartrate (Dick and Tabatabai 1977; He et al. 1998). This is especially important considering that competing ligands are present in heterogeneous soil solutions, impeding accurate orthophosphate detection in these mediums. Several modifications have been made to the original Murphy and Riley (1962) method to address these issues, although not in conjunction.

To overcome ligand interference (e.g., oxalate, citrate, tartrate, and fluoride), He et al. (1998) modified the Murphy and Riley (1962) method to include an excess amount of ammonium molybdate. This modification increases sensitivity to orthophosphate concentration by reducing the interference from competing ligands.

Dick and Tabatabai (1977) addressed the issue of organic and polyphosphate hydrolysis in the acidic color forming media by modifying the ascorbic acid technique to contain a citrate-arsenite-acetic acid reagent to remove excess molybdate ions. By adding citrate and arsenite ligands, orthophosphate derived from hydrolysis in the acidic color forming media cannot complex with molybdate to intensify the blue color of the sample, which allows more accurate measurements of dissolved orthophosphate when hydrolysable P species are present. Asher (1980) modified this method by including HCl instead of trichloroacetic acid in the reducing reagent (Asher 1980), and recommending a wavelength of 660 nm, whereas Dick and Tabatabai (1977) postulated that at lower acidities, the absorbance of molybdenum blue is maximized around 700 nm.

While these P spectrophotometric methods were developed using ultrapure water (18.2 MΩ) as the background medium, the sensitivity and optimum wavelength of each method have never been compared and evaluated for an important agronomic purpose such as Soil Test Phosphorus (STP). Common STP extractants like Bray and Kurtz P-1 (Bray and Kurtz 1945) and Mehlich III (Mehlich 1984) contain an abundance of competing ligands (Bray P-1: 0.025 M HCl and 0.03 M NH_4F, Mehlich III: 0.2 N acetic acid, 0.25 N NH_4NO_3, 0.015 NH_4F; 0.013 N HNO_3, 0.001 M and ethylenediaminetetraacetic (EDTA) acid). It is not well understood how recommended wavelengths and detection limits in ultrapure water compare to STP extracting solutions. The objective of this study is to evaluate the detection limits and efficacy of these methods in common STP extracts as background media.

2.3 Materials and methods

2.3a Materials

In all experiments, ACS grade chemicals and ultrapure water (18.2 MΩ) were used unless otherwise mentioned in the text.

2.3b Phosphate spectrophotometric analysis

The absorbance spectra of phosphate standards (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2, 5, 10, 15, 20, and 25 mg/L) in ultrapure water, Bray P-1 extracting solution, and Mehlich III extracting solution were evaluated at 500-950 nm wavelength using the molybdenum blue colorimetric methods of Murphy and Riley (1962), He et al. (1998), Dick and Tabatabai (1977) and Asher (1980). Hereafter, all concentrations are referred to as dissolved phosphate. Methodological details are summarized below. An Ocean Optics QE Pro spectrometer, an Ocean Optics DT-Mini-2 light source, and Ocean Optics software were used to acquire the spectra. Integration time varied with the medium and method used: 130 ms with ultrapure water, 140 ms with Bray P-1 extractant, and 146 ms with Mehlich III extract solution as background media. Scans to average were set to 20 and boxcar width to 5 over all trials. Assuming that absorption is proportional to concentration according to Beer-Lambert's law, linear regression analyses were performed at recommended and/or optimum wavelengths (nm).

2.3c Murphy and Riley (1962) method

The three reagents used are: 1) Reagent A – sulfuric acid (2.5 M)-ammonium molybdate (0.01 M)-potassium antimonyl tartrate (0.001 M), 2) Reagent B – Ascorbic acid (0.1 M), and 3) Reagent C – Mixed reagent consisting of 35 mL reagent A and 215 mL reagent B. The procedure is as follows: 0.5 mL of sample is added to a cuvette followed by 2 mL of reagent C. The contents of the cuvette are mixed by inversion and the color is left to develop for 10 min followed by measurement of absorbance at 882 nm (Murphy and Riley 1962).

2.3d He et al. (1998) method

The reagents and procedure for He et al. (1998) are the same as Murphy and Riley (1962), with the exception of reagent A, which has additional ammonium molybdate to limit interference from other ligands, such as fluoride and tartrate. The three reagents used are: 1) Reagent A – sulfuric acid (2.5 M)-ammonium molybdate (0.02 M)-potassium antimonyl tartrate (0.001 M), 2) Reagent B – Ascorbic acid (0.1 M), and 3) Reagent C – Mixed reagent consisting of 35 mL reagent A and 215 mL reagent B. The procedure is as follows: 0.5 mL of sample is added to a cuvette followed by 2 mL of reagent C. The contents of the cuvette are mixed by inversion and the color is left to develop for 10 min followed by measurement of absorbance at 882 nm.

2.3e Dick and Tabatabai (1977) method

A modified ammonium molybdate-ascorbic acid method by Dick and Tabatabai (1977) was developed to reduce the over quantification of phosphate that is released from the acid hydrolysis reactions of organic P and poly P in the color forming agent. In this method, excess molybdate ions are complexed by citrate and arsenite ligands so that orthophosphate produced in the color-forming solution will not be detected. Sodium bisulfite is added to reagent C to reduce any arsenate present to arsenite. Three reagents are used in this procedure: 1) Reagent A - Ascorbic acid (0.1 M)-trichloroacetic acid (0.5 M), 2) Reagent B - Ammonium molybdate (0.01 M), and 3) Reagent C - Sodium citrate (0.1 M)-sodium arsenite (0.2 M)-sodium bisulfite (0.03 M) -acetic acid (5%). 1.4 mL of reagent A is added to a cuvette followed by 0.14 mL of aqueous sample, 0.28 mL reagent B, and 0.7 mL reagent C. The sample is diluted with 0.98 mL of ultrapure water, mixed by inversion, and left for at least 10 min of color development.

2.3f Asher (1980) method

Analogous to Dick and Tabatabai (1977), poly-P and organic-P hydrolysis in the color forming agent is addressed by the addition of a citrate-arsenite-sodium bisulfite reagent. Reagents A and B, however, differ by the addition of hydrochloric acid and lack of trichloroacetic acid. The three reagents used are: 1) Reagent A – Ascorbic acid (0.1 M)-hydrochloric acid (0.38 M), 2) Reagent B – Ammonium molybdate (0.01 M)-hydrochloric acid (0.48 M), and 3) Reagent C – Sodium citrate (0.1 M)-sodium arsenite (0.2 M)-sodium bisulfite (0.03 M)-acetic acid (5%). The procedure is the same as that of Dick and Tabatabai (1977), except for the optimal wavelength.

2.3g Statistical Evaluation of methods

The analytical methods were validated according to the International Conference for Harmonization (ICH) guidelines (Miller and Miller 2005) under optimized experimental conditions. Linearity was evaluated by linear regression analysis ($Y=mx+b$). Molar absorptivity was calculated from the slope of said regressions and the limit of detection (LOD) and limit of quantification (LOQ) were calculated via the formulas $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, where σ is the standard deviation of blank response and S is the slope of the calibration curve. The limits of detection and limits of quantification illustrate the concentration of phosphate that gives an instrument signal significantly different than the blank and the lowest concentration of phosphate that can be detected by the proposed methods with confidence, respectively. Sandell's sensitivity, which is the concentration of analyte that gives an absorbance of 0.001 in a cell of path length 1 cm, and is expressed as $\mu g\ cm^{-2}$. Sandell's sensitivity values were calculated by dividing $[PO_4^{3-}]$ in µg/mL by absorbance and multiplying by 0.001.

2.4 Results and discussion

Spectra comparison of spectrophotometric methods (Figs 2.1 and 2.5) and the results of regression analysis are summarized in Figs 2.4 and 2.6-2.11 and Table 2.1. The comparison was made in two groups based on the method with or without additional chemical reagents (e.g., arsenite, citrate) to scavenge excess ammonium molybdate: 1) Murphy and Riley (1962) VS. He et al. (1998) and 2) Dick and Tabatabai (1977) VS. Asher (1980).

2.4a Results of statistical evaluation

The results of statistical evaluations in Table 2.1 reflect the efficacy and sensitivity of the proposed methods. The Sandell's sensitivity values for the methods of Murphy and Riley (1962) and He et al. (1998) show that the minimum amount of detectable phosphate is much lower than with the methods of Dick and Tabatabai (1977) or Asher (1980). The method of He et al. (1998) is slightly more sensitive, on average, than that of Murphy and Riley (1962) as is the technique of Asher (1980) when compared to Dick and Tabatabai (1977), which is shown by their lower LODs and LOQs (Table 2.1).

2.4b Comparison of the methods by Murphy & Riley (1962) and He et al. (1998)

The spectral features produced by using the Murphy and Riley (1962) molybdenum blue method are relatively similar in all background media (Figs 2.1a-2.1c). All regression analysis in different concentration ranges and background media are summarized in Figs 2.4 and Table 2.1. Maximum absorbance peaks are at approximately 720 nm and 882 nm (Figs 2.1a-2.1c), though there are bathochromic shifts in absorbance peaks with increasing phosphate concentration. The spectra compare favorably with the spectra produced in the original paper, which shows 882 nm as the optimal wavelength for linear regression as long as [P] is below 2 mg/L. The absorbance peaks at > 2 mg/L are shifted to 888 nm in ultrapure water (Fig 2.1a), 891 nm in Bray P-1 (Fig 2.1b), and 893 nm in Mehlich III (Fig 2.1c). The R^2 value for 0-2 mg/L at 882 nm are 0.999, 0.993, and 0.999 with ultrapure water, Bray P-1, and Mehlich III, respectively (Figs 2.2a, 2.3a, and 2.4a). Due to the bathochromic shifts, correlation using linear regression is not satisfied over the entire data range (0-25 mg/L) (Figs 2.2b, 2.3b, and 2.4b). The Murphy and Riley (1962) method has been used extensively in phosphorus research, and as such there are many examples of its sensitivity and accuracy with 0-2 mg/L phosphate concentration in water (Andraski and Bundy 2001; Jørgensen et al. 2015; Westermann et al. 2001), Bray P-1 extractant (Menon, Chien, and Hammond 1989; Sharpley, Jones, and Gray 1984), and Mehlich III extractant (Cox and Hendricks 2000; Tarkalson and Mikkelsen 2004), though its inefficacy at higher [PO_4^{3-}] (mg/L) in Bray P-1 and Mehlich III extractants have not been shown until now.

The method designed by He et al. (1998) produces markedly different absorbance spectra compared to the Murphy and Riley (1962) method (Fig 2.1). Excess molybdate results in greater color intensity when analyzing standards between 0-25 mg/L phosphate. Absorbance values are consistently higher over the spectral range and hypsochromic shift occurs with increasing P concentration (Figs 2.1d-2.1f). The absorbance peak present at ~720 nm in the Murphy and Riley method (Figs 2.1a-2.1c) is much less prominent in the spectra produced by the He et al. (1998) method (Figs 2.1d-2.1f). At lower concentrations (0-2 mg/L) the maximum absorbance peaks are approximately 882- 888 nm in ultrapure water, Bray P-1, and Mehlich III (Figs 2.1d-2.1f).

Between 2-25 mg/L in all three media (Figs 2.1d, 2.1e and 2.1f), the maximum peak absorbance shifts to lower wavelengths, ~832 nm. For this reason, the regressions analysis was conducted at 882nm using the spectra at 0-2 mg/L. 882 nm presents the best fit for linear correlation. Between 0-2 mg/L, the R^2 values are 0.998, 0.998, and 0.999, with ultrapure water, Bray P-1, and Mehlich III, respectively (Figs 2.2c, 2.3c, and 2.4c). When the entire concentration range (0-25 mg/L) was assessed, linear regression did not provide a suitable fit due to a non-linear relationship between concentration and absorbance. Effective use of this method in the range of 0-2 mg/L phosphate has been reported with water (Kowalenko and Babuin 2007; Mathews et al. 2005; Wei, Chen, and Xu 2009), and Bray P-1 (He et al., 1998) as background media, but not with Mehlich III extractant.

2.4c Comparison of the methods by Dick and Tabatabai (1977) and Asher (1980)

Unlike in the previous two methods, the method by Dick and Tabatabai showed some blue color in the blank. This is due to 1) phosphate impurity in trichloroacetic acid

(Amresco Inc.), arising from use of organic components in its manufacturing process and 2) arsenate impurity in sodium arsenite. Although ACS grade chemicals were used, these contaminations were unavoidable. Addition of bisulphite reduced the color intensity arising from arsenate, but the background phosphate contamination from trichloroacetic acid still remained. The method by Asher (1980) produced a colorless blank since trichloroacetic acid was replaced with HCl.

Figs 2.5a, 2.5b, and 2.5c show the spectra obtained using the method of Dick and Tabatabai (1977) in ultrapure water, Bray P-1, and Mehlich III extractants. In Fig 2.5a, the highest peak occurred at approximately 850 nm with a smaller peak at 700 nm. The results of regression analyses are summarized in Figs 2.7-2.11.

With ultrapure water as the background medium, Dick and Tabatabai's (1977) publication recommended a wavelength of 700 nm. The same optimal wavelength was found in this study, having an R^2 value of 0.996 between 2-25 mg/L (Fig 2.6b). Below 2 mg/L, correlation rapidly decreased to < 0.95 R^2 (Fig 2.7a), suggesting that this method is not suited for [P] < 2 mg/L. Regression analysis was also conducted at 850 nm (Fig 2.5a), however, hypsochromic shift reduced the R^2 to 0.981 (not shown).

In Bray P-1 extractant, the optimal wavelength for linear regression was 886 nm instead of 700 nm (Fig 2.5b), giving an R^2 of 0.998 between 2-25 mg/L (Fig 2.8a) and 0.973 between 0-2 mg/L (Fig 2.8a). Sensitivity suffers at [P] < 2 mg/L in Bray P-1 extractant. At the recommended 700 nm in Bray P-1 extractant, the R^2 value was 0.973 between 0-2 mg/L (Fig 2.9a) and 0.994 between 2-25 mg/L (Fig 2.9b). In Mehlich III extractant, the optimal wavelength for regressions was 888 nm (Fig 2.1c) with an R^2 of 0.993 between 2-25 mg/L (Fig 2.10b) and 0.787 between 0-2 mg/L (Fig 2.10a). It is clear that this method is not sensitive to [P] < 2 mg/L in Mehlich III extractant.

At 700 nm, the R^2 value between 2-25 mg/L was 0.996 (Fig 2.11b) and 0.873 (Fig 2.11a) between 0-2 mg/L. Overall, 888 nm is the better wavelength to estimate dissolved orthophosphate at > 2 mg/L. Multiple studies have utilized this method to determine phosphate concentrations between 2-25 mg/L with water (Griffin, Honeycutt, and He 2003; Waldrip et al. 2015), Bray P-1 extractant (Dick and Tabatabai 1987; Jacoby 2005), or Mehlich III (Jacoby 2005; Waldrip et al. 2015) as a background medium.

Figs 2.5d, 2.5e, and 2.5f show the spectra obtained using the method of Asher (1980) in ultrapure water, Bray P-1, and Mehlich III extractants, respectively. The highest absorbance peak occurs at approximately 888 nm with a smaller peak at 660 nm in all background media (Figs 2.5d, 2.5e, and 2.5f). The results of regression analyses are summarized in Figs 2.7-2.11 and Table 2.1. Using the Asher (1980) method, the optimal wavelength was 888 nm across all media (Figs 2.5d, 2.5e, and 2.5f). Prior to the assessment at 888 nm, the recommended 660 nm was used in regression analysis. In ultrapure water, the R^2 values between 0-2 mg/L were 0.830 (Fig 2.6a) and 0.984 between 2-25 mg/L (Fig 2.6b). At 888 nm in ultrapure water, the R^2 value between 0-2 mg/L was 0.927, but was 0.997 between 2-25 mg/L (Figs 2.7c and 2.7d). Like the method by Dick and Tabatabai (1977), this method is more sensitive at higher phosphate concentration.

At 660 nm with Bray P-1 as the background medium, R^2 values were 0.928 between 0-2 mg/L (Fig 2.9c) and 0.996 between 2-25 mg/L (Fig 2.9d). At 888 nm however, the R^2 values improve to 0.975 between 0-2 mg/L (Fig 2.8c) and 0.998 between 2-25 mg/L (Fig 2.8d). 888 nm seems to be a better wavelength to evaluate the concentration at 2-25 mg/L and Bray P-1 as a background medium.

At 660 nm with Mehlich III extractant as the background medium, the R^2 values were 0.954 between 0-2 mg/L (Fig 2.11c) and 0.996 between 2-25 mg/L (Fig 2.11d). The R^2 values at 888 nm were 0.755 between 0-2 mg/L (Fig 2.10c) and 0.998 between 2-25 mg/L (Fig 2.10d). 888 nm again appears to be a more suitable wavelength for evaluation of samples between 2-25 mg/L phosphate. Used less extensively than the other methods, there is evidence for accurate determination of phosphate between 2-25 mg/L with water as a background medium (Mueller-Harvey and Wild 1986; Xie and MacKenzie 1988), but not with Bray P-1 or Mehlich III extractants as background media.

2.5 Conclusion

The results of this study have several important implications for the users of the molybdenum blue-ascorbic acid methods for phosphate determination designed by Murphy and Riley (1962), He et al. (1998), Dick and Tabatabai (1977), and Asher (1980). Though the absorbance spectra are markedly different, the recommended wavelength of

882 nm is suitable for both the methods of Murphy and Riley (1962) and He et al. (1998) regardless of whether the background medium is ultrapure water, Bray P-1 extractant, or Mehlich III extractant. The inclusion of excess molybdate in He et al. (1998) drastically reduces the peak observed at ~720 nm in the spectra of Murphy and Riley (1962), which can be attributed to interfering ligands. The elimination of interfering ligands also makes He et al. (1998) a slightly more sensitive and better correlated method than Murphy and Riley (1962). The methods by Murphy and Riley (1962) and He et al. (1998) are best used at 882 nm for evaluation of phosphate at less than 2 mg/L. The decrease in linearity at > 2 mg/L can be attributed to the hypsochromic shift in the absorbance spectra with increasing phosphate concentration.

In the method described by Dick and Tabatabai (1977), the inclusion of trichloroacetic acid in the reducing agent has the consequence of introducing phosphate contamination to any sample, including the blank. For this reason, sensitivity at low phosphate concentrations (0-2 mg/L) is poor. If the method of Dick and Tabatabai (1977) is to be used for evaluation of samples above 2 mg/L, the suggested wavelength of 700 nm is optimal in ultrapure water, but with Bray P-1 extractant or Mehlich III extractant as background media, 886 and 888 nm are the optimal wavelengths, respectively. The method designed by Asher (1980) produces a colorless blank due to the substitution of P-contaminated trochloroacetic acid with HCl, but like Dick and Tabatabai (1977), there is a lack of sensitivity at < 2 mg/L and the method is only suitable for samples containing > 2 mg/L phosphate. The recommended wavelength of 660 nm in the original paper was attributable to instrumental limitations. In this study, however, it was found that 888 nm provides better correlation whether ultrapure water, Bray P-1 extractant, or Mehlich III extractant is the background media. This study suggests that the use of specific wavelengths in each P spectrophotometric method becomes critical in detecting different concentration ranges of orthophosphate in Bray P-1 and Mehlich III extractants.

2.6 Figures

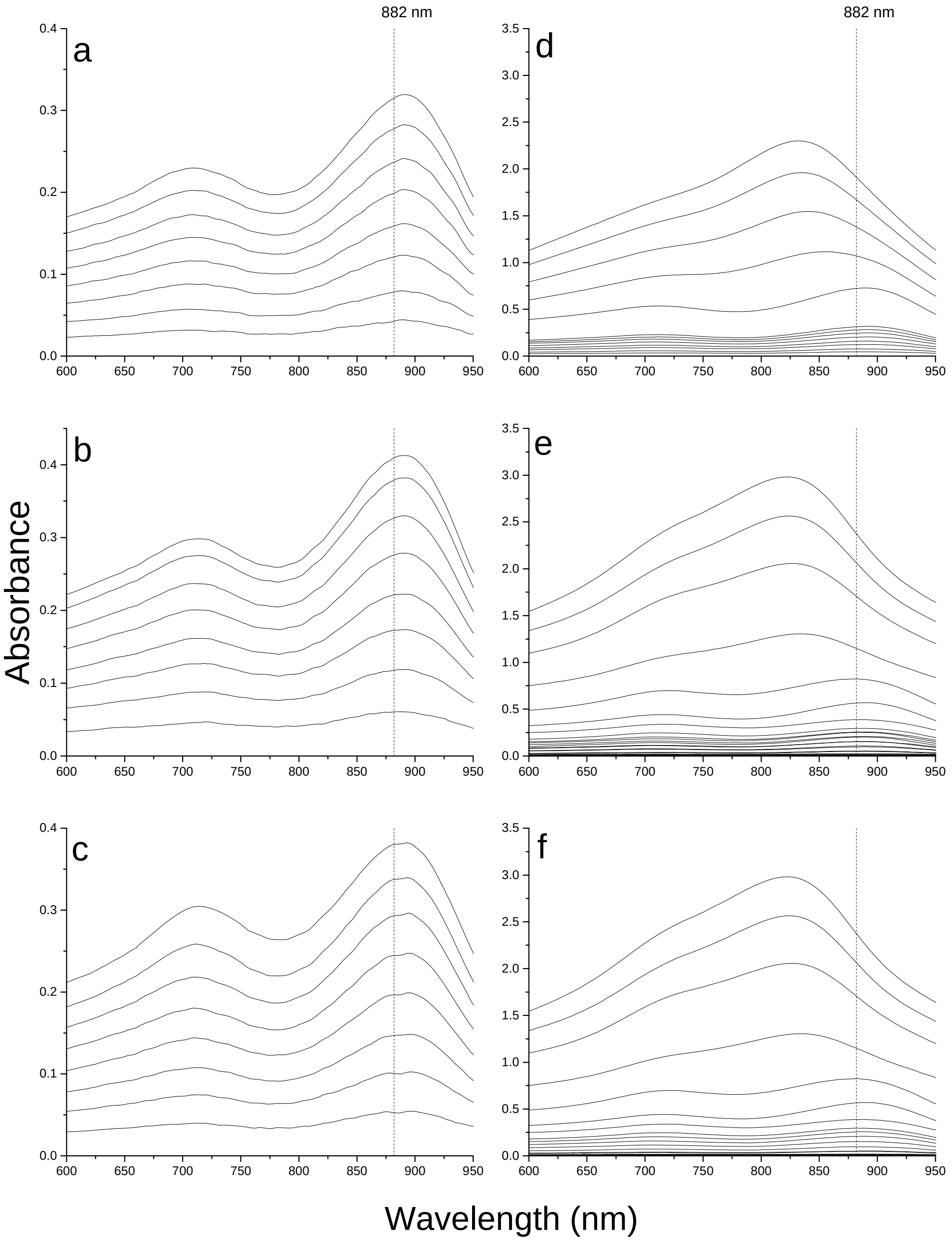

Figure 2.1: Absorbance spectra of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 5.0, 10.0, 15.0, 20.0, and 25.0 ppm phosphate in: a) Milli-Q water, b) Bray P-1 extracting solution, c) Mehlich III extracting solution. Plots a-c utilized the molybdenum blue method designed by Murphy and Riley (1962). Plots d-f utilized He et al. (1998) Vertical dashed lines represent wavelength (nm) recommended in method.

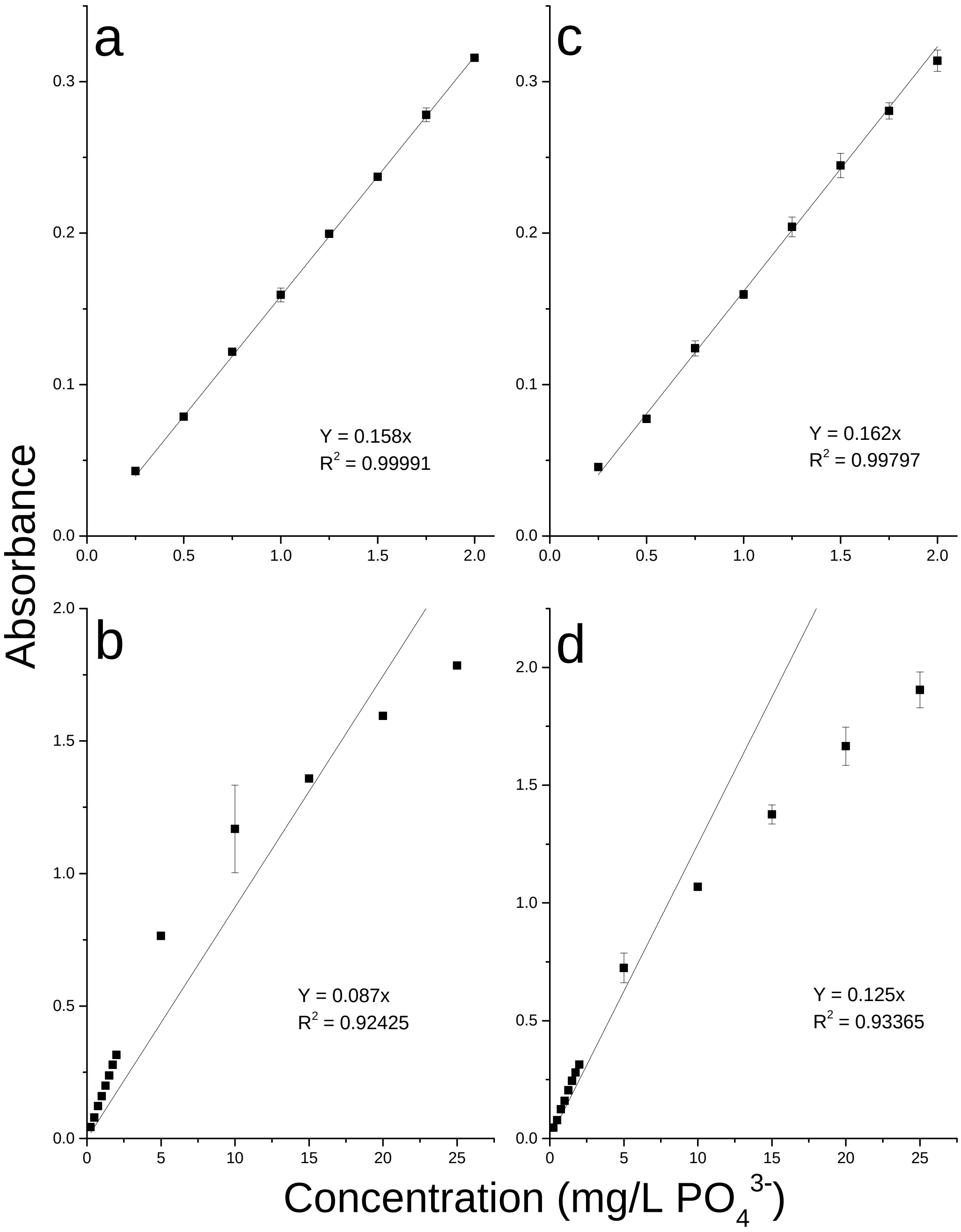

Figure 2.2: Regression analyses using the molybdenum blue methods by Murphy and Riley (1962) at 882 nm in ultrapure water (a and b) and by He et al. (1998) at 882 nm in ultrapure water (c and d). [P] at 0-2 mg/L (a and c) and [P] at 0-25 mg/L (b and d).

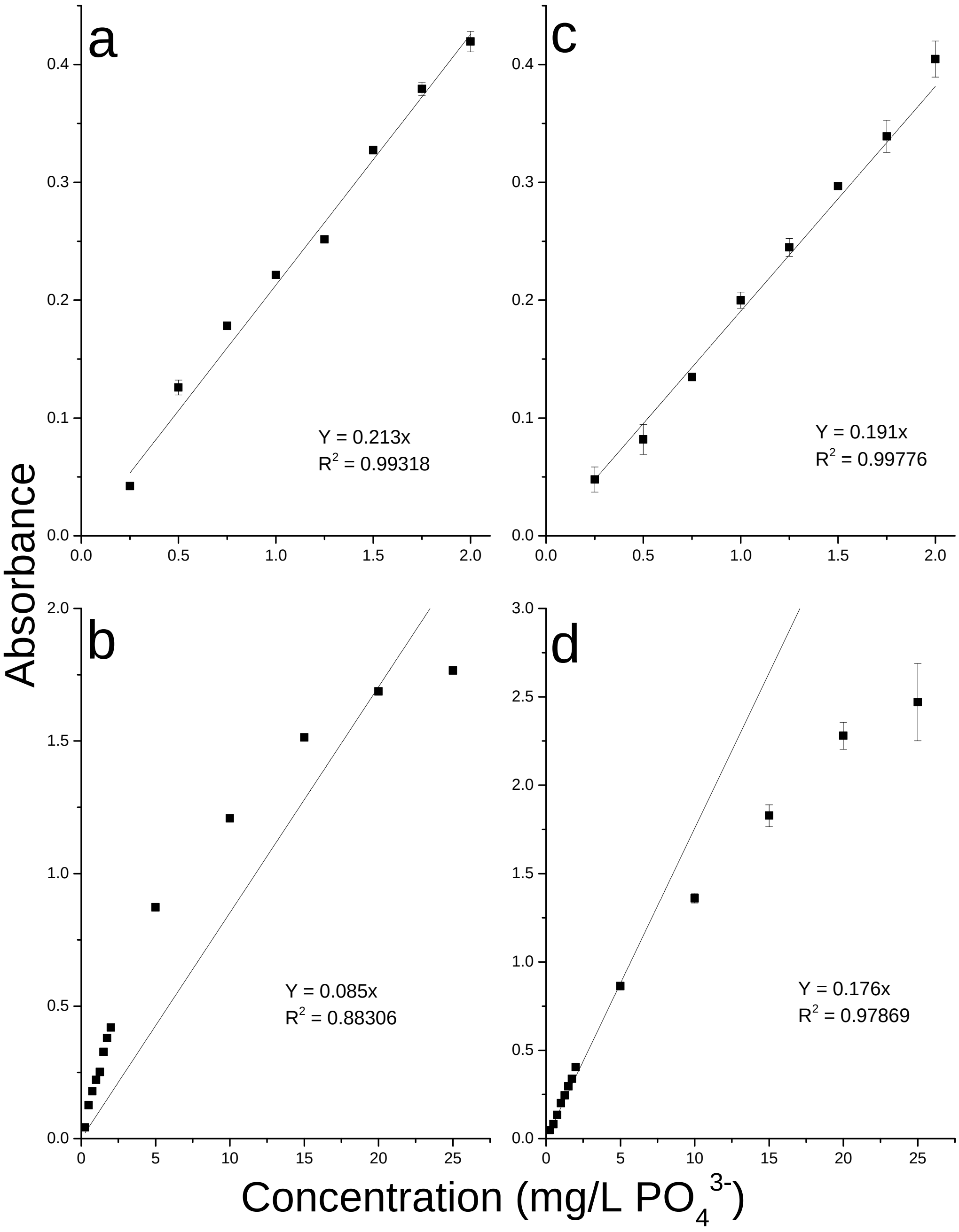

Figure 2.3: Regression analyses using the molybdenum blue methods by Murphy and Riley (1962) at 882 nm in Bray P-1 (a and b) and by He et al. (1998) at 882 nm in Bray P-1 (c and d). [P] at 0-2 mg/L (a and c) and [P] at 0-25 mg/L (b and d).

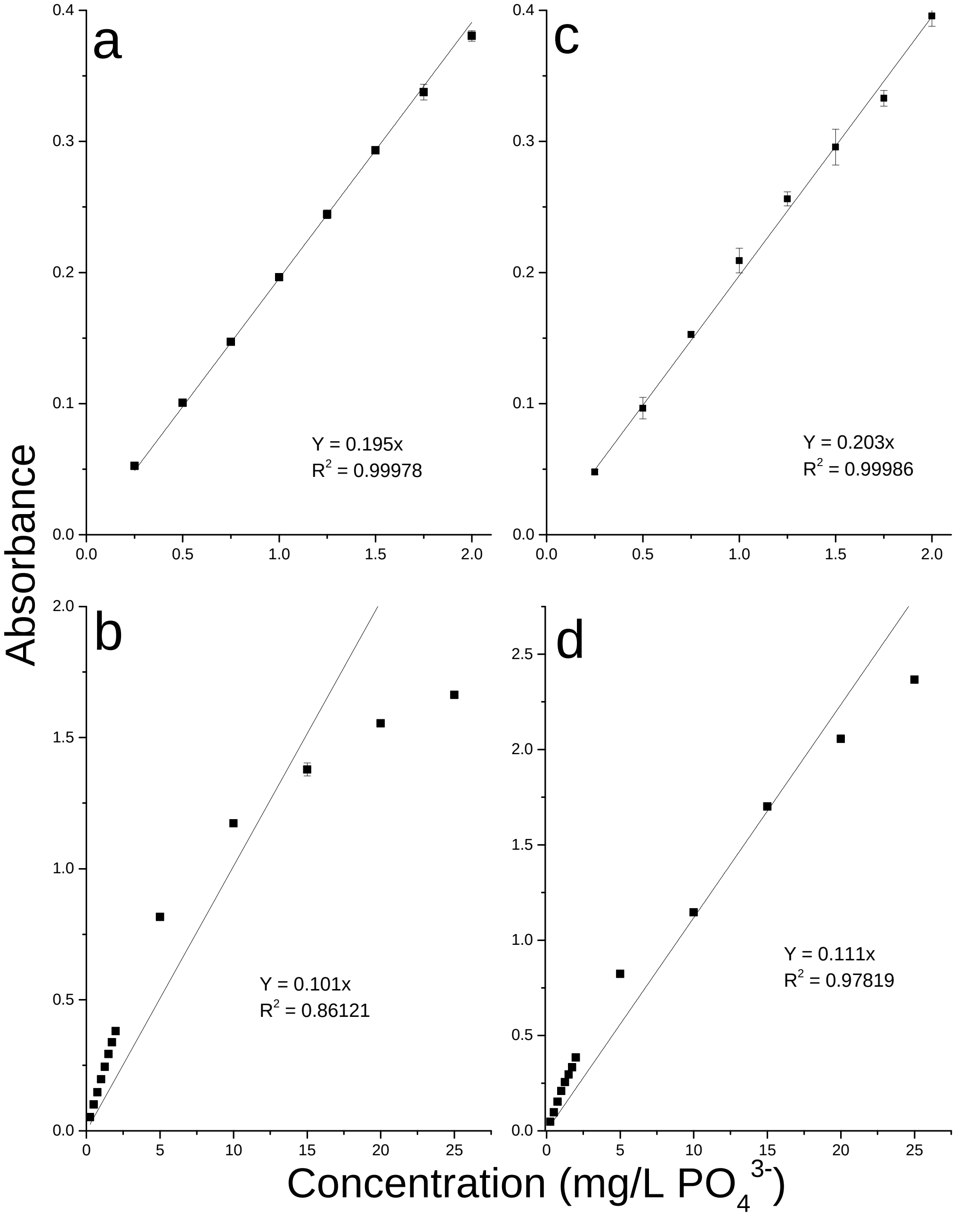

Figure 2.4: Regression analyses using the molybdenum blue methods by Murphy and Riley (1962) at 882 nm in Mehlich III (a and b) and by He et al. (1998) at 882 nm in Mehlich III (c and d). [P] at 0-2 mg/L (a and c) and [P] at 0-25 mg/L (b and d).

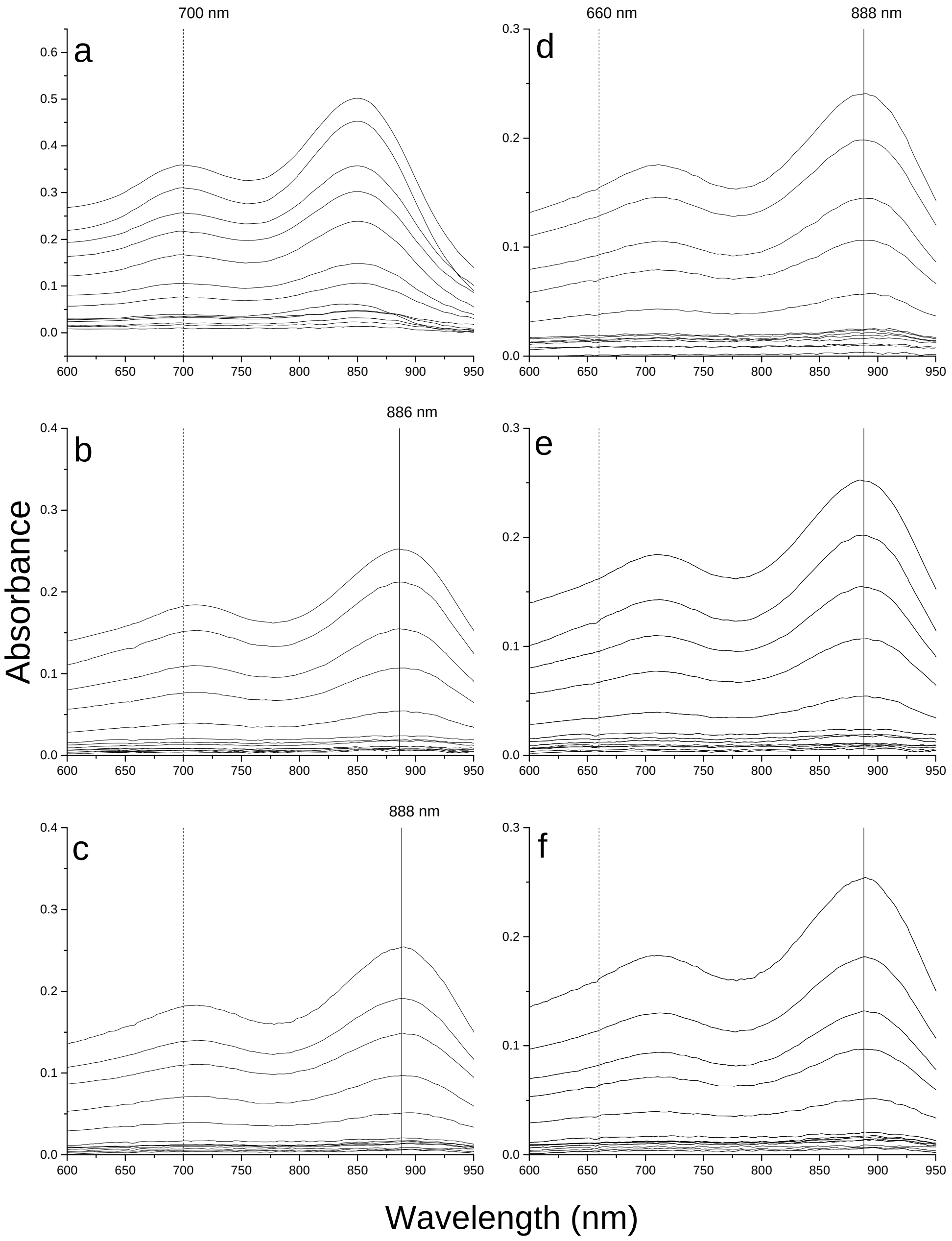

Figure 2.5: Absorbance spectra of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 5.0, 10.0, 15.0, 20.0, and 25.0 ppm phosphate in: a) Milli-Q water, b) Bray P-1 extracting solution, c) Mehlich III extracting solution. Plots a-c utilized the molybdenum blue technique designed by Dick and Tabatabai (1977). Plots d-f utilized Asher (1980). Vertical dashed lines represent wavelength (nm) recommended in method. Vertical solid lines represent optimal wavelength suggested by linear regression trials.

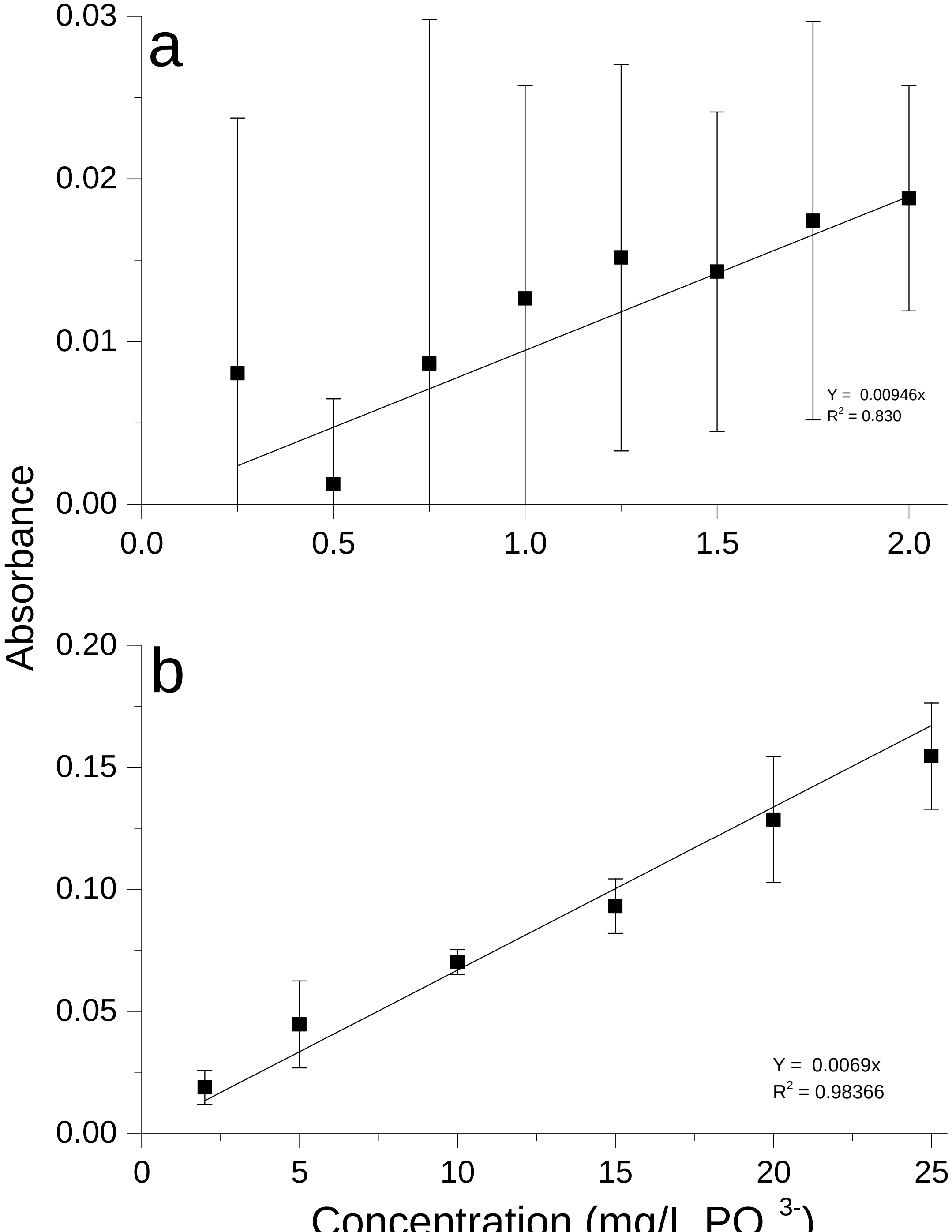

Figure 2.6: Regression analyses using the molybdenum blue methods Asher (1980) at 660 nm in ultrapure water (c and d). [P] at 0-2 mg/L (a) and [P] at 0-25 mg/L (b).

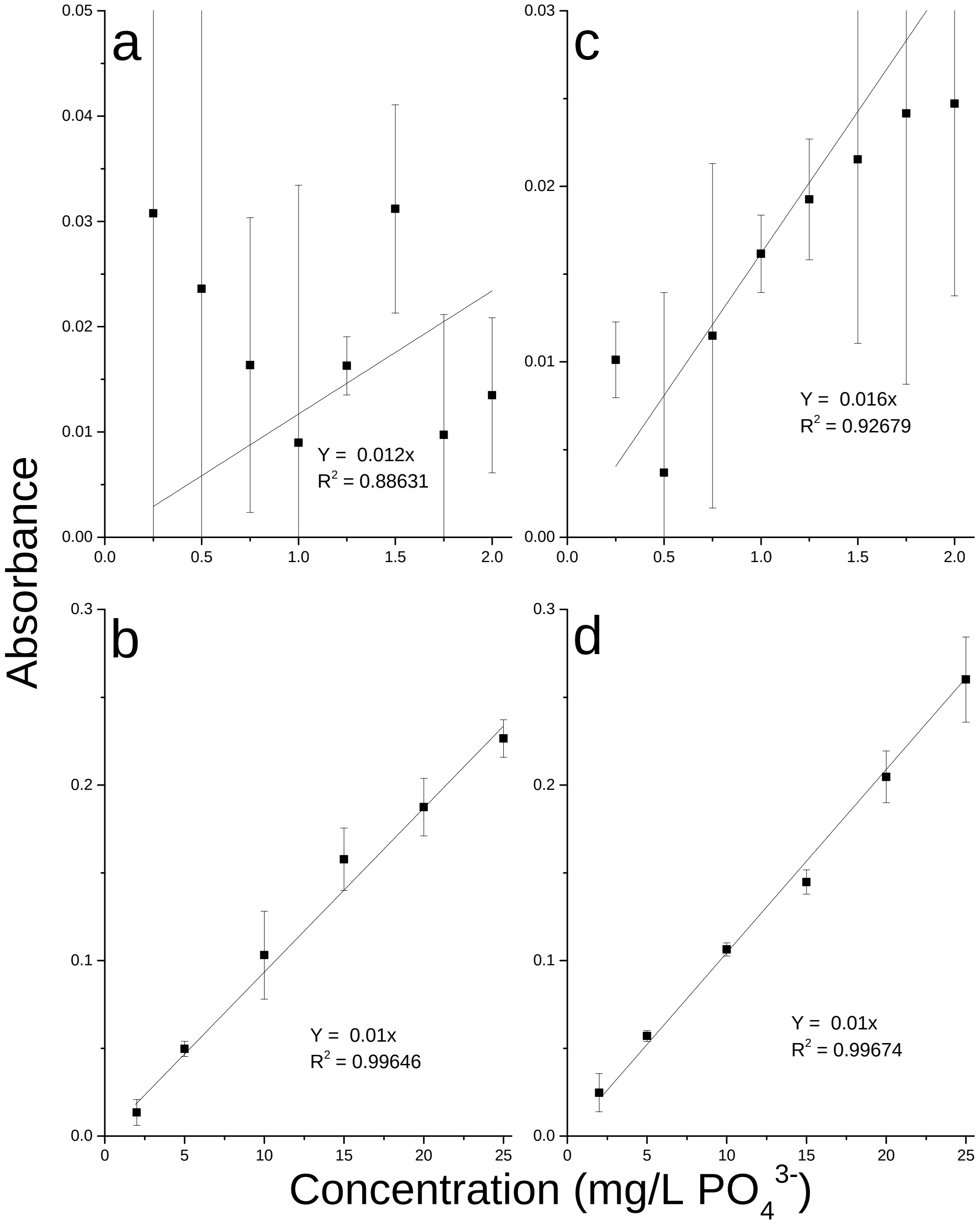

Figure 2.7: Regression analyses using the molybdenum blue methods by Dick and Tabatabai (1977) at 700 nm in ultrapure water (a and b) and by Asher (1980) at 888 nm in ultrapure water (c and d). [P] at 0-2 mg/L (a and c) and [P] at 0-25 mg/L (b and d).

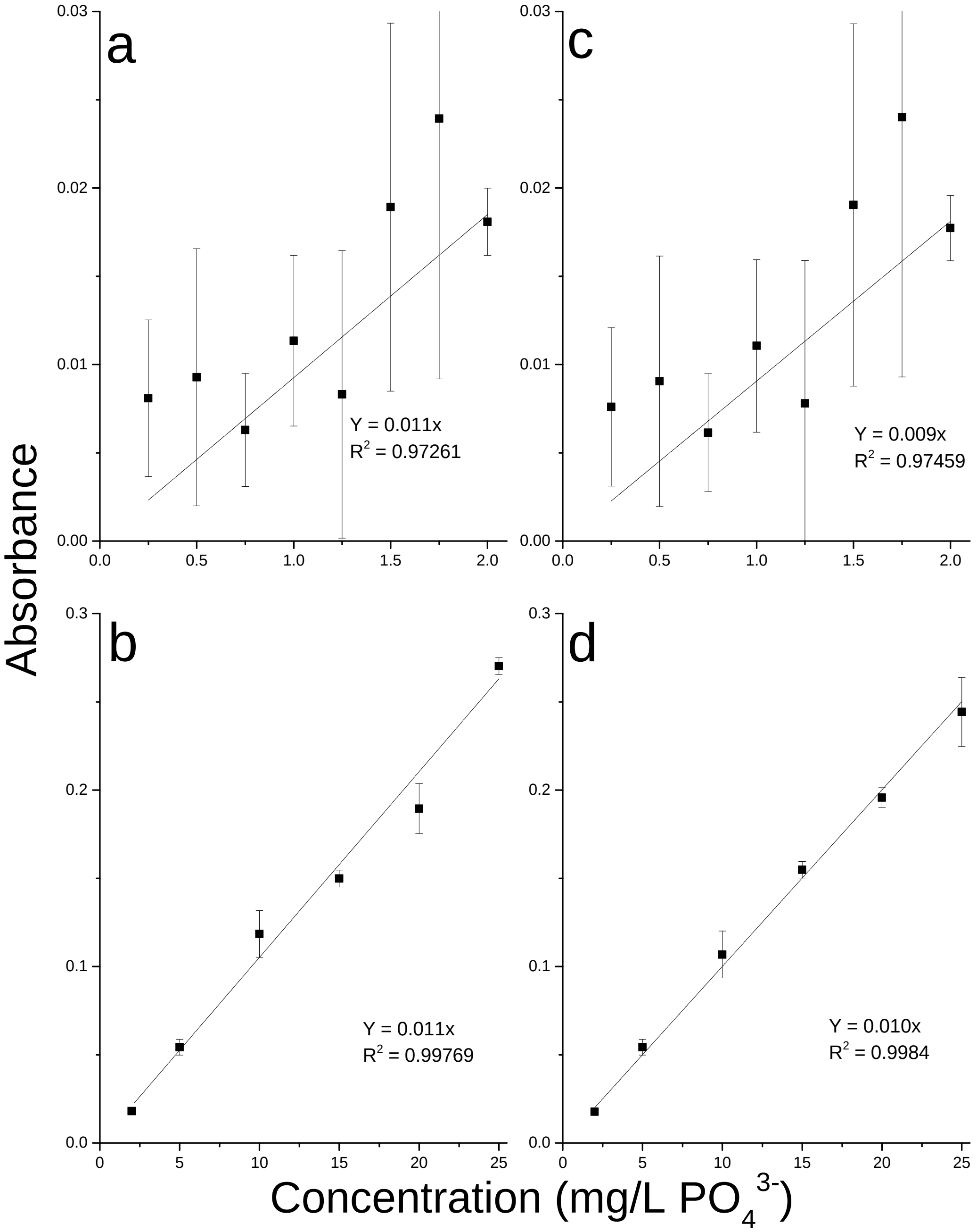

Figure 2.8: Regression analyses using the molybdenum blue methods by Dick and Tabatabai (1977) at 886 nm in Bray P-1 (a and b) and using Asher (1980) at 888 nm in Bray P-1 (c and d). [P] at 0-2 mg/L (a and c) and [P] at 0-25 mg/L (b and d).

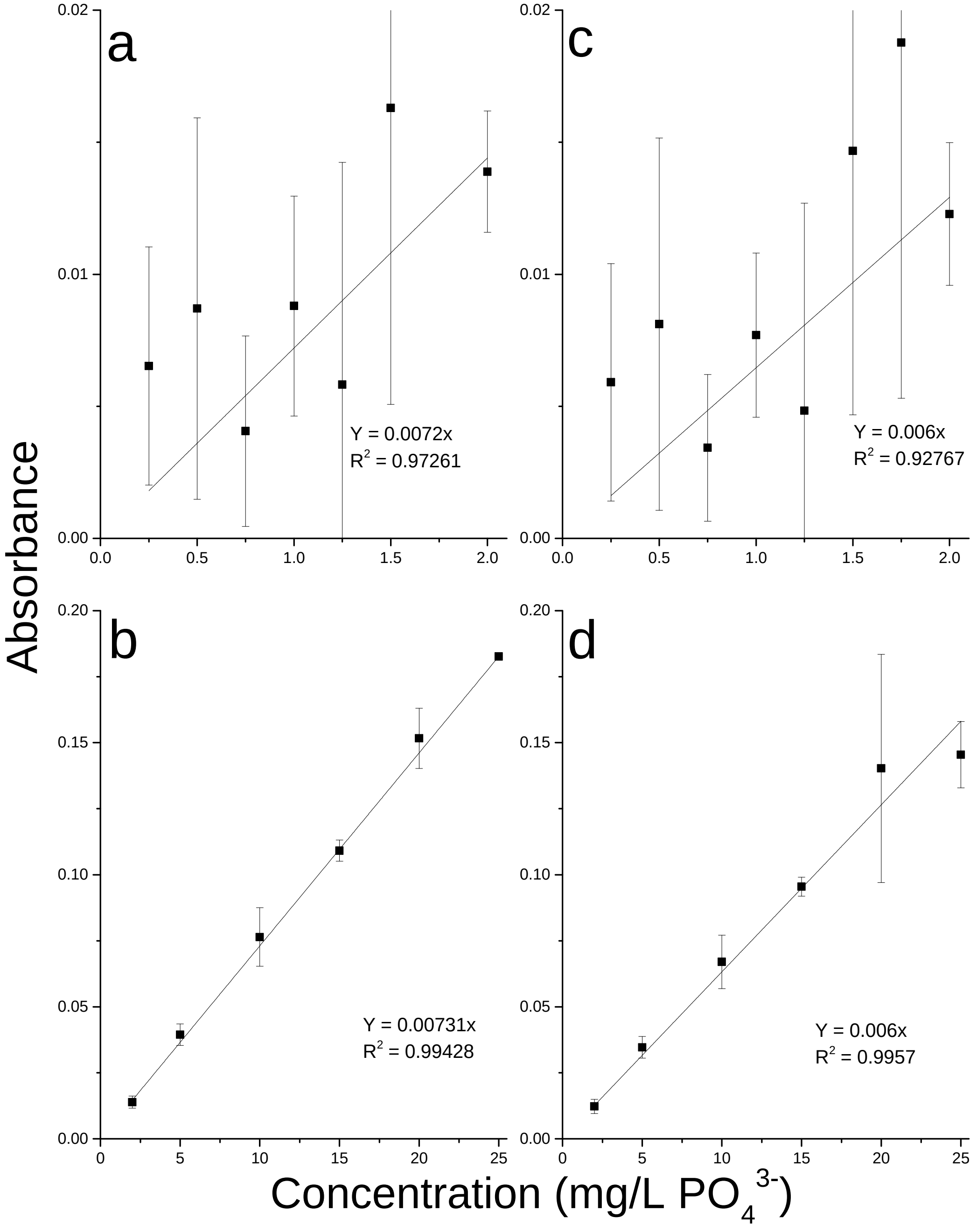

Figure 2.9: Regression analyses using the molybdenum blue methods by Dick and Tabatabai (1977) at 700 nm in Bray P-1 (a and b) and using Asher (1980) at 660nm in Bray P-1 (c and d). [P] at 0-2 mg/L (a and c) and [P] at 0-25 mg/L (b and d).

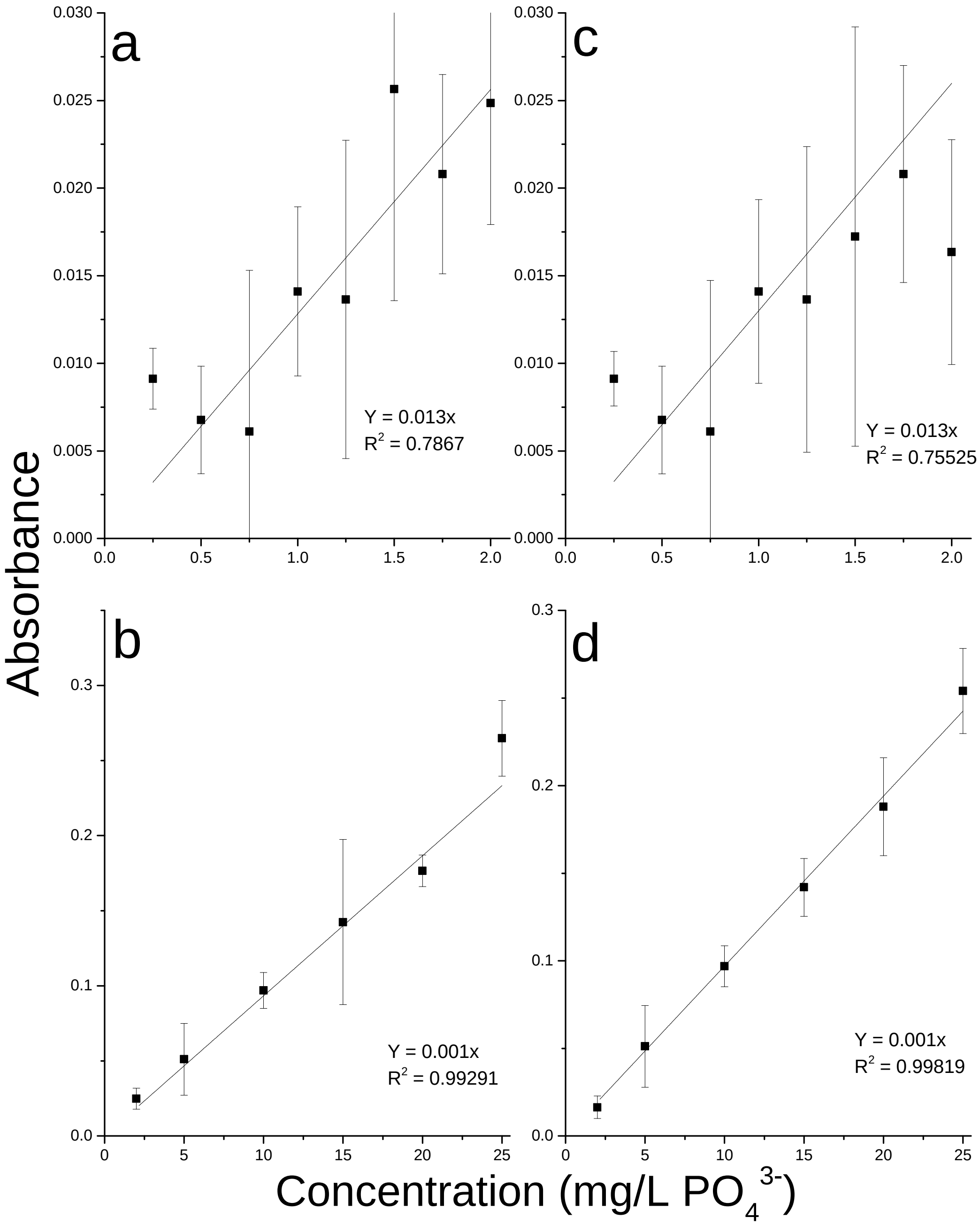

Figure 2.10: Regression analyses using the molybdenum blue methods by Dick and Tabatabai (1977) at 888nm in Mehlich III (a and b) and by Asher (1980) at 888 nm in Mehlich III (c and d). [P] at 0-2 mg/L (a and c) and [P] at 0-25 mg/L (b and d).

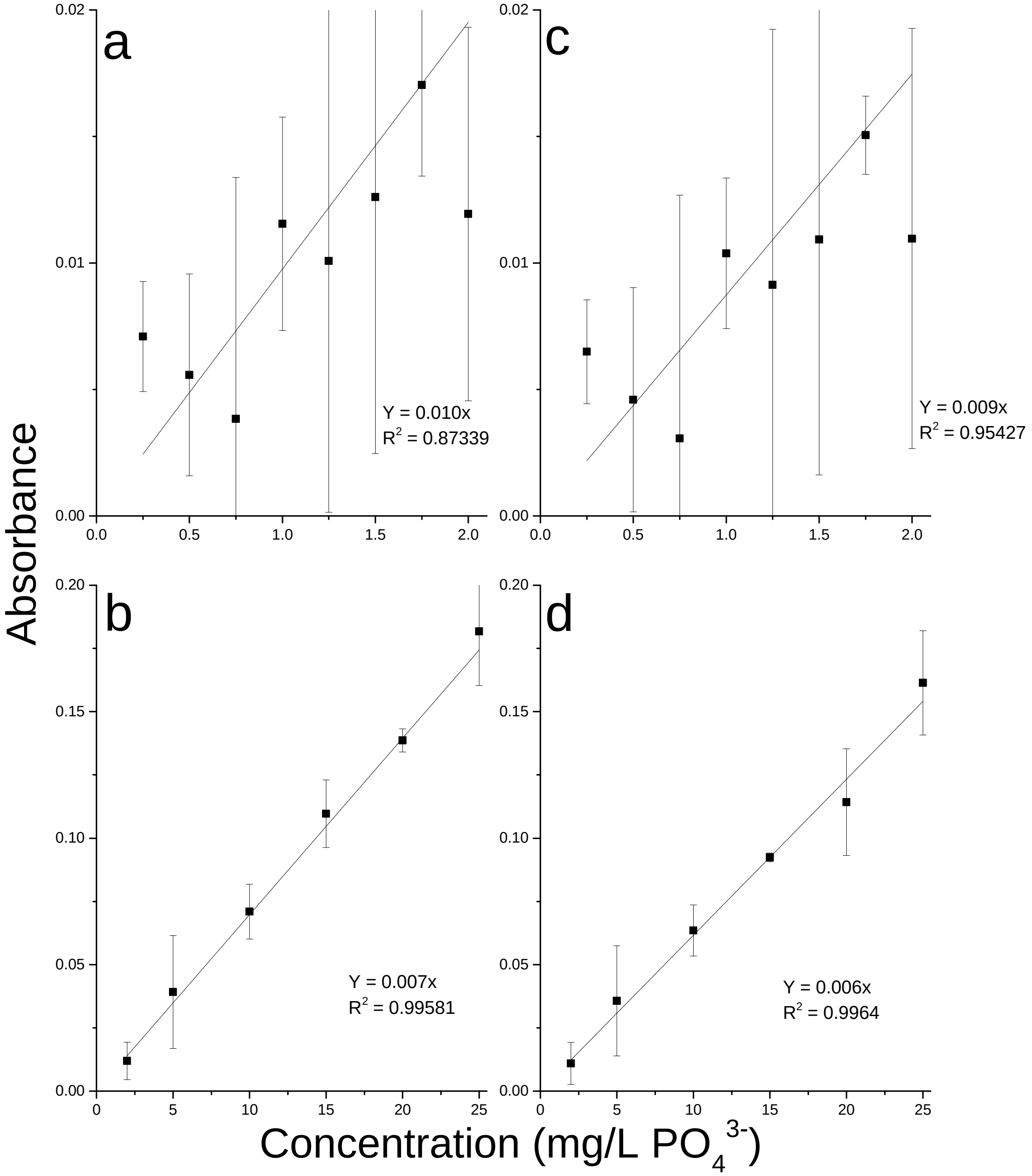

Figure 2.11: Regression analyses using the molybdenum blue methods by Dick and Tabatabai (1977) at 700nm in Mehlich III (a and b) and by Asher (1980) at 660 nm in Mehlich III (c and d). [P] at 0-2 mg/L (a and c) and [P] at 0-25 mg/L (b and d).

2.7 Table

Table 2.1: Summary of linear regression and analytical parameters of selected molybdenum blue spectrophotometric analyses

Method	Background Medium	Wavenumber in nm from original methods (R^2)	Optimal wavenumber in nm from this study (R^2)	Molar Absorptivity (l $mole^{-1}$ cm^{-1})	Sandell's sensitivity (µg cm^{-2})	0-2 mg/l		2-25mg/l	
						LOD§	LOQ§	LOD§	LOQ§
Murphy and Riley (1962)	Ultrapure Water	882 (0.999†, 0.924‡)	-	1.50×10^4	0.0159	0.026	0.079	-	-
	Bray P-1	-	882 (0.993†, 0.883‡)	2.02×10^4	0.0117	0.039	0.118	-	-
	Mehlich III	-	882 (0.999†, 0.861‡)	1.85×10^4	0.0128	0.043	0.130	-	-
He et al. (1999)	Ultrapure Water	882 (0.998†, 0.934‡)	-	1.54×10^4	0.0154	0.022	0.067	-	-
	Bray P-1	-	882 (0.998†, 0.979‡)	1.81×10^4	0.0131	0.032	0.097	-	-
	Mehlich III	-	882 (0.999†, 0.978‡)	1.93×10^4	0.0123	0.034	0.103	-	-
Dick and Tabatabai (1977)	Ultrapure Water	700 (0.886†, 0.996‡)	-	9.50×10^3	0.250	-	-	1.332	4.037
	Bray P-1	-	886 (0.972†, 0.998‡)	1.04×10^4	0.227	-	-	1.431	4.338
	Mehlich III	-	888 (0.787†, 0.993‡)	6.94×10^3	0.342	-	-	1.426	4.327
Asher (1980)	Ultrapure Water	660 (0.830†, 0.984‡)	888 (0.927†, 0.998‡)	6.55×10^3¶ 1.05×10^4#	0.362¶ 0.158#	-	-	0.712¶ 0.517#	2.159¶ 1.534#
	Bray P-1	-	888 (0.975†, 0.998‡)	1.28×10^4	0.172	-	-	0.570	1.726
	Mehlich III	-	888 (0.775†, 0.998‡)	9.93×10^3	0.240	-	-	0.580	1.757

†R^2 for regression at 0-2 mg l^{-1}, ‡R^2 for regression at 2-25 mg l^{-1}, §All units are in mg l^{-1}, ¶ for measurements at 660nm, # for measurements at 888nm.

2.8 References

Andraski, Todd W. and Larry G. Bundy. 2001. “Management Practice Effects on Phosphorus Losses in Runoff in Corn Production Systems.” *Journal of Environment Quality* 30(5):1822–28.

Asher, L. E. 1980. “An Automated Method for the Determination of Orthophosphate in the Presence of Labile Polyphosphates.” *Soil Science Society of America Journal* 44(1):173–75.

Berenblum, Isaac and Ernst Chain. 1938. “An Improved Method for the Colorimetric Determination of Phosphate.” *Biochemical Journal* 32(2):295.

Bray, Roger H. and L. T. Kurtz. 1945. “Determination of Total, Organic, and Available Forms of Phosphorus in Soils.” *Soil Science* 59(1):39–46.

Burkart, M. R., W. W. Simpkins, A. J. Morrow, and J. M. Gannon. 2004. “Occurrence of Total Dissolved Phosphorus in Unconsolidated Aquifers and Aquitards in Iowa.” *Journal of the American Water Resources Association* 40(3):827–34.

Correll, David L. 1998. “The Role of Phosphorus in the Eutrophication of Receiving Waters: A Review.” *Journal of Environment Quality* 27(2):261.

Cox, F. R. and S. E. Hendricks. 2000. “Soil Test Phosphorus and Clay Content Effects on Runoff Water Quality.” *Journal of Environmental Quality* 29(5):1582–86. Retrieved (https://dl.sciencesocieties.org/publications/jeq/abstracts/29/5/1582).

Dick, R. P. and M. A. Tabatabai. 1987. “Polyphosphates as Sources of Phosphorus for Plants.” *Fertilizer Research* 12(2):107–18.

Dick, W. A. and M. A. Tabatabai. 1977. “Determination of Orthophosphate in Aqueous Solutions Containing Labile Organic and Inorganic Phosphorus Compounds.” *Journal of Environmental Quality* 6(1):82–85.

Dolah, Frances M. Van. 2000. “Marine Algal Toxins: Origins, Health Effects, and Their Increased Occurrence.” *Environmental Health Perspectives* 108(SUPPL. 1):133–41.

Griffin, T. S., C. W. Honeycutt, and Z. He. 2003. “Changes in Soil Phosphorus from Manure Application.” *Soil Science Society of America Journal* 67(2):645–53.

He, Z. L., V. C. Baligar, D. C. Martens, K. D. Ritchey, and M. A. Elrashidi. 1999. “Relationship of Ryegrass Growth to Extractable Phosphorus in Acidic Soil Amended with Phosphate Rock, Coal Combustion by-Product, Limestone, and Cellulose.” *Communications in Soil Science and Plant Analysis* 30(3–4):457–70.

He, Z. L., V. C. Baligar, K. D. Ritchey, and D. C. Martens. 1998. “Determination of Soluble Phosphorus in the Presence of Organic Ligands or Fluoride.” *Soil Science Society of America Journal* 62(6):1538–41.

Jacoby, Freddy J. 2005. “Extractable Soil Phosphorus, Correlation with P Forms in Soil Runoff, and Relationships with the Texas P Index as a Nutrient Management Tool for CAFOs.” Texas A&M.

Jørgensen, C., K. S. Inglett, Henning S. Jensen, Kasper Reitzel, and K. R. Reddy. 2015. “Characterization of Biogenic Phosphorus in Outflow Water from Constructed Wetlands.” *Geoderma* 257–258:58–66. Retrieved (http://www.sciencedirect.com/science/article/pii/S0016706115000373).

Kleinman, Peter J. a et al. 2011. “Managing Agricultural Phosphorus for Water Quality Protection: Principles for Progress.” *Plant and Soil* 349(1–2):169–82.

Kowalenko, C. G. and D. Babuin. 2007. “Interference Problems with

Phosphoantimonylmolybdenum Colorimetric Measurement of Phosphorus in Soil and Plant Materials." *Communications in Soil Science and Plant Analysis* 38(9–10):1299–1316.

Kuo, S. 1996. "Phosphorus." Pp. 869–919 in *Methods of Soil Analysis Part 3 - Chemical Methods*, vol. 3, edited by P. A. Helmke and R. H. Loeppert. Madison, WI: SSSA.

Mathews, B. W., J. R. Carpenter, L. E. Sollenberger, and S. Tsang. 2005. "Phosphorus in Hawaiian Kikuyugrass Pastures and Potential Phosphorus Release to Water." *Journal of Environmental Quality* 34:1214–23. Retrieved (http://dx.doi.org/10.2134/jeq2004.0393).

Mehlich, Adolf. 1984. "Mehlich 3 Soil Test Extractant: A Modification of Mehlich 2 Extractant." *Communications in Soil Science & Plant Analysis* 15(12):1409–16.

Menon, R. G., S. H. Chien, and L. L. Hammond. 1989. "Comparison of Bray I and Pi Tests for Evaluating Plant-Available Phosphorus from Soils Treated with Different Partially Acidulated Phosphate Rocks." *Plant and soil* 114(2):211–16.

Miller, James N. and Jane Charlotte Miller. 2005. *Statistics and Chemometrics for Analytical Chemistry*. Pearson Education.

Mueller-Harvey, I. and A. Wild. 1986. "The Nature and Stability of Organic Phosphates in Leaf Litter and Soil Organic Matter in Nigeria." *Soil Biology and Biochemistry* 18(6):643–47.

Murphy, James and J. P. Riley. 1962. "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters." *Analytica chimica acta* 27:31–36.

Nolan, Bt and Jd Stoner. 2000. "Nutrients in Groundwaters of the Conterminous United States." *Environmental Science & Technology* 34(7):1156–65. Retrieved (http://pubs.acs.org/doi/abs/10.1021/es9907663).

Pierzynski, Gary M. 2000. "Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters." *Southern cooperative series bulletin* 110.

Sharpley, A. N., C. A. Jones, and C. Gray. 1984. "Relationships among Soil-P Test Values for Soils of Differing Pedogenesis." *Communications in Soil Science and Plant Analysis* 15(8):985–95.

Tarkalson, D. D. and R. L. Mikkelsen. 2004. "Runoff Phosphorus Losses as Related to Phosphorus Source, Application Method, and Application Rate on a Piedmont Soil." *Journal of Environmental Quality* 33(4):1424–30. Retrieved (http://dx.doi.org/10.2134/jeq2004.1424).

Waldrip, Heidi M. et al. 2015. "Legacy Phosphorus in Calcareous Soils: Effects of Long-Term Poultry Litter Application." *Soil Science Society of America Journal* 79(6):1601. Retrieved (https://dl.sciencesocieties.org/publications/sssaj/abstracts/79/6/1601).

Wei, L. L., C. R. Chen, and Z. H. Xu. 2009. "The Effect of Low-Molecular-Weight Organic Acids and Inorganic Phosphorus Concentration on the Determination of Soil Phosphorus by the Molybdenum Blue Reaction." *Biology and Fertility of Soils* 45(7):775–79.

Westermann, D. T., D. L. Bjorneberg, J. K. Aase, and C. W. Robbins. 2001. "Phosphorus Losses in Furrow Irrigation Runoff." *Journal of Environment Quality* 30(3):1009–15.

Xie, R. J. and A. F. MacKenzie. 1988. "The pH Effect on Sdrption-desorption and Fractions of Zinc in Phosphate Treated Soils." *Communications in Soil Science and Plant Analysis* 19(7–12):873–86. Retrieved (http://www.tandfonline.com/doi/abs/10.1080/00103628809367981).

Chapter 3: Investigation of acid hydrolysis reactions of polyphosphates and phytic acid in Bray P-1 and Mehlich III extracting solutions

3.1 Abstract

Soil test phosphorus (STP) (Bray P-1, Mehlich III) was developed to predict plant-available P in agricultural soils in the United States. The acidic STP extracting solutions were intended to extract phosphate from alkaline earth metal based solubility products and or adsorbed species on amorphous metal hydroxides or phyllosilicates in soils. However, the accuracy of STP in predicting plant-available phosphate has often been questioned due to potential acid hydrolysis of poly- and organic-P in soils during the STP extraction. The objective of the study was to investigate the acid hydrolysis reaction of reference poly- and organic-P compounds (1.25-3.75 mM) in Bray and Kurtz P-1 (Bray P-1) and Mehlich III extraction solutions using spectrophotometric methods. The results showed that tripolyphosphate and pyrophosphate have readily undergone acid hydrolysis reaction over 2 hr, whereas phytic acid did not hydrolyze. The results suggest an overestimation of dissolved reactive phosphate from these polyphosphates should be considered when evaluating the STP data.

3.2 Introduction

Phosphorus (P) is an essential element for plant growth, playing an important role in a variety of processes (e.g., photosynthesis, enzyme activation/deactivation) (Vance et al., 2003). However, the bulk of soil orthophosphate (hereafter, referred to as phosphate) is not readily available to plants because it forms stable solubility products with alkaline earth metals and undergoes inner-sphere surface complexation in soil inorganic and organic components (Arai and Livi, 2013; Arai and Sparks, 2001; Arai and Sparks, 2007; Freeman and Rowell, 1981; Lindsay, 1979; Rick and Arai, 2010). Depending on the C/P ratio, orthophosphate can be immobilized to organic P such as inositol P (White and Ayoub, 1983; Stewart and Sharpley, 1987; Turner et al., 2002). All of these biogeochemical processes contribute to the slow P release from soils (Parfitt et al., 1975; Sanyal and De Datta, 1991; Staats et al., 2004; Vitousek et al., 2015), making the prediction of plant-available P for optimum crop production difficult. In agronomic settings, rapid soil test P (STP) extraction methods such as Bray P-1 (Bray and Kurtz, 1945), and Mehlich III

(Mehlich, 1984) have been widely used to quantify plant-available P. However, concerns have been raised about the accuracy of these methods because the acidity of STP extracting solutions could hydrolyze organic- and poly-P species, resulting in overestimation of the concentration of phosphate in STP extracts (Busman and Tabatabai, 1985; Masson et al., 2001; He and Honeycutt, 2005; Kulaev et al., 2005; McBeath et al., 2007). No scientific data have thus far been reported to evaluate this possibility; therefore, the present study was undertaken to investigate the acid hydrolysis reactions of reference polyphosphates and an organic phosphate compound in Bray P-1 and Mehlich III extracting solutions using two spectrophotometric methods.

3.3 Materials

In all experiments, ACS grade chemicals and ultrapure water (18.2 MΩ) were used unless otherwise mentioned in the text.

3.4 Methods

3.4a Batch acid hydrolysis experiments

Acid hydrolysis experiments of reference poly-P and organic-P in Bray P-1 (0.025 M HCl and 0.03 M NH_4F) (Bray and Kurtz, 1945) and Mehlich III (0.2 M acetic acid, 0.25 M NH_4NO_3, 0.015 M NH_4F; 0.013 M HNO_3, 0.001 M ethylenediaminetetraacetic (EDTA) acid) (Mehlich, 1984) STP extracting solutions were conducted in duplicate at room temperature ($22^oC\pm0.3$) under atmospheric conditions. Sodium pyrophosphate decahydrate (JT Baker Chem, Phillipsburg, NJ), sodium tripolyphosphate (Spectrum Chem & Lab Products, Gardena, CA), and phytic acid (Sigma-Aldrich co., Billerica, MA) were used in these experiments. The stock solutions of poly-P and organic-P were freshly made in ultrapure water. At the beginning of experiments, they were mixed with the STP extracting solutions to assure the final poly-P and phytic acid concentration to be 1.25, 2.5, and 3.75 mM. The three concentrations were chosen to observe the effects of concentration and these concentrations were needed to reach the detection limit of the colorimetric methods. Reaction vessels were continually stirred at 150 rpm with a magnetic stirrer. Samples were taken at 1, 10, 30, 60, 120, 150, and 180 min. Aliquots were immediately analyzed for orthophosphate concentration via two modified molybdenum blue colorimetric procedures (Asher, 1980; He et al., 1998) described below. The justification for the use of two spectrophotometric methods is described below.

3.4b Phosphate spectrophotometric analysis

Because we are interested in detecting a subtle change in [phosphate], the analytical methods focused on total P (e.g., ICP-AES) were not considered in this study. Instead, the following two spectrophotometric methods are used. The molybdenum blue colorimetric methods of He et al. (1998) and Asher (1980) both hold merit in this study and provide valuable information about the hydrolysis of tripolyphosphate, pyrophosphate, and phytic acid in Bray P-1 and Mehlich III STP extracts. He's method is a modification of the technique originally described by Murphy and Riley (1962), in which excess ammonium molybdenum is added to effectively detect small changes in [phosphate] generated by hydrolysis of poly- or organic-P in STP extracting solutions. Asher's method, analogous to that of Dick and Tabatabai (1977), is designed to scavenge excess molybdenum with citrate (i.e., through formation of citrate-Mo aqueous complexes) so as to prevent overestimation of [phosphate] originating from acid hydrolysis of organic- or poly-P. The advantage of Asher's (1980) method over that of Dick and Tabatabai (1977) is discussed in detail in Chapter two and in a recent paper by Miller and Arai (2016).

3.4c Statistical evaluation of methods

Independent-samples t-tests were conducted to assess the difference in [phosphate] hydrolyzed from 1.25 mM-3.75 mM poly-P/phytic acid measured by the He et al. (1998) method and the background [phosphate] measured by the method of Asher (1980). Sample means were compared at each time interval (1, 5, 10, 30, 60, 90, 120, 150, and 180 min) and p values reported. The statistical analysis was conducted as long as the background [phosphate] exceeded the limit of detection by Asher's method.

3.5 Results and discussion

3.5a Acid hydrolysis reactions in Bray P-1

Changes in [phosphate] in tripolyphosphate, pyrophosphate, and phytic acid in Bray P-1 and Mehlich III solutions are presented as a function of time in Figures 3.1-3.6. Figure 3.1 shows the changes in [phosphate] when 1.25, 2.5, and 3.75 mM tripolyphosphate was mixed with Bray P-1 extracting solution. Two horizontal lines in each panel indicate the release of phosphate analyzed using two different spectrophotometric methods. The line with He's method describes phosphate generated during the acid hydrolysis reactions. The line with Asher's method describes the background concentration of orthophosphate (i.e., orthophosphate impurity in stock chemical). In Figure 3.1a, [phosphate] by the Asher's method was below detection limit (0.57 mg/L), suggesting the background orthophosphate (i.e., an impurity in the stock chemical) is lower than 0.57 mg/L. The concentration of phosphate after 1 min by He's method (dashed line in Figure 3.1a) is ~1 mg/L, which is already greater than the detection limit of the Asher's method (0.57 mg/L), suggesting that tripolyphosphate was already hydrolyzed after 1 min. The concentration continually increased up to 180 min. The [phosphate] increased to ~1.2 mg/L in the 1.25 mM tripolyphosphate solution. The trends remain relatively consistent in the 2.5 mM system (Figure 3.1b). The background [phosphate] by the Asher method is ~0.75 mg/L. Increasing [phosphate] by He's method suggests that tripoly-P was acid hydrolyzed. The same trend is observed in the 3.75 mM system (Figure 3.1c). The results of independent-samples t-tests for the 2.5 and 3.75 mM systems are shown in Table 3.1. Please note that the t-test was unable to be conducted for the 1.25 mM system because no background phosphate was detected by Asher's method. P values greater than 0.1 indicate that the mean phosphate concentration measured by He et al. (1998) did not vary significantly from the mean [phosphate] measured by Asher (1980). All means measured for [phosphate] by He's method (hydrolyzed species) in Bray P-1 extractant in Figure 3.1 are significantly different from the background phosphate, confirming the acid hydrolysis reaction.

In the case of pyrophosphate at 1.25-3.75 mM, we observed the same trend (Figures 3.2a-c). No [phosphate] detected using Asher's method is reported, suggesting the background [phosphate] is lower than 0.57 mg/L. After 1 min, [phosphate] measured by

He's method is much greater than 0.57 mg/L in all three systems (Figures 3.2a, 3.2b, and 3.2c). This indicates that an acid hydrolysis reaction was occurring after mixing with Bray P-1 extracting solution. There is an increasing trend in all three concentrations. The hydrolysis of pyrophosphate in acidic colorimetric media has been well documented (Bell, 1947; Fogg and Wilkinson, 1958; Worsfold et al., 2005; McBeath et al., 2007), but not specifically in Bray P-1 extracts.

In the case of phytic acid, the baseline is difficult to assess since the lowest [phosphate] is ~0.039 mg/L which is far below the limit of detection of Asher's method (0.57 mg/L). For this reason, the degree of acid hydrolysis after 1 min could not be evaluated. In contrast to the other poly-P cases, [phosphate] is stable with increasing time. This is consistent with all three concentrations. No hydrolysis reaction of phytic acid was observed with the Bray P-1 extractant.

3.5b Acid hydrolysis reactions in Mehlich III

The acid hydrolysis reaction of tripolyphosphate in Mehlich III extractant was similar to those with the Bray P-1 extractant, but the hydrolysis reaction was more rapid at the 2.5 and 3.75 mM concentrations, producing a greater change in [phosphate] over the experimental timeframe (Figure 3.4). This can be attributed to Mehlich III extractant being more strongly buffered at pH ~2.5 ± 0.4. Tripolyphosphate hydrolysis was surprisingly more rapid in Bray P-1 than Mehlich III at the 1.25 mM concentration (Figure 3.4). To distinguish that the increasing [phosphate] trend detected by He's method is different from the background phosphate, a t-test was conducted using [phosphate] measured using both methods at the same sampling periods. The results of the t-test showed that the difference was significant at most of the time intervals except for the data points at 30 min in the 2.5 mM system (Table 3.1), suggesting that the increasing [phosphate] originated from acid hydrolysis reactions and not from background phosphate.

Results of pyrophosphate hydrolysis for Mehlich III (Figure 3.5) are similar to the results with the Bray P-1 extractant. The background [phosphate] detected by Asher (1980) is below the method's detection limit. For this reason, a t-test was not conducted. However, it is clear that the extent of hydrolysis is increasing with increasing time in all three concentrations.

Similarly, only the He et al. (1998) method was used to detect the acid hydrolysis of 1.25-3.75 mM phytic acid due to [phosphate] being well below Asher's detection limit (0.57 mg/L) (Figure 3.6). The concentration of orthophosphate stays near the measurement taken at 1 min over 180 min. Phytic acid at 1.25-3.75 mM does not appear to undergo hydrolysis during Mehlich III extractions.

3.6 Conclusion

We investigated the acid hydrolysis reaction of reference polyphosphates and organic P compounds in Bray P-1 and Mehlich III extracting solutions using two specific P spectrophotometric methods. Tripolyphosphate immediately hydrolyzed in Bray P-1 and Mehlich III extracting solutions, and the reaction continued after 2 hrs. Pyrophosphate also readily hydrolyzed in these STP solutions. The acid hydrolysis reaction in Mehlich III seems faster than in the Bray P-1 extractant. Interestingly, phytic acid did not get hydrolyzed under the reaction conditions we studied. Considering the STP extraction times (5-10 min) for Bray P-1 and Mehlich III, one should be cautioned that tripolyphosphates and pyrophosphates in soils could largely contribute to excess [phosphate] during STP extractions. Longer extraction times also lead to greater overestimation of [phosphate].

3.7 Figures

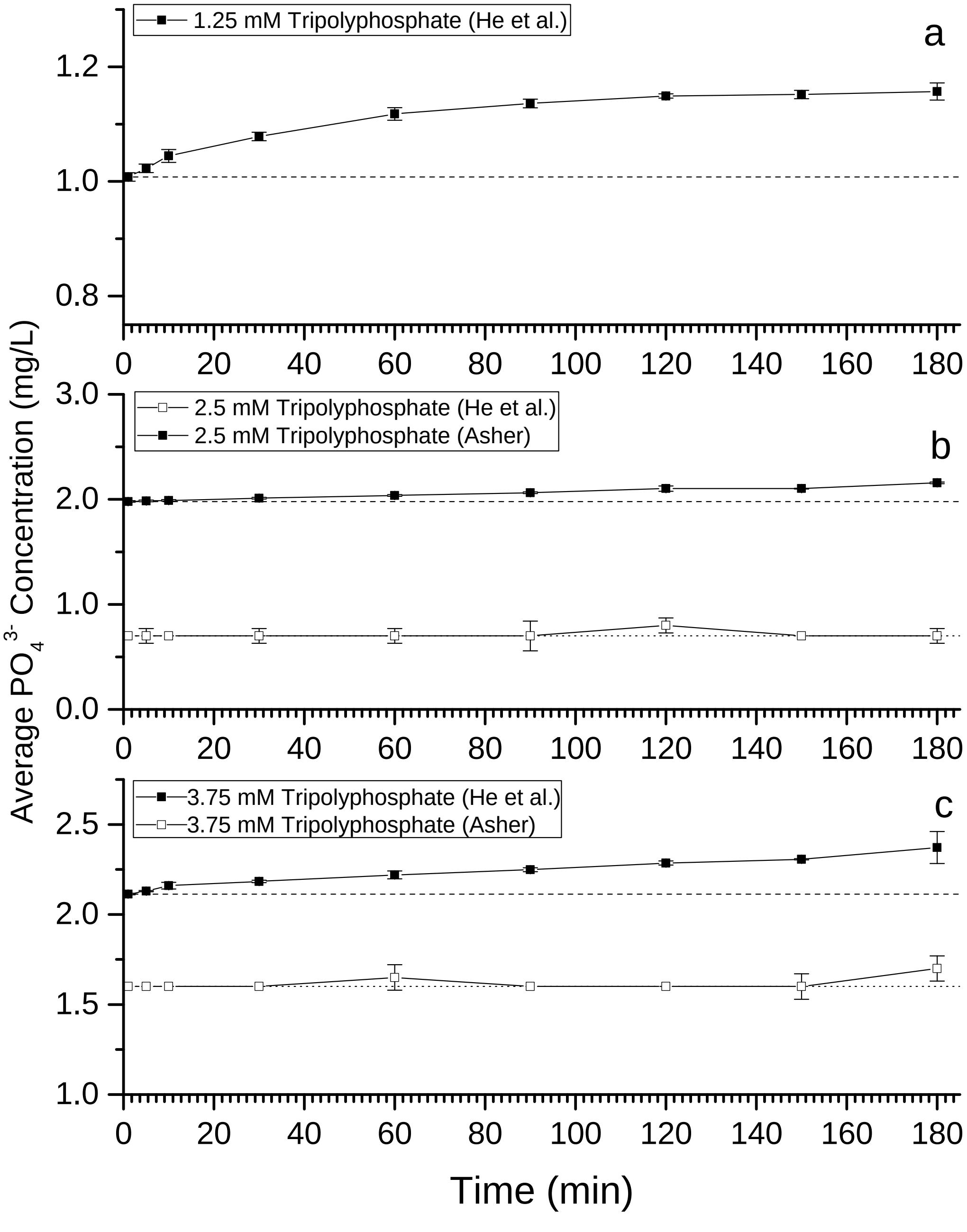

Figure 3.1: Changes in [phosphate] in a mixture of Bray P-1 and (a) 1.25 mM, (b) 2.5 mM, and (c) 3.75 mM tripolyphosphate. Dashed line corresponds to 1-min reading of concentration according to the He et al. (1998) method. Dotted line corresponds to 1-min reading of concentration according to the Asher (1980) method. No baseline for the 1.25 mM system is presented due to BDL: 0.57 mg/L by Asher's method.

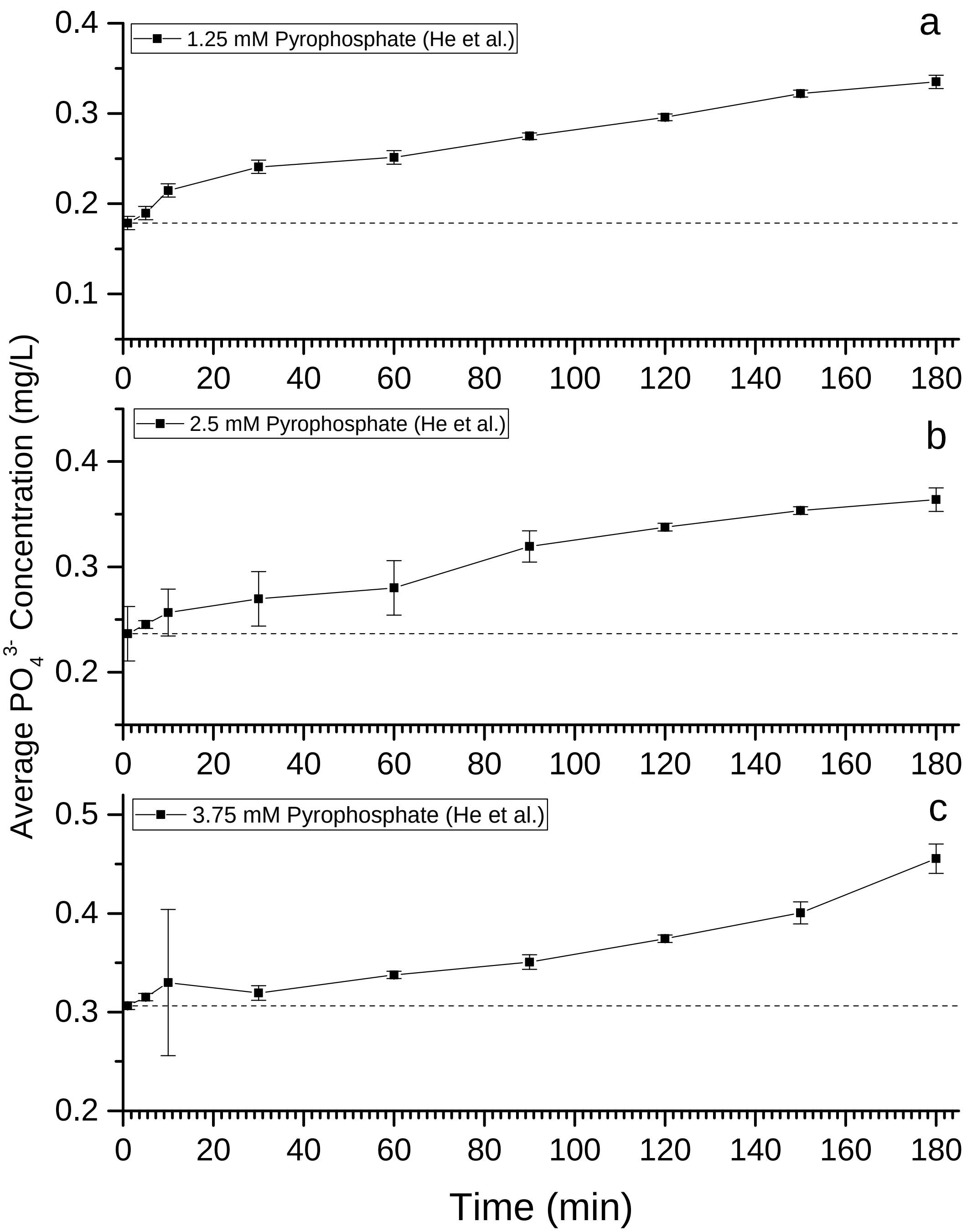

Figure 3.2: Changes in [phosphate] in a mixture of Bray P-1 and (a) 1.25 mM, (b) 2.5 mM, and (c) 3.75 mM pyrophosphate. Dashed line corresponds to 1-min reading of concentration according to the He et al. (1998) method. No baseline is presented due to BDL: 0.57 mg/L by Asher's method.

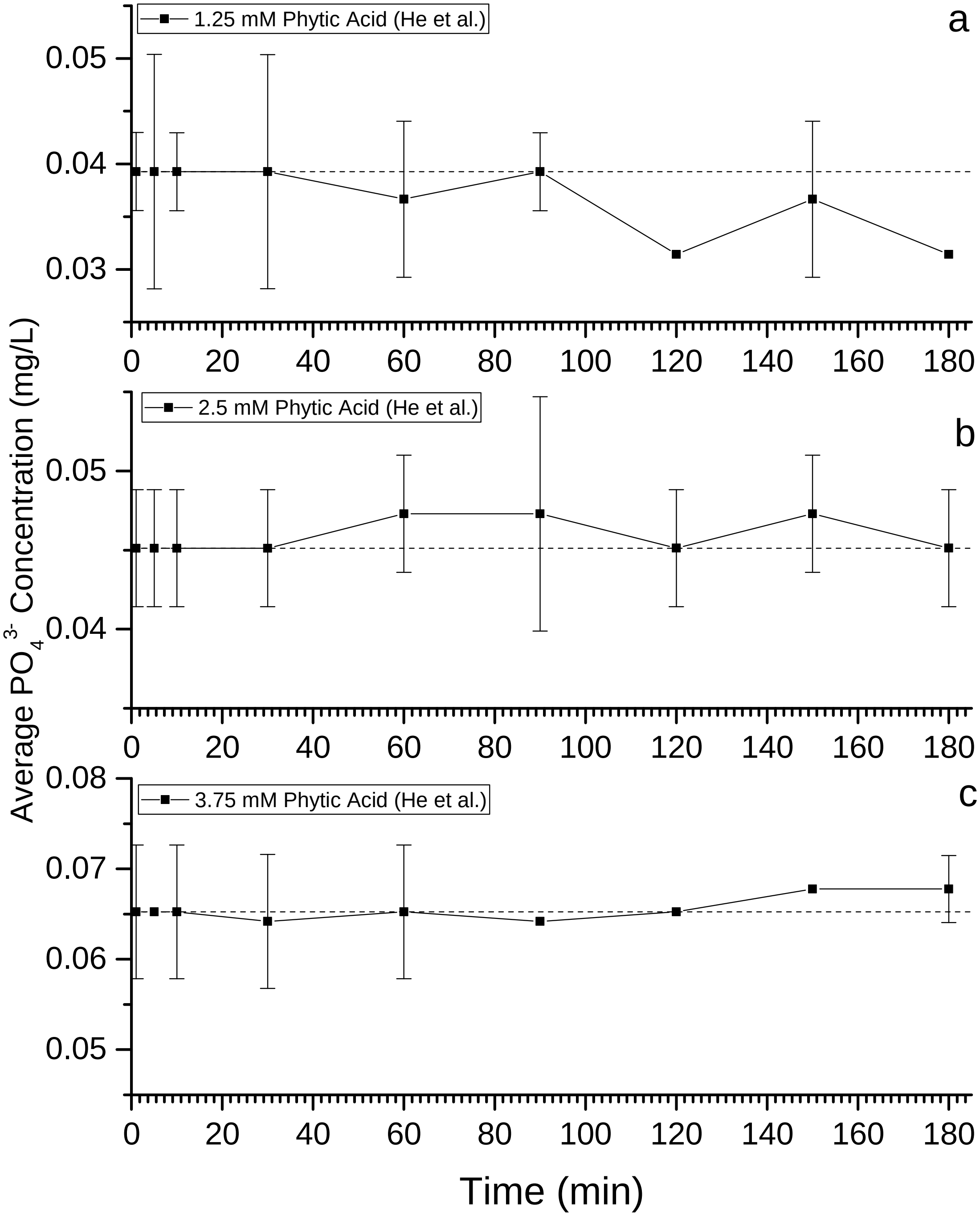

Figure 3.3: Changes in [phosphate] in a mixture of Bray P-1 and (a) 1.25, (b) 2.5 mM, and (c) 3.75 mM phytic acid. Dashed line corresponds to 1-min reading of concentration according to the He et al. (1998) method. No baseline is presented by the method by Asher due to BDL: 0.57 mg/L.

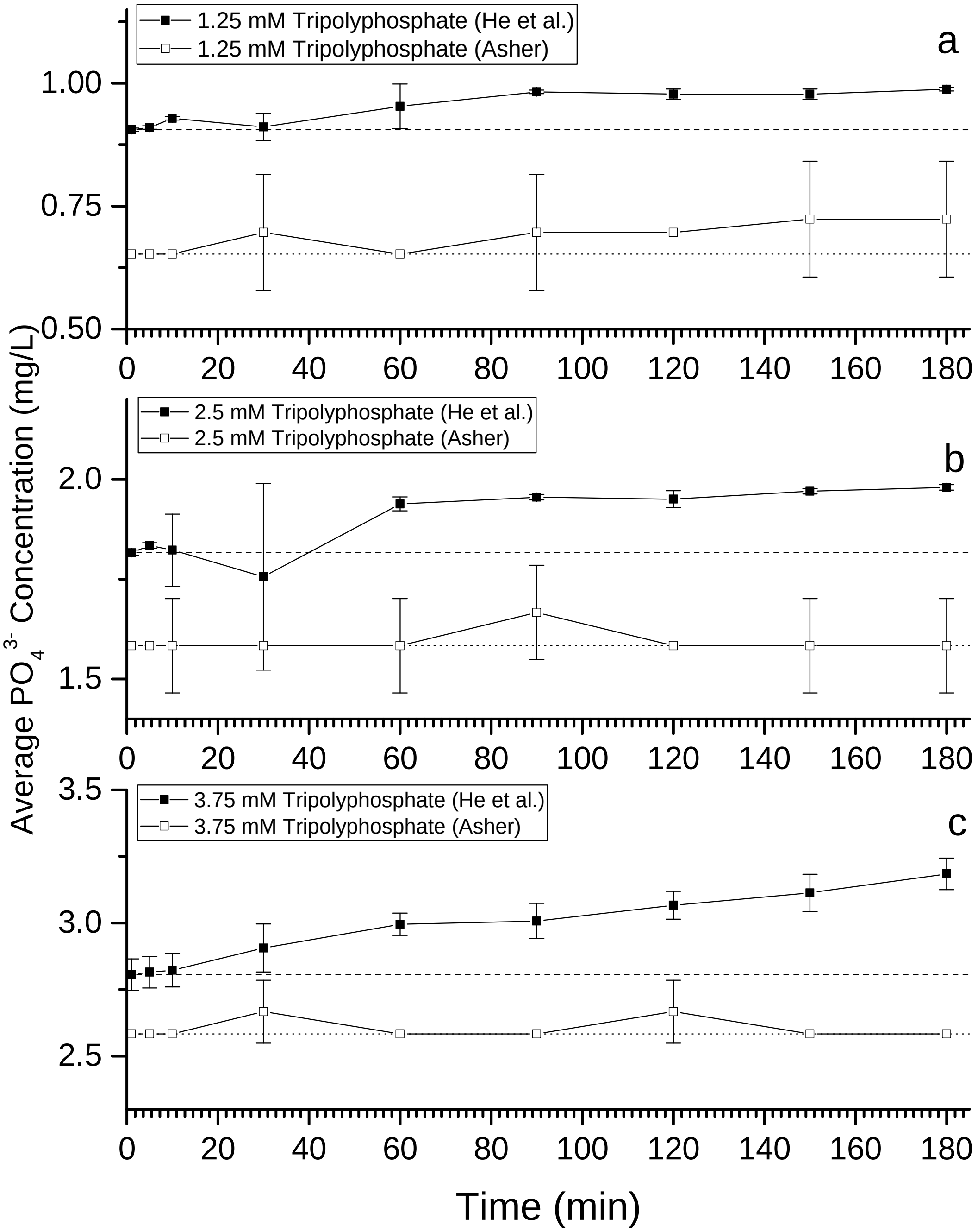

Figure 3.4: Changes in [phosphate] in a mixture of Mehlich III and (a) 1.25 mM, (b) 2.5 mM, and (c) 3.75 mM tripolyphosphate. Dashed line corresponds to 1-min reading of concentration according to the He et al. (1998) method. Dotted line corresponds to 1-min reading of concentration according to the Asher (1980) method.

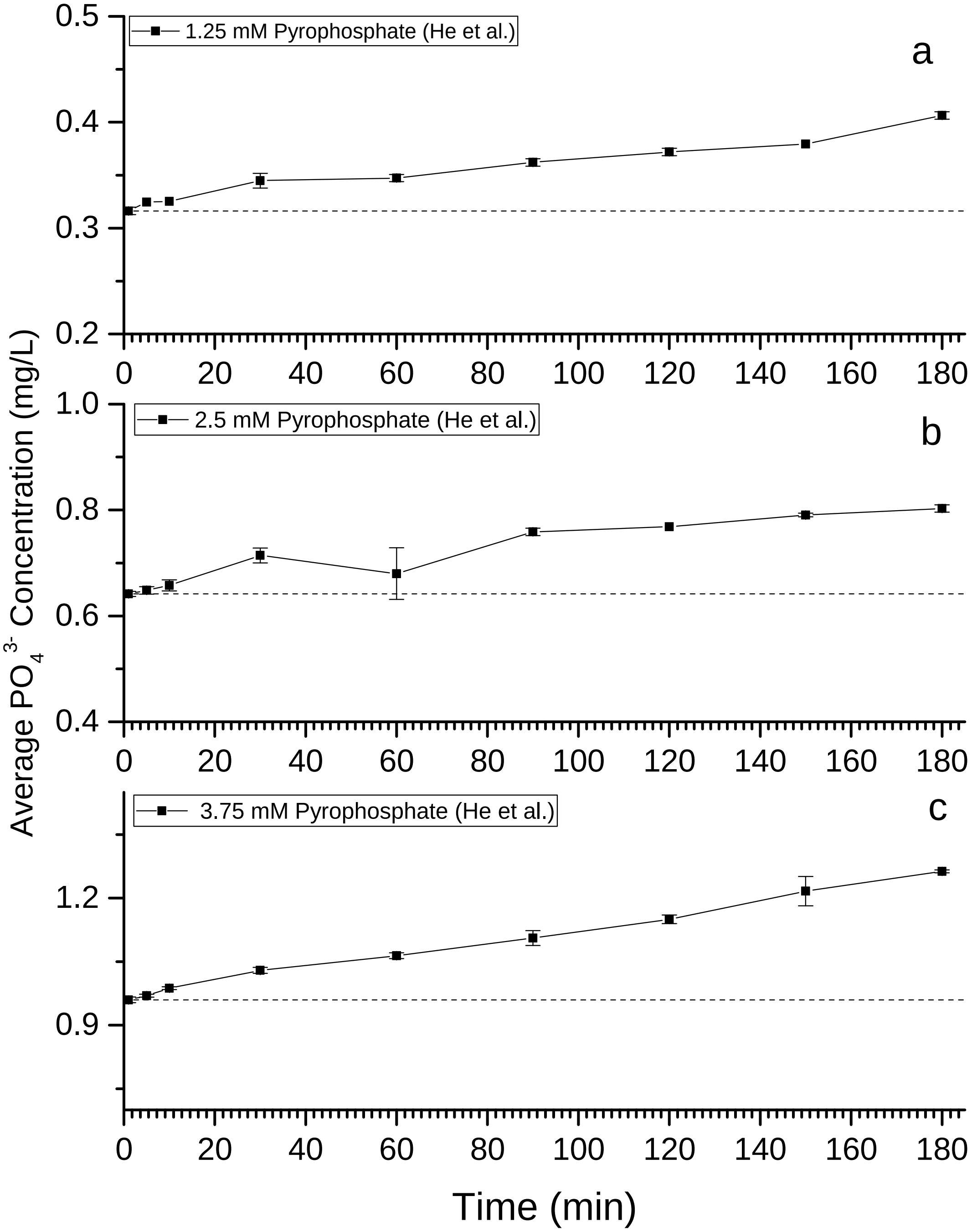

Figure 3.5: Changes in [phosphate] in a mixture of Mehlich III and (a) 1.25 mM, (b) 2.5 mM, and (c) 3.75 mM pyrophosphate. Dashed line corresponds to 1-min reading of concentration according to the He et al. (1998) method. No baseline is presented by the method by Asher due to BDL: 0.57 mg/L.

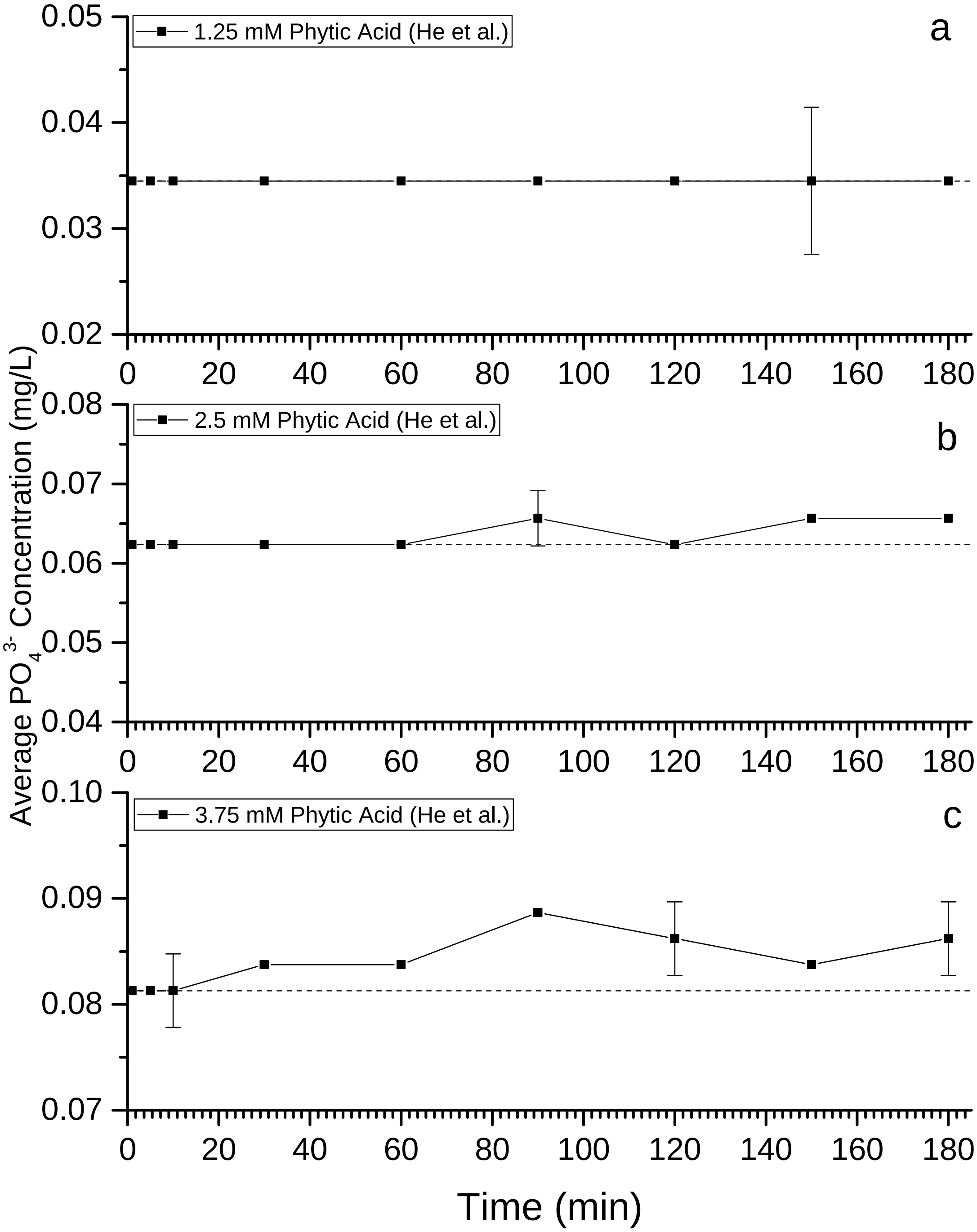

Figure 3.6: Changes in [phosphate] in a mixture of Mehlich III and (a) 1.25 mM, (b) 2.5 mM, and (c) 3.75 mM phytic acid. Dashed line corresponds to 1-min reading of concentration according to the He et al. (1998) method. No baseline is presented by the method by Asher due to BDL: 0.57 mg/L.

3.8 Table

Table 3.1: *p* values from a t-test matrix of P concentration averages hydrolyzed from tripolyphosphate in Bray P-1 and Mehlich III extractants measured by the methods of He et al. (1998) and Asher (1980). Values not included for pyrophosphate or phytic acid due to concentrations BDL (0.57 mg/L) for Asher (1980). For the same reason, no data are presented for the 1.25 mM-Bray P-1.

	Bray P-1		Mehlich III		
Time (min)	2.5 mM	3.75 mM	1.25 mM	2.5 mM	3.75 mM
1	<0.001	<0.001	<0.001	<0.001	0.017
5	0.001	<0.001	<0.001	<0.001	0.016
10	<0.001	<0.001	<0.001	0.076	0.017
30	0.001	<0.001	0.065	0.203	0.076
60	0.001	0.004	0.006	0.026	0.003
90	0.003	<0.001	0.038	0.037	0.006
120	0.001	<0.001	<0.001	0.001	0.024
150	<0.001	0.003	0.047	0.022	0.005
180	<0.001	0.007	0.044	0.021	0.003

3.9 References

Arai, Yuji and Ken J. Livi. 2013. "Underassessed Phosphorus Fixation Mechanisms in Soil Sand Fraction." *Geoderma* 192:422–29.

Arai, Yuji and D. L. Sparks. 2001. "ATR–FTIR Spectroscopic Investigation on Phosphate Adsorption Mechanisms at the Ferrihydrite–Water Interface." *Journal of Colloid and Interface Science* 241(2):317–26. Retrieved May 17, 2016 (http://www.sciencedirect.com/science/article/pii/S0021979701977732).

Arai, Yuji and D. L. Sparks. 2007. "Phosphate Reaction Dynamics in Soils and Soil Components: A Multiscale Approach." *Advances in Agronomy* 94(6):135–79. Retrieved (http://linkinghub.elsevier.com/retrieve/pii/S0065211306940036).

Asher, L. E. 1980. "An Automated Method for the Determination of Orthophosphate in the Presence of Labile Polyphosphates." *Soil Science Society of America Journal* 44(1):173–75.

Bell, Russell N. 1947. "Hydrolysis of Dehydrated Sodium Phosphates." *Industrial & Engineering Chemistry* 39(2):136–40.

Bray, Roger H. and L. T. Kurtz. 1945. "Determination of Total, Organic, and Available Forms of Phosphorus in Soils." *Soil Science* 59(1):39–46.

Busman, L. M. and M. A. Tabatabai. 1985. "Hydrolysis of Trimetaphosphate in Soils." *Soil Science Society of America Journal* 49(3):630–36.

Dick, W. A. and M. A. Tabatabai. 1977. "Determination of Orthophosphate in Aqueous Solutions Containing Labile Organic and Inorganic Phosphorus Compounds." *Journal of Environmental Quality* 6(1):82–85.

Fogg, D. N. and N. T. Wilkinson. 1958. "The Colorimetric Determination of Phosphorus." *Analyst* 83(988):406–14.

Freeman, J. S. and D. L. Rowell. 1981. "The Adsorption and Precipitation of Phosphate onto Calcite." *Journal of Soil Science* 32(1):75–84.

He, Z. L., V. C. Baligar, K. D. Ritchey, and D. C. Martens. 1998. "Determination of Soluble Phosphorus in the Presence of Organic Ligands or Fluoride." *Soil Science Society of America Journal* 62(6):1538–41.

He, Zhongqi and C.Wayne Honeycutt. 2005. "A Modified Molybdenum Blue Method for Orthophosphate Determination Suitable for Investigating Enzymatic Hydrolysis of Organic Phosphates." *Communications in Soil Science and Plant Analysis* 36(9–10):1373–83.

Kulaev, I. S. et al. 2004. "Peculiarities of Metabolism and Functions of High-Molecular Inorganic Polyphosphates in Yeasts as Representatives of Lower Eukaryotes." *Molekuliarnaia biologiia* 39(4):567–80.

Lindsay, Willard Lyman. 1979. *Chemical Equilibria in Soils.* 1st ed. Chichester, West Sussex: John Wiley and Sons Ltd.

Masson, Pierre, Christian Morel, Eric Martin, Astrid Oberson, and Dennis Friesen. 2001. "Comparison of Soluble P in Soil Water Extracts Determined by Ion Chromatography, Colorimetric, and Inductively Coupled Plasma Techniques in PPB Range." *Communications in soil science and plant analysis* 32(13–14):2241–53.

McBeath, Therese M., Enzo Lombi, Michael J. McLaughlin, and Else K. B??nemann. 2007. "Polyphosphate-Fertilizer Solution Stability with Time, Temperature, and pH." *Journal of Plant Nutrition and Soil Science* 170(3):387–91.

Mehlich, Adolf. 1984. “Mehlich 3 Soil Test Extractant: A Modification of Mehlich 2 Extractant.” *Communications in Soil Science & Plant Analysis* 15(12):1409–16.

Miller, Anthony P. and Yuji Arai. 2016. “Comparative Evaluation of Phosphate Spectrophotometric Methods in Soil Test Phosphorus Extracting Solutions.” *Soil Science Society of America Journal* 80(6):1543–50.

Murphy, James and J. P. Riley. 1962. “A Modified Single Solution Method for the Determination of Phosphate in Natural Waters.” *Analytica chimica acta* 27:31–36.

Parfitt, Roger L., Roger J. Atkinson, and Roger S. C. Smart. 1975. “The Mechanism of Phosphate Fixation by Iron Oxides.” *Soil Science Society of America* 39(3):838–41.

Rick, Allison R. and Yuji Arai. 2011. “Role of Natural Nanoparticles in Phosphorus Transport Processes in Ultisols.” *Soil Science Society of America Journal* 75(2):335–47.

Sanyal, S. K. and S. K. De Datta. 1991. “Chemistry of Phosphorus Transformations in Soil.” Pp. 1–120 in *Advances in soil science*. New York, NY: Springer.

Staats, Kristin E., Yuji Arai, and Donald L. Sparks. 2004. “Alum Amendment Effects on Phosphorus Release and Distribution in Poultry Litter–amended Sandy Soils.” *Journal of environmental quality* 33(5):1904–11.

Stewart, J. W. B. and A. N. Sharpley. 1987. “Controls on Dynamics of Soil and Fertilizer Phosphorus and Sulfur.” *Soil fertility and organic matter as critical components of production system, S* 101–21.

Turner, B. L., M. J. Paphazy, P. M. Haygarth, and I. D. Mckelvie. 2002. “Inositol Phosphates in the Environment.” *Philosophical Transactions of the Royal Society: Biological Sciences* 357(1420):449–69. Retrieved (http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2001.0837).

Vance, Carroll P., Claudia Uhde-Stone, and Deborah L. Allan. 2003. “Phosphorus Acquisition and Use, Critical Adaptations by Plants for Securing a Nonrenewable Resource.” *New Phytologist* 157(3):423–47.

Vitousek, Peter M., Stephen Porder, Benjamin Z. Houlton, Oliver A. Chadwick, and Z. Houlton. 2010. “Terrestrial Phosphorus Limitation: Mechanisms, Implications, and Nitrogen-Phosphorus Interactions.” *Ecological Applications* 20(1):5–15.

White, R. E., & Ayoub, A. T. (1983). Decomposition of plant residues of variable C/P ratio and the effect on soil phosphate availability. *Plant and Soil*, *74*(2), 163-173.

Worsfold, Paul J. et al. 2005. “Sampling, Sample Treatment and Quality Assurance Issues for the Determination of Phosphorus Species in Natural Waters and Soils.” *Talanta* 66(2 SPEC. ISS.):273–93.

Chapter 4: Effects of extraction time and phosphorus speciation on soil test phosphorus data: a case study of Illinois agricultural soils

4.1 Abstract

Bray P-1 and Mehlich III STP extractions aim to assess the concentrations of labile P in soils. It is unknown, however, how the observed quantity of labile P is affected by the release of variable P species. The kinetics of orthophosphate (P_i), organic-P (P_o), and colloidal P were evaluated in manure-amended and chemical fertilizer-amended Central Illinois agricultural soils using Bray P-1 and Mehlich III extractants. Comparing the extent of long-term P release and STP, long term P desorption experiments were also conducted using the same soils in 0.01 M $CaCl_2$. In both soils, Bray and Mehlich III extractable P increased with increasing time. The extracted P species are not only P_i but also P_o and colloidal P. Between 10-15% of total P_o was extracted from each soil with manure-amended soils releasing more total P_o than fertilizer amended soils. Bray P-1 and Mehlich III extracted colloidal P from manure-amended and fertilizer-amended soils, but concentrations were only significant after 1-3 h. Soils with high STP values released more P for a long period, indicating a proportional relationship between STP and the total desorbable P in 0.01 M $CaCl_2$. The results indicate that P speciation and extraction time have major impacts on the results of STP extractions.

4.2 Introduction

Phosphorus (P) is an essential element for plant growth, playing an important role in a variety of processes (e.g., nucleic acid synthesis, photosynthesis, glycolysis, respiration, enzyme activation/deactivation, carbohydrate metabolism, and nitrogen fixation) (Vance, Uhde-Stone, and Allan 2003). However, the bulk of soil P is not readily available to plants due to being in very stable associations with soil inorganic and organic components (Arai and Sparks 2007). Soil pH and the activity of soluble cations like aluminum, iron, and calcium often control the formation of P precipitation reactions (Freeman and Rowell 1981; Lindsay 1979). Inner sphere-surface complexation of P on variable-charge mineral surfaces makes P less soluble in soil solutions (Arai and Livi 2013; Arai and Sparks 2001, 2007) Depending on the C/P ratio, orthophosphate can be immobilized to organic P such as inositol P (Stewart and Sharpley 1987; Turner et al. 2002; White and Ayoub 1983). All of these biogeochemical processes contribute to the slow P release from soils (Parfitt, Atkinson, and Smart 1975; Sanyal and De Datta 1991; Vitousek et al. 2010), complicating the prediction of plant-available P for optimum crop production.

In agronomic settings, rapid soil extraction methods such as Bray and Kurtz P-1 (Bray and Kurtz 1945), Mehlich III (Mehlich 1984), and Olsen (Olsen 1954) have been used to quantify plant-available P. However, it has been a challenging task to manage soil P levels for sustainable agricultural practices, largely due to the promotion of P inputs by sales-based fertilizer management and manure disposal from confinement livestock operations. In the North Central region of the United States, both fertilizers and manures are commonly applied to agricultural soils. As a result, a wide variety of inorganic and organic P forms can be found (Leikam et al. 2005). Other regions of the US use less manure than the North Central due to a lower density of animal production, and therefore rely more heavily on chemical fertilizers (Potter et al. 2010). Due to fertilizer applications and manure applications/disposal, P concentrations in agricultural soils greatly exceed indigenous soil concentrations. In soils unaffected by P fertilizer addition, the average concentration of P is approximately 7.28-13 mg/kg (Hedley, Kirk, and Santos 1994; Sims, Simard, and Joern 1998). The current average concentration of P in all soils of North America is ~25 mg P/kg (Fixen et al. 2010), which illustrates how anthropogenic input to agricultural soils has drastically elevated average P concentrations.

Plant-available forms of P are not easy to quantify due to the low solubility of P and the multiple reaction pathways for release from organically and colloidal bound forms into the soil solution (Fernández and Hoeft 2009). Labile organic P from manure amendments cannot be ignored due to the use of animal-based fertilizers (Maguire, Chardon, and Simard 2005). This leads to a main issue with STP in which it cannot speciate among the various forms of soil P that may play significant roles in supplying P to crops. Previous studies have evaluated whether organic-P is extracted by STP procedures (Messiga et al. 2014; Shang et al. 2013; Steffens et al. 2010), or have used data analysis to compare between P fractions and STP methods (Herlihy and McCarthy 2006), but no attempts have been made to fractionate inorganic and organic P in STP extracts. Colloidal P, traditionally defined as P associated with colloids larger than 0.45 μm, may also be present in STP extracts. If an STP methodology requires the evaluation of TP via ICP, the presence of colloidal P would increase concentration measurements (Filella et al. 2006; Haygarth, Warwick, and House 1997). The identification of P species is critical in assessing the actual orthophosphate concentration instead of total P that is currently analyzed by ICP-AES in many soil testing laboratories. If the presence of these various forms of P are found, acidity of STP extracting solutions could influence the extraction of orthophosphate in the STP extracts. Several researchers have reported the acid hydrolysis reaction of organic P and poly P (Busman and Tabatabai 1985; He and Honeycutt 2005; Kulaev, Vagabov, and Kulakovskaya 2005; Masson et al. 2001; McBeath et al. 2007). If these organic- and poly-P forms are present in soil samples, they will be hydrolyzed during the extraction, resulting in the overestimated concentration of orthophosphate.

Another issue lies in the fact that STP extraction times can be varied during the peak spring season at soil testing laboratories. The original Bray P-1 and Mehlich III methods suggest that extraction time be 1 and 5 min, respectively (Bray and Kurtz 1945; Mehlich 1984). It is difficult to follow the exact protocol (i.e., short extraction time) when thousands of soil samples are processed in a day. It is likely that extraction time will be varied and/or extended. Interestingly, different soil testing laboratories have longer extraction times than the original methods (Department of Sustainable Natural Resources 1995; Ketterings and Barney 2010; Mallarino 1995; Wuenscher et al. 2015). For example, the standard soil testing procedures for the North Central region suggest a shaking time of 5 min. for Bray P-1 extractions (Brown 2015). Furthermore, the relationship between equilibrium-based STP extractions and the extent of long-term P release from soils is not clearly understood. A slow continuum of P release in agricultural soils has been frequently documented by several investigators (Elkhatib and Hern 1988; Fekri, Gorgin, and Sadegh 2011; McDowell and Sharpley 2003), suggesting the importance of temporal-scale P availability in soils.

The objectives of this study are 1) to examine the impact of extraction time and P speciation on STP extracts and 2) to compare the results of STP with short- and long-term $CaCl_2$-desorbable P. The results are also compared with operationally defined organic and inorganic P fractions in soils. Bray and Kurtz P-1 and Mehlich III methods were chosen for this study since they are commonly used to test IL agricultural soils with high base saturation.

4.3 Materials

A total of four topsoil (top 30 cm) samples were collected from two Central Illinois agricultural fields consisting of soils from the Sable and Drummer series (fine-silty, mixed, superactive, mesic, Typic Endoaquolls). One site is dominated by cattle manure slurry amendments since the 1950's, (31,832 kg dairy manure/ha/yr) (From hereafter, these are abbreviated to Mp). The other site predominantly received diammonium phosphate (DAP) fertilizer at a variable-rate application of DAP (Avg: 386 kg/ha/yr) (From hereafter, these are abbreviated to Fp). Soils were air-dried and passed through a 2 mm sieve prior to experiments. Soil pH_{water} was determined in deionized water using a soil/solution ratio of 1:1 (McLean 1982). Loss-on-ignition and hydrometer methods were used to measure percentage organic matter (OM) and particle size, respectively (Schulte and Hopkins 1996; Sims and Heckendorn 1991). Total exchange capacity (TEC) by summation was conducted according to Ross (1995).

4.4 Methods

4.4a Total inorganic and organic-P determination in soils

Total P fractions (i.e., organic and inorganic P phases) were differentiated using an acid–base extraction method described by Bowman (1989). Approximately 2.0 g of air-dried soil was mixed with 3 mL of 18 M H_2SO_4 in 50-mL volumetric flasks. All samples were measured in triplicate. The mixtures were gently swirled for 10 min with addition of 4 mL of deionized water (1 mL at a time). After cooling, the mixture was diluted to volume and filtered through Whatman No. 1 filter paper. Phosphate concentrations in the acid extracts were measured using an ascorbic acid-molybdate colorimetric method (Asher 1980). After the acid extraction, soils were further extracted with 98 mL of 0.5 M NaOH. The mixtures were shaken at 150 rpm for 2 h in an end-over-end shaker, and then filtered through Whatman No. 1 filter paper. Extracts were analyzed for phosphate using the Murphy and Riley ascorbic acid-molybdate colorimetric method (Murphy and Riley 1962). Acid and base filtrates were analyzed for total P via persulfate digestion (Eisenreich, Bannerman, and Armstrong 1975). Briefly, 1 mL of acid or base extract was combined with 1 g potassium persulfate and 2 mL 5.5 M sulfuric acid in 20-mL scintillation vials. The samples were digested on a hot plate at 160°C for 30 min, cooled, and adjusted to pH ~5 using 10 M NaOH and p-nitrophenol as an indicator. Phosphate concentration was then determined (Murphy and Riley 1962).

4.4b Inorganic-P fractionation

An inorganic-P sequential fractionation method proposed by Jiang and Gu (1989) designed specifically for high base saturation soils was used. Amounts of various inorganic-P fractions in each soil were extracted as follows: 1 g (oven-dry weight) of soil was weighed into a 50-mL Nalgene high-speed centrifuge tube. A volume of 25 mL of the first extractant, $NaHCO_3$ at pH 7.5 (Table 4.1), was added. Samples were processed in triplicate. The centrifuge tube was placed in an orbital shaker for 1 h at 20-25 °C, followed by centrifugation at 6000 x g for 15 min. The supernatant was removed and filtered through Whatman No. 42 filter paper (~2.5 μm pore size). This extraction procedure was repeated sequentially with the six extractants listed in Table 4.1. The filtered extracts were analyzed for [P]. The Asher (1980) and Murphy and Riley (1962) Mo-blue colorimetric methods were used for [P] > 2 mg/L and [P] < 2 mg/L, respectively. Asher's method prevents the overestimation of acid hydrolysable organic P.

4.4c STP kinetic fractionations

For Bray P-1 extractions, 3 g of soil were weighed into 50-mL pyrex centrifuge tubes and combined with 21 mL of extractant. For Mehlich III extractions, 2 g of soil were combined with 20 mL of extractant. Triplicate samples were prepared for shaking times of 1 min, 10 min, 30 min, 1 h, 2 h, 3 h, and 1 d (Bray P-1) and 5 min, 10 min, 30 min, 1 h, 2 h, 3 h, and 1 d (Mehlich III). Samples were shaken in end-over-end shaker, removed, filtered through Whatman 42 filter paper, and evaluated for P_i using the method of Asher (1980). Approximately 2-mL aliquots were removed and filtered through 0.45 μm PVDF filters into scintillation vials. Additional 2 mL aliquots were removed and placed into scintillation vials without filtration. Both filtered and unfiltered scintillation samples were digested for TP using an acid-persulfate digestion procedure (Nelson 1987). Aliquots were taken from the scintillation vials and evaluated for P_i using the Murphy and Riley (1962) method. Organic-P was calculated as the difference between the initial P_i measurement and P_i from the 0.45

μm filtered TP samples. Colloidal P was calculated as the difference between P_i in the 0.45 μm filtered TP samples and unfiltered TP samples.

4.4d Long-term batch desorption

One gram of soil was transferred into a 50-mL pyrex centrifuge tube along with 30 mL of 0.01 M $CaCl_2$. All samples were analyzed in duplicate. Tubes were placed in an end-over-end shaker at 200 rpm and room temperature. At time intervals of 1, 5, 12, 18, 32, 40, and 62 d, a set of duplicate tubes was sacrificed. Samples were analyzed for total P, colloidal P, organic P, and dissolved orthophosphate according to the method described above.

Results were plotted according to the Elovich equation and model parameters calculated. The Elovich equation ($q = (1/\beta)\ln(\alpha\beta) + (1/\beta)\ln(t)$), where q (mg/kg) represents quantity desorbed at time t (h) and α (mg/kg/h) and β $(\text{mg/kg})^{-1}$ are constants defined experimentally, is a widely used kinetic model to describe sorption and desorption, resulting from heterogeneous diffusion of inorganic species from soil materials (Shin et al. 2006). It has been observed to aptly describe bulk and surface diffusion and the activation and deactivation of catalytic surfaces (Chien and Clayton 1980; Sparks 2003). Phosphorus desorption can be said to follow the Elovich equation when a plot of q vs. $\ln(t)$ yields a straight line with a slope of $(1/\beta)$ and an intercept of $(1/\beta)\ln(\alpha\beta)$. The α and β parameters have been used to estimate reaction rates where a decrease in β and an increase in α is indicative of an increase in reaction rate (Sparks 1998).

4.5 Results and discussion

4.5a Soil characterization

Table 4.2 shows the results of physicochemical characterization and STP of the selected soils. The manure-amended soils from the Drummer series ranged in pH from 6.8-6.9 while the fertilizer-amended Sable series soil measured a pH of ~5.5. Previous studies have shown that manure additions can increase soil pH, especially that of cattle manure, in which $CaCO_3$ added to the cattle diet is excreted and has a buffering effect on soil acidity (Eghball 1999; Whalen et al. 2000). Calcium accounts for a much higher percentage of the base saturation in the manure-amended Drummer soil than in the fertilizer-amended Sable soil. The manure-amended soils contained a higher organic matter content than the fertilizer-amended soils with values from loss-on-ignition being consistent with values found in studies using similar manure-amended soils (Fernández, Farmaha, and Nafziger 2012; Kanissery, Welsh, and Sims 2015; Sassman and Lee 2005). Numerous studies have shown that organic manure additions to soils cause large increases in soil organic matter due to the organic content of manure (Haynes and Naidu 1998; Khaleel, Reddy, and Overcash 1981; Lal and Kang 1982). Both Bray P-1 and Mehlich III values are much higher in the manure-amended soils than those in the fertilizer-amended soils. Multiple studies have documented an increase in STP following manure amendments due to an increase in both inorganic and organic P content (Laboski and Lamb 2003; Pierzynski, McDowell, and Sims 2005; Sharpley, McDowell, and Kleinman 2004).

4.5b Phosphorus fractionations

Total P content of the selected soils varied from 387 to 826 mg kg^{-1} (Table 4.3). As expected, the manure-amended soils showed a much higher TP content than the fertilizer-amended soils. In the fertilizer-amended soils, organic P accounts for approximately 30% of TP, while in the manure-amended soils, organic P accounts for approximately 40% of TP. Past studies have shown that manure amendments increase organic P, in some cases by up to 40% (Anderson 1980; Reddy, Rao, and Rupa 2000). Direct inputs of organic P through manure application along with the stimulation of microbial populations could be responsible for an increase in organic P. Inorganic P can be taken up by microbes to form organic P compounds, thus enriching soil organic P fractions (McLaughlin, Alston, and Martin 1988). The manure-amended soils also contain about twice the amount of inorganic P as the fertilizer-amended soils, pointing to the fact that manures contain an abundance of inorganic P as well (Smith et al. 1998). Application rates also play a substantial role in the inorganic and organic P contents of the fertilizer and manure-amended soils. If the application rate of DAP was higher on the fertilizer-amended soils, then there would likely be less discrepancy in P contents between the two soils.

4.5c Inorganic phosphorus fractionations

The results of inorganic P fractionation are summarized in Table 4.4. The fractionation is designed for high base saturation soils, such as the Central Illinois Mollisols used in this study. The first Ca_2-P fraction, extractable by 0.25 M $NaHCO_3$, represents labile P species adsorbed onto the mineral surfaces and easily dissolved Ca-P minerals such as brushite and monetite (Adhami et al. 2006; Chang and Jackson 1957; Jiang and Gu 1989). In the chemical fertilizer-amended soils, Ca_2-P comprises approximately 30% of inorganic P while in the manure-amended soils it comprises approximately 20%. The second Ca_8-P fraction, extracted using NH_4Ac represent octacalcium P (OCP) and tricalcium P (TCP). Results indicate that these are the highest of all fractions in each soil, comprising approximately 30% of P_i in the fertilizer-amended soils and approximately 40% of P_i in the manure-amended soils. Manured soils have been shown to contain elevated contents of these moderately labile Ca-Ps, which readily dissolve in acidic extractants (Tran et al. 1990). The manure-amended soils are also at higher pH than the fertilizer-amended soils, making fixation of P by Ca^{2+} more favorable (Hemwall 1957).

The Al-P fraction is shown to comprise a very low percentage of P_i (< 1%) in the selected soils. Previous studies have shown that similar soils are low in Al-P and that Mollisols often contain more Ca-P than sesquioxide or reductant soluble P (Ippolito et al. 2010; Mausbach 1969; Tembhare 1973). It is well known that the dominant soil cation exerts strong control over the mobility and retention of P. Results from the base saturation analysis (Table 4.2) show that Ca^{2+} is the dominant cation in these soils, and as such, it is reasonable to expect that Ca-P fractions are dominant among the P_i fractions. The low amount of Al-P detected could also be a product of underestimation in the experimental procedure, an operationally defined fraction. Free Ca^{2+} can react with F^- ions from the NH_4F extractant to form CaF_2, which is known to strongly sorb P (Pierzynski et al. 2005). The re-adsorbed P is not recovered until extraction with the reductant-soluble or acid-soluble P fractions, making it possible that these are overestimated and Al-P underestimated (Audette et al. 2016).

The Fe-P fraction, extracted by $NaOH$-Na_2CO_3 is shown to be the second highest P_i fraction in the selected soils, comprising approximately 30% of P_i in all soils. At acidic to neutral pH, orthophosphate is known to be sorbed to Fe-oxides on the surfaces of variable charge minerals (Beauchemin, Simard, and Cluis 1996; Singh and Gilkes 1991; Torrent 1987), and is a significant P retention mechanism in these soils. The fifth extraction using citrate-dithionite, targeted occluded P, which refers to P fractions associated with crystalline Fe-oxides such as goethite (Audette et al. 2016). This fraction comprises only 1-2% of these soils, and is commonly higher in more weathered soils with lower base saturation (Barroso and Nahas 2005). It is commonly found to be negligible in less weathered, high base saturation soils, such as those used in this study (Mausbach 1969; Smeck 1985; Uriyo and Kesseba 1973).

The last extraction targeted stable Ca_{10}-P type minerals (i.e. apatite), considered to be unavailable to plants. This fraction ranged from 5-10% of P_i in the soils and is the most prone of the fractions to be overestimated by the hydrolysis of organic P because of the use of 0.5 M H_2SO_4 and the inclusion of Al-P re-adsorbed in the NH_4F extractant (Audette et al. 2016; Pierzynski et al. 2005). Hydrolysis of organic P was corroborated by the observation that the color of samples analyzed by the Murphy and Riley (1962) technique became increasingly dark over a period of one hour. Samples evaluated by the Asher (1980) method, which were stable in color, are the ones reported in Table 4.4.

Overall, results of the P_i fractionation show that Ca-P fractions (a sum of Ca_2-P, Ca_8-P, Ca_{10}-P) comprise approximately 70% of P_i in the selected soils. This is consistent with other studies showing that slightly weathered Mollisols are dominated by acid-soluble Ca-P species (Sharpley, Tiessen, and Cole 1987; Tiessen, Stewart, and Cole 1984).

4.5d STP kinetic fractionations

Labile P species

Figures 4.1 and 4.2 show the quantities of P released during a 1-d period (1440 min.) in Bray P-1 and Mehlich III STP extractants. Though empirical in nature, the Bray P-1 extractant targets P bound to Al and Fe hydroxides, while the multi-element Mehlich III extractant targets Ca-P species as well. P content in extractant solutions was fractionated into total P, inorganic P (P_i), and organic P (P_o). In Figures 4.1 and 4.2, there is a line denoted as "Total P < 0.45 µm". This is to observe the impact of colloidal P (0.45 µm-2.5µm) on the STP extractants. The manure-amended soils (Mp_1, Mp_2) contain approximately twice the amount of P_i, P_o, and TP as the fertilizer-amended soils (Fp_1, Fp_2) (Table 4.3). Approximately 36, 20, 30, and 20% P_i, P_o, TP, and colloidal P, respectively, was extracted from the fertilizer-amended soils in comparison to the manured soils in the STP extractants after 1-d (Figures 4.2 & 4.3). With respect to P_i, results of the fractionation shown in Table 4.4 suggest that labile/moderately-labile Ca-P species (Ca_2-P and Ca_8-P) constitute a large proportion of P_i in both soils and were likely contributors to the observed trend of increasing P_i over time. Shen et al. (2004) found that Ca_2-P and Ca_8-P were significantly correlated to P uptake by crops. Other studies have suggested that Ca_2-P and Ca_8-P are readily available and partly available, respectively (Song, Han, and Tang 2007; Wang et al. 2010).

Release of Inorganic P Species

The inorganic P (orthophosphate, DRP) is shown by filled triangles in both figures. In both Bray P-1 and Mehlich III solutions, extraction of P_i was rapid over the initial 120 min., and then slowed for the remainder (1320 min.). Mehlich III extracted more P_i than Bray P-1 in all soils, due to it being a more aggressive and buffered extractant (Ebeling et al. 2008; Ketterings and Flock 2005; Mallarino and Blackmer 1992; Tucker 1992). For both Bray P-1 and Mehlich III samples, the pH after 24 h remained under 4 (initial pH ~2.5), with Mehlich III maintaining slightly more acidic conditions at pH ~2.5. Dissolution of Ca-P species over time in acidic conditions and in the presence of competitive ligands like EDTA resulted in the observed trend of increasing P_i (Figures 4.1 and 4.2), along with the desorption/diffusion of less labile P species (Baginski, Foa, and Zak 1967; Otani and Ae 1999; Tiessen and Moir 1993). The increase in P_i over the experimental timeframe can also be attributed in part to the presence of hydrolysable P species.

Release of Organic P

Organic P is known to be an important P fraction in soils and can constitute up to 75% of TP in surface soils (Stevenson 1982). Organic P is a major P fraction in soils used in this study, as it was found to comprise ~30% of total P in Fp soils and ~40% of total P in Mp soils (Table 4.3). Multiple organic P species have been identified in soils including, but not limited to nucleic acids, phospholipids, and inositol phosphates (Makarov, Haumaier, and Zech 2002; Turner, Frossard, and Baldwin 2005). Inositol phosphates comprise the greatest proportion of soil P_o. Due to their strong sorption to Fe, Al, and Ca-oxides, they are likely the main species released during extraction (Celi et al. 1999; Turner et al. 2002). Despite the fact that the most common extractants for soil P_o are alkaline, multiple studies have documented P_o release in acidic media (Hayes, Richardson, and Simpson 2000; Makarov et al. 2002; Turner, Cade-Menun, et al. 2005). Acidity of the STP extractants, along with the inclusion of competitor ions and chelating agents, resulted in significant P_o release from the soils being examined. A gradual increase in P_o over the experimental timeframe was observed with more P_o release from the manure-amended soils. Organic P accounts for approximately 10-15% of TP extracted from fertilizer-amended soils in the STP extracts, whereas P_o accounts for 47-50% of TP extracted from manured-amended soils in the STP extracts. On average, Mehlich III extracted slightly more P_o than Bray P-1 from all soils. Along with having a higher buffering capacity, Mehlich III contains EDTA, a reagent often used in the extraction of P_o (Bowman and Moir 1993).

Impact of Labile Colloidal P

There is evidence that indicates colloidal P transport is an important mechanism of the transfer of P from agricultural to aquatic systems (Heathwaite et al. 2005; Schelde et al. 2006; Sims et al. 1998). Colloidal P compounds can be present as P occluded within mineral or organic compounds, primary or secondary P minerals, P sorbed to soil particles, such as clay or metal-oxides, or P within organic molecules, such as live bacterial cells (Broberg and Persson 1988; Buffle and van Leeuwen 1993; Hens and Merckx 2001; Turner, Kay, and Westermann 2004). The primary colloidal P species in acidic to neutral soils with high OM content occurs as humic-metal-phosphate complexes (Gerke 1992; Hens and Merckx 2001).

Operationally defined colloidal P in extracts was detected as the difference between "TP < 2.5 μm" and TP < 0.45 μm". A gap between the two TP values was consistently observed over the experimental timeframe in all soils, but was only significantly detectable after ~1 h for the manure-amended soils and after ~3 h for the fertilizer-amended soils. Slightly more colloidal P was released from soils by Mehlich III extraction than by Bray P-1 extraction. No significant colloidal P could be detected for Fp_2 in Bray P-1 (Table 4.5), while colloidal P could be detected for this soil after 1 d in Mehlich III (Table 4.6). Because these lines are close to each other, independent samples t-tests were conducted. The results summarized in tables 4.5 and 4.6 show that the differences between two TP values were not always significant.

4.5e Long-term P desorption

When discussing long-term desorption results in comparison to rapid STP extraction results, it is important to consider the intended purpose of the extractants being used. 0.01M $CaCl_2$-extractable P has been described as P found in soil solution in addition to a minor portion of P that is bound to the soil-solid phase (Hylander, Svensson, and Simán 1995). This easily extractable P fraction is often correlated with DRP in runoff (Maguire and Sims 2002; Pote et al. 1996) and serves as a useful index of P availability (Olsen, Sommers, and Page 1982). Bray P-1 and Mehlich III STP extractants are used in the Midwestern region to assess the fertility status of agricultural soils for fertilizer recommendations. Bray and Kurtz (1945) designed their extractant to dissolve and remove only the forms of P that are of immediate significance to plant growth, or whose variations are responsible for variations in crop growth and response to added phosphates. As described by Sims (2000), the fundamental goal of soil P testing is to identify the "optimum" soil test P concentration required for plant growth. The P fraction extracted by STP extractants is also commonly referred to as plant-available P (Gartley and Sims 1994; Menon, Chien, and Hammond 1989; Watson and Mullen 2007).

Plants are known to principally take up dissolved orthophosphate (P_i) to fuel critical biological processes (Schachtman, Reid, and Ayling 1998). As such, STP extractants aim to extract only P_i from soil in order to more accurately determine the plant-available P content of a soil. As previously shown, Bray P-1 and Mehlich III extracts contained P_o and colloidal P in addition to P_i. Extracts evaluated by ICP will include P_o and colloidal P species in the calculated concentration of plant-available P.

As such, P desorption in 0.01M $CaCl_2$ was evaluated for TP for comparison of long-term desorption concentrations against STP (Figure 4.3). Desorption followed a biphasic trend in all soils, with a more rapid initial release over the first ~15 d and a slower release for the remainder. A similar trend has been observed in other studies evaluating long-term desorption kinetics (Maguire, Sims, and Foy 2001; Sharpley and Ahuja 1982; Staats, Arai, and Sparks 2004). The manure-amended soils desorbed a much greater amount of TP than the fertilizer-amended soils. This trend is similar to what was observed in the previous section, however fertilizer-amended soils desorbed only ~5% the amount of TP as the manure-amended soils in the long-term desorption, while they desorbed ~30% the amount in the STP extractants.

Reaction pH, [P_i], and [Ca^{2+}] from background electrolytes and/or exchangeable cations were input into Visual Minteq software (Gustafsson 2001) to calculate saturation indices for hydroxyapatite related species in order to better interpret the release of P_i. Saturation indices of hydroxyapatite in the fertilizer-amended soils were undersaturated, suggesting that P_i release is controlled by desorption processes over the experimental timeframe. However, the manure-amended soil system was oversaturated with respect to hydroxyapatite, suggesting that P_i release was controlled by the mineral phase.

Results of long-term desorption was fit to several kinetic models for comparison including the Elovich model, the parabolic diffusion model, and the power function model. Relationships of specific soil constituents and desorption properties can be of great importance to fertility management. Rates of P reactions with soils influence the fate of P fertilizer added to soils, the release of P at the rhizosphere, and equilibration between P on soil solid phases and in percolating water (Raven and Hossner 1994). The Elovich model presented the best fit for all soils with an average R^2 of 0.977 (Table 4.7). The α and β parameters of the Elovich equation can be used for comparison of the reaction rates of P release in different soils (Chien and Clayton 1980). The manure-amended soils have a much higher α value than the fertilizer-amended soils, reflecting that the initial desorption rate from these soils occurred more rapidly (Table 4.7). Previous studies have shown that higher SOM content enhances P desorption rates due to increased net negative surface charge on soil particles (Jiao, Whalen, and Hendershot 2007). Organic matter also competes with and displaces P from anion exchange sites (Shariatmadari, Shirvani, and Jafari 2006). The β parameter of the Elovich equation has an inverse relationship to α and is proportionally larger when compared to α in the fertilizer-amended soils than in the manure-amended soils.

Figure 4.3 illustrates the results of P species-specific desorption over 62 d in 0.01 M $CaCl_2$. Trends were similar to those observed in STP desorptions, with manure-amended soils consistently higher in desorption values for P_i, P_o, and TP. The fertilizer-amended soils surprisingly desorbed a greater amount of P_o than P_i over the experimental timeframe, which is consistent with other studies that have assessed the effect of fertilizer additions on P_o fractions (Condron and Goh 1989; Zhang and MacKenzie 1997), while in STP extractants they desorbed a greater amount of P_i than P_o. This indicates that they contain a higher fraction of loosely-bound P_o than P_i, but contain a higher fraction of extractable P_i than P_o when subjected to the conditions of the STP extractants. In a study comparing virgin and cultivated soils, Sharpley and Smith (1985) found that fertilizer amendment increased the labile P_o fraction in a Vertic Haplaquoll (Fargo silty clay loam), which can be attributed to priming of soil microbes by fertilizer addition. The difference between TP in 0.45 μm filtered extracts and 2.5 μm filtered extracts was not great enough to be significantly different making it inconclusive that colloidal P was extracted during the long-term desorption (Table 4.8)

Finally, the relationship between the STP values and the extent of P desorption was evaluated using two end-points, P desorbed after 1 d and 62 d. Selected P species are TP and P_i because these species are commonly measured in STP using ICP-AES/OES and/or Mo-blue colorimetric methods. Figures 4.4 and 4.5 illustrate the relationship between short-term (1 d) and long-term (62 d) 0.01 M $CaCl_2$ desorption values and Bray P-1 (Figure 4.4) and Mehlich III (Figure 4.5) STP values. It is reasonable to say that soils with high STP release more P. However, this might not be the case if irreversible/insoluble P species are present in soils. In the case of calcareous soils, there is a positive slope correlation between the STP values and P_i desorbed after 1 d and 62 d. The proportional relationship shows that as soils increase in STP, temporal-scale P_i release also increases. The value of slope increases from the 1 d samples (Fig 4.4a and Fig 4.5a) to the 62 d samples (Figs 4.4b and Fig 4.5b). The same trend is also observed when TP is compared. In both STP procedures, the slope of P_i is always greater than the slope of TP. This suggests that STP will more accurately predict the release of P_i.

4.6 Conclusion

This study was conducted to address knowledge gaps in the use of Bray P-1 and Mehlich III STP extractants for predicting plant-available P. Calcareous agricultural soils that have a history of long-term manure and fertilizer amendments were chosen because these represent common agricultural soils in the Midwestern U.S.

STP procedures do not speciate among desorbed P species. Manure and chemically fertilized soils were used to evaluate P-species specific desorption in Bray P-1 and Mehlich III extractants over a period of one day. The manure-amended soils desorbed a much greater concentration of P_o and a slightly greater concentration of P_i and colloidal P than the fertilizer-amended soils. Results for both soil types show that the inclusion of P_o and colloidal P increases the results of STP. Identification of P species is important when attempting to assess orthophosphate concentration of extracts. If ICP is used, as is often the case with Mehlich III extracts, P_o and colloidal P will contribute to a TP measurement higher than if orthophosphate is measured alone, especially in manure-amended soils. Further research is needed in assessing P species for plant availability and whether species besides orthophosphate that are desorbed in extracts should be included in the measurement of available P.

Results indicate that time of extraction has a major impact on STP results, whether samples are evaluated colorimetrically or by ICP. Biphasic increases in P_i, TP, and colloidal P were observed, with an initial fast desorption occurring over ~2 h and a slower desorption for the remainder. Mehlich III consistently extracted more of all P species owing to the fact that it is a more buffered extractant. It is likely that extraction times vary in soil testing labs, due to inconsistent methodology, or difficulties in following protocol with thousands of soil samples to process. Extension of extraction time greatly increased the amounts of all P species for both the fertilizer- and manure-amended soils. This can lead to overestimation of available P, lowering the fertilizer recommendations. Kinetic limitations of STP are often questioned as whether they are reliable to account for the P requirement for a long growing season. It is unclear how it is correlated to temporal-scale P availability. Short- and long-term end points of the long-term desorption experiment were correlated with STP values. In these calcareous soils, it is the case that soils higher in STP can reasonably be expected to release more P_i over a growing season.

4.7 Figures

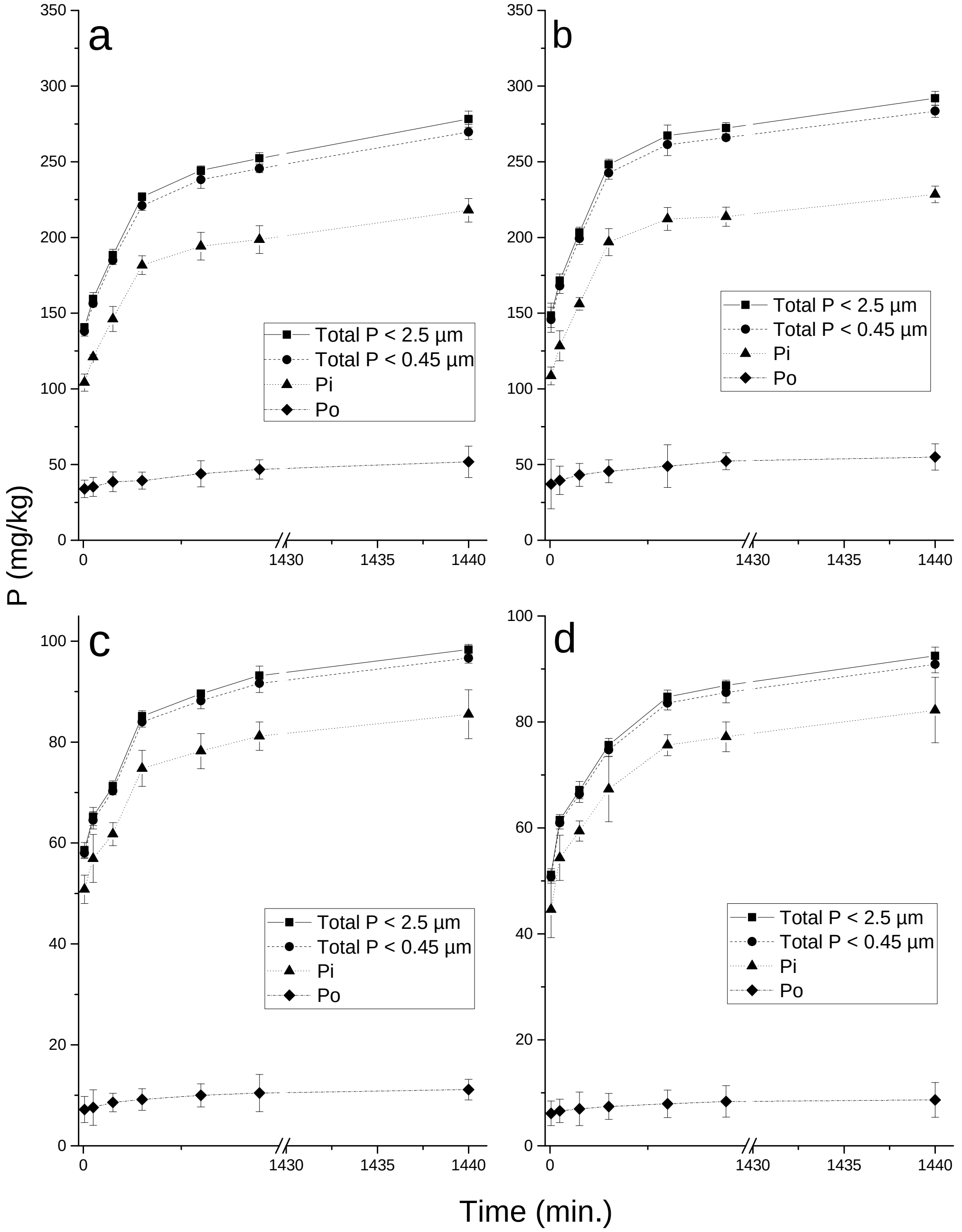

Figure 4.1: Trends of organic P, inorganic P, TP < 0.45 µm, and TP < 2.5 µm over 1 d period (1440 min) in Bray P-1 extracting solution, soil are: a) Mp_1, b) Mp_2, c) Fp_1, and d) Fp_2.

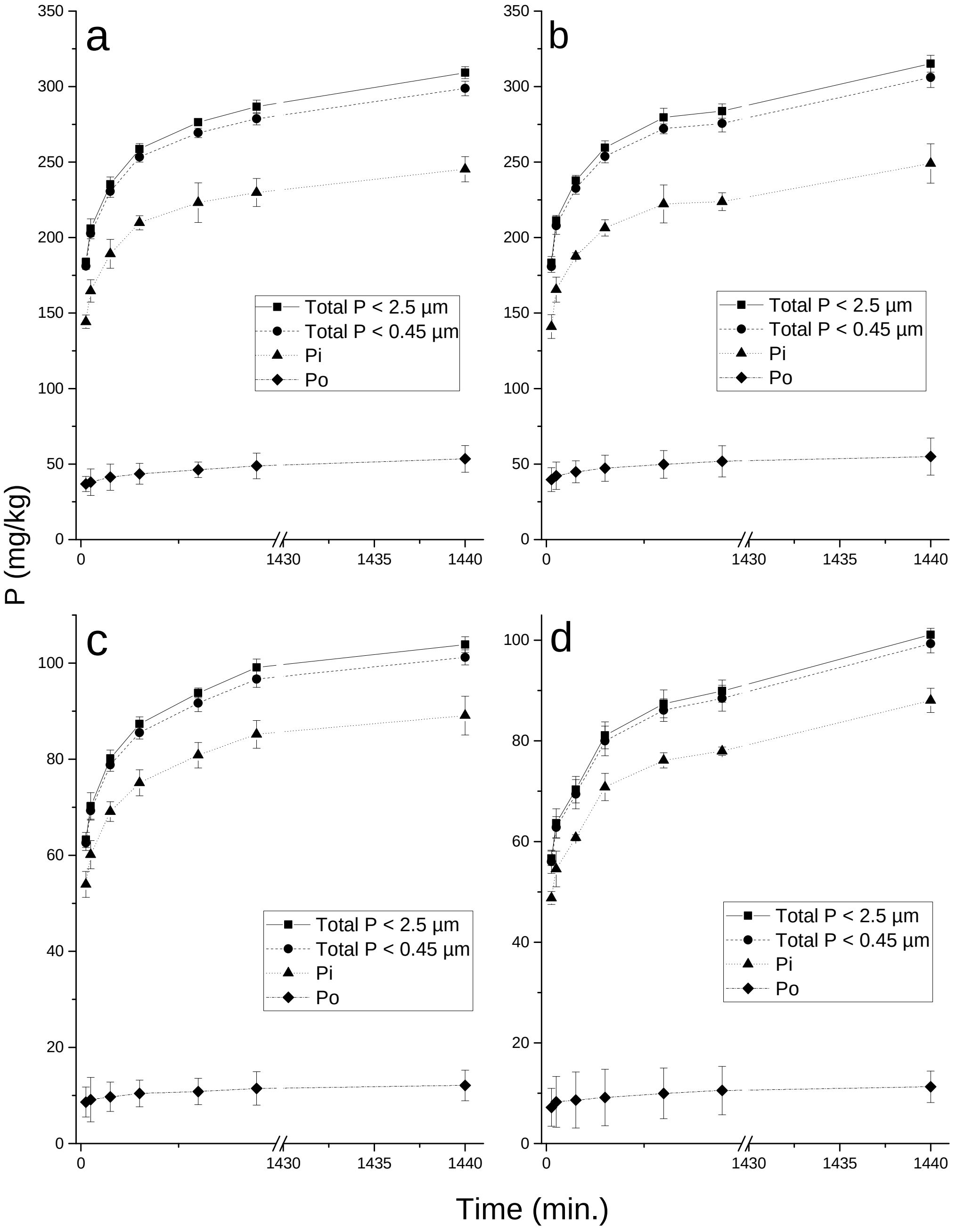

Figure 4.2: Trends of organic P, inorganic P, TP < 0.45 µm, and TP < 2.5 µm over 1 d period (1440 min) in Mehlich III extracting solution, soil are: a) Mp_1, b) Mp_2, c) Fp_1, and d) Fp_2.

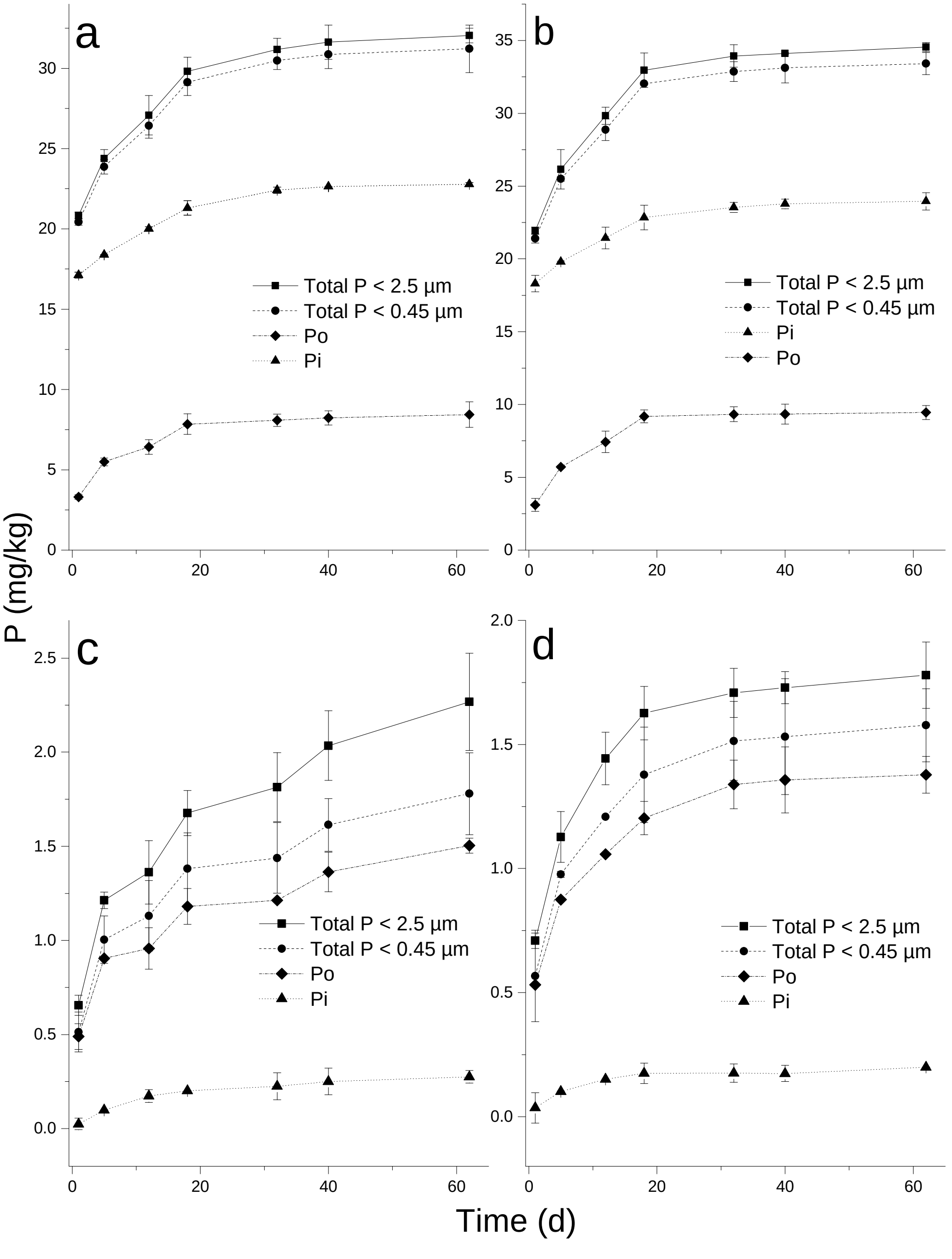

Figure 4.3: Long-term (62 d) P desorption as a function of P species (organic P, inorganic P, TP < 0.45 µm, and TP < 2.5 µm) in 0.01 M $CaCl_2$ solution. a) Manure-amended P rich (Mp) soil #1 (Mp_1), b) Mp_2, c) Fertilizer-amended P rich soils #1 (Fp_1), and d) Fp_2.

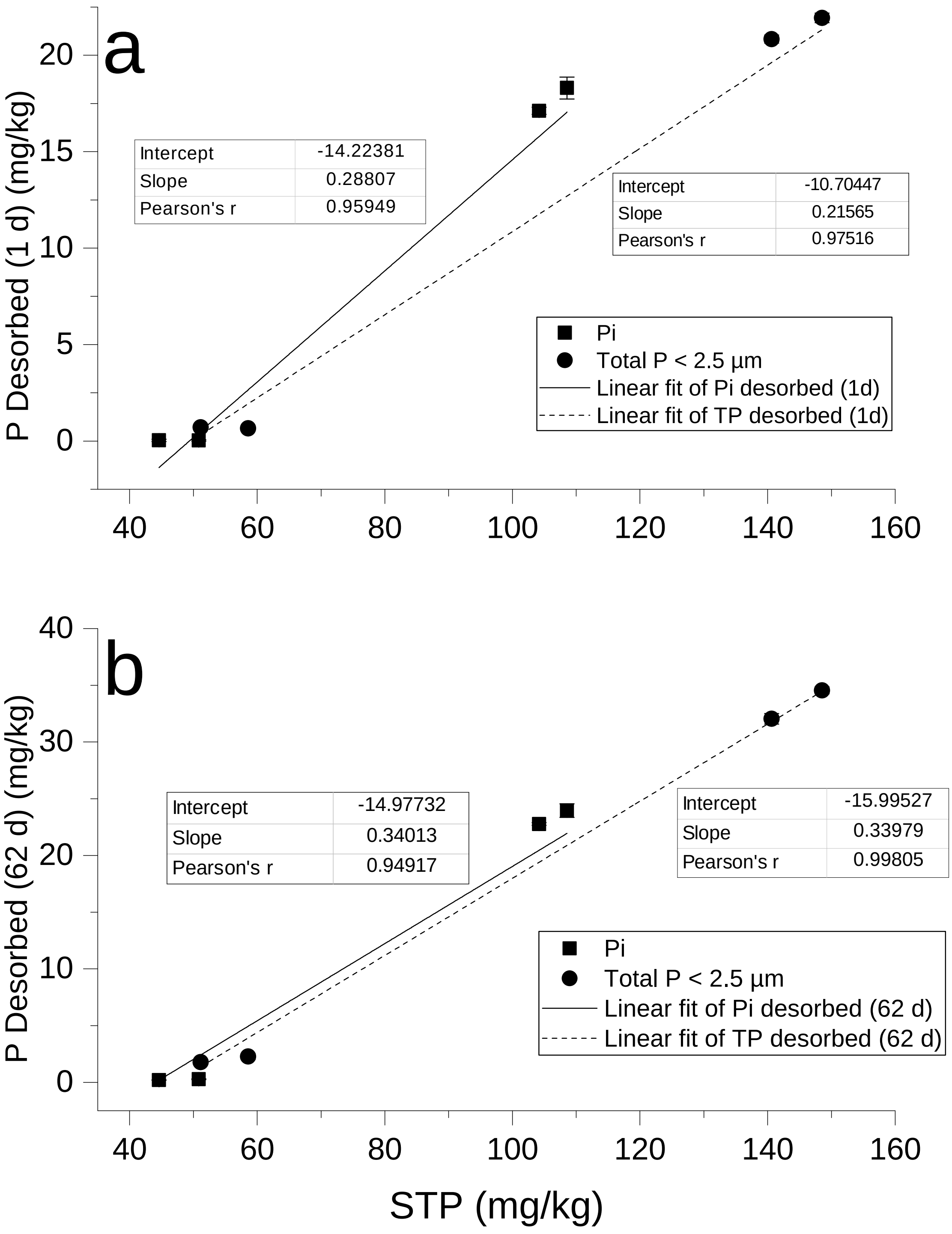

Figure 4.4: Relationship between Bray P-1 STP of four soils in Table 4.2 and total desorbed P (TP < 2.5 μm and P_i) after a) 1 d and b) 62 d shown in Figure 4.3.

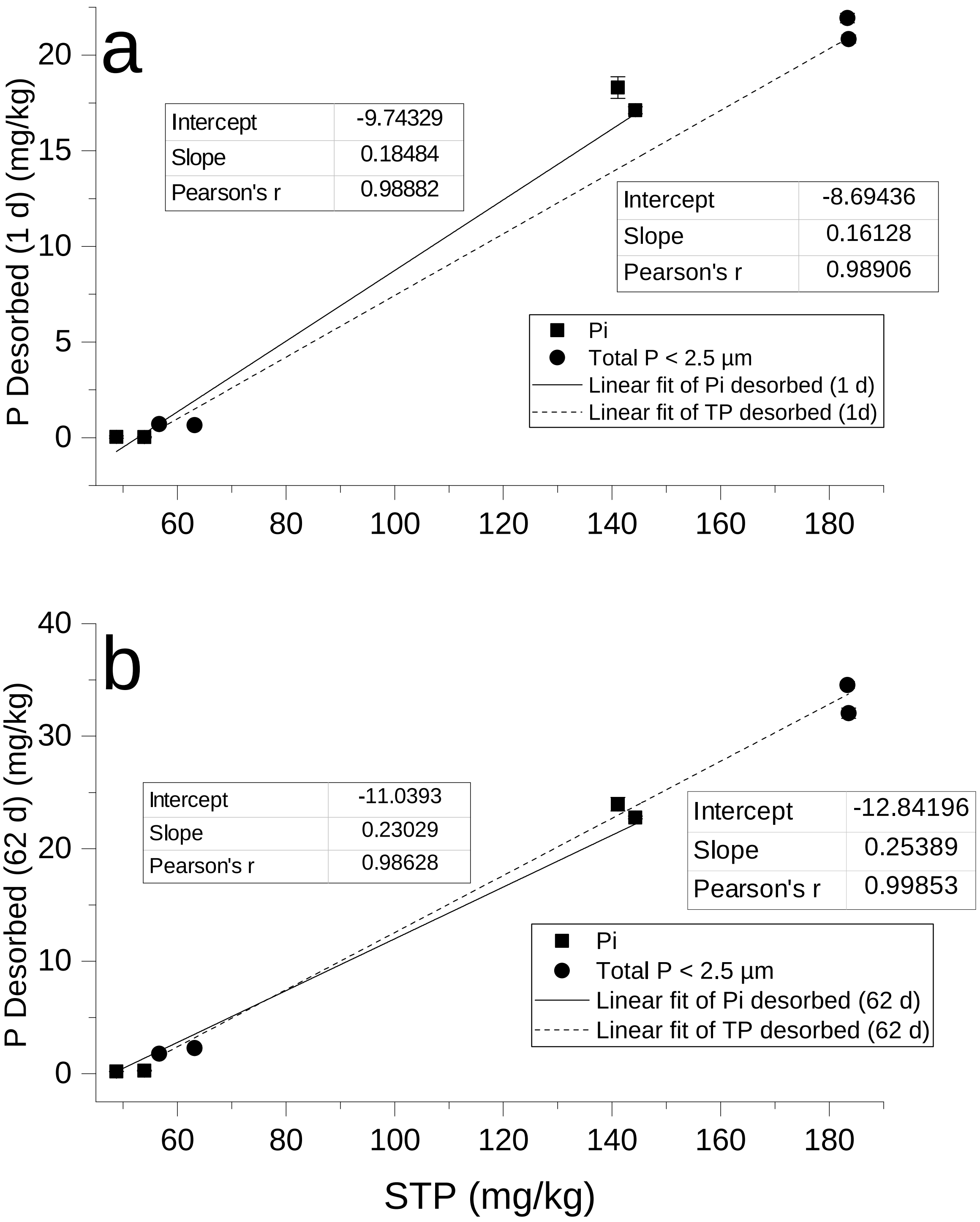

Figure 4.5: Relationship between Mehlich III STP of four soils in Table 4.2 and total desorbed P (TP < 2.5 µm and P_i) after a) 1 d and b) 62 d shown in Figure 4.3.

4.8 Tables

Table 4.1: A sequential fractionation method for inorganic-P in high base saturation soils (Jiang and Gu; 1989). Occluded P was extracted with 0.3 M sodium citrate (20 mL)-dithionite (1.0 g)-1.0 M sodium hydroxide (5 mL).

Step	Inorganic-P Fraction	Extractant	pH	Mo-Blue Method
1	Labile P/Ca_2-P	0.25 M $NaHCO_3$	7.5	(Asher 1980)
2	Ca-P (i.e. Ca_8-P, Ca_{10}-P)	0.5 M NH_4Ac	4.2	(Asher 1980)
3	Al-P	0.5 M NH_4F	8.2	(Murphy and Riley 1962)
4	Fe-P	0.1 M NaOH-Na_2CO_3	12.0	(Asher 1980)
5	Occluded P	0.3 M CD	13.0	(Murphy and Riley 1962)
6	Stable Ca-P	0.25 M H_2SO_4	1.0	(Asher 1980)

Table 4.2: Results of STP and physicochemical characteristics in phosphorus rich agricultural soils. Manure-amended soils indicated by MP, fertilizer-amended soils indicated by FP.

Sample ID	Soil series	Designation	pH†	% Organic matter‡	Mehlich III P (mg/kg)	Bray P-1 (mg/kg)	CEC ($cmol_c$/kg)	% base saturation		
								Ca	Mg	K
Fp_1	Sable silty clay loam	Typic Endoaquoll	5.55	3.62	53.94 ± 2.69	50.83 ± 2.83	29.00	44.97	13.97	1.15
Fp_2	Sable silty clay loam	Typic Endoaquoll	5.55	3.66	48.79 ± 1.30	44.61 ± 5.37	31.81	47.22	14.91	1.18
Mp_1	Drummer silty clay loam	Typic Endoaquoll	6.9	4.37	144.30 ± 4.52	104.17 ± 5.65	22.58	63.95	17.46	10.34
Mp_2	Drummer silty clay loam	Typic Endoaquoll	6.8	5.90	141.08 ± 7.92	108.57 ± 5.83	25.04	70.87	14.64	4.95

†Measured in H_2O, ‡Measured by loss on ignition

Table 4.3: Inorganic, organic, and total P in soils studied. Manure-amended soils indicated by MP, fertilizer-amended soils indicated by FP.

Soil	**P_i**	**P_o**	**TP**
		(mg P kg^{-1})	
Fp_1	272.02 ± 1.96	115.61 ± 0.87	387.02 ± 2.01
Fp_2	283.25 ± 0.77	108.15 ± 0.56	391.41 ± 1.22
Mp_1	432.05 ± 0.23	328.05 ± 1.45	760.10 ± 1.89
Mp_2	470.87 ± 1.14	355.32 ± 1.42	826.19 ± 2.31

Table 4.4: Inorganic P fractions sequentially extracted from soil samples. Manure-amended soils indicated by MP, fertilizer-amended soils indicated by FP. Numbers in parentheses are percentage of P_i fraction with respect to total P_i (does not take into account standard deviation).

Soil	**Soil inorganic P fractions (mg P kg^{-1})**					
	Ca_2-P	**Ca_8-P**	**Al-P**	**Fe-P**	**O-P**	**Ca_{10}-P**
Fp_1	68.07 ± 1.80 (30)	71.73 ± 2.75 (31)	0.75 ± 0.22 (< 1)	70.89 ± 8.00 (31)	3.37 ± 1.23 (1.5)	15.73 ± 5.45 (6.2)
Fp_2	67.82 ± 2.30 (27)	78.88 ± 4.82 (32)	0.85 ± 0.10 (< 1)	69.55 ± 1.43 (28)	3.42 ± 1.02 (2.3)	28.29 ± 0.16 (10.4)
Mp_1	81.27 ± 13.92 (19)	181.21 ± 12.60 (42)	0.80 ± 0.34 (< 1)	137.40 ± 31.64 (32)	9.75 ± 7.24 (2.3)	21.16 ± 18.33 (4.6)
Mp_2	92.83 ± 10.09 (20)	185.38 ± 25.99 (41)	0.85 ± 0.25 (< 1)	135.22 ± 7.54 (30)	1.83 ± 0.30 (< 1)	37.47 ± 6.33 (8.5)

Table 4.5: *p* values from a t-test matrix of P concentration averages in Bray P-1 extractant over a time period of 1 d.

Soil	Time (min)	P_i vs. TP < 0.45 µm	TP < 0.45 µm vs. TP < 2.5µm
Fp_1	1	0.01723	0.31502
	10	0.04822	0.32059
	30	0.00755	0.14717
	60	0.01916	0.12697
	120	0.01152	0.13023
	180	0.0044	0.07183
	1440	0.02616	0.05631
Fp_2	1	0.09113	0.35077
	10	0.0538	0.29562
	30	0.00433	0.29097
	60	0.08386	0.20528
	120	0.00341	0.16938
	180	0.00848	0.19012
	1440	0.06403	0.15525
Mp_1	1	0.00114	0.21549
	10	<0.001	0.16699
	30	0.00459	0.13222
	60	0.00112	0.07265
	120	0.00193	0.09669
	180	0.00425	0.03273
	1440	<0.001	0.05727
Mp_2	1	0.00216	0.34576
	10	0.00448	0.2041
	30	<0.001	0.13199
	60	0.0026	0.07223
	120	<0.001	0.0826
	180	0.00131	0.03916
	1440	<0.001	0.0184

Table 4.6: *p* values from a t-test matrix of P concentration averages in Mehlich III extractant over a time period of 1 d.

Soil	Time (min)	P_i vs. TP < 0.45 µm	TP < 0.45 µm vs. TP < 2.5µm
Fp_1	1	0.0154	0.32755
	10	0.01774	0.33214
	30	0.01586	0.17349
	60	0.04526	0.16683
	120	0.00245	0.07992
	180	0.00646	0.07982
	1440	0.00186	0.05446
Fp_2	1	0.00811	0.34743
	10	0.01774	0.35854
	30	0.01586	0.35335
	60	0.00848	0.32802
	120	0.00245	0.2874
	180	0.00646	0.12521
	1440	0.00186	0.07012
Mp_1	1	<0.001	0.15331
	10	0.00433	0.25329
	30	0.00451	0.13177
	60	<0.001	0.06705
	120	0.01048	0.0197
	180	0.00246	0.04262
	1440	<0.001	0.02336
Mp_2	1	0.00257	0.23022
	10	0.00142	0.21214
	30	<0.001	0.07817
	60	<0.001	0.08683
	120	0.00787	0.07294
	180	<0.001	0.0622
	1440	0.00378	0.04773

Table 4.7: Elovich parameters and model correlations.

P fraction	**Soil**	**α (mg/kg/h)**	**β $(mg/kg)^{-1}$**	**R^2**
TP	Fp_1	1.15E-4	2500	0.9715
	Fp_2	1.54E-4	3333	0.9861
	Mp_1	0.0947	322.6	0.9757
	Mp_2	0.0664	285.7	0.9731
P_i	Fp_1	2.14E-6	5000	0.9898
	Fp_2	5.41E-6	2.5E4	0.9628
	Mp_1	3.913	666.7	0.9548
	Mp_2	2.814	588.2	0.9596
P_o	Fp_1	7.36E-5	1.67E4	0.963
	Fp_2	7.36E-5	5000	0.9951
	Mp_1	6.38E-4	714.3	0.9772
	Mp_2	4.94E-4	588.2	0.9413

Table 4.8: *p* values from a t-test matrix of P concentration averages in 0.01 M $CaCl_2$ over a time period of 62 d.

Soil	**Time (d)**	**TP < 0.45 µm vs. TP < 2.5µm**
Fp_1	1	0.13802
	5	0.11478
	12	0.16276
	18	0.11332
	32	0.08981
	40	0.06673
	62	0.09046
Fp_2	1	0.23493
	5	0.14063
	12	0.09791
	18	0.14222
	32	0.15253
	40	0.21691
	62	0.14453
Mp_1	1	0.18901
	5	0.21489
	12	0.30306
	18	0.25951
	32	0.19847
	40	0.25902
	62	0.28536
Mp_2	1	0.10663
	5	0.30916
	12	0.14565
	18	0.2326
	32	0.13871
	40	0.20352
	62	0.12552

4.9 References

Adhami, E. et al. 2006. “Inorganic Phosphorus Fractionation of Highly Calcareous Soils of Iran.” *Communications in soil science and plant analysis* 37(13–14):1877–88.

Anderson, George. 1980. “Assessing Organic Phosphorus in Soils.” Pp. 411–31 in *The role of phosphorus in agriculture*, edited by F. E. Khasawneh, E. C. Sample, and E. J. Kamprath. Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Arai, Yuji and Ken J. Livi. 2013. “Underassessed Phosphorus Fixation Mechanisms in Soil Sand Fraction.” *Geoderma* 192:422–29.

Arai, Yuji and D. L. Sparks. 2001. “ATR–FTIR Spectroscopic Investigation on Phosphate Adsorption Mechanisms at the Ferrihydrite–Water Interface.” *Journal of Colloid and Interface Science* 241(2):317–26. Retrieved May 17, 2016 (http://www.sciencedirect.com/science/article/pii/S0021979701977732).

Arai, Yuji and D. L. Sparks. 2007. “Phosphate Reaction Dynamics in Soils and Soil Components: A Multiscale Approach.” *Advances in Agronomy* 94(6):135–79. Retrieved (http://linkinghub.elsevier.com/retrieve/pii/S0065211306940036).

Asher, L. E. 1980. “An Automated Method for the Determination of Orthophosphate in the Presence of Labile Polyphosphates.” *Soil Science Society of America Journal* 44(1):173–75.

Audette, Yuki, Ivan P. O’Halloran, Les J. Evans, and R.Paul Voroney. 2016. “Preliminary Validation of a Sequential Fractionation Method to Study Phosphorus Chemistry in a Calcareous Soil.” *Chemosphere* 152:369–75.

Baginski, E. S., P. P. Foa, and B. Zak. 1967. “Determination of Phosphate: Study of Labile Organic Phosphate Interference.” *Clinica Chimica Acta* 15(1):155–58.

Barroso, C. B. and E. Nahas. 2005. “The Status of Soil Phosphate Fractions and the Ability of Fungi to Dissolve Hardly Soluble Phosphates.” *Applied Soil Ecology* 29(1):73–83.

Beauchemin, S., R. R. Simard, and D. Cluis. 1996. “Phosphorus Sorption-Desorption Kinetics of Soil under Contrasting Land Uses.” *Journal of Environmental Quality* 25(6):1317–25.

Bowman, R. A. 1989. “A Sequential Extraction Procedure with Concentrated Sulfuric Acid and Dilute Base for Soil Organic Phosphorus.” *Soil Science Society of America Journal* 53(2):362–66. Retrieved (http://dx.doi.org/10.2136/sssaj1989.03615995005300020008x).

Bowman, R. A. and J. O. Moir. 1993. “Basic EDTA as an Extractant for Soil Organic Phosphorus.” *Soil Science Society of America Journal* 57(6):1516–18.

Bray, Roger H. and L. T. Kurtz. 1945. “Determination of Total, Organic, and Available Forms of Phosphorus in Soils.” *Soil Science* 59(1):39–46.

Broberg, Ola and Gunnar Persson. 1988. “Particulate and Dissolved Phosphorus Forms in Freshwater: Composition and Analysis.” Pp. 61–90 in *Phosphorus in Freshwater Ecosystems*, edited by G. Persson and M. Jansson. Rotterdam: Springer Netherlands.

Brown, J. .. 2015. “Recommended Chemical Soil Test Procedures for the North Central Region.” 221(221):76. Retrieved (http://extension.missouri.edu/explorepdf/specialb/sb1001.pdf)

Buffle, Jacques and Herman P. van Leeuwen. 1993. *Environmental Particles*. 2nd ed. Boca Raton, FL: Lewis publishers.

Busman, L. M. and M. A. Tabatabai. 1985. “Hydrolysis of Trimetaphosphate in Soils.” *Soil*

Science Society of America Journal 49(3):630–36.

Celi, Luisella, Sebastiano Lamacchia, Franco Ajmone Marsan, and Elisabetta Barberis. 1999. "Interaction of Inositol Hexaphosphate on Clays: Adsorption and Charging Phenomena." *Soil Science* 164(8):574–85. Retrieved (http://www.scopus.com/inward/record.url?eid=2-s2.0-0032826509&partnerID=tZOtx3y1).

Chang, S. C. and Mo L. Jackson. 1957. "Fractionation of Soil Phosphorus." *Soil science* 84(2):133–44.

Chien, S. H. and W. R. Clayton. 1980. "Application of Elovich Equation to the Kinetics of Phosphate Release and Sorption in Soils." *Soil Science Society of America Journal* 44(2):265–68.

Condron, L. M. and K. M. Goh. 1989. "Effects of Long-term Phosphatic Fertilizer Applications on Amounts and Forms of Phosphorus in Soils under Irrigated Pasture in New Zealand." *Journal of soil science* 40(2):383–95.

Department of Sustainable Natural Resources. 1995. "Soil Survey Standard Test Method: Available Phosphorus: Bray No 1 Extract." (1):1–4. Retrieved (http://www.environment.nsw.gov.au/soils/testmethods.htm).

Ebeling, Angela M., Larry G. Bundy, Aaron W. Kittell, and Daniel D. Ebeling. 2008. "Evaluating the Bray P1 Test on Alkaline, Calcareous Soils." *Soil Science Society of America Journal* 72(4):985. Retrieved (https://www.soils.org/publications/sssaj/abstracts/72/4/985).

Eghball, Bahman. 1999. "Liming Effects of Beef Cattle Feedlot Manure or Compost." *Communications in Soil Science & Plant Analysis* 30(19–20):2563–70.

Eisenreich, S. J., R. T. Bannerman, and D. E. Armstrong. 1975. "A Simplified Phosphorus Analysis Technique." *Environmental letters* 9(1):43–53.

Elkhatib, E. A. and J. L. Hern. 1988. "Kinetics of Phosphorus Desorption from Appalachian Soils." *Soil Science* 145(3):222–29.

Fekri, M., N. Gorgin, and L. Sadegh. 2011. "Phosphorus Desorption Kinetics in Two Calcareous Soils Amended with P Fertilizer and Organic Matter." *Environmental Earth Sciences* 64(3):721–29.

Fernández, F. G., B. S. Farmaha, and Emerson D. Nafziger. 2012. "Soil Fertility Status of Soils in Illinois." *Communications in Soil Science and Plant Analysis* 43(22):2897–2914. Retrieved (http://proxy.timbo.org.uy:443/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=lbh&AN=20123415277&lang=es&site=ehost-live%5Cnhttp://www.tandfonline.com/loi/lcss20%5Cnemail: fernande@uiuc.edu).

Fernández, Fg and Rg Hoeft. 2009. "Managing Soil pH and Crop Nutrients." *... agronomy handbook. 24th ed. Univ. of ...* 91–112. Retrieved (http://www.ebooksmagz.com/pdf/managing-soil-ph-and-crop-nutrients-129919.pdf).

Filella, Montserrat, Claire Deville, Vincent Chanudet, and Davide Vignati. 2006. "Variability of the Colloidal Molybdate Reactive Phosphorous Concentrations in Freshwaters." *Water research* 40(17):3185–92.

Fixen, P. E., Bruulsema, T. W., Jensen, T. L., Mikkelsen, R., Murrell, T. S., Phillips, S. B., ... & Stewart, W. M. (2010). The fertility of North American soils, 2010. *Better Crops with Plant Food*, *94*(4), 6-8.

Freeman, J. S. and D. L. Rowell. 1981. "The Adsorption and Precipitation of Phosphate onto Calcite." *Journal of Soil Science* 32(1):75–84.

Gartley, K. L. and J. T. Sims. 1994. "Phosphorus Soil Testing: Environmental Uses and Implications." *Communications in Soil Science & Plant Analysis* 25(9–10):1565–82.

Gerke, J. 1992. "Orthophosphate and Organic Phosphate in the Soil Solution of Four Sandy Soils in Relation to pH Evidence for Humic FE (AL) Phosphate Complexes." *Communications in Soil Science & Plant Analysis* 23(5–6):601–12.

Gustafsson, J. P. (2011). Visual MINTEQ ver. 3.0. *Department of Land and Water Resources Engineering, Royal Institute of Technology: Stokholm, Sweden*.

Hayes, J. E., A. E. Richardson, and R. J. Simpson. 2000. "Components of Organic Phosphorus in Soil Extracts That Are Hydrolysed by Phytase and Acid Phosphatase." *Biology and Fertility of Soils* 32(4):279–86.

Haygarth, Philip M., Melanie S. Warwick, and W.Alan House. 1997. "Size Distribution of Colloidal Molybdate Reactive Phosphorus in River Waters and Soil Solution." *Water Research* 31(3):439–48.

Haynes, Richard J. and R. Naidu. 1998. "Influence of Lime, Fertilizer and Manure Applications on Soil Organic Matter Content and Soil Physical Conditions: A Review." *Nutrient cycling in agroecosystems* 51(2):123–37.

He, Zhongqi and C.Wayne Honeycutt. 2005. "A Modified Molybdenum Blue Method for Orthophosphate Determination Suitable for Investigating Enzymatic Hydrolysis of Organic Phosphates." *Communications in Soil Science and Plant Analysis* 36(9–10):1373–83.

Heathwaite, Louise, Phil Haygarth, Rachel Matthews, Neil Preedy, and Patricia Butler. 2005. "Evaluating Colloidal Phosphorus Delivery to Surface Waters from Diffuse Agricultural Sources." *Journal of environmental quality* 34(1):287–98.

Hedley, M. J., G. J. R. Kirk, and M. B. Santos. 1994. "Phosphorus Efficiency and the Forms of Soil Phosphorus Utilized by Upland Rice Cultivars." *Plant and Soil* 158(1):53–62.

Hemwall, John B. 1957. "The Role of Soil Clay Minerals in Phosphorus Fixation." *Soil Science* 83(2):101–8.

Hens, Maarten and Roel Merckx. 2001. "Functional Characterization of Colloidal Phosphorus Species in the Soil Solution of Sandy Soils." *Environmental science & technology* 35(3):493–500.

Herlihy, M. and J. McCarthy. 2006. "Association of Soil-Test Phosphorus with Phosphorus Fractions and Adsorption Characteristics." *Nutrient Cycling in Agroecosystems* 75(1–3):79–90.

Hylander, Lars D., Hans-Ivar Svensson, and Gyula Simán. 1995. "Comparison of Different Methods for Determination of Phosphorus in Calcium Chloride Extracts for Prediction of Availability to Plants." *Communications in Soil Science & Plant Analysis* 26(5–6):913–25.

Ippolito, J. A. et al. 2010. "Phosphorus Biogeochemistry across a Precipitation Gradient in Grasslands of Central North America." *Journal of Arid Environments* 74(8):954–61.

Jiang, Baifan and Yichu Gu. 1989. "A Suggested Fractionation Scheme of Inorganic Phosphorus in Calcareous Soils." *Fertilizer Research* 20(3):159–65.

Jiao, You, Joann K. Whalen, and William H. Hendershot. 2007. "Phosphate Sorption and Release in a Sandy-Loam Soil as Influenced by Fertilizer Sources." *Soil Science Society of America Journal* 71(1):118–24.

Kanissery, Ramdas G., Allana Welsh, and Gerald K. Sims. 2015. “Effect of Soil Aeration and Phosphate Addition on the Microbial Bioavailability of Carbon-14-Glyphosate.” *Journal of environmental quality* 44(1):137–44.

Ketterings, Q. M. and M. Flock. 2005. “Comparison of Bray-1 and Mehlich-3 Tests in High Phosphorus Soils.” *Soil Science* 170(3):212–19. Retrieved (http://www.scopus.com/inward/record.url?eid=2-s2.0-15244341148&partnerID=40&md5=3aaddac4f8fb31152c4a6081a997ceb4).

Ketterings, Quirine M. and Pete Barney. 2010. “Phosphorus Soil Testing Methods Agronomy Factsheet Series.” Factsheet 15. Retrieved (http://nmsp.cals.cornell.edu).

Khaleel, R., K. R. Reddy, and M. R. Overcash. 1981. “Changes in Soil Physical Properties due to Organic Waste Applications: A Review.” *Journal of Environmental Quality* 10(2):133–41.

Kulaev, Igor S., Vladimir Vagabov, and Tatiana Kulakovskaya. 2005. *The Biochemistry of Inorganic Polyphosphates*. 2nd ed. Chichester, West Sussex: John Wiley & Sons.

Laboski, Carrie A. M. and John A. Lamb. 2003. “Changes in Soil Test Phosphorus Concentration after Application of Manure or Fertilizer.” *Soil Science Society of America Journal* 67(2):544–54.

Lal, R. and B. T. Kang. 1982. “Management of Organic Matter in Soils of the Tropics and Subtropics.” Pp. 152–78 in *Non Symbiotic Nitrogen Fixation and Organic Matter in the Tropics. Symp. Papers I. Trans. 12th Int. Cong. Soil Sci. New Delhi*.

Leikam, Dale F., Frank P. Achorn, J. T. Sims, and A. N. Sharpley. 2005. “Phosphate Fertilizers: Production, Characteristics, and Technologies.” Pp. 23–50 in *Phosphorus: agriculture and the environment*. Madison, WI: ASA, CSSA, SSSA.

Lindsay, Willard Lyman. 1979. *Chemical Equilibria in Soils.* 1st ed. Chichester, West Sussex: John Wiley and Sons Ltd.

Maguire, R. O., J. T. Sims, and R. H. Foy. 2001. “Long-Term Kinetics for Phosphorus Sorption-Desorption by High Phosphorus Soils from Ireland and the Delmarva Peninsula, USA.” *Soil science* 166(8):557–65.

Maguire, Rory O., Wim J. Chardon, and Regis R. Simard. 2005. "Assessing potential environmental impacts of soil phosphorus by soil testing." *Phosphorus: agriculture and the environment*: 145-180.

Maguire, Rory O. and J.Thomas Sims. 2002. “Soil Testing to Predict Phosphorus Leaching.” *Journal of Environmental Quality* 31(5):1601–9.

Makarov, M. I., L. Haumaier, and W. Zech. 2002. “Nature of Soil Organic Phosphorus: An Assessment of Peak Assignments in the Diester Region of 31 P NMR Spectra.” *Soil Biology and Biochemistry* 34(10):1467–77.

Mallarino, A. P. and A. M. Blackmer. 1992. “Comparison of Methods for Determining Critical Concentrations of Soil Test Phosphorus for Corn.” *Agronomy Journal* 84(5):850–56.

Mallarino, Antonio P. 1995. “Comparison of Mehlich-3, Olsen, and Bray-P1 Procedures for Phosphorus in Calcareous Soils.” *Proceedings of the twenty-fifth North Central Extension-Industry soil fertility conference* (11):96–101.

Masson, Pierre, Christian Morel, Eric Martin, Astrid Oberson, and Dennis Friesen. 2001. “Comparison of Soluble P in Soil Water Extracts Determined by Ion Chromatography, Colorimetric, and Inductively Coupled Plasma Techniques in PPB Range.” *Communications in soil science and plant analysis* 32(13–14):2241–53.

Mausbach, Maurice J. 1969. “Inorganic Phosphorus Fractions of Some Iowa Soil Profiles.”

(Unpublished M.S. thesis). Iowa State University, Ames, Iowa.

McBeath, Therese M., Enzo Lombi, Michael J. McLaughlin, and Else K. Bunemann. 2007. "Polyphosphate-Fertilizer Solution Stability with Time, Temperature, and pH." *Journal of Plant Nutrition and Soil Science* 170(3):387–91.

McDowell, R. W. and A. N. Sharpley. 2003. "Phosphorus Solubility and Release Kinetics as a Function of Soil Test P Concentration." *Geoderma* 112(1):143–54.

McLaughlin, Michael J., A. M. Alston, and J. K. Martin. 1988. "Phosphorus Cycling in Wheat Pasture Rotations. II. The Role of the Microbial Biomass in Phosphorus Cycling." *Soil Research* 26(2):333–42.

McLean, E. O. "Soil pH and lime requirement." *Methods of soil analysis. Part 2. Chemical and microbiological properties* (1982): 199-224.

Mehlich, Adolf. 1984. "Mehlich 3 Soil Test Extractant: A Modification of Mehlich 2 Extractant." *Communications in Soil Science & Plant Analysis* 15(12):1409–16.

Menon, R. G., S. H. Chien, and L. L. Hammond. 1989. "Comparison of Bray I and Pi Tests for Evaluating Plant-Available Phosphorus from Soils Treated with Different Partially Acidulated Phosphate Rocks." *Plant and soil* 114(2):211–16.

Messiga, A. J., Y. Ba, N. Ziadi, G. Bélanger, and J. Lafond. 2014. "Assessing the Depletion of Soil P Following Sequential Extractions with Mehlich-3 and Olsen Solutions." *Archives of Agronomy and Soil Science* 60(10):1445–58. Retrieved (http://www.tandfonline.com/doi/abs/10.1080/03650340.2014.884709).

Murphy, James and J. P. Riley. 1962. "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters." *Analytica chimica acta* 27:31–36.

Nelson, Norman S. 1987. "An Acid-persulfate Digestion Procedure for Determination of Phosphorus in Sediments." *Communications in Soil Science & Plant Analysis* 18(4):359–69.

Olsen, S. R., L. E. Sommers, and A. L. Page. 1982. "Methods of Soil Analysis. Part 2." *Chemical and microbiological properties of Phosphorus. ASA Monograph* (9):403–30.

Olsen, Sterling Robertson. 1954. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*. US Government Printing Office.

Otani, Takashi and Noriharu Ae. 1999. "Extraction of Organic Phosphorus in Andosols by Various Methods." *Soil Science and Plant Nutrition* 45(1):151–61.

Parfitt, Roger L., Roger J. Atkinson, and Roger S. C. Smart. 1975. "The Mechanism of Phosphate Fixation by Iron Oxides." *Soil Science Society of America* 39(3):838–41.

Pierzynski, G. M., R. W. McDowell, and J. M. Sims. 2005. "Phosphorus Reactions and Cycling in Soils." *Phosphorus: agriculture and the environment. Agronomy Monograph* (46):53–57.

Pote, D. H. et al. 1996. "Relating Extractable Soil Phosphorus to Phosphorus Losses in Runoff." *Soil Science Society of America Journal* 60(3):855–59.

Potter, Philip, Navin Ramankutty, Elena M. Bennett, and Simon D. Donner. 2010. "Characterizing the Spatial Patterns of Global Fertilizer Application and Manure Production." *Earth Interactions* 14(2):1–22.

Raven, K. P. and L. R. Hossner. 1994. "Soil Phosphorus Desorption Kinetics and Its Relationship with Plant Growth." *Soil Science Society of America Journal* 58(2):416–23.

Reddy, D.Damodar, A.Subba Rao, and T. R. Rupa. 2000. "Effects of Continuous Use of Cattle Manure and Fertilizer Phosphorus on Crop Yields and Soil Organic Phosphorus in a Vertisol." *Bioresource Technology* 75(2):113–18.

Ross, Donald S., and Quirine Ketterings. 1995. "Recommended methods for determining soil cation exchange capacity." *Recommended soil testing procedures for the northeastern United States* 2: 62-70.

Sanyal, S. K. and S. K. De Datta. 1991. "Chemistry of Phosphorus Transformations in Soil." Pp. 1–120 in *Advances in soil science*. New York, NY: Springer.

Sassman, Stephen A. and Linda S. Lee. 2005. "Sorption of Three Tetracyclines by Several Soils: Assessing the Role of pH and Cation Exchange." *Environmental Science & Technology* 39(19):7452–59.

Schachtman, Daniel P., Robert J. Reid, and Sarah M. Ayling. 1998. "Phosphorus Uptake by Plants: From Soil to Cell." *Plant physiology* 116(2):447–53.

Schelde, Kirsten, Lis W. de Jonge, Charlotte Kjaergaard, Mette Laegdsmand, and Gitte H. Rubæk. 2006. "Effects of Manure Application and Plowing on Transport of Colloids and Phosphorus to Tile Drains." *Vadose Zone Journal* 5(1):445–58.

Schulte, E. E., and B. G. Hopkins. "Estimation of soil organic matter by weight loss-on-ignition." *Soil organic matter: analysis and interpretation* (1996): 21-31.

Shang, C., L. W. Zelazny, D. F. Berry, and R. O. Maguire. 2013. "Orthophosphate and Phytate Extraction from Soil Components by Common Soil Phosphorus Tests." *Geoderma* 209–210:22–30. Retrieved (http://www.sciencedirect.com/science/article/pii/S0016706113001973).

Shariatmadari, H., M. Shirvani, and A. Jafari. 2006. "Phosphorus Release Kinetics and Availability in Calcareous Soils of Selected Arid and Semiarid Toposequences." *Geoderma* 132(3):261–72.

Sharpley, A. N. and L. R. Ahuja. 1982. "Effects of Temperature and Soil-Water Content during Incubation on the Desorption of Phosphorus from Soil." *Soil Science* 133(6):350–55.

Sharpley, A. N. and S. J. Smith. 1985. "Fractionation of Inorganic and Organic Phosphorus in Virgin and Cultivated Soils." *Soil Science Society of America Journal* 49(1):127–30. Retrieved (http://dx.doi.org/10.2136/sssaj1985.03615995004900010025x).

Sharpley, A. N., H. Tiessen, and C. V Cole. 1987. "Soil Phosphorus Forms Extracted by Soil Tests as a Function of Pedogenesis." *Soil Science Society of America Journal* 51(2):362–65.

Sharpley, Andrew N., Richard W. McDowell, and Peter J. A. Kleinman. 2004. "Amounts, Forms, and Solubility of Phosphorus in Soils Receiving Manure." *Soil science society of America journal* 68(6):2048–57.

Shen, J. et al. 2004. "Crop Yields, Soil Fertility and Phosphorus Fractions in Response to Long-Term Fertilization Under the Rice Monoculture System on a Calcareous Soil." *Field Crops Research* 86(2):225–38.

Shin, Mari, Suzelle F. Barrington, William D. Marshall, and Jin-Woo Kim. 2006. "Kinetics of Metal Desorption from Soil with Nonionic Micelle-Solubilized Ligands." *Journal of Environmental Engineering and Science* 5(2):163–73.

Sims, J. T. and S. E. Heckendorn. 1991. "Methods of Analysis of the University of Delaware Soil Testing Laboratory." *Bull* 10.

Sims, J. T., R. R. Simard, and B. C. Joern. 1998. "Phosphorus Loss in Agricultural Drainage: Historical Perspective and Current Research." *Journal of Environment Quality* 27(2):277.

Singh, Balwant and R. J. Gilkes. 1991. "Phosphorus Sorption in Relation to Soil Properties for the Major Soil Types of South-Western Australia." *Soil Research* 29(5):603–18.

Smeck, Neil E. 1985. "Phosphorus Dynamics in Soils and Landscapes." *Geoderma* 36(3–4):185–99.

Smith, K. A., A. G. Chalmers, B. J. Chambers, and P. Christie. 1998. "Organic Manure Phosphorus Accumulation, Mobility and Management." *Soil Use and Management* 14(s4):154–59.

Song, C., Xiao-Zeng Han, and C. Tang. 2007. "Changes in Phosphorus Fractions, Sorption and Release in Udic Mollisols under Different Ecosystems." *Biology and fertility of Soils* 44(1):37–47.

Sparks, Donald L. 1998. *Soil Physical Chemistry*. 2nd ed. Boca Raton, FL: CRC press.

Sparks, Donald L. 2003. *Environmental Soil Chemistry*. 2nd ed. San Diego, CA: Elsevier. Retrieved November 7, 2015 (http://www.sciencedirect.com/science/article/pii/B9780126564464500153).

Staats, Kristin E., Yuji Arai, and Donald L. Sparks. 2004. "Alum Amendment Effects on Phosphorus Release and Distribution in Poultry Litter–amended Sandy Soils." *Journal of environmental quality* 33(5):1904–11.

Steffens, Diedrich, Thomas Leppin, Nora Luschin-Ebengreuth, Zhi Min Yang, and Sven Schubert. 2010. "Organic Soil Phosphorus Considerably Contributes to Plant Nutrition but Is Neglected by Routine Soil-Testing Methods." *Journal of Plant Nutrition and Soil Science* 173(5):765–71.

Stevenson, F. J. 1982. "Organic Phosphorus and Sulfur Compounds." *Humic chemistry: Genesis, composition, reactions. John Wiley & Sons, New York* 120–145.

Stewart, J. W. B. and A. N. Sharpley. 1987. "Controls on Dynamics of Soil and Fertilizer Phosphorus and Sulfur." *Soil fertility and organic matter as critical components of production system, S* 101–21.

Tembhare, Bhojraj. 1973. "Available and Inorganic Forms of Phosphorus in Selected Alfisols and Mollisols." Digital Repository@ Iowa State University, http://lib. dr. iastate. edu/.

Tiessen, H. and J. O. Moir. 1993. "Characterization of Available P by Sequential Extraction." Pp. 293–306 in *Soil sampling and methods of analysis*, vol. 7, edited by M. Carter and E. Gregorich. Boca Raton, FL: Lewis Publishers.

Tiessen, H., J. W. B. Stewart, and C. V. Cole. 1984. "Pathways of Phosphorus Transformations in Soils of Differing Pedogenesis." *Soil Science Society of America Journal* 48(4):853–58.

Torrent, J. 1987. "Rapid and Slow Phosphate Sorption by Mediterranean Soils: Effect of Iron Oxides." *Soil Science Society of America Journal* 51(1):78–82.

Tran, T. Sen, M. Giroux, J. Guilbeault, and P. Audesse. 1990. "Evaluation of Mehlich-III Extractant to Estimate the Available P in Quebec Soils." *Communications in Soil Science & Plant Analysis* 21(1–2):1–28.

Tucker, M. R. 1992. "Determination of Phosphorus by Mehlich 3 Extractant." *Reference Soil and Media Diagnostic procedure for the southern region of the United States. So. Coop. Series Bulletin* 374:9–12.

Turner, B. L., M. J. Paphazy, P. M. Haygarth, and I. D. Mckelvie. 2002. "Inositol Phosphates in the Environment." *Philosophical Transactions of the Royal Society: Biological Sciences* 357(1420):449–69. Retrieved (http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2001.0837).

Turner, Benjamin L., Barbara J. Cade-Menun, Leo M. Condron, and Susan Newman. 2005.

“Extraction of Soil Organic Phosphorus.” *Talanta* 66(2):294–306.

Turner, Benjamin L., Emmanuel Frossard, and Darren S. Baldwin. 2005. *Organic Phosphorus in the Environment*. 1st ed. Cambridge, MA: CABI.

Turner, Benjamin L., Mary A. Kay, and Dale T. Westermann. 2004. “Colloidal Phosphorus in Surface Runoff and Water Extracts from Semiarid Soils of the Western United States.” *Journal of environmental quality* 33(4):1464–72.

Uriyo, A. P. and A. Kesseba. 1973. “Phosphate Fractions in Some Tanzania Soils.” *Geoderma* 10(3):181–92.

Vance, Carroll P., Claudia Uhde-Stone, and Deborah L. Allan. 2003. “Phosphorus Acquisition and Use: Critical Adaptations by Plants for Securing a Nonrenewable Resource.” *New Phytologist* 157(3):423–47.

Vitousek, Peter M., Stephen Porder, Benjamin Z. Houlton, Oliver A. Chadwick, and Z. Houlton. 2010. “Terrestrial Phosphorus Limitation: Mechanisms, Implications, and Nitrogen-Phosphorus Interactions.” *Ecological Applications* 20(1):5–15.

Wang, Jun, Wen-Zhao Liu, Han-Feng Mu, and Ting-Hui Dang. 2010. “Inorganic Phosphorus Fractions and Phosphorus Availability in a Calcareous Soil Receiving 21-Year Superphosphate Application.” *Pedosphere* 20(3):304–10.

Watson, Maurice and Robert Mullen. 2007. “Understanding Soil Tests for Plant-Available Phosphorus.” *School Environ Natur Resour, The Ohio State University, Columbus-OH, Fact Sheet*.

Whalen, Joann K., Chi Chang, George W. Clayton, and Janna P. Carefoot. 2000. “Cattle Manure Amendments Can Increase the pH of Acid Soils.” *Soil Science Society of America Journal* 64(3):962–66.

White, R. E., and A. T. Ayoub. "Decomposition of plant residues of variable C/P ratio and the effect on soil phosphate availability." *Plant and Soil* 74.2 (1983): 163-173.

Wuenscher, R., H. Unterfrauner, R. Peticzka, and F. Zehetner. 2015. “A Comparison of 14 Soil Phosphorus Extraction Methods Applied to 50 Agricultural Soils from Central Europe.” *Plant Soil Environ.* 61(2):86–96. Retrieved (http://www.agriculturejournals.cz/publicFiles/144700.pdf).

Zhang, T. Q. and A. F. MacKenzie. 1997. “Changes of Soil Phosphorous Fractions under Long-Term Corn Monoculture.” *Soil Science Society of America Journal* 61(2):485–93.

Chapter 5: Summary and conclusion

5.1 Summary and conclusion

An overall goal of this study was to investigate the effects of variable extraction time and P species on STP using appropriate P colorimetric techniques and data interpretation.

The experiments in Chapter two were conducted to compare the detection limit and the sensitivity of four colorimetric methods. The methods designed by Murphy and Riley (1962) and He et al. (1998) use an excess of molybdate, which will complex with hydrolyzed PO_4^{3-} and affect absorbance readings. The Dick and Tabatabai (1977) and Asher (1980) methods were developed to include scavenger ions that complex with excess molybdate, thus eliminating the detection of hydrolyzed PO_4^{3-}. All methods were evaluated in ultrapure water, Bray P-1 STP extractant, and Mehlich III STP extractant and compared on the basis of similar methodologies (i.e. Murphy and Riley (1962) vs. He et al. (1998) and Dick and Tabatabai (1977) vs. Asher (1980)). Through linear regressions and statistical analyses of absorbance spectra, the sensitivity and optimal wavelengths (nm) for each method were found. It was concluded that the optimal wavelengths for the Murphy and Riley (1962) and He et al. (1998) methods is 882 nm, regardless of the background medium. Hypsochromic shift of absorbance spectra beyond 2 mg/L resulted in loss of linear correlation for both the Murphy and Riley (1962) and He et al. (1998) methods and the conclusion that they are best suited for use with samples containing < 2 mg/L PO_4^{3-}.

Variance in the absorbance spectra of Dick and Tabatabai (1977) based on background media resulted in optimal wavelengths of 700 nm in ultrapure water, 886 nm in Bray P-1 extractant, and 888 nm in Mehlich III extractant, while the Asher (1980) method was optimal at 888 nm in all background media. Trichloroacetic acid used in the Dick and Tabatabai (1977) procedure resulted in the inability to produce a colorless blank, a significant issue when attempting to analyze samples for DRP. As such, it was concluded that in forthcoming experiments, the Asher (1980) method would be used when possible to limit over-quantification of orthophosphate. The inclusion of scavenger ions decreased the sensitivity of DRP detection, with linear correlation for the Asher (1980) method being optimal at > 2 mg/L PO_4^{3-}. Overall, the study suggests or confirms the optimal wavelengths (nm) for each spectrophotometric method and indicates that methods with excess molybdate are best used with samples < 2 mg/L PO_4^{3-} and methods containing scavenger ions are best used with samples > 2 mg/L PO_4^{3-}.

Chapter three addresses the concern that acid hydrolysis of organic- or poly-P species might cause overestimation of the concentration of orthophosphate in acidic Mo-blue color forming media. As part of this, acid hydrolysis of polyphosphate (pyrophosphate and tripolyphosphate) and organic P (phytic acid) was evaluated in Bray P-1 and Mehlich III solutions over a period of 1 d. The Asher (1980) and He et al. (1998) colorimetric methods were used concurrently to evaluate PO_4^{3-} in samples, in case samples were < 2 mg/L PO_4^{3-}. Continued hydrolysis of pyrophosphate and tripolyphosphate over a period of 3 h was detected by the He et al. (1998) method in both extractants. Only the background PO_4^{3-} concentration from tripolyphosphate was detectable by the Asher (1980) method due to its higher limit of detection and inclusion of scavenger ions. Phytic acid did not seem to undergo hydrolysis within the STP extractants. The findings indicate that if hydrolysable polyphosphate compounds are present in soil samples they could lead to overestimation of orthophosphate during the STP extraction.

In Chapter four, the effects of P speciation and extraction time on the results of STP procedures were evaluated in manure- and fertilizer-amended Central Illinois agricultural soils. The manure-amended soils desorbed a much greater concentration of P_o than the fertilizer-amended soils and a slightly greater concentration of P_i and colloidal P. Results indicate that inclusion of P_o and colloidal P species in STP procedures when ICP-AES is used greatly increases the P concentration detected. Extraction time also played a major role in P concentration detected by the STP procedures. Both soil types exhibited a biphasic increase in P_i desorption occurring at a faster rate over ~2 h and a slower rate for the remainder. Organic P and colloidal P gradually increased over the entire time period. Mehlich III extracted greater concentrations of all P species, due to being more highly buffered than Bray P-1. If extraction time varies in soil testing labs, results of STP can be easily overestimated and alter fertilizer recommendations. In terms of build-up maintenance recommendations, such as those used in Illinois, a longer STP extraction time could place a soil into the “no fertilization needed category”, when if taken at the recommended extraction time the soil would be in the “maintenance required” category. This effect may actually be desirable, considering the widespread over-application of P fertilizer. Further research should be conducted about whether longer STP extraction times may actually correlate better to plant-available P.

Soil test phosphorus procedures are designed to identify “optimum” available-P concentrations required for plant growth. Kinetic limitation is often questioned whether STP is reliable to account for the plant P requirement over a long growing season. It was unclear how STP extractions are correlated to temporal-scale P availability for the high base saturation Illinois agricultural soils used in this study. Manure-amended soils released a greater concentration of P_i, P_o, and colloidal P than the fertilizer-amended soils, which was proportional to the trends observed in the STP values. Trends of P species release in 0.01 M $CaCl_2$ were similar to those observed in STP desorption, the only inconsistencies being that the fertilizer-amended soils released more P_o than P_i, and that colloidal P could not be detected. Short- and long-term end points of the long-term desorption experiment were correlated with STP values. In these soils, it is the case that soils higher in STP can reasonably be expected to release more P over a growing season.

The results of this study have several important implications regarding the use of STP extractants. Interpretation of STP results can be improved by employing the findings of the aforementioned studies. Optimal wavelengths for the Dick and Tabatabai (1977) and Asher (1980) colorimetric methods have been refined, taking into consideration the use of Bray P-1 and Mehlich III STP extractants as the background media. Optimal wavelengths for the Murphy and Riley (1962) and He et al. (1998) methods have been confirmed for use with STP extractants as the background medium. It was shown that hydrolysable P species can influence the concentration of DRP detected in STP extracts. It was also shown that organic and colloidal P species will increase the amount of DRP detected if methods such as ICP are used. Manure-amended soils appear to contain a greater concentration of P_i, P_o, and colloidal P than fertilizer-amended soils. Finally, it was shown that extraction time has a major impact on STP extracts. It is imperative that extraction times are strictly uniform, any deviance will impact results and make comparisons between results invalid.

In addition to our recommendation above, there are still many opportunities for future research and improvements to STP procedures. Calibrations are ideally carried out in experimental cultivation fields under conditions similar to where the STP will be put to use (Dahnke and Olson 1990). Soil critical test levels can be determined, displaying the point where correlation between STP values and crop yield increase flattens, making it obvious that P would no longer be the limiting nutrient (Black 1993). There is a need however for updated calibrations between STP and crop yields, as the ones currently in use by the Illinois agronomy handbook are “limited” (Fernández and Hoeft 2009). Soil properties have likely changed since the last set of calibrations were completed due to fertilizer and manure applications and land management strategies. In addition, calibrations should be done using soils of differing chemical properties and native P availability, so that correlations between STP values and % yields can be applied on a more localized basis.

Soil test P critical test levels are evaluated by multiple statistical methods, and often do not agree with one another, showing different correlation factors. It is important to choose a single correlation within a region and apply it from lab to lab, so that there is consistency in what the soil critical test level is (Mallarino and Blackmer 1992). STP values are further used to divide soils into several interpretation categories based on “P-supplying power of the soil.” P supplying power refers to the P buffering capacity of a soil, or the intensity with which it is able to replace soil solution P from labile P sources such as colloidal and organic P (McCollum 1991). P-buffering shows variability on a localized scale based on clay content and composition, pH, and amount of organic matter present. P supplying power in the state of Illinois is split up into the categories of low, medium, and high. Low, medium, and high regions of P supplying power cover large vague areas of the state, creating doubt that interpretations can be valid due to the fact that P-buffering characteristics are much more variable soil to soil than the broad regions proposed by the Illinois Agronomy Handbook (Fernández and Hoeft 2009). Due to these differences the recommended application rate of 9 lb P_2O_5 to increase STP by 1 lb should not be considered valid for all Illinois soils.

The gap between use of ICP-AES/OES and colorimetric techniques for STP needs to be addressed. ICP-AES/OES use yields an estimation of total P within an extract, but calibration for plant uptake and response is far less developed for this method than for colorimetric methods. If ICP use is to supplant colorimetric analysis as the primary method of evaluating P content of STP extracts, extensive studies should be conducted to correlate crop growth to the STP concentrations. A shift towards ICP may be ideal as available inorganic and organic P are quantified instead of solely inorganic and plants have been shown to utilize organic P via enzymatic activity.

Sampling to plow depth (surface 7 in) is an unrealistic illustration of the rooting depth of commonly grown crops. Corn and soybean have been shown to root to depths of ~5 and ~4 ft, respectively (Allmaras, Nelson, and Voorhees 1975). To more accurately assess available P, studies should be conducted that sample to the rooting depth of crops being grown in that region. Calibration to results of P extracted from these samples would allow for a greater understanding of crop needs.

Though STP is considered to be a more reliable test than counterparts for many other soil elements (Fernández and Hoeft 2009), there is still much to be desired. Environmental impacts of excess P such as hypoxia and eutrophication exhibit that sizable amounts of P are being lost from agricultural soils. STP measurements should account for both agronomic and environmental impacts, not one or the other. This can be accomplished by using an extraction methodology that identifies plant-available forms of P (orthophosphates) and labile P including organic and particulate P that becomes available to crops as plant-available P is depleted in the soil solution (Eliason, Lamb, and Rehm 2001). Agronomic field collections of samples for STP must also be examined, as the current methods for collection of STP samples has many inadequacies.

5.2 References

Allmaras, R. R., W. W. Nelson, and W. B. Voorhees. 1975. “Soybean and Corn Rooting in Southwestern Minnesota: II. Root Distributions and Related Water Inflow1.” *Soil Science Society of America Journal* 39(1):771–77. Retrieved (http://dx.doi.org/10.2136/sssaj1975.03615995003900040046x).

Asher, L. E. 1980. “An Automated Method for the Determination of Orthophosphate in the Presence of Labile Polyphosphates.” *Soil Science Society of America Journal* 44(1):173–75.

Black, C. A. (1993). *Soil Fertility Evaluation and Control*. Boca Raton, FL: CRC Press.

Dahnke, W. C. and R. A. Olson. 1990. “Soil Test Correlation, Calibration, and Recommendation.” *Soil Testing and Plant Analysis, 3rd ed. SSSA, Madison, WI* 45–71.

Dick, W. A. and M. A. Tabatabai. 1977. “Determination of Orthophosphate in Aqueous Solutions Containing Labile Organic and Inorganic Phosphorus Compounds.” *Journal of Environmental Quality* 6(1):82–85.

Eliason, R., J. L. Lamb, and G. W. Rehm. 2001. “Colorimetric and ICP Measurement of P Extracted by the Mehlich-3 Procedure.” *Agron. Abstracts [CD-ROM], ASA, CSSA, and SSSA, Madison, WI*.

Fernández, Fg and Rg Hoeft. 2009. “Managing Soil pH and Crop Nutrients.” *… agronomy handbook. 24th ed. Univ. of …* 91–112. Retrieved (http://www.ebooksmagz.com/pdf/managing-soil-ph-and-crop-nutrients-129919.pdf).

He, Z. L., V. C. Baligar, K. D. Ritchey, and D. C. Martens. 1998. “Determination of Soluble Phosphorus in the Presence of Organic Ligands or Fluoride.” *Soil Science Society of America Journal* 62(6):1538–41.

Mallarino, A. P. and A. M. Blackmer. 1992. “Comparison of Methods for Determining Critical Concentrations of Soil Test Phosphorus for Corn.” *Agronomy Journal* 84(5):850–56.

McCollum, R. E. 1991. “Buildup and Decline in Soil Phosphorus: 30-Year Trends on a Typic Umprabuult.” *Agronomy Journal* 83(1):77–85.

Murphy, James and J. P. Riley. 1962. “A Modified Single Solution Method for the Determination of Phosphate in Natural Waters.” *Analytica chimica acta* 27:31–36.