Phosphorus Speciation in Dairy Manure-Amended Calcareous Soils

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Authorization to Submit Thesis

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Abstract

Growth in the dairy industry in Idaho has created a surplus of manure that may be a useful source of fertilizer for crops. The purpose of this project was to determine the effects of manure amendments on phosphorus (P) speciation in the calcareous soils of southern Idaho. Sequential extraction, nuclear magnetic resonance (^{31}P-NMR) spectroscopy, and x-ray absorbance near-edge spectroscopy (XANES) analysis were used to analyze changes in the soil caused from manure amendments over a three-year period. Results show that annual manure amendments of 17 Mg/ha and 52 Mg/ha (dry weight) over three years will cause an increase in total soil P concentration. XANES linear combination fitting results show that all soil samples collected were comprised of majority apatite (54-74%), along with adsorbed phosphorus (25-35%) and organic phosphorus. NMR results showed that organic P in the soil consisted of primarily monoesters and diesters. Adsorption isotherms were also prepared to examine the impact of manure on P adsorption to calcite. Results showed that percent P adsorbed dropped significantly in the presence of manure. XANES analysis also found distinct differences in the P minerals formed in the presence of manure, suggesting manure affects P solubility and mineralization. Increases in total soil phosphorus, as well as overall solubility, may affect phosphorus loading to nearby surface waters if not carefully monitored. The results also show the benefits of using multiple analytical methods to provide an in-depth profile of the phosphorus species present.

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Chapter 1: The Effects of Manure Amendments on Soil Phosphorus and its Relationship to Water Quality

1.1 Introduction

1.1.1 Background

Phosphorus (P) is a key nutrient for agricultural production, and is regularly added to soils in a variety of forms to improve crop yields. However, runoff of P to aquatic systems via surface erosion is a major contributor to eutrophication of freshwater systems (Carpenter et al., 1998; McDowell et al. 2004). Thus, monitoring and regulating the release of P into surface waters from watersheds is an important component of water quality protection. Since land supplied P is a non-point source to surface waters, it is critical that the processes surrounding P uptake and storage within the soil be understood to allow prevention of P loading to surface waters.

In southern Idaho, dairy operations are a rapidly growing industry, and the manure waste produced is a major source of P to nearby soils as well as surface waters (Manning, 2014). In 2015, about 587,000 dairy cows were in the state of Idaho (USDA, 2015), with an estimated 75% residing within the Snake River watershed (Carey et al., 2011). This rapid expansion of dairy production necessitates increased research on the potential regional impacts to better understand the industry’s effect on the local environment. Fully comprehending the relationship between CAFOs, agricultural production, and water quality would help refine best management practices within the Snake River watershed. Improving policies regionally will help limit surface water degradation from P loading caused by repetitive or excessive manure applications.

To define best management practices for limiting P loading from the Snake River watershed, a complete understanding of P build-up and speciation within the soil profile is needed. Understanding the relationship between long-term dairy manure amendments, soil properties, and soil P speciation will provide the necessary insight into best management practices to reduce P release into nearby surface waters.

In nature, P occurs in a variety of organic and inorganic chemical forms. Plants can absorb P through their roots as orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) (Shen et al., 2011). Other P forms are not directly accessible to plants, and are retained in the soils as a reserve pool that may be transformed to plant available P at a later time, or transported out of the soil (Shen et al., 2011). Some P species are more easily converted to usable forms, and are considered bioavailable or labile (Audette et al., 2016; Li and Brett, 2013). For example, many organic P species can be readily converted into soluble orthophosphate available for plant uptake, while calcium-P minerals generally have low solubility and are considered to be an unavailable soil P species (Audette et al., 2016; Li and Brett, 2013). P availability is in constant flux within the soil profile, becoming bioavailable through weathering and mineralization, and becoming unavailable by adsorption, precipitation and biological fixation (Shen et al., 2011). However, research indicates that the majority of soil P exists in phases that are slowly available or unavailable (Liu et al., 2015).

1.1.2 Agricultural Land Use and Eutrophication

Biogeochemical movement of P in the environment is a key nutrient cycle, and extensive research has been conducted to understand it. Of particular interest is the role soil P plays in freshwater eutrophication. It is well established that inputs of P to aquatic systems are a leading cause of eutrophication (Bechmann et al, 2005; Carpenter et al., 1998; Dodds et al., 2009; Sharpley et al., 1995). Point sources of these nutrients have been heavily regulated over the years, and non-point pollution is now considered the primary source of nitrogen and P loading in freshwater systems (Carpenter, 2005; Carpenter et al., 1998). In the past, agricultural inputs have been found to account for 57% of the total non-point contributions to waterways in the United States (Carpenter et al., 1998). More recently, the United States Geological Survey (USGS) determined that between 1994 and 2004, animal manure alone was responsible for 40% of the P inputs to agricultural water basins (Dubrovsky et al., 2010).

Type and intensity of land use can affect the rate of P loading in waterways. Manures are commonly applied at rates that meet the nitrogen requirements for crops, which results in over-application of P; up to six times as much P as needed for crop production may be applied if manure is applied at crop N rates (Carey et al., 2011; Leytem et al., 2011). Continuous applications of fertilizers and manures has led to a buildup of P in many agricultural soils, well past the point of plant growth needs (McDowell and Sharpley, 2001). These high concentrations of P in the environment lead to increased P transport into nearby surface waters via runoff, erosion, and leaching.

Increased production from dairies has also been linked to a corresponding increase in the amount of P found in the surrounding streams (Palmer-Felgate et al., 2009). For example, Smith et al. (2013) observed that increased rates of milk production in Victoria, Australia caused increasing concentrations of P in streams adjacent to the farms. Research has found a clear correlation between rates of manure production and the total P loaded to the surrounding aquatic systems (Carpenter et al., 1998; Sharpley et al., 2004). A southern Norway study found a reduction in manure application resulted in a decline in the total P concentration in the neighboring stream (Bechmann et al., 2005).

Recent research has also found that cessation of fertilizer application to soils with high levels of accumulated P in the soil profile did not lead to a decline in crop yields. Thus, persistent over-application of P, a common agricultural practice, may create a reserve pool of P in the soils leading to long lasting problems with P loading to surface waters (Liu et al., 2015). To remedy this, P fertilizer application rates need to change to ensure adequate nutrient supplementation to maximize crop yields without developing environmentally harmful buildup of P in the soil. Withers et al. (2014) argued that a revision to the current fertilizer application rates and methods (such as applying to the entire field rather than spot application) to minimize environmental degradation and reduce costs to the farmer is needed. Mined rock phosphate is a finite resource, and continuous over-consumption is driving an increase in prices (Withers et al., 2014). More efficient agricultural practices could not only lead to economic gains to crop producers, but also result in a decline in environmental degradation.

1.1.3 Phosphorus in the Water Column

Phosphorus in surface waters is particularly troublesome to regulate. Evidence shows that once P enters into a freshwater system, it can remain for extensive periods of time as bed sediments. Bjorneberg et al. (2015) found a strong correlation between a reduction in the total suspended solids in irrigation ponds and a decrease in the amount of total P found in the water. This demonstrates the importance of soil-bound P contributing to eutrophication. Jarvie et al. (2010) found evidence of in-stream uptake of soluble reactive P, and determined the most likely cause was the sorption of P to streambed sediments. While surface adsorption to soil particles can serve as a temporary P sink, it can become a long-term source of P within the water column via chemical reactions and species transformation (Carpenter, 2005; Jarvie et al., 2010). Carpenter (2005) also found that P is recycled from lake sediments, extending the period of eutrophication and delaying the reduction in measured P despite no additional P inputs to the system. A 2008 study found that the majority of P inputs to rivers are in particulate form. A small portion of the particulate P is initially bioavailable, and the remaining P may be released over time via desorption and dissolution processes (James and Larson, 2008). The release rate depends on the speciation of the particulate P in the sediments. P inputs to freshwater systems deposited today could impact water quality long into the future.

1.1.4 Phosphorus Speciation in Soils

Manure application to soils leads to an increase in total P present in the soil (McDowell and Sharpley, 2001; Ojekami et al., 2011; Somenahally et al., 2009). The types of P added can vary, depending on the type of manure being used and how it is processed (Hansen et al., 2004; Leytem and Westermann, 2005; Turner and Leytem, 2004). For example, He et al. (2007) found that fresh dairy manure consisted of predominately inorganic P (62% orthophosphate), with the remainder of P present in various organic forms (monoesters and diesters). Leytem and Westermann (2005) found liquid dairy manure to be 90% inorganic P. A wide range of total P concentrations in different animal manures is observed; cattle manure typically contains 4,943 mg/kg of P compared to 15,953 mg/kg present in poultry litter (Turner and Leytem, 2004). Once introduced to the soil, the manure P undergoes a number of chemical transformations. Factors such as climate, topography, and the soil's chemical and physical properties affect the soil P species that develop (McDowell and Sharpley, 2001; Ojekami et al., 2011; Somenahally et al., 2009).

Manure amendments also affect P cycling by supplying increased carbon to the soils. An increased carbon to P ratio in soils has been linked to a greater pool of soluble P, expected to be caused by increased microbial activity (Leytem and Westermann, 2005; Tarkalson and Leytem, 2009). The presence of dissolved organic carbon in a system has been found to have an inhibitory effect on calcium and P precipitation, potentially affecting Ca-P mineral formation (Sindelar et al., 2015). Research has also found that greater amounts of negatively charged organic acids increase the rate of P desorption in soils (Jiao et al., 2007). Sharpley et al. (2004) found that soils amended with manure had an increased amount of total P solubility, comparable to crystalline octacalcium phosphate (OCP) and tricalcium phosphate (TCP), while untreated soils had a P solubility comparable to hydroxyapatite (HAP), suggesting an inhibitory effect on crystallization of P minerals. Sims et al., (1998) also observed increased rates of P leaching through the soil profile in soils with a high composition of organic matter. Thus, adding organic matter in the form of manure or compost may further affect P availability in soils, as well as eutrophication rates in freshwater systems.

The long-term additions of organic matter can also affect the pH of soils, further altering P speciation (Sharpley et al., 2004; Whalen et al., 2000). Long-term manure application can raise pH in acidic soils, but has been found to lower pH in highly alkaline soils (Chang et al., 1990; Whalen et al., 2000). Rates of P adsorption were found to increase with decreasing pH when sorbing to ferrihydrite, a trend commonly observed for most oxides (Arai and Sparks, 2007; Goldberg, 2010). In contrast, Sø et al. (2011) found that lowering pH reduces the rate of phosphate sorption on calcite. Sindelar et al. (2015) found greater amounts of calcium-P precipitated in a system with pH 9.5 compared to a pH of 8.5. Thus, it can be concluded that pH is an important factor in P adsorption processes, and indicates that over many years of amendments, it may be possible to alter the pH conditions of a soil.

Soil physico-chemical properties play a significant role in P speciation. For example, in the eastern United States, soils are typically acidic to neutral, and P availability is regulated by aluminum and iron oxides in the soil, which bind P and remove it from the labile pool (Carey et al., 2011; Leytem and Westermann, 2005; Shen et al., 2011). When P is added to alkaline soils, common in arid regions of the western United States, calcium carbonate minerals interact with P via surface adsorption or mineral precipitation, removing the labile P from the plant-available pool (Leytem and Mikkelsen, 2005). As calcium-P minerals age, they are converted from more readily soluble minerals such as dicalcium phosphate dihydrate (DCPD), to more crystalline, stable minerals such as OCP, and eventually convert to HAP, which is the most thermodynamically stable calcium-P mineral (Alvarez et al., 2004; Arai and Sparks, 2007; Lindsay, 1979). These transformations further reduce the P found in soil solution and lower plant availability, which then requires additional fertilizer amendments to overcome the soil calcium sink (Carey et al., 2011). If calcium carbonates are present in the soil, it is estimated that crop producers have to apply an additional 0.011 Mg/ha of fertilizer or manure for every percent increase (Leytem and Mikkelsen, 2005).

1.1.5 Phosphorus Research and Regulation in Southern Idaho

In the Snake River Watershed, soils are calcareous, and soil pH is typically greater than 7.5. Regional growth of the dairy industry has created an ample supply of manure that may be used in place of chemical fertilizers on crops (Carey et al., 2011). Regional concerns over P loading to surface waters have led to the development of guidelines and limits to minimize the environmental impacts of agriculture on water quality. The Idaho Department of Environmental Quality (DEQ) has set a total maximum daily load (TMDL) of 0.075 mg L^{-1} for P in impaired segments of the Snake River (Buhidar, 2005). To help meet this guideline, the Natural Resources Conservation Service (NRCS) set a maximum concentration of 40 mg kg^{-1} Olsen extractable P in the first foot of soils, (Carey et al., 2011; Hines et al., 2012). Manure and fertilizer application rates to soils with Olsen-P concentrations above 40 mg kg^{-1} are restricted, and only the amount that will be utilized by the crop may be applied (Hines et al., 2012). To help meet these environmental regulations without impairing crop production, extensive research has been conducted in the region to understand P cycling in the alkaline soils of southern Idaho.

Irrigation return flows within the Snake River watershed are the primary source of P loading to surface waters, in both sediment-bound and dissolved P form (Bjorneberg et al., 2015). Installation of settling ponds has reduced total P concentrations in return flows by an average of 27%, mainly by allowing suspended sediments to settle out of the water column (Bjorneberg et al., 2015). Further research on water quality in the region observed that treating irrigation water with alum removed as much as 95% of the soluble P present through flocculation, although extensive use of this treatment is not prevalent due to operational costs (Leytem and Bjorneberg, 2005).

The interaction between calcium carbonates and P in alkaline soils is a significant factor in P availability in the region (Leytem and Mikkelsen, 2005). However, the presence of oxides, clay surfaces, and organically complexed metals can also play a significant role in the rate of P adsorption in the region (Leytem and Westermann, 2003). Research on soils in the western United States found that the majority of soil P is inorganic (49 – 90%), while the organic P that is present is comprised mainly of orthophosphate monoesters (Turner et al., 2003). Turner et al., (2003) found that alkaline soils in the western United States have a lower concentration of organic P compared to cultivated soils in other regions of North America (11 – 127 mg kg^{-1} compared to 122 – 212 mg kg^{-1}), possibly due to differences in mean temperature and precipitation rates.

Previous research has investigated the effect of manure amendments on the calcareous soils in southern Idaho. Studies have found that compared to manure amendments, chemical fertilizers (including ammonium phosphate, mono ammonium phosphate, and mono calcium phosphate) produced the greatest proportional increase in soluble P (Leytem et al., 2011; Leytem et al., 2005; Leytem and Westermann, 2005). Calcareous soils found in semiarid regions typically have low rates of organic carbon, and the increased supply of carbon provided by the manure positively impacts the rate of P assimilation by microbes (Leytem et al., 2005). However, the increased organic matter may increase the rate of P leaching downward through the soil profile. Soil column studies using calcareous soil found a greater rate of downward movement of P in columns treated with dairy manure compared to chemical fertilizer (Tarkalson and Leytem, 2009). Similar results were found in a field study, where greater concentrations of subsurface P were found in soils amended with water from dairy lagoons (Hansen et al., 2004). Tarkalson et al. (2009) theorized that increased carbon from manure coated or otherwise interfered with adsorption sites in the surface horizon, allowing for increased movement through the soil. Hansen et al. (2004) suggested that saturation of P sorption sites might cause increased downward movement of P, allowing for accumulation in the subsurface.

1.2 Research Goals and Objectives

The complexity of P movement and cycling within the soil profile is affected by a number of factors. While manure can be a cost effective method of supplying key plant nutrients to agricultural fields, changes in the soil characteristics may affect long-term P availability and concentrations in the soil, which will impact crop production as well as water quality in southern Idaho.

The goal of this project was to measure the initial soil speciation of P in a long-term manure amendment study in southern Idaho. The objectives of the research presented in this thesis were to, 1) quantify the changes in soil P speciation caused by manure amendments using sequential chemical extraction, 2) use advanced analysis including ^{31}P-NMR and XANES spectroscopy to determine the differences in P speciation in manure-amended soils, and 3) measure how soluble organic carbon from manure affects P adsorption on calcite. By studying the initial conditions in the soil profile, future changes to the soil caused by long-term manure amendments will be better understood. This information will help inform future management guidelines for the control of P to freshwater systems in southern Idaho.

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Chapter 2: Phosphorus Speciation in Calcareous Soils Following Annual Dairy Manure Amendments

Abstract

Applying manure to crops in place of chemical fertilizer may alter phosphorus (P) concentrations and speciation in the soil profile. The objective of this research was to determine how annual manure amendments affect P concentrations and speciation within the soil profile. Pre-plant soil samples were collected in early spring of 2013, 2014, and 2015 from an experimental field in Kimberly, Idaho to analyze differences in P composition following annual applications of 17 Mg ha^{-1} manure, 52 Mg ha^{-1} manure, mono-ammonium phosphate fertilizer, and control plots receiving no P source applications in the fall of 2012, 2013, and 2014. Sequential chemical extraction, P K-edge X-ray adsorption near edge structure (XANES) spectroscopy, nuclear magnetic resonance (^{31}P-NMR) spectroscopy, and microprobe element mapping were all used to provide a comprehensive analysis of changes to P in the soils. Results showed significant increases in the total P concentration in the manure-amended soils (284 mg kg^{-1} additional total P in 52 Mg ha^{-1} after three years, 0-30 cm depth compared to control plot). Extractable P via Olsen bicarbonate extraction were greatest in the high manure amendment rate plots. The majority of extractable P in the sequential extraction procedure was removed in extract C, suggesting that P associated with Ca minerals is the predominant form in the soil. XANES results showed that amongst all soil amendment types, P species were similar, containing 54 – 74% hydroxyapatite, 25 – 35% adsorbed P, and 0 - 19% organic P that was identified using ^{31}P-NMR as predominantly phytic acid. Despite the poorly soluble Ca-P species being a predominant phase in the manure-amended soils, plant-available P tests predicted increases in available P with

repeated application and application rate. The P speciation information discovered in this study provide a baseline to compare how long-term changes affect P availability, and will be useful for designing long-term management scenarios in manure-amended soils.

2.1 Introduction

Since 1993, the dairy industry in Idaho has tripled in size, becoming the third largest dairy producer in the United States (Idaho Dairymen's Association, 2016; Manning, 2014). The majority of dairy production occurs within the Snake River Watershed (Watson et al., 2014). In 2015, approximately 587,000 head of dairy cows existed in the state of Idaho (NASS USDA, 2015). The NRCS estimates that the typical dairy cow produces an average of 36 kg of manure per day (wet weight), resulting in an estimated 7.7 billion kilograms of wet manure produced in 2015 in the state of Idaho alone from dairy cows ("Animal Manure Management | NRCS New Jersey," 1995). In an attempt to recycle nutrients, crop producers are increasingly using manure as a source of nitrogen (N) and phosphorus (P) fertilizer on their fields in place of chemical fertilizers (Leytem et al., 2011). This shift in regional agricultural practices may alter P application rates to the soil and P species within the soil, with long-term consequences on P accumulation and bioavailability within the soil profile, as well as nutrient loading to the regional aquatic systems.

The calcareous properties of southern Idaho soils play an important role in controlling the fate of P in the soil because high carbonate concentrations significantly impact the availability of P for crop uptake (Carreira et al., 2006). For example, when P is added to alkaline soils, calcium carbonate minerals fix P via surface adsorption or mineral precipitation, removing it from the plant available pool (Leytem and Mikkelsen, 2005). This is distinctly different from acidic soils, where P availability is largely controlled by the presence of aluminum and iron oxides, where P adsorbs as inner-sphere complexes, especially in low pH soils (Carey et al., 2011; Leytem and Westermann, 2005; Shen et al., 2011). The formation of calcium-phosphate (Ca-P) minerals decreases P availability, and often requires over-application of fertilizers to overcome the soil-calcium binding (Westermann, 1992). It is estimated that crop producers have to apply an additional 0.011 Mg ha^{-1} (10 lb acre^{-1}) of fertilizer or manure for every percent increase in calcium carbonate mineral present in the soil (Leytem and Mikkelsen, 2005).

Continuous manure application to soils has the potential to alter soil P species, both by adjusting the types of P initially added to the soil, as well as altering the physical and chemical properties of the soil over time. Different manure types and processing methods affect the types and concentrations of P that occur in soils (Hansen et al., 2004; Leytem and Westermann, 2005; Turner and Leytem, 2004). For example, He et al., (2007) observed that fresh dairy manure consisted of mainly inorganic P (62% orthophosphate), with the remainder in various organic forms (monoesters and diesters). Comparatively, Leytem and Westermann (2005) found liquid dairy manure to be 90% inorganic P.

Compared to commercial fertilizer applications, manure amendments also supply increased carbon to the soils. Research has found that an increased carbon to P ratio results in greater amounts of soluble P, possibly due to increased microbial activity (Leytem and Westermann, 2005; Tarkalson and Leytem, 2009). Other research has found that the increased presence of negatively charged organic acids also contributes to increases in the rate of P desorption in soils (Jiao et al., 2007), likely by anion exchange. Sims et al. (1998) observed a link between increased soil organic matter and rates of P leaching through the soil profile, which would increase P loading rates to freshwater systems.

Long-term manure additions may also influence soil pH, which can affect P speciation (Alvarez et al., 2004; Sharpley et al., 2004). For example, manure application can raise pH in acidic soils, but lower pH in highly alkaline soils (Chang et al., 1990; Whalen et al., 2000). However, in calcareous soils pH was lowered after over a decade of annual manure amendments, suggesting a long-term, cumulative effect on soil conditions (Chang et al., 1990). These shifts in soil pH affect mobility and solubility of P in the soil profile by impacting Ca-P mineral precipitation as well as adsorption (McDowell and Sharpley, 2003; Sharpley et al., 2004). Past studies, using both sequential chemical extraction procedures and XANES analysis, have found soils amended with manure lack crystalline hydroxyapatite (HAP), considered the most stable Ca-P mineral, instead finding the Ca-P minerals tricalcium phosphate (TCP) and octacalcium phosphate (OCP), which have greater solubility than HAP (Audette et al., 2016; Sato et al., 2005).

Measurement of soil P speciation requires both macroscopic extraction methods and molecular methods. Solution nuclear magnetic resonance (^{31}P-NMR) spectroscopy is one of the most commonly used methods for determining soil P composition (Abdi et al., 2014; Cade-Menun and Liu, 2013). ^{31}P-NMR spectroscopy is capable of identifying molecular species of P in soils, and is especially suited for characterizing organic P species (Turner et al., 2003). However, there are challenges in the application of NMR analysis of soils since some orthophosphate diesters may degrade when exposed to the extraction solution, and overlapping peak positions can prevent identification of certain forms. However, with careful data processing these side effects can be minimized (Cade-Menun and Liu, 2013; Hansen et al., 2004; He et al., 2007; Liu et al., 2015).

Although ^{31}P-NMR spectroscopy is a powerful method for analyzing P species in soils, inorganic solid phase species are not directly observed. In soils the predominant P species is orthophosphate, which can be either adsorbed onto mineral and clay surfaces, or precipitated as minerals such as Ca-phosphate minerals (e.g., hydroxyapatite) or other metal-phosphate minerals, e.g, Fe- or Al-phosphate minerals. To detect the species of P in soils requires methods capable of directly probing P speciation, such as X-ray spectroscopy.

XANES analysis provides insight not offered by sequential extraction methods (Liu et al., 2013; Negassa et al., 2010). Phosphorus K-edge X-ray absorption near-edge structure (XANES) spectroscopy provides detailed information about P speciation at the molecular level (Eriksson et al., 2015a; Liu et al., 2013; Peak et al., 2012). Previous studies have found that XANES analysis can distinguish between precipitated and adsorbed P stages, and that each species of P has its own unique signature, allowing for accurate identification of various P species present in soils (Ajiboye et al., 2007). Ajiboye et al. (2007) observed that the different P species in standards of known mixtures (25% hydroxyapatite; 75% variscite) could be quantitatively measured to within 17% accuracy using linear combination fitting (LCF) of the P K-edge XANES. In a study comparing soils amended with biosolids or chemical fertilizers, sequential fractionation of P in the soils found that calcium phosphates were present. Using XANES analysis, the specific Ca-P minerals present in the soils were discovered to be poorly crystalline dicalcium phosphate (DCP) minerals in the biosolids and apatite in the fertilizer-amended soils (Kar et al., 2011). The increased information provided by XANES showed the affects of different soil amendments on P speciation.

An advantage of P XANES is that there are no alterations to the samples via the introduction of chemical solutions (as with sequential extractions). Used together with other methods, XANES analysis greatly improves the level of speciation information in soils (Ingall et al., 2011). XANES has also been successfully used in conjunction with sequential extractions as a way to confirm the general conclusions drawn regarding the forms of P extracted at each phase of the sequential extraction (Kruse et al. 2010). Using linear combination fitting (LCF) from XANES spectra, Kruse et al. (2010) found a clear, though not exact, correlation between the acid treatment in a fractionation procedure, and the proportion of Ca-P minerals measured in the XANES spectrum.

XANES spectroscopy is generally non-destructive to the sample (Beauchemin et al., 2003; Liu et al., 2015), although some radiation damage is possible on high flux beamlines. Although P XANES is a powerful method for speciation analysis, it is limited in detecting exact species of organic P in soils (Prietzel et al., 2013). Thus, utilizing XANES spectroscopy in conjunction with chemical extractions and NMR spectroscopy can provide a more complete picture of the P speciation that occurs within the soil profile, and the changes occurring as a result of manure amendments.

The goal of this project is to investigate how manure amendments affect P speciation and availability in a manure amendment study on a calcareous soil. The experiment began in 2012, and is scheduled to continue as a long-term experiment. Our objectives were to, 1) quantify the annual changes in total P from manure amendments using sequential chemical extraction methods, and 2) use ^{31}P-NMR and XANES spectroscopy to determine the P species in the manure-amended soil. The focus of this project was to capture the initial soil P speciation and any early observable changes taking place to provide baseline information for future comparison.

2.2 Materials and Methods

2.2.1 Study Design

The soil samples analyzed in this study were collected from the experimental fields at the United States Department of Agriculture Agricultural Research Service (USDA-ARS) Northwest Irrigation and Soils Research Laboratory (NWISRL) station located in Kimberly, Idaho (42°33’N; 114°21’W). The soil is Portneuf silt loam, a coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid (USDA NRCS, 2016). The site receives an average of 203.7 mm rainfall annually, with a mean air temperature of 7.7°C. The experimental plots (12 m x 18 m) were designed as a randomized block design for a total of 8 treatments, with each treatment having four replications (Figure 2.1). This study analyzed the plots receiving 17 Mg ha^{-1} and 52 Mg ha^{-1} (dry weight) annual manure treatments, annual chemical fertilizer treatments, and the control plots.

The Magic Valley Compost Facility, located in Jerome, Idaho, provided non-composted dairy manure for the plot amendments. Manure P loading rates are listed in Table 2.1. Total P supplied by the manure, which was applied to the soil, was determined using nitric/perchloric acid digestion method (Gavlak et al., 2005), and analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Perkin-Elmer Optima 7300 DV). The rates of chemical fertilizer additions varied annually according to crop needs, except for the control plot, which did not receive any fertilizer (Table 2.1).

The experiment had a yearly crop rotation of wheat (2013), potato (2014), and barley (2015). The samples were collected over three years (2013–2015) in March, before crop planting. Soil samples were collected for each plot by Dr. Amber Moore at 0-30 and 30-60 cm depths, with 10 samples per plot collected and composited for each depth to create a representative sample. Thus, 16 soil samples were collected from 0-30 cm, and another 16 samples were collected from 30-60 cm depth. Samples were air dried before P analysis.

Table 2.1 – Annual P supplied by the manure applied to plots at the experimental field at the USDA-ARS, NWISRL station in Kimberly, Idaho.

Plot Type	Year	Crop	Annual P Added	Cumulative P Added
			Mg ha^{-1}	
Fertilizer	2013	Wheat	0.040	0.040
	2014	Potato	0.089	0.129
	2015	Barley	0.020	0.149
17 Mg/ha	2013	Wheat	0.095	0.095
	2014	Potato	0.141	0.236
	2015	Barley	0.097	0.333
52 Mg/ha	2013	Wheat	0.286	0.286
	2014	Potato	0.424	0.710
	2015	Barley	0.294	1.004

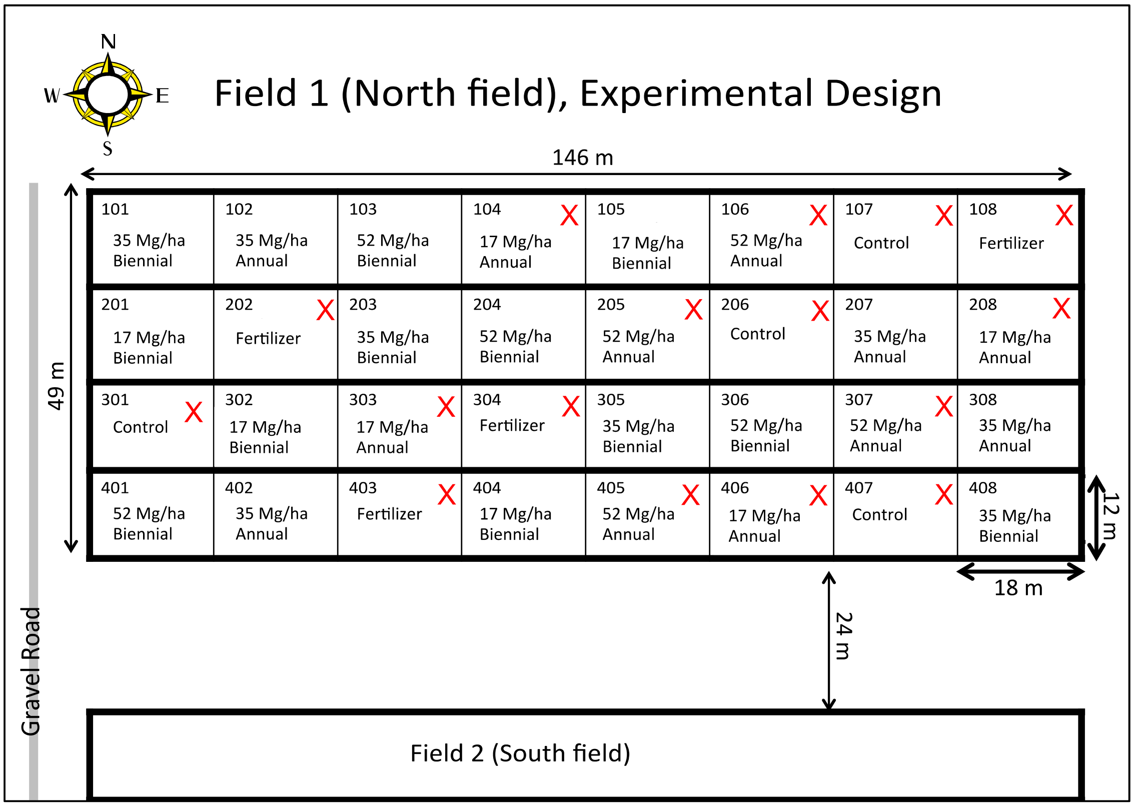

Figure 2.1 - Experimental field setup and rate of amendments (plots marked with "X" denote plots being tested in this project) (Kimberly, ID; USDA-ARS, NWISRL). Rates describe dry weight of manure added to the plot, and whether applied annually or biennially, from 2013 - 2015.

2.2.2 Sequential Extraction

A modified extraction procedure was developed from the "P Fractionation" method described by Pierzynski (2000) to extract P from calcareous soils. The extraction targets operationally defined fractions as follows: Extract A: labile P (readily plant available or leachable P); Extract B: Fe- and Al-bound P; Extract C: Ca-bound P. Samples were tested in duplicate, with at least one sample run in triplicate in each batch of extractions. A standard soil was analyzed along with all extractions as a quality control process to test accuracy of the results (Appendix Table A.1).

Soil samples weighing 0.5 g were placed in 50 mL centrifuge tubes, mixed with 25 mL of 0.1 M NaOH + 1 M NaCl solution (Extract A), and placed on the end-over-end shaker for 17 hours. The samples were then centrifuged (IEC Centra-8; International Equipment Company; Needham Heights, Massachusetts) at approximately 1000 x g for 15 minutes, and the solution decanted. Between all three extractions, the soil samples were washed with 25 mL of 0.1 M NaCl solution to remove entrained solution from the previous extractant. The salt wash and extractant solution were combined and filtered through 0.2 μm filter paper (Merck Millipore, Ltd.; Cork, Ireland). Extract B was 20 mL 0.3 M sodium citrate, 2.5 mL 1 M $NaHCO_3$, and 0.5 g sodium dithionate added to the soil sample, mixed thoroughly, and placed in a hot water bath for 15 minutes. Extract C was 25 mL 0.5 M HCl solution added to the soil samples and shaken for 1 hour. The final filtered supernatants were diluted and analyzed for P concentration using ICP-AES (iCAP 6000 Series, Thermo Scientific; Waltham, Massachusetts).

2.2.3 Element Mapping by Electron Microprobe

Microprobe images were collected to investigate P-mineral associations within the soil. Four soil samples (52 Mg ha^{-1} annual manure application and the control plots for both 2013 and 2014) were mapped using the JEOL 8500F field emission electron microprobe located at Washington State University geo-analytical laboratory in Pullman, Washington. The soil samples were saturated with epoxy and vacuum dried, then polished with 0.05 μm polishing solution (Buehler; Lake Bluff, Illinois). Samples were carbon coated. Element maps were collected using wavelength dispersive spectrometers. The elements Si, Al, Fe, P, and Ca were mapped at three different locations within each sample, resulting in 12 total maps. Map images were processed with Image J software (NIH, v. 1.48) to convert them to

500 µm x 500 µm intensity matrices, where each cell represents element intensity at a pixel (0.2 µm x 0.2 µm).

2.2.4 ^{31}P-NMR Spectroscopy

Speciation of molecular P in soils was analyzed using nuclear magnetic resonance (^{31}P NMR) spectroscopy. Samples from plots 104 (17 Mg ha^{-1}), 106 (52 Mg ha^{-1}), 107 (Control), and 108 (Fertilizer) (0-30 cm depth) for 2013 and 2014 were extracted using the methods described in Cade-Menun et al. (2002). Two grams of each soil sample were placed in 50 mL centrifuge tubes, and 25 mL of 0.25 M NaOH + 0.05 M Na_2EDTA solution was added. The samples were placed on an end-over-end shaker for four hours, and then centrifuged for 40 minutes at 1000 x g. One milliliter of extractant was decanted from each sample, filtered through 0.2 µm paper, diluted 1:10 with deionized water, and analyzed by ICP-AES for total P concentration (Appendix Table A.2). The remaining solution for each sample was freeze-dried and sent to the Stanford Magnetic Resonance Laboratory (Palo Alto, California) for NMR analysis. Dr. Barbara Cade-Menun at Agriculture and Agri-Food Canada conducted the NMR analysis. The samples were mixed with 0.4 mL of 10 M NaOH and 2.6 mL D_2O, and allowed to sit for 30 minutes. Samples were then centrifuged at 1500 x g and the supernatants were transferred to NMR tubes for analysis.

NMR spectra were obtained at 202.45 MHz, using a Varian INOVA 500 MHz spectrometer equipped with a 10-mm broadband probe. Spectra were analyzed according to the methods outlined in Cade-Menun et al. (2002).

2.2.5 Phosphorus K-Edge XANES Analysis

Synchrotron based X-ray absorption near edge structure (XANES) spectroscopy was used to determine the P species present in the soil. XANES spectra from three manure samples were also collected. The manure samples were taken from the 2012, 2013, and 2014 manures applied to the long-term study sites.

2.2.5.1 XANES Data Collection

XANES spectra for soil samples of the four amendment types at both depths (0-30 and 30-60 cm) were collected. All soil samples were sieved to <60 μm before being brought to the beamline for analysis. P K-edge XANES spectra of the soil samples and P standards were collected at the Soft X-ray Micro-characterization Beamline (SXRMB) at the Canadian Light Source (CLS), Saskatoon, Canada. Dr. Derek Peak at the University of Saskatchewan assisted with data collection. The beamline uses an InSb(III) monochromator with a 300 μm × 300 μm beam size. The beamline was calibrated to 2158 eV using $ZnPO_4$ powder. Soil sample spectra were collected using a Vortex detector. Spectra were collected from 2135–2190 eV with a step size of 1 eV on the pre edge region (2110–2145 eV), 0.25 eV in near edge region (2145.25–2180 eV), and 0.5 eV in the post edge region (2180.5–2200 eV). A minimum of ten spectra were collected for each sample.

2.2.5.2 XANES Data Analysis

The collected scans were analyzed using Six-Pack (Webb, 2005) and Athena (Ravel, 2009) software programs. The spectra for each sample were first averaged together to improve the signal to noise ratio. The averaged spectra underwent background subtraction at the pre-edge and post-edge regions, and normalized to an edge jump of one (Kar et al., 2012).

Variances in P K-edge spectra normalization affects the white line intensity, which can affect linear combination fitting (LCF) results (Werner and Prietzel, 2015). Werner et al. (2015) used a background subtraction optimization routine that chose normalization parameters based on the lowest residual from LCF of a select set of standards. They report a 6% increase in accuracy from this strategy (Werner and Prietzel, 2015). In this study, the same set of background subtraction parameters were used on all spectra, thus avoiding bias in fitting a standard set that the unknowns were optimized to fit. Based on similarity of features, but differences in white line intensity, a few spectra required small changes in the background subtraction parameters to make the normalized spectrum white line intensity consistent with all of the other spectra in the set.

Principle component analysis (PCA) was done on the 24 normalized spectra using Six Pack to determine the number of independent components in the spectra (Ajiboye et al., 2007; Eriksson et al., 2015b). Target transformation was applied to the PCA using a base set of standards (Appendix Table A.3) to identify the chemical species that best reproduce the component set (Ajiboye et al., 2007). Accuracy is 17% minimum error (Ajiboye et al., 2007). The Spoil factors were used to determine possible end members in the principal components, where Spoil factors less than 3 are judged to be acceptable standards (Beauchemin et al., 2003). Acceptable standards from the Target Transform (TT) were then used for linear combination fitting in Athena. Combinatorial fitting of three samples using five standards from TT was done to determine the best fitting routine. The R-factor (a measure of mean square sum of the misfit at each data point) was used to evaluate fit quality. If a standard had less than 10% contribution, it was eliminated from the standard set and the fitting was repeated. Because all spectra from the soils were very similar (Figure 2.10), we used the same standard set to fit all 24 spectra.

2.2.6 Available Phosphorous and Soil Physicochemical Properties

Percent organic matter was determined using the Sims and Haby colorimetric method (J. R. Sims & Haby, 1971). Olsen extractable P (P extracted using 0.5 M $NaHCO_3$) and pH were measured using standard procedures found in Gavlak et al., (2005), and following federal Good Laboratory Practices (40 CFR Part 160) (US Code of Federal Regulations, 2011). Total Ca, Fe, Al, and P were determined by Aqua Regis digestion and analyzed with ICP-AES by Bureau Veritas Minerals Laboratory (Vancouver, Canada; ISO9001 certified).

2.2.7 Statistical Analysis

Results from the sequential extraction were analyzed by Bill Price, UI CALS statistical consultant, using SAS (SAS Institute, Cary, NC) to determine statistical significance. The experimental plot design includes only a single crop for each year, which is changed on an annual rotation; thus because the experiment was not designed to test for crop plant influences, statistical analysis was done only for the treatments within each year. The data was log-transformed before analysis to normalize the data distribution (Stroup, 2014). Both standard analysis of variance (ANOVA) and Tukey-HSD tests were performed. P-values less than 0.05 were considered significant. Repeated measures analysis was also conducted to compare significance across treatments and years.

2.3 Results

2.3.1 Soil Properties

Properties of the soil samples changed across amendment types and years (Table 2.3). Samples from both manure-amended plots showed an increase in the percent organic matter present, as well as a slightly higher percent composition of calcium in the soil (Table 2.3).

Table 2.2 - Summary of soil characteristics (± standard deviation) of samples collected from Kimberly, Idaho. Values are averaged from all four plots (n=4). Percent Ca, Al, and Fe are total soil concentrations. Amendments are annual additions to soil, and manure values listed are dry weight.

Depth	Amendment	Year	OM	pH	Olsen P	Total P	Ca	Al	Fe
cm			%		mg kg^{-1}		%		
0-30	Control	2013	1.4 (±0.08)	7.9 (±0.10)	14 (±1.39)	813 (±22.17)	2.09 (±0.29)	1.40 (±0.02)	1.66 (±0.05)
		2014	1.4 (±0.05)	7.8 (±0.00)	12 (±2.25)	843 (±28.72)	2.13 (±0.36)	1.46 (±0.05)	1.70 (±0.08)
		2015	1.3 (±0.10)	7.8 (±0.04)	13 (±2.63)	803 (±38.62)	1.99 (±0.37)	1.30 (±0.14)	1.60 (±0.10)
	Fertilizer	2013	1.4 (±0.13)	7.8 (±0.10)	12 (±2.55)	788 (±32.02)	2.01 (±0.35)	1.39 (±0.08)	1.65 (±0.05)
		2014	1.4 (±0.08)	7.9 (±0.06)	13 (±3.10)	800 (±20.00)	2.03 (±0.22)	1.41 (±0.06)	1.69 (±0.06)
		2015	1.4 (±0.10)	7.8 (±0.06)	17 (±1.50)	770 (±31.62)	1.86 (±0.27)	1.21 (±0.04)	1.56 (±0.08)
	17 Mg ha^{-1}	2013	1.6 (±0.10)	7.8 (±0.10)	21 (±6.84)	833 (±55.00)	2.20 (±0.28)	1.39 (±0.03)	1.68 (±0.03)
		2014	1.6 (±0.21)	7.7 (±0.08)	22 (±4.27)	843 (±26.30)	2.15 (±0.22)	1.42 (±0.03)	1.70 (±0.02)
		2015	2.0 (±0.31)	7.8 (±0.02)	42 (±9.38)	890 (±72.57)	2.20 (±0.48)	1.28 (±0.13)	1.58 (±0.09)
	52 Mg ha^{-1}	2013	1.8 (±0.15)	7.7 (±0.13)	34 (±8.53)	870 (±23.09)	2.31 (±0.21)	1.39 (±0.07)	1.70 (±0.16)
		2014	2.0 (±0.19)	7.7 (±0.05)	46 (±4.12)	928 (±17.08)	2.36 (±0.19)	1.36 (±0.09)	1.64 (±0.08)
		2015	2.8 (±0.31)	7.8 (±0.04)	115 (±24.16)	1080 (±82.87)	2.31 (±0.38)	1.22 (±0.12)	1.53 (±0.11)
30-60	Control	2013	0.8 (±0.07)	7.8 (±0.05)	3 (±0.46)	680 (±62.18)	2.50 (±2.47)	1.36 (±0.18)	1.63 (±0.19)
		2014	0.8 (±0.05)	7.8 (±0.19)	2 (±1.66)	688 (±55.60)	2.50 (±1.64)	1.46 (±0.12)	1.71 (±0.12)
		2015	0.8 (±0.09)	7.7 (±0.10)	4 (±0.88)	648 (±61.85)	2.57 (±2.66)	1.26 (±0.18)	1.56 (±0.17)
	Fertilizer	2013	0.8 (±0.09)	7.9 (±0.06)	4 (±0.84)	685 (±45.09)	2.45 (±2.18)	1.35 (±0.12)	1.61 (±0.14)
		2014	0.9 (±0.25)	7.9 (±0.21)	3 (±4.16)	698 (±45.00)	1.83 (±0.59)	1.46 (±0.03)	1.75 (±0.04)
		2015	0.8 (±0.11)	7.8 (±0.10)	4 (±0.87)	638 (±49.92)	2.23 (±2.00)	1.22 (±0.13)	1.54 (±0.15)
	17 Mg ha^{-1}	2013	0.9 (±0.07)	7.8 (±0.06)	9 (±5.68)	703 (±40.31)	2.36 (±0.95)	1.40 (±0.09)	1.69 (±0.10)
		2014	0.8 (±0.07)	7.7 (±0.10)	3 (±0.79)	675 (±20.82)	1.92 (±0.32)	1.45 (±0.05)	1.74 (±0.03)
		2015	1.1 (±0.18)	7.7 (±0.05)	10 (±3.16)	675 (±52.60)	1.92 (±0.45)	1.30 (±0.15)	1.62 (±0.10)
	52 Mg ha^{-1}	2013	1.0 (±0.28)	7.7 (±0.10)	10 (±8.30)	683 (±72.28)	1.56 (±0.77)	1.42 (±0.04)	1.69 (±0.04)
		2014	0.9 (±0.05)	7.6 (±0.05)	6 (±2.93)	680 (±21.60)	1.91 (±0.94)	1.48 (±0.03)	1.74 (±0.04)
		2015	1.2 (±0.22)	7.7 (±0.05)	28 (±10.20)	703 (±59.09)	1.30 (±0.31)	1.30 (±0.15)	1.61 (±0.11)

In the 52 Mg ha^{-1} manure-amended samples, average Olsen extractable P levels over the three years of amendment increased from 34 (±8.53) to 115 (±24.16) mg kg^{-1} in the surface soil sample (Table 2.3). Trends in the subsurface of the highest manure-amendment rate were similar. At the lower depth (30-60 cm) in both the manure-amended soil samples, Olsen P was greater after three years compared to the control and fertilizer amendments, indicating an increase in plant available P at lower depths (Table 2.3).

2.3.2 Sequential Extraction

The total amount of P extracted in all phases of the sequential extraction significantly increased in the 52 Mg ha^{-1} amendments compared to all other plot types across all three years (Figure 2.2). The 52 Mg ha^{-1} samples increased from 772 mg kg^{-1} to 1049 mg kg^{-1} in total extractable P (sum of all fractions) compared to the control plots, which had negligible gains (Table 2.2)

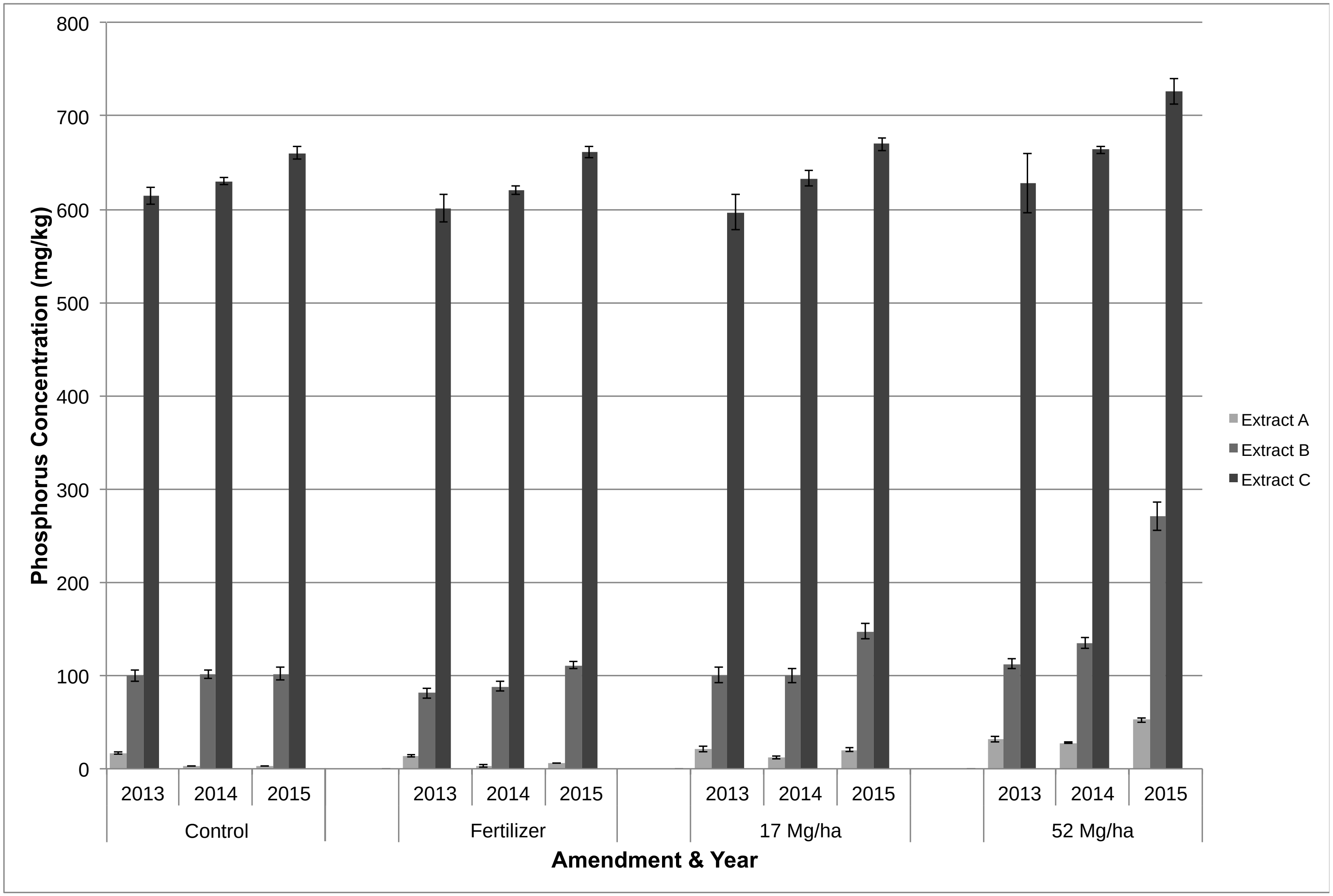

Figure 2.2 - Total phosphorus extracted from the 0-30 cm depth of calcareous soils from Kimberly, ID after annual amendments. Extracts are operationally defined to be Extract A = labile P; Extract B = Fe- and Al-bound P; Extract C = Ca-bound P. Error bars denote standard error (n=4).

Table 2.3 - Average phosphorus concentrations (± standard deviation) determined for each extraction for all four annual treatments (manure values are dry weight basis) in the 0-30 cm depth, for 2013-2015, collected from Kimberly, Idaho. Extracts are operationally defined to be Extract A = labile P; Extract B = Fe- and Al-bound P; Extract C = Ca-bound P. Total phosphorus determined by adding the averages for Extracts A, B, and C. Letters indicate significantly different values ($p<0.05$) between extracts of the different amendments within each year, determined using Tukey-HSD analysis.

Year	**Amendment**	**Extract A**	**Extract B**	**Extract C**	**Total P**
		mg kg^{-1}			
2013	Control	17 (±2.35) a	99 (±12.40) a	614 (±18.00) a	730 (±32.21) ab
	Fertilizer	14 (±2.50) a	81 (±11.13) b	601 (±29.08) a	696 (±32.10) a
	17 Mg ha^{-1}	21 (±5.84) b	100 (±17.22) a	596 (±37.87) a	718 (±52.21) ab
	52 Mg ha^{-1}	32 (±6.99) c	112 (±10.06) a	628 (±63.36) a	772 (±71.39) b
2014	Control	3 (±2.01) a	101 (±13.67) a	630 (±11.19) a	734 (±19.98) a
	Fertilizer	3 (±3.29) a	88 (±22.84) a	620 (±14.22) a	712 (±30.68) a
	17 Mg ha^{-1}	12 (±4.47) b	100 (±14.53) a	633 (±23.59) a	745 (±24.95) a
	52 Mg ha^{-1}	28 (±2.72) b	135 (±16.08) b	664 (±11.81) b	826 (±24.98) b
2015	Control	3 (±1.78) a	102 (±20.01) a	661 (±20.41) a	765 (±27.52) a
	Fertilizer	6 (±1.25) a	111 (±10.63) a	661 (±19.08) a	778 (±23.26) a
	17 Mg ha^{-1}	20 (±4.95) b	147 (±22.42) b	670 (±20.27) a	838 (±28.78) b
	52 Mg ha^{-1}	52 (±8.02) b	270 (±45.66) c	726 (±39.99) b	1049 (±58.19) c

Extract C removed the largest fraction of P from all soil samples under all amendments, while Extract A removed the smallest fraction (Figure 2.2). The fertilizer and control plots had similar P concentrations at every extraction phase for all years, except for Extract B in the first year, which was lower (Table 2.2). After 3 years of amendments (2015 samples), the 17 Mg/ha samples had significantly greater Fe- and Al bound (Extract B) P compared to the control and fertilizer plots (Table 2.2). The 52 Mg ha^{-1} amended samples had significantly greater Fe- and Al bound P in the second and third years (2014 and 2015), as well as significant increases in Ca bound P (Extract C) for year two and three (Table 2.2).

2.3.3 Element Mapping by Electron Microprobe

The majority of the element maps collected showed low *visible* concentrations of P. Several of the element maps, however, did show a clear association of P and calcium within the soil (Figure 2.3, Figure 2.4). No differences were observed between the control and manure-amended soils. The element maps showed that within the soil, Ca and P were correlated, and P distribution was not correlated with Al or Fe element distributions in the soils.

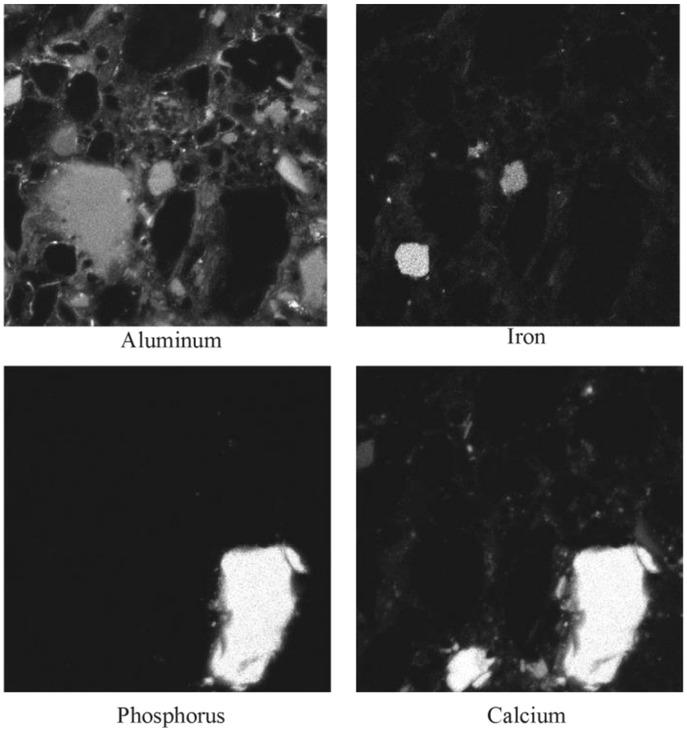

Figure 2.3 - Microprobe imaging showing mineral-phosphorus associations in a control soil (500 µm x 500 µm) (plot 107, 0-30 cm depth, 2014).

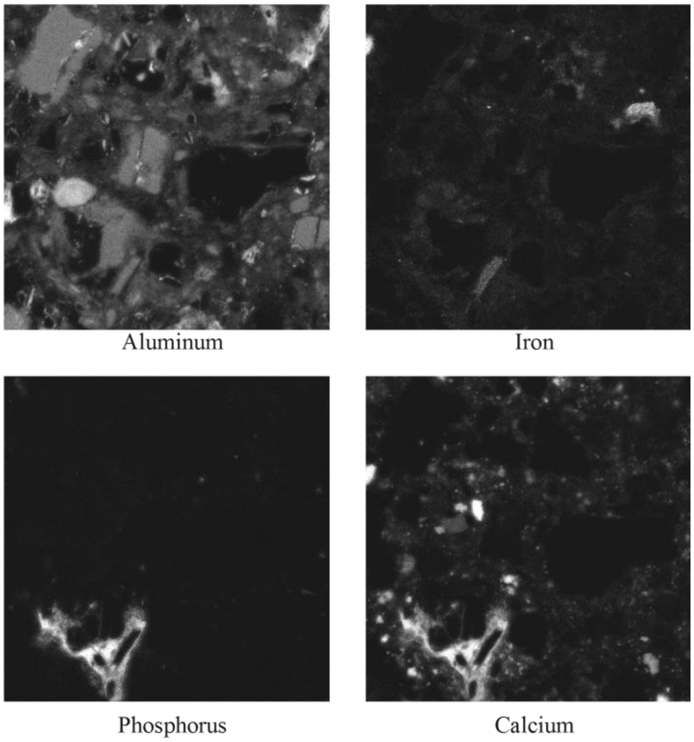

Figure 2.4 - Microprobe imaging showing mineral-phosphorus associations in a 52 Mg ha^{-1} soil (500 µm x 500 µm) (plot 106, 0-30 cm depth, 2014).

Because the low P concentrations in the microprobe scans do not always plot on a visual map, correlation graphs provide a more precise way of investigating element associations than the visual plots of the element distributions. Plotting element associations within pixels shows correlations between P and Ca (Figure 2.5). Investigating all samples for correlations between P and Fe or Al shows these associations are rare; Figure 2.6 provides an example of the lack of correlations that were typical in all element maps.

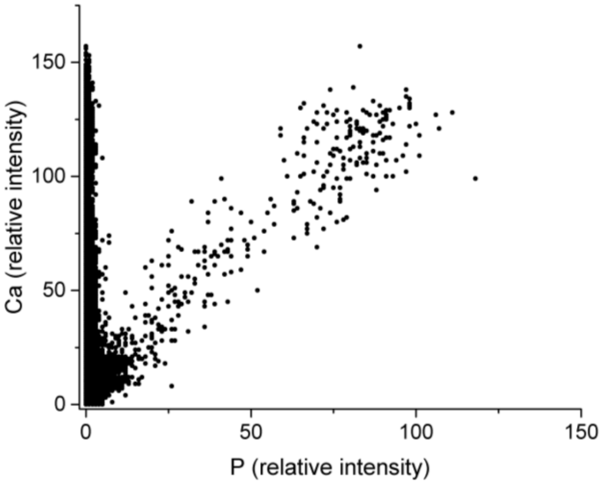

Figure 2.5 - Ca and P relative concentrations in pixels from a microprobe image of 52 Mg ha^{-1} soil, 0-30 cm depth (plot 106, 2014). Units are counts received by detector, and thus are a relative scale.

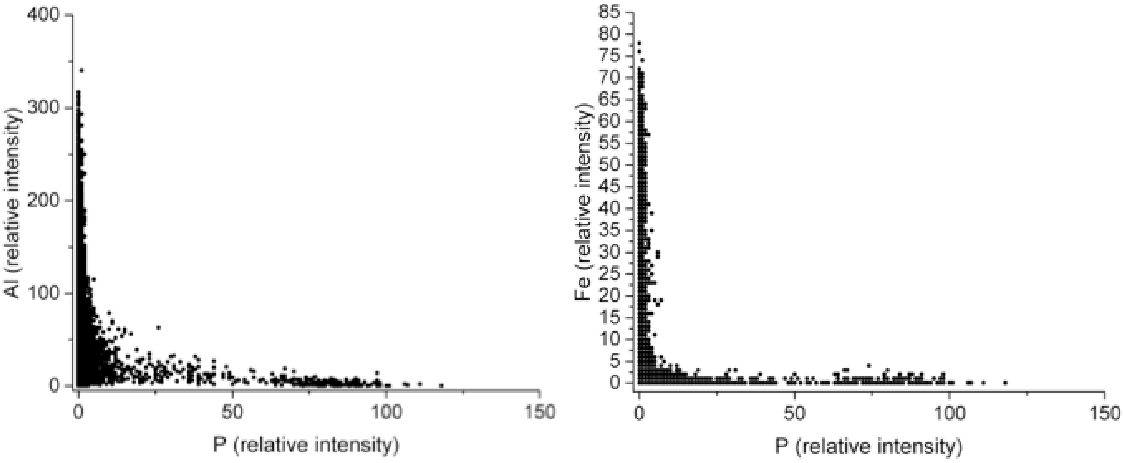

Figure 2.6 - Al and P relative concentrations in pixels (left), and Fe and P relative concentrations in pixels (right) from a microprobe image of 52 Mg ha^{-1} soil, 0-30 cm depth (plot 106, 2014).

2.3.4 ^{31}P-NMR Spectroscopy

NMR spectroscopic analysis showed similar peaks between all soil types (Figure 2.7). In the regions from 5 to -4 ppm (Figure 2.8), spectra show a small peak immediately following the orthophosphate peak that indicates the presence of monoesters, as well as diesters and some pyrophosphate.

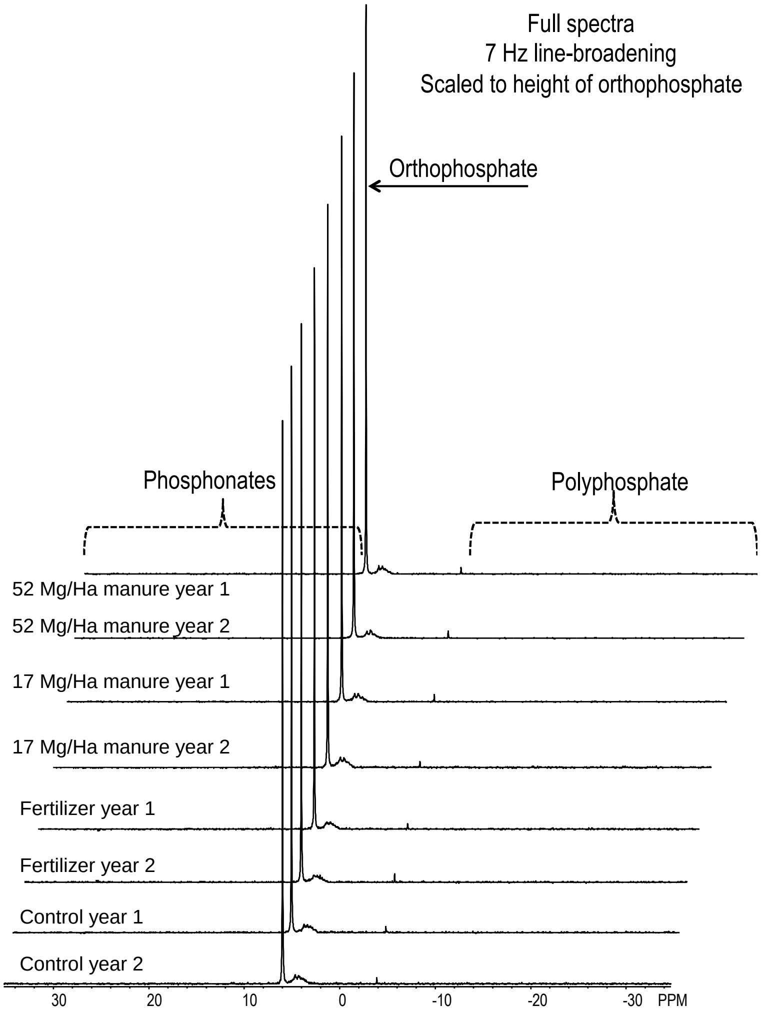

Figure 2.7 - Spectra of soil samples from all four amendment plots (0-30 cm, years 2013 and 2014) collected using ^{31}P NMR spectroscopy.

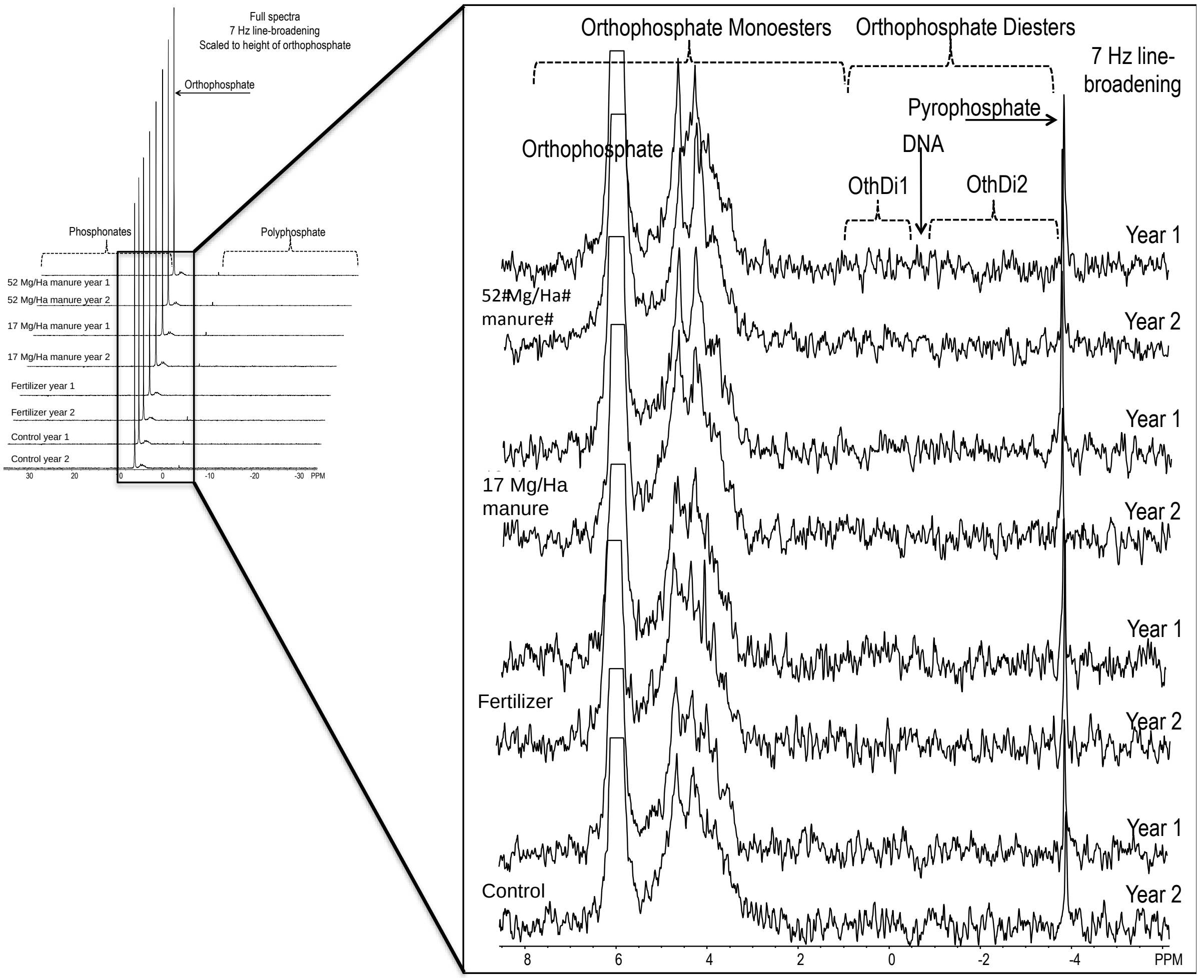

Figure 2.8 - Image of ^{31}P NMR spectra, magnified to show monoester and diester phosphate peaks. Samples are from 2013 and 2014, 0-30 cm soil depth, for all amendment types.

Analysis of the percent composition of the NMR spectra determined all samples had a soil P composition of majority orthophosphate, with smaller contributions of monoester and diester phosphates (Figure 2.9). Both the 17 Mg ha^{-1} and 52 Mg ha^{-1} samples had slightly higher amounts of orthophosphate compared to the control and fertilizer samples (Figure 2.9). The control and fertilizer samples had slightly higher amounts of monoester P (Figure 2.9).

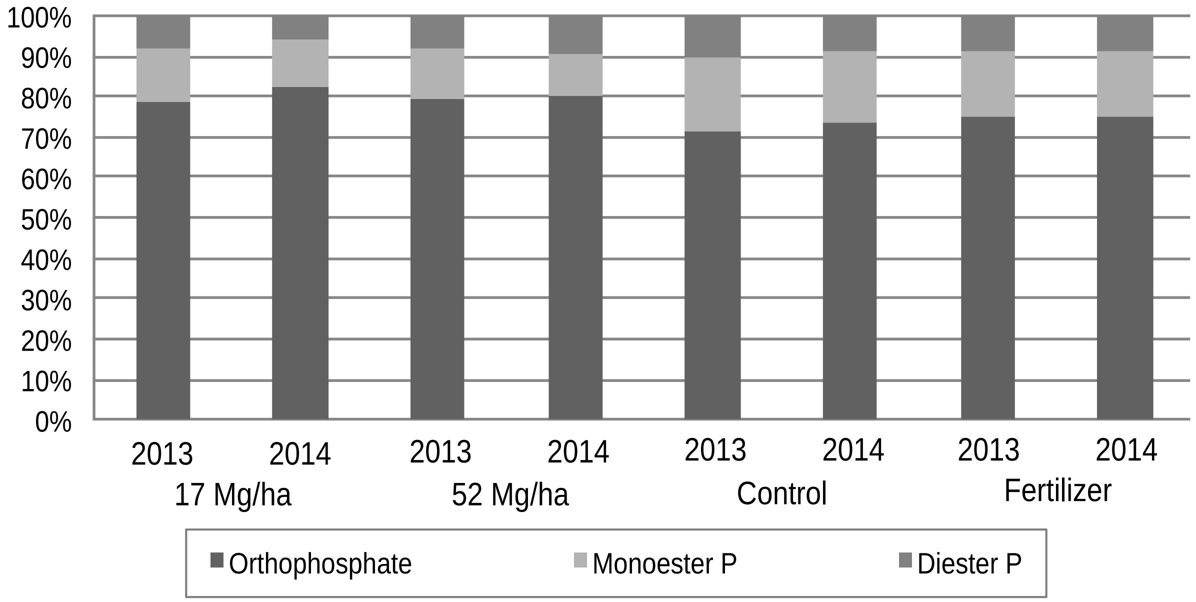

Figure 2.9 - Phosphorus speciation determined by ^{31}P-NMR spectroscopy for soil samples from Kimberly, Idaho; samples are from 0-30 cm profile (fit data are presented in Table 2 in Appendix A).

2.3.5 XAFS Fitting Results

Normalized XANES spectra have similar features across all three years and all amendments (Figure 2.10).

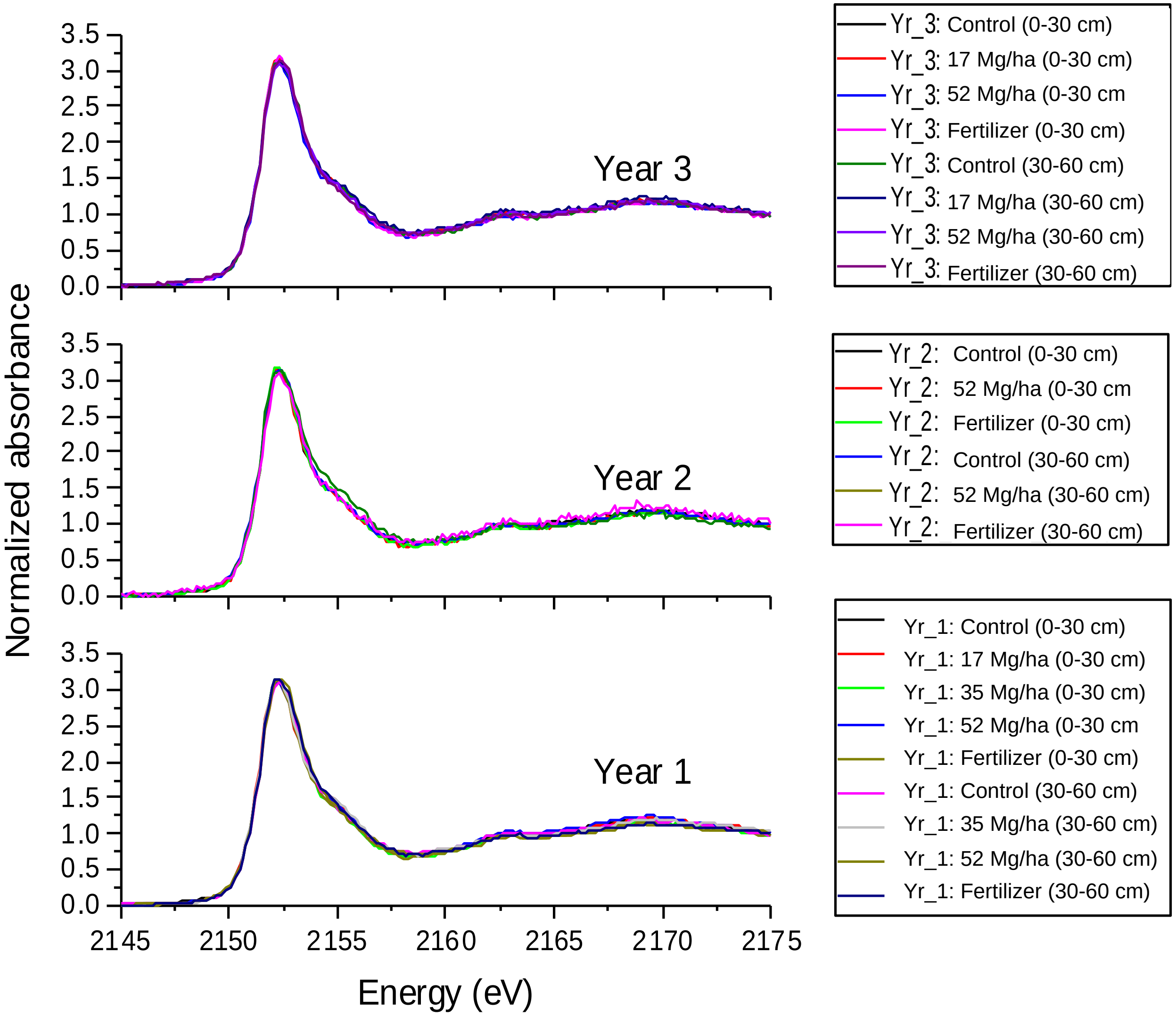

Figure 2.10 - P K-edge XANES spectra of all plots collected for soil samples from Kimberly, ID from years 2013–2015.

Principal component analysis of the 24 spectra resulted in a minimum of the indicator function (IND) at 5 components, suggesting that this is the number of significant components needed to recreate the 24 different spectra (Table 2.5) (Beauchemin et al., 2003). The first component is the main edge-jump that comprises 91.6% of the variance. The second and third components comprise 1-2 percent of the variance each. The remaining components comprise less than 1% of the variance each. Visual observation shows that the five components represent distinct differences between the spectra (Figure 2.11); i.e., features are present that are larger than the background.

Table 2.2 - Indicator Function (IND) determined by principle component analysis from the 24 spectra plotted in Figure 2.10,.

	Standard Type	Eigen Value	Indicator Function (IND)
1	Component1	99.333	0.00101
2	Component2	1.427	0.00093
3	Component3	1.359	0.00081
4	Component4	0.958	0.00074
5	Component5	0.746	0.00069
6	Component6	0.449	0.00072
7	Component7	0.393	0.00077

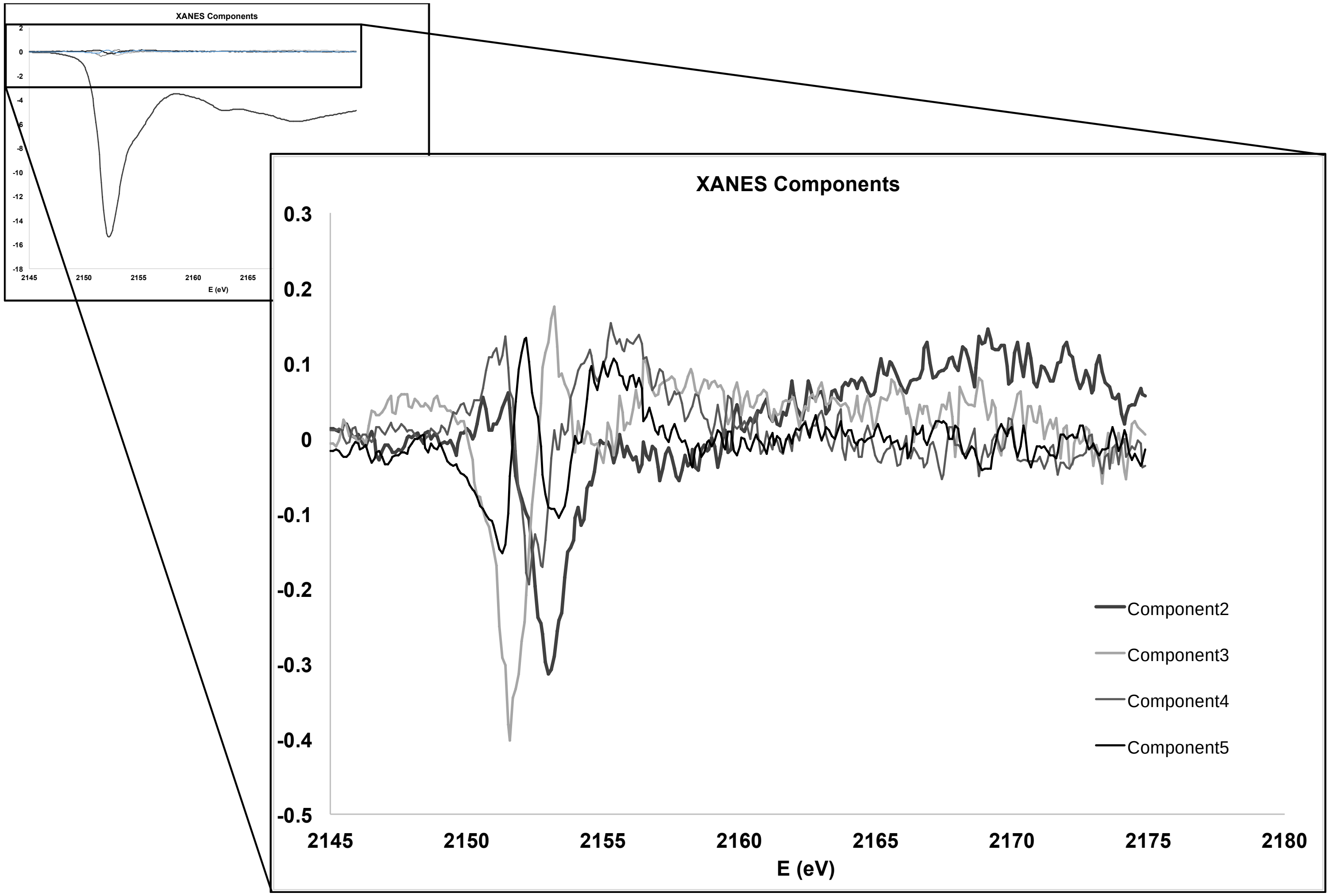

Figure 2.11 - Principal components derived from PCA analysis of the 24 soil XANES spectra. Upper left offset shows relative contribution of first component.

To determine standards needed to reproduce the 24 spectra, target transform using the five components to a set of nine standards chosen as likely soil phases was done. Results showed that several standards were acceptable or moderately acceptable (Table 2.6). The standards with the lowest SPOIL factors have distinct XANES features that can account for the features in the soil XANES spectra (Figure 2.12). Hydroxyapatite had the lowest SPOIL value, indicating that the soil very likely has hydroxyapatite present in it. Other, less crystalline Ca-P phases were also possible standards that could account for the features in the principal components, but their SPOIL factors were smaller than hydroxyapatite, suggesting they are not as likely to fit to the soil data.

Table 2.3 - List of standards and their SPOIL factors from Target Transform of five components. A lower SPOIL value indicates greater agreement between the principal components and the standard.

Standard	**SPOIL Factor**
Hydroxyapatite	1.011
Phytic Acid	1.078
Aluminum Phosphate	1.240
Ferric Phosphate	1.969
DCPD	2.173
Monetite	2.560
Brushite	2.914
Adsorbed P (pH 6.5)	2.915
DCPD (50% sub Mg)	4.342

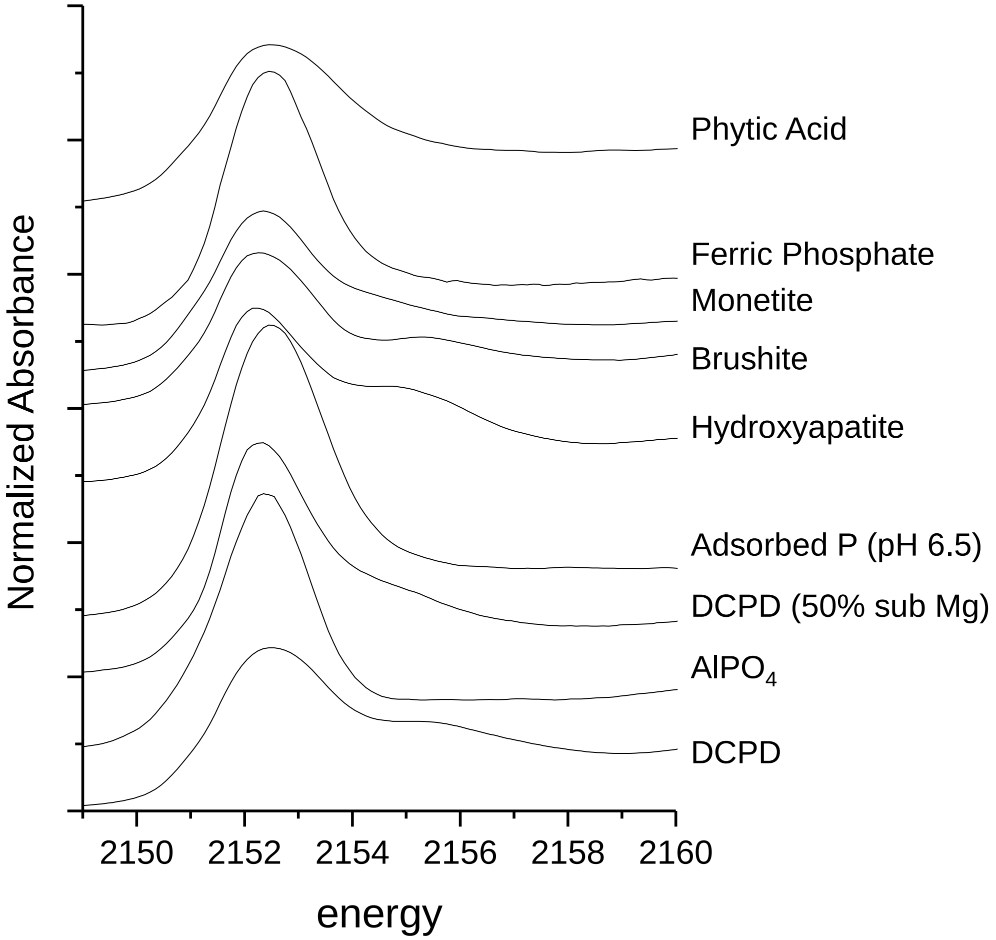

Figure 2.12 – P K-edge XANES spectra for nine standards used in linear combination fitting for soil samples. Adsorbed P standard is P adsorbed on goethite.

For initial linear combination fitting (LCF), all of the standards identified as possible species present in the soil from PCA and target transform were used, however, they were only fit five at a time. Standards fit with contributions less than 10% were eliminated from the fit set, and replaced with another possible standard. Using a combinatronics approach, it became apparent that the hydroxyapatite and phosphate adsorbed on iron oxide standards were required. Figure 2.13 shows the LCF to a soil sample using hydroxyapatite and adsorbed P only. The fit quality is very good (R-factor = 0.00459), with slight mismatch of white line intensity, which could be an artifact of the background subtraction. From comparison of the XANES in Figure 2.13, it is evident that hydroxyapatite is not the only phase in the soil because the sharp peak/shoulder at 2155 eV in hydroxyapatite is not as prominent in the soil spectra.

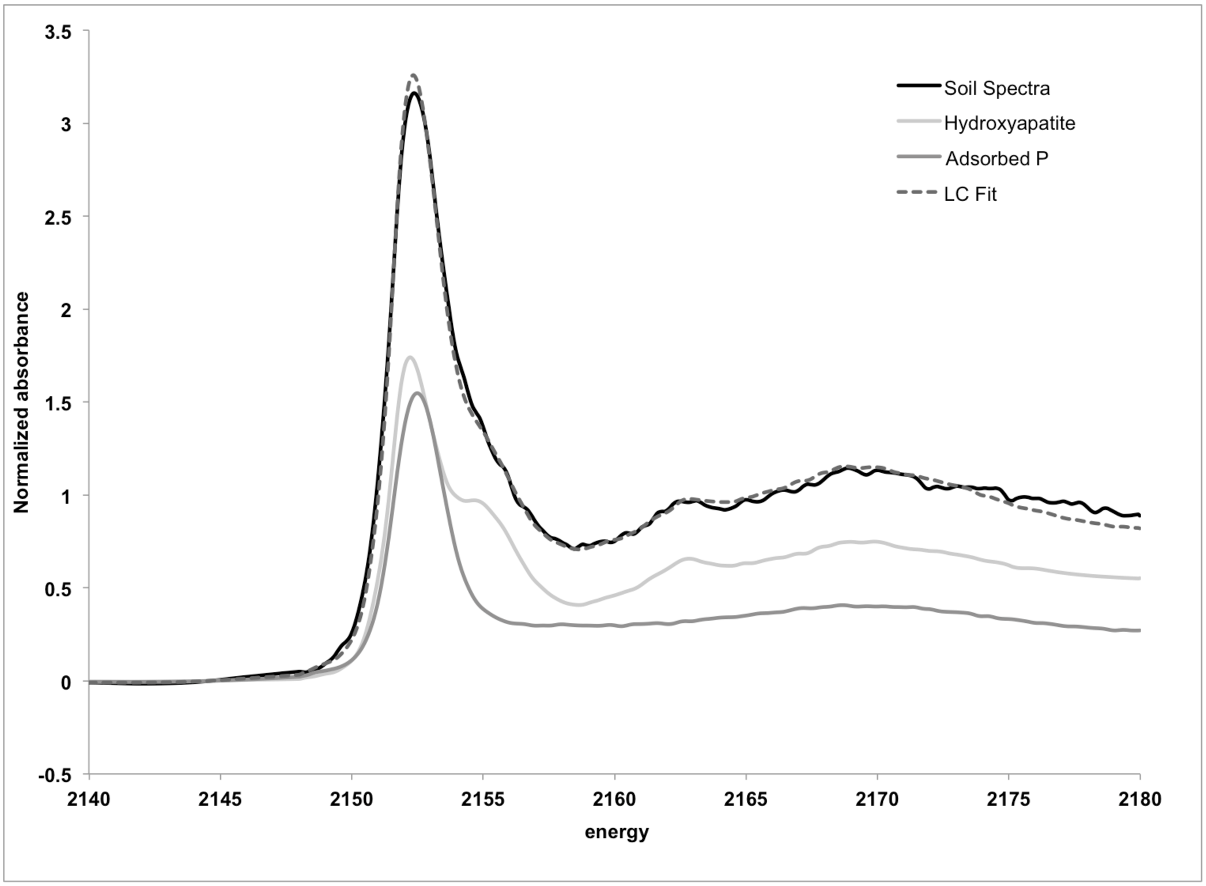

Figure 2.13 - Linear combination fitting of the 30 - 60 cm, 52 Mg ha^{-1} soil sample from 2013, showing the best fit from using only hydroxyapatite (66%) and adsorbed phosphorus (34%) standards to the soil spectrum.

The DCPD and phytic acid could also be fit in the sample spectra. The DCPD directly reduced the concentration of hydroxyapatite, but only minimally improved the fit residual (e.g., 0.00131 with DCPD and 0.00138 without DCPD; a 5% relative improvement in fit). Thus, DCPD was deemed unnecessary in the fitting routine. It appears that LCF of the soil spectra are indiscriminate for the DCPD and hydroxyapatite. This may be an artifact of the fact that in soils there are multiple phases present, some less than 10% that are difficult to isolate in linear combination fitting of the XANES spectra, and these phases create an overall decrease in the level of accuracy in the LCF.

The phytic acid standard fit less than 10% in all but 4 samples. Figure 2.14 shows the fit of the 2014 52 Mg ha^{-1} manure-amended soil (30 - 60 cm) using hydroxyapatite, adsorbed P, and phytic acid standards. To test for possible organic P presence, phytic acid was used as a standard in the samples. In some cases, the phytic acid standard was rejected completely by the LCF, and the soil spectra was fit with hydroxyapatite and adsorbed P only.

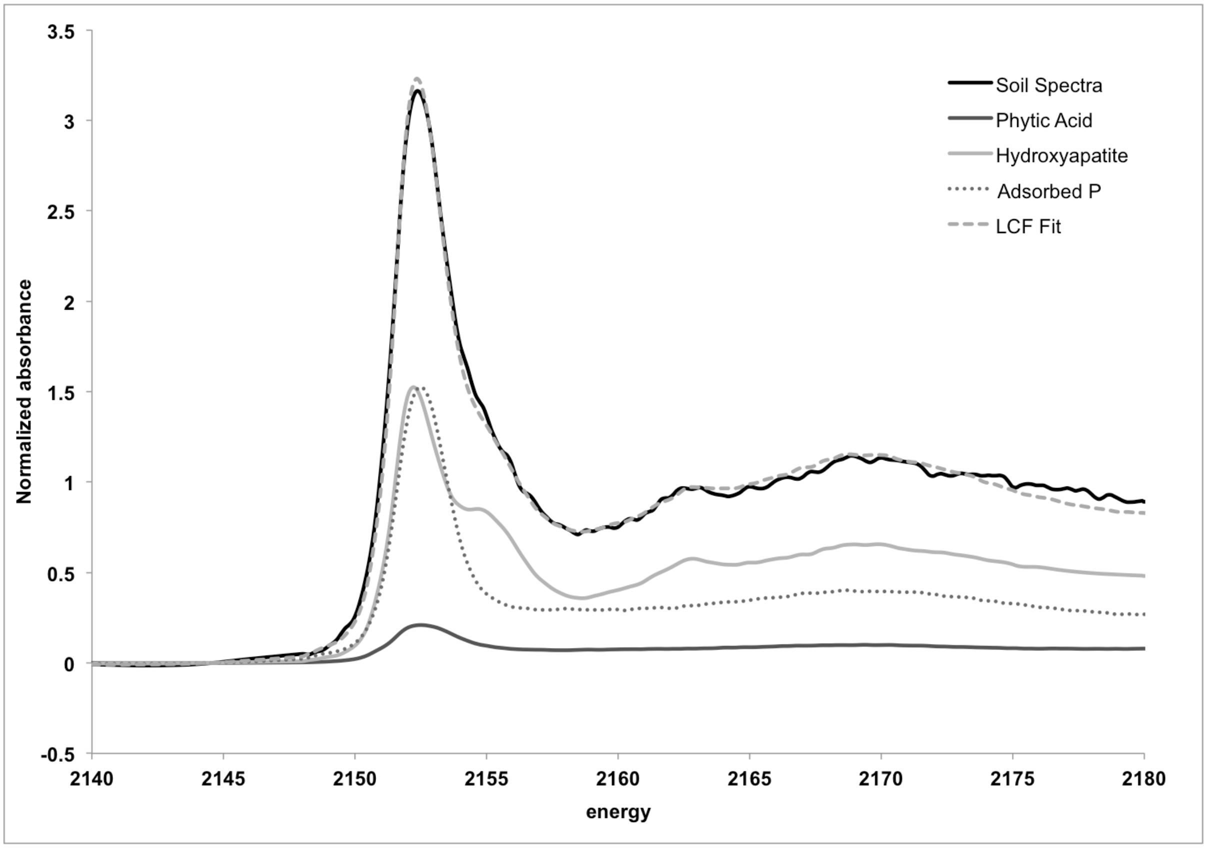

Figure 2.14 - Linear combination fitting of the 30-60 cm, 52 Mg ha^{-1} soil sample from 2013, showing the best fit using hydroxyapatite (57%), adsorbed P (34%), and phytic acid (9%) standards.

Linear combination fitting resulted in hydroxyapatite as the predominant P species in all soils. As much as 35% P adsorbed on mineral surfaces was also fit (Figure 2.15, Table 2.7). Organic P was fit as zero to 18.8% of the P in the samples (Table 2.7). The subsurface samples had more organic P as determined by XANES LCF. The strong hydroxyapatite LCF results in the samples (54% to 74%) agrees with the target transform results (Table 2.6). Of the more soluble calcium phosphate minerals, only DCPD was able to be fit in the soil spectra when hydroxyapatite was also fit; and, as discussed above, it was not required to achieve a good fit. The percent DCPD fit directly reduced the percent hydroxyapatite fit, suggesting that the features of the two spectra are coincident in the LCF. Thus, we conclude that it is possible that DCPD is present in the soil samples and that the percent hydroxyapatite fit represents a combination of the two Ca-P minerals.

The adsorbed P standard was phosphate adsorbed on goethite, however, phosphate adsorbed on other soil minerals is likely similar, with only small variances in XANES features that cannot be distinguished in a soil XANES spectra where subtle features are difficult to detect in mixed samples. LCF results show a greater initial amount of phytic acid in the 30-60 cm depth of the control sample, steadily declining in 2014 and 2015 (Figure 2.15). In the 0-30 cm fertilizer samples, phytic acid composition increased in 2014, with a slight decline the following year (Figure 2.15). No trends were apparent in the manure-amended soil samples (Figure 2.15).

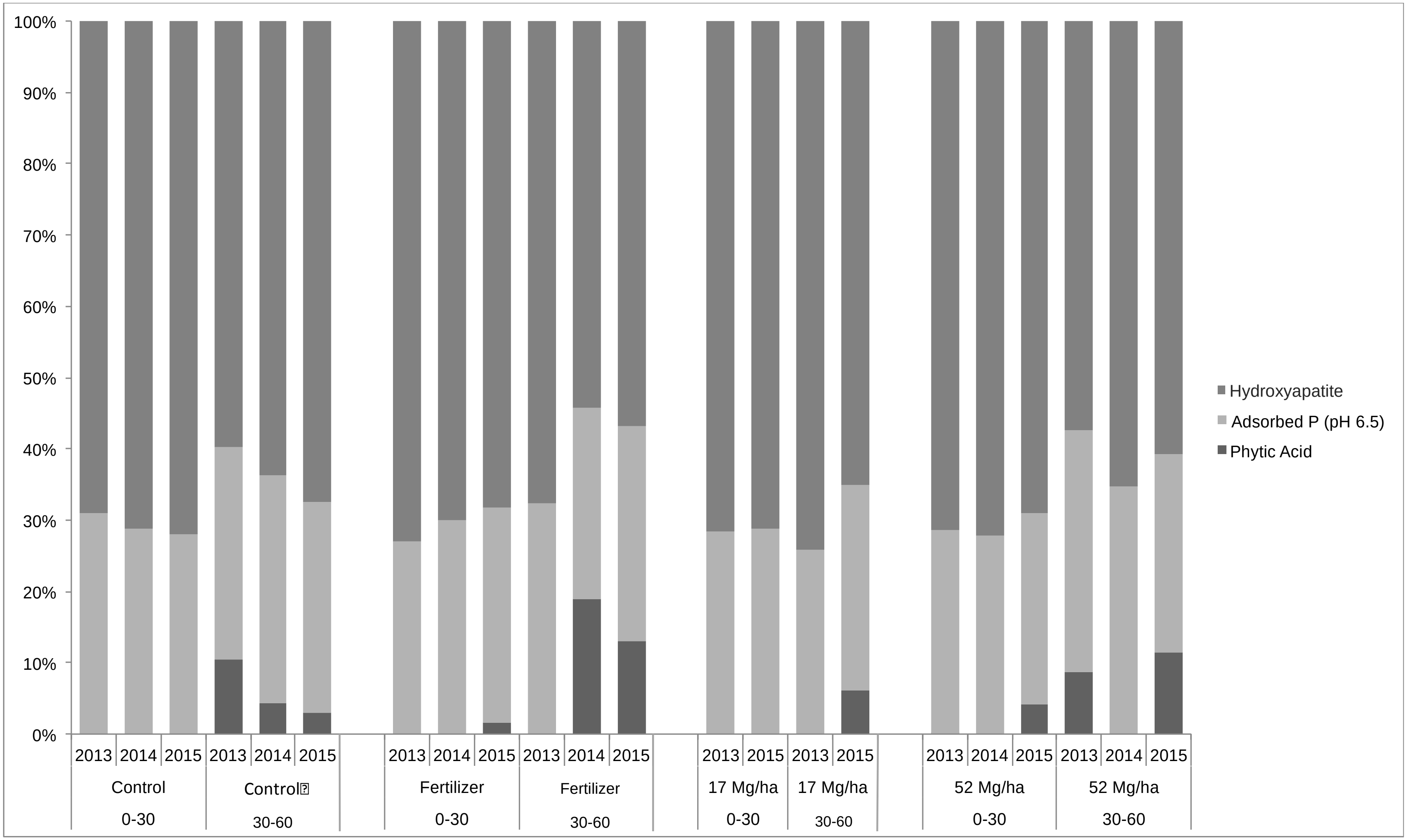

Figure 2.15 - Phosphorus species composition of soil samples from Kimberly, Idaho determined by linear combination fitting of K-edge XANES spectra. Soil phosphorus was fit with phytic acid, phosphorus adsorbed on goethite, and hydroxyapatite. All samples were from the first row of amendments (plots 101-108). Samples from 17 Mg ha^{-1} plot for 2014 were not analyzed.

Table 2.4 - Percent composition of phosphorus species of soil samples from Kimberly, Idaho determined by linear combination fitting of K-edge XANES spectra. Samples from 17 Mg ha^{-1} plot for 2014 were not analyzed.

Amendment	Depth (cm)	Year	Phytic Acid	Adsorbed P (pH 6.5)	Hydroxyapatite
				(%)	
Control	**0-30**	**2013**	0.0	31.0	69.0
		2014	0.0	28.9	71.1
		2015	0.0	28.1	71.9
	30-60	**2013**	10.5	29.8	59.7
		2014	4.3	31.9	63.8
		2015	3.0	29.5	67.5
Fertilizer	**0-30**	**2013**	0.0	27.1	72.9
		2014	0.0	30.0	70.0
		2015	1.5	30.2	68.2
	30-60	**2013**	0.0	32.4	67.6
		2014	18.9	27.0	54.2
		2015	13.1	30.2	56.7
17 Mg/ha	**0-30**	**2013**	0.0	28.4	71.6
		2015	0.0	28.8	71.2
	30-60	**2013**	0.0	25.9	74.1
		2015	6.2	28.7	65.0
52 Mg/ha	**0-30**	**2013**	0.1	28.6	71.3
		2014	0.0	27.9	72.1
		2015	4.1	26.9	69.0
	30-60	**2013**	8.7	33.9	57.4
		2014	0.0	34.7	65.3
		2015	11.4	27.8	60.8

LCF results for the P K-edge XANES from the 2013 manure sample suggest a composition of phytic acid (15%), hydroxyapatite (35%), and adsorbed P (50%) (Figure 2.16). The manure was found to have a greater percent composition of phytic acid and adsorbed P than the soil samples, and less hydroxyapatite.

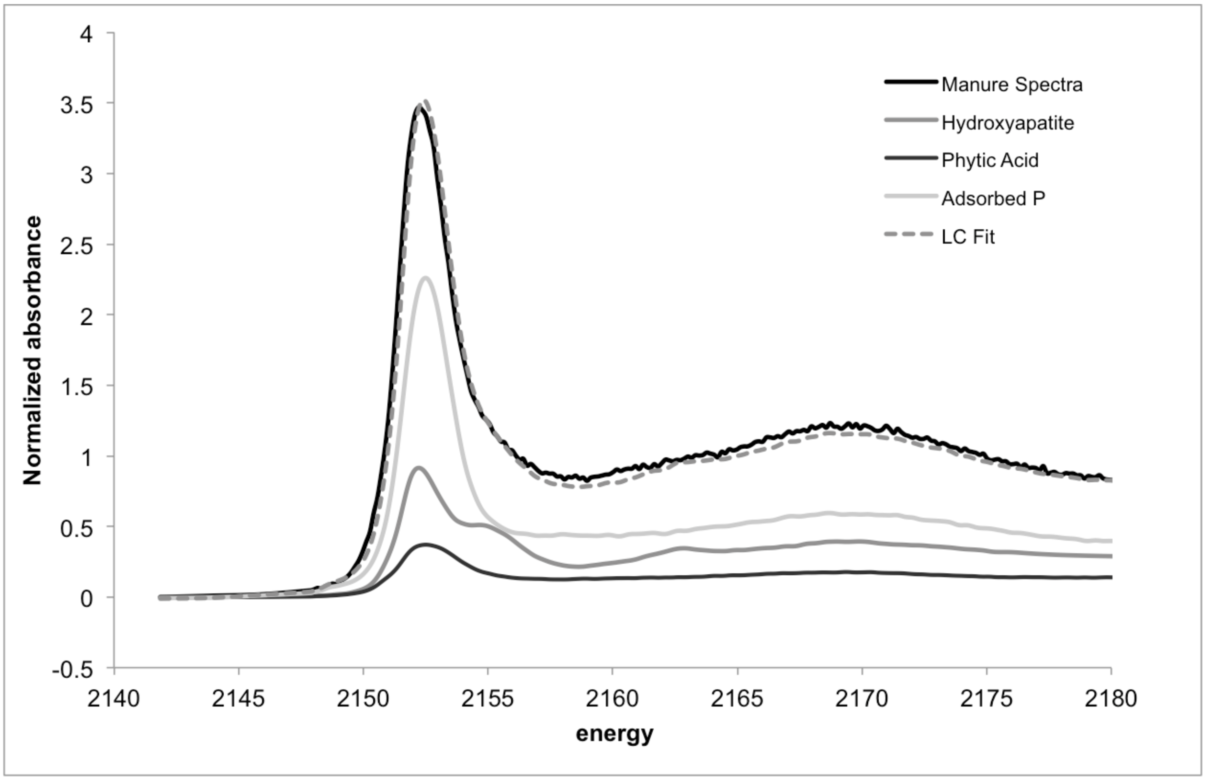

Figure 2.16 - Linear combination fitting results for a subsample of manure applied to the experimental field in 2013. Results show the contribution of hydroxyapatite (35%), phytic acid (15%), and adsorbed phosphorus (50%) to the spectrum.

2.4 Discussion

After three years of manure amendment, total P in the 0-30 cm depth of the 52 Mg ha^{-1} amended samples increased (Table 2.2). The annual manure amendments supplied a greater cumulative amount of P to the soil than the cumulative fertilizer treatments (Table 2.1). This may occur because the P present in chemical fertilizer is mostly, by design, accessible for immediate crop uptake upon application to the soil (Shen et al., 2011). In contrast, dairy manure contains a variety of P species that may not all be readily utilized by crops. The additional carbon supplied from manure (Table 2.3) may also increase the rate of immobilization via microbial uptake P in the soil, reducing soluble P available for plant uptake (Leytem et al., 2005), and thus causing soil phosphorus concentrations to increase.

The immobilized P can exist as organic P species, such as phytic acid, which can be strongly adsorbed (Celi et al., 2000), further decreasing the P in the soil labile pool. Supplying P to the soil as manure rather than chemical fertilizer may also affect plant uptake of other micronutrients, impacting crop production (Leytem et al., 2011).

The increase in calcium associated P in the manure-amended soils, shown via sequential extraction (Extract C, Figure 2.2), suggests that Ca-P mineral precipitation may occur at a greater rate in manure-amended soils than those amended with chemical fertilizer (Figure 2.2). The manure amendments may be supplying additional calcium to the soil, increasing the potential precipitation of low solubility Ca-P mineral phases in the soil. Past research has found calcium to be an important component of animal manures, affecting the soil pH and P species found in soils (Leytem et al., 2005; Sharpley et al., 2004). Calcium concentrations of soil samples were greater in the manured soils compared to the control plots (Table 2.3). LC fitting of XANES spectra for the manure shows 34% hydroxyapatite or Ca-P mineral present in the manure samples (Figure 2.16). The addition of Ca to the soil from the manure amendment may explain the increase in both the percent soil Ca, as well as the greater Ca-P fraction extracted from the manure amended soils in the sequential extraction (Figure 2.2, Table 2.3).

In addition to increased Olsen P concentrations in the manure-amended surface soil, the subsurface samples also had greater Olsen extractable P concentrations. In the 52 Mg ha^{-1} manure-amended soil at the 30-60 cm depth, the 2015 samples had an Olsen P value of 28 mg kg^{-1}, nearing the NRCS regulatory limit (40 mg kg^{-1}) (Hines et al., 2012). Past research has shown that manure applications cause increases in P concentration in the lower soil horizons, potentially due to the saturation of adsorption sites in the upper regions of the soil profile (Hansen et al., 2004). The high Olsen P value shown for 2015 analysis of the 30-60 cm depth in the 52 Mg ha^{-1} manure-amended soil may be an indicator of saturation of surface soil adsorption capacity occurring.

Extract A and Olsen bicarbonate extractions are operationally defined as labile P, however both are macroscopic analytical methods, and may extract unintended species from the soil (Negassa et al., 2010; Pierzynski, 2000). The results from this study show that over the years, the concentrations of P extracted by each are different, underscoring the fact that plant bio-accessibility methods such as Olsen bicarbonate extractable P and sequential extraction labile fractions are not necessarily comparable and may not be accurate for predicting P leaching and plant available P assessment. After the first year, P concentrations from Extract A were lower than the P concentrations determined by Olsen extraction (Table 2.7). The difference in results serves to demonstrate the imprecision in assigning P *speciation* to the different extractions, and the usefulness in combining sequential extraction results with other analytical methods to clarify P speciation in soils.

Table 2.5 - Comparison of average Olsen extractable phosphorus (0.5M $NaHCO_3$) and average phosphorus removed by extract A (Pa) (0.1M NaOH+1M NaCl) from upper soil profile (0-30 cm) samples.

	2013			**2014**			**2015**		
Amendment	**Pa**	**Olsen P**	**Difference**	**Pa**	**Olsen P**	**Difference**	**Pa**	**Olsen P**	**Difference**
	mg kg^{-1}		**%**	**mg kg^{-1}**		**%**	**mg kg^{-1}**		**%**
Control	17	14	14%	3	12	318%	3	13	328%
Fertilizer	14	12	15%	3	13	340%	6	17	203%
17 Mg/ha	21	21	2%	12	22	82%	20	42	108%
52 Mg/ha	32	34	5%	28	46	65%	52	115	122%

Percent organic P determined in the soil samples by NMR analysis ranged from 17.5 – 28.2% (Figure 2.9). Hansen et al. (2004) found similar results from NMR analysis of calcareous soils from the same region as this study. In contrast, analysis of the soil by LCF XANES spectra determined that organic P (fit using phytic acid) was present in the upper soil profile at concentrations 0 – 4.1% (Table 2.6). An important difference in the findings is that the NMR extractant removed only a fraction of the total P (202 – 310 mg kg^{-1} compared to total soil P 770 – 1080 mg kg^{-1}) (Appendix Table A.4; Table 2.3), while XANES spectroscopy probes the entire soil sample. The NaOH + EDTA extraction used to prepare the NMR samples decouples the cations associated with orthophosphate, preventing determination of the different inorganic P species, principally cation-P minerals such as hydroxyapatite or P associated with Fe and Al oxide minerals (Liu et al., 2013). Thus, the NMR results represent relative percent distribution of extracted P (Appendix Table A.4), not total soil P speciation.

Another important consideration in assessing organic P in the soils is the fact that organic P K-edge XANES do not have distinct features, which makes fitting them in complex matrices such as soils difficult (Prietzel et al., 2013). This is also compounded by a 17% error in P XANES LCF (Ajiboye et al., 2007). The organic P component could therefore be underestimated from the LCF of the soil XANES spectra. Consequently, NMR analysis provides insight into the composition of the organic P fraction in the soils, and XANES analysis determines the in-situ inorganic fractions not identified by NMR spectroscopy. For the soils examined in this study, Ca-P precipitates and adsorbed species are the dominant forms present in the soil, confirmed by both the sequential extractions and XANES LCF (Figure 2.2, Table 2.8). The results from multiple analytical methods show the benefits of using a variety of techniques to accurately quantify and categorize soil P (Ajiboye et al., 2007; Peak et al., 2012).

Long-term studies have found manure applications result in higher organic P values in the soil (Dodd and Sharpley, 2015; Pizzeghello et al., 2011). In this study, however, the results of both the NMR and XANES analysis did not show any trends in organic P concentration changes with annual manure amendments, The presence of phosphate diesters, shown in the NMR results (Figure 2.9), are indicative of microbial activity within the soil. Dodd and Sharpley (2015) found that soils with a higher organic P concentration may have a larger labile fraction of the total soil P pool. It is possible that continued manure amendments would result in an increased organic P fraction in the soil, potentially altering the overall plant availability and solubility of the P soil pool.

Although all of the XANES data from the samples appear to be similar, the relative error associated with XANES processing and analysis make detecting gradual shifts of P speciation in the soil difficult. Previous studies investigating long-term organic amendments (> 10 years) determined soil P speciation using P K-edge XANES analysis, sequential extraction, or chemical modeling, observed distinct differences in P speciation in amended and control soils (Peak et al., 2012; Sharpley et al., 2004). Results from our sequential extraction study suggests that manure amendments have a cumulative effect on soil P, which may become more pronounced over time, and will be observable using molecular-scale analytical tools.

2.5 Conclusion

This study used a combination of analytical methods to characterize P speciation in calcareous soils amended with dairy manure. After three years of amendments, increases in the total concentrations of P in manure-amended soils were significant compared to chemical fertilizer. Manure amendment also led to increased plant-available P as assessed by the Olsen extract in the 17 Mg ha^{-1} and 52 Mg ha^{-1} soil samples. However, changes in the dominant P species detected using XANES spectroscopic analysis were minimal. NMR analysis also showed similar composition of organic P across amendment types, indicating no significant changes in organic P speciation after 3 years of amendments. Together, the bulk, microscopic, and spectroscopic data suggest that portions of the P present in manure-amended soils are being converted to insoluble forms through adsorption and precipitation processes, and thus not available for plant use. P speciation information should be used to understand how to better interpret P availability as measured by the Olsen bicarbonate extract availability indices, or any other P management method.

The buildup of P in the soil may negatively impact water quality if sediments from the field reach freshwater ecosystems carrying the particulate-bound P. The future availability of the bound P to become bioavailable in the surface water is dependent on the P speciation. Our speciation results suggest that the majority of P in manure-amended soils exists as low solubility Ca-P minerals, such as hydroxyapatite. Phosphorus release from Ca-P mineral bound phosphate in the eroded soil particles should be minimal. However, there is a significant fraction of adsorbed P in the manure-amended soil samples, which has a greater potential to be released via desorption than Ca-P bound phosphate. Also, in the manure-amended soil samples, the absolute amount of “available P” assessed as either Extract A from the sequential extract experiment or Olsen bicarbonate extractable P is greater than the control and fertilizer samples — especially in year 3 high application rate samples — suggesting that risks form soils to surface waters in manure-amended soils is significant.

Results from this study provide an important baseline of soil P speciation. As the long-term study continues, manure amendment will likely cause changes in soil P speciation. Future studies will be designed to investigate these changes and compare to the initial P speciation results reported in this study.

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Chapter 3: Dairy Manure Impacts on Phosphorus Adsorption Rates and Speciation on Calcite

Abstract

In calcareous soils, phosphorus (P) adsorption and mineral precipitation with calcium (Ca) is an important reaction process that controls P availability to plants, and leaching potential. Past research has shown that the presence of organic matter can lower phosphorus adsorption, and alter the Ca-P minerals that form. To measure the effects of cow manure on Ca-P mineral precipitation rate and speciation, two calcite-phosphorus adsorption experiments were done to compare the amount of phosphorus adsorbed to calcite at different incubation times in a manure solution extracted at 1% and 10% (mass:volume) dairy manure. A non-dairy manure sample was also incubated for comparison. Compared to samples in deionized water, a significant reduction in the percent phosphorus adsorbed to calcite occurred when manure was present. XANES K-edge spectroscopic analysis showed that hydroxyapatite was the dominant P species formed in all samples, except for the samples incubated in the 10% manure solution. In the 10% manure solution, the P K-edge XANES were different than the XANES from hydroxyapatite and other Ca-P minerals, suggesting that the organic acids in the manure extract inhibited the formation of Ca-P minerals. Results indicate that manure amendment to calcareous soils alters the amount, speciation and overall solubility of Ca-P minerals in the soils, which will affect plant uptake and surface water quality.

3.1 Introduction

Phosphorus (P) is a key nutrient for plant growth, and is regularly applied to the soil to improve crop yields (Withers et al., 2014). As a result of confined animal feeding operations (CAFO), farmers are increasingly applying animal manure to fields surrounding the CAFOs to dispose of the excess manure, and to utilize the nutrients in the manures for crop growth (Leytem et al., 2011). However, once applied to the soil, P adsorption or precipitation reactions will reduce its availability for plant uptake (Shen et al., 2011). Continued additions of P to the soil to overcome the immobilized P cause total soil P to increase (Liu et al., 2015; McDowell and Sharpley, 2001). Thus, eroded soils amended with manure carry a higher concentration of P into surface waters, increasing the risk of eutrophication (Dupas et al., 2015). The availability of eroded particulate P to aquatic plant growth depends on the speciation of P in the eroded soil.

The dominant P species in soils are dependent on the soil physicochemical properties. In highly weathered soils, P predominantly reacts with aluminum and iron oxides. In alkaline soils, P primarily reacts with calcium to form Ca-P minerals, and adsorbs on calcite mineral surfaces (Cross and Schlesinger, 2001; Shen et al., 2011). Over time, initially formed Ca-P precipitates transform from readily soluble minerals, such as dicalcium phosphate dihydrate (DCPD), to more crystalline minerals, such as octacalcium phosphate (OCP) or hydroxyapatite (HAP) that are less soluble (Alvarez et al., 2004; Arai and Sparks, 2007; Lindsay and Moreno, 1960).

Phosphorus interactions within soils are complex, and can be altered by a variety of factors. For example, addition of organic matter to the soil can affect P adsorption and precipitation mechanisms. Soluble organic acids provided by organic matter have been shown to inhibit the precipitation rates of a range of calcium-phosphate minerals (Alvarez et al., 2004). Previous studies have found that negatively charged organic acids provided by manure amendments resulted in higher rates of P desorption in soils (Brauer et al., 2007; Jiao et al., 2007). The presence of dissolved organic carbon in soils has also been found to have an inhibitory effect on calcium and P co-precipitation rates (Sindelar et al., 2015). Since organic acids are elevated in manure-amended soils, understanding their effect on phosphate precipitation is critical. Organic acids may inhibit P adsorption by outcompeting for adsorption sites on mineral surfaces (Perassi and Borgnino, 2014). Organic acids have been shown to inhibit precipitation of hydroxyapatite, instead promoting the precipitation of metastable calcium P minerals such as OCP (Cooperband and Good, 2002; Grossl and Inskeep, 1992; Harris et al., 1994; Inskeep and Silvertooth, 1998; LeGeros et al., 1989).

The addition of organic matter can alter the pH of the soil, which also impacts P adsorption (Whalen et al., 2000). Rates of P adsorption to ferrihydrite were found to increase as pH decreased (Arai and Sparks, 2007). However, Sø et al. (2011) found that lowering pH can decrease phosphate sorption onto calcite. Sindelar et al. (2015), showed that calcium-P co-precipitation rates increased at pH 9.5 versus 8.5. An increase in soil organic matter has been found to lower the pH in alkaline soils, which could lead to a reduction in the amount of Ca-P mineral precipitation (Whalen et al., 2000).

Past research has found that changes in the soil organic matter can affect the type of Ca-P minerals that form (Sharpley et al., 2004). Sharpley et al. (2004) used sequential fractionation and thermodynamic solubility modeling of common P species to determine that extract solutions from soils located in central and eastern United States amended with various manure types contained more of the less crystalline OCP and β-tricalcium phosphate than untreated soils, which were dominated by HAP (Sharpley et al., 2004). Alvarez et al. (2004)

used Fourier transform infrared spectroscopy, laser Raman spectroscopy, and X-ray diffraction to analyze calcium-phosphate precipitation experiments in the presence of humic acids and observed that the organic matter delayed transformation of Ca-P minerals to more thermodynamically stable minerals.

Apart from organic matter and pH, several other variables can affect rates of P adsorption and precipitation. This includes temperature, time, surface charge, and phosphate concentration (Arai and Sparks, 2007; Lin and Singer, 2006; Sø et al., 2011). While most adsorption experiments of P on soils and soil minerals show rapid uptake of P in the first 4-24 hours, continuous slow adsorption has been observed for up to 90 days (Arai and Sparks, 2007).

Most research has used macroscopic data from batch adsorption or precipitation experiments to determine how soil variables impact calcite-P interactions. The research presented in this chapter used spectroscopic methods to measure the effects of soluble organic matter extracted from manure on P sorption kinetics on calcite. Phosphorus adsorbed on calcite samples were analyzed using K-edge X-ray absorption near edge structure (XANES) spectroscopy to discern the species of the sorbed P. Results will provide insights into how soluble-organic manure extract affects P sorption behavior and species on calcite, which are directly applicable to understanding manure-sourced P cycling in calcareous soils and predicting P availability for desorption or dissolution.

3.2 Methods

3.2.1 Calcite Adsorption Isotherms

3.2.1.1 One Percent Manure Extract Solution Experiment

A 49 day kinetic study was done to observe the effects of manure on calcite-P adsorption over time. Samples were prepared at different time intervals, ranging from 24 hours to 7 weeks (1176 hours) of incubation, with the 1176 hour incubation prepared first, and the 24 hour incubation prepared last (Table 3.1); this allowed for all the samples to complete their incubation period just prior to spectroscopic analysis via P K-edge XANES.

0.4 g of Multifex-MM precipitated calcium carbonate (Specialty Minerals, Inc; Adams, Massachusetts) was weighed into eighteen 50 mL centrifuge tubes. Experiments were run in duplicate or triplicate. Half of the samples had deionized water added, while the rest received manure extract solution.

The manure extract solution was prepared by combining 4.74 g of dairy manure (Magic Valley Compost Facility, Magic Valley, Idaho) in 415 mL of deionized water, shaking the mixture for 4 hours in the end-over-end shaker (Glas-Col; Terre Haute, Indiana), then centrifuging at approximately 1000 x g for 30 minutes. The manure solution was filtered through sterile 0.2 μm disposable vacuum filter units (Thermo Scientific; Waltham, Massachusetts) to remove microbes. Fresh manure solution was prepared for each incubation period. During the adsorption experiment, agar plates were used to spot check samples and confirm sterilization was successful (See Appendix B, Figure 1). The pH of both the deionized water and manure solution were adjusted to 8 (± 0.1) using 1 M NaOH or 0.5 M HCl solution as needed.

After the manure extract solutions were added to centrifuge tubes, 0, 0.5, or 1.5 mL of 10,000 mg L^{-1} P (NaH_2PO_4) were added along with deionized water to reach a total volume of 40 mL (Table 3.1). Total initial P concentrations for the manure solution samples were determined by adding the P supplied by the manure solution (determined via ICP-AES) to the P supplied by the added NaH_2PO_4 (Table 3.1). The samples were placed in the end-over-end shaker for 24 hours to thoroughly mix, then left at room temperature to incubate. Every 48 hours throughout the incubation, samples were mixed. Each sample type was prepared in triplicate.

A subsample of each manure stock solution was analyzed colorimetrically on a spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific; Waltham, Massachusetts) using the molybdenum blue method (Pierzynski, 2000), and by inductively coupled plasma atomic-emission spectroscopy (ICP-AES) (iCAP 6000 Series, Thermo Scientific; Waltham, Massachusetts) to determine inorganic and total P concentrations, respectively (Appendix B, Table 1).

Table 3.1 - List of samples prepared for the long-term incubation study, showing solution types, incubation times, volume of 10,000 mg L^{-1} phosphorus (NaH_2PO_4) added, total solution volume, and initial P concentrations. Both solutions adjusted to pH 8.

Deionized Water Solution (DI)				**Manure Solution (ME)**				
Incubation Time	**Added P**	**Added DI**	**Initial P**	**Incubation Time**	**Added P**	**Added ME**	**Added DI**	**Initial P**
(Hours)	**(ml)**		**(mg/L)**	**(Hours)**	**(ml)**			**(mg/L)**
24	0.0	40.0	0	24	0.0	35	5.0	13
	0.5	39.5	125		0.5	35	4.5	138
	1.5	38.5	375		1.5	35	3.5	388
72	0.0	40.0	0	72	0.0	35	5.0	13
	0.5	39.5	125		0.5	35	4.5	138
	1.5	38.5	375		1.5	35	3.5	388
96	0.0	40.0	0	96	0.0	35	5.0	12
	0.5	39.5	125		0.5	35	4.5	137
	1.5	38.5	375		1.5	35	3.5	387
336	0.0	40.0	0	336	0.0	35	5.0	13
	0.5	39.5	125		0.5	35	4.5	138
	1.5	38.5	375		1.5	35	3.5	388
504	0.0	40.0	0	504	0.0	35	5.0	13
	0.5	39.5	125		0.5	35	4.5	138
	1.5	38.5	375		1.5	35	3.5	388
840	0.0	40.0	0	840	0.0	35	5.0	13
	0.5	39.5	125		0.5	35	4.5	138
	1.5	38.5	375		1.5	35	3.5	388
1176	0.0	40.0	0	1176	0.0	35	5.0	13
	0.5	39.5	125		0.5	35	4.5	138
	1.5	38.5	375		1.5	35	3.5	388

After incubation, the samples were centrifuged at 1000 x g for 15 minutes to settle the calcite, and pH was recorded. The solution was then decanted and filtered with 0.2 μm paper (Merck Millipore, Ltd.; Cork, Ireland). The calcite was freeze-dried and subsampled to collect XANES spectra. The solutions were analyzed on the ICP-AES for total P, and colorimetrically for inorganic P on a spectrophotometer using the molybdenum blue method (Pierzynski, 2000) (Appendix B, Table 2). The total amount of P adsorbed to the calcite was calculated by comparing the differences between the calculated initial P solution concentration and the final solution concentration for each sample (initial P was adjusted to include the amount of P supplied by the manure).

3.2.1.2 Ten Percent Manure Extract Solution Experiment

A second incubation study was prepared to investigate the effects of a roughly 10% manure solution concentration on P adsorption kinetics and speciation. This study utilized the same methods and manure as the long-term adsorption isotherm, but the manure extract solutions were prepared with 50 g manure in 415 mL of deionized water, for a ratio roughly ten times greater than the solution used in the first experiment (Appendix B, Table 3). Samples were incubated for 24 hours, 5 days (120 hours), and 9 days (216 hours) following the same preparation and analysis used in the long-term study, except samples were prepared in duplicates, not triplicates (Table 3.2) (Appendix B, Table 4).

Table 3.2 - List of samples prepared for the 10% manure extract incubation study, showing solution types, incubation times, volume of 10,000 mg L^{-1} phosphorus (NaH_2PO_4) added, total solution volume, and initial P concentrations. Both solutions adjusted to pH 8.

Solution	**Incubation Time**	**Added P**	**Added ME**	**Added DI**	**Initial P**
	(Hours)		**(ml)**		**(mg/L)**
Deionized Water (DI)	24	0.0	0	40.0	0
		0.5	0	39.5	125
		1.5	0	38.5	375
	120	0.0	0	40.0	0
		0.5	0	39.5	125
		1.5	0	38.5	375
	216	0.0	0	40.0	0
		0.5	0	39.5	125
		1.5	0	38.5	375
Manure Solution (ME)	24	0.0	35	5.0	18
		0.5	35	4.5	143
		1.5	35	3.5	393
	120	0.0	35	5.0	14
		0.5	35	4.5	139
		1.5	35	3.5	389
	216	0.0	35	5.0	14
		0.5	35	4.5	139
		1.5	35	3.5	389

3.2.2 Sterilization

To prevent interference from microbial activity, sterile centrifuge tubes were used for all samples; pipette tips, plastic storage containers, and glassware were all autoclaved before use. Devices used to dispense the calcite were rinsed with ethanol before and between uses, as was the pH probe used during pH adjustment.

3.2.3 XANES Analysis

K-edge XANES spectra were collected for the freeze-dried calcite samples (one replicate for each incubation time) at both the Stanford Synchrotron Radiation Light Source (SSRL) laboratory in Stanford, CA and at the Canadian Light Source (CLS) in Saskatoon, SK. Spectra for the long-term isotherm were collected at SSRL in May 2015, using Beamline 14.3, a bending magnet beamline that is energy focused using a Si (111) monochromator. Samples were placed on plastic tape and placed in a He purged sample chamber. Fluorescence was detected from the samples using either a large area PIPS detector or 4-element Vortex detector. Step size across the edge was 0.15 eV. Three to five scans of each sample were collected. Spectra were normalized by subtracting a pre-edge and post edge, and aligned so the peaks in their first derivative were at 2151.2 eV.

P K-edge XANES spectra of the 10% manure solution calcite samples and P standards were collected at the Soft X-ray Micro-characterization beamline (SXRMB) at the Canadian Light Source (CLS), Saskatoon, Canada. All samples were mounted using carbon tape on a copper sample holder. The beamline uses an InSb(III) monochromator with a 300 μm × 300 μm beam size. The beamline was calibrated to 2158 eV using $ZnPO_4$ powder. Soil sample spectra were collected using a Vortex detector. Spectra were collected from 2135–2190 eV, with a step size of 1 eV on the pre edge region (2110–2145 eV), 0.25 eV in near edge region (2145.25–2180 eV), and 0.5 eV in the post edge region (2180.5–2200 eV). A minimum of four spectra were collected for each sample. Replicate spectra were averaged for data analysis. Spectra were normalized by subtracting a pre-edge and post edge, and aligned so the peaks in their first derivative were at 2151.2 eV.

3.2.4 Statistical Analysis

Statistical significance of the adsorption rates of each solution type was determined by running standard two-way ANOVA in R (R Core Team, 2013). P values of <0.05 were considered significant.

3.2.5 Organic Carbon Analysis

Non-purgable organic carbon was determined for both the 1% and 10% manure solution concentrations using a total organic carbon (TOC) analyzer (TOC-L analyzer, Shimadzu Corporation; Canby, Oregon). Sample solutions were prepared in triplicate, using the same methods described above, with the pH adjusted to 2.7 using 0.5 M HCl for analysis on the TOC-L analyzer.

3.3 Results

3.3.1 One Percent Manure Extract Solution

The 1% manure solutions prepared for each incubation provided additional P, ranging from 11.59 – 13.45 mg L^{-1} (Appendix Table B.1). Total dissolved organic C in the 1% manure solution was 43.59 mg L^{-1} (Table xxx).

The additional P contributed by the manure solution increased the total initial concentrations of P in the manure incubations compared to the deionized water samples. This increase in available P allowed for a slight increase in the overall adsorption of P in the manure samples (Figure 3.1). The majority of P adsorption in both the 1% manure solution and the DI sample occurred within the first 24 hours (Figure 3.1; Figure 3.2). However, the overall adsorption rate in the manure solution samples was less than the deionized water samples, when 0.5 ml of P was added (Figure 3.1).

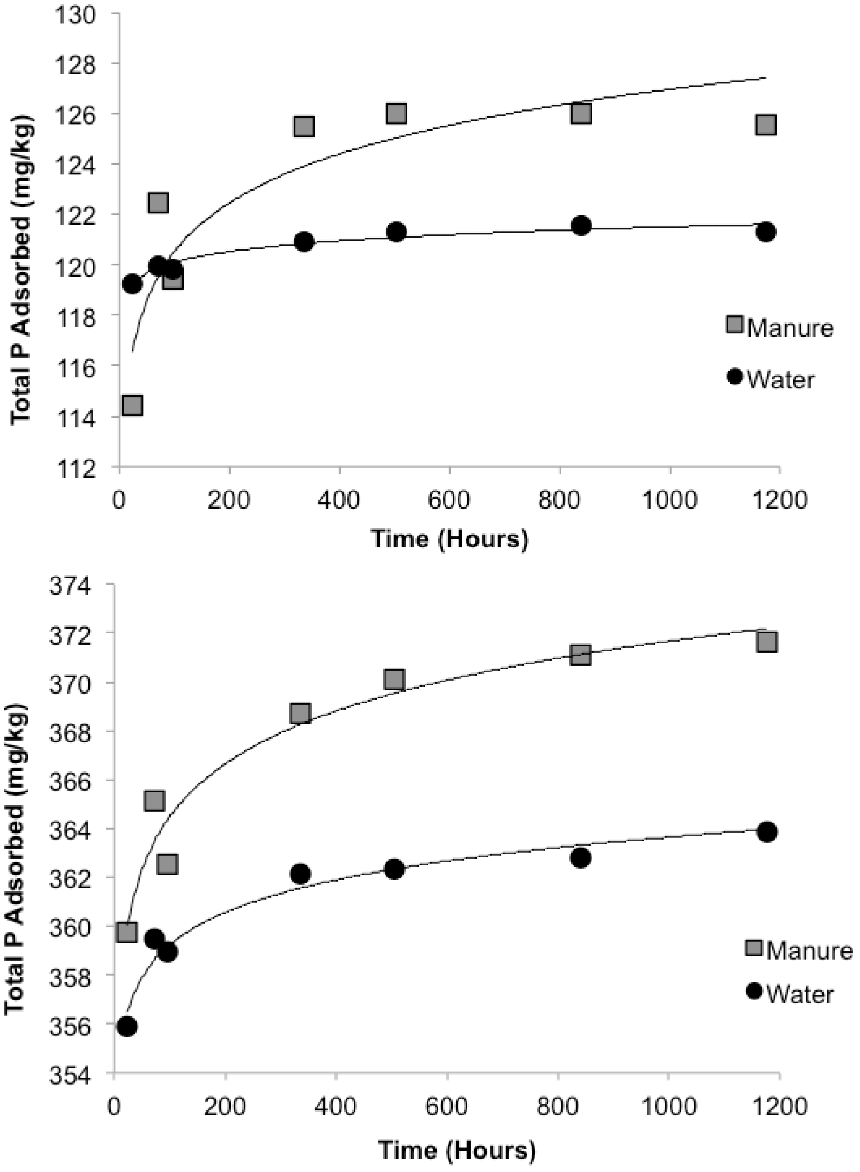

Figure 3.1 - Comparison of total phosphorus adsorbed on calcite over time between deionized water and 1% manure solution, with 0.5 mL of 10,000 mg L^{-1} P (NaH_2PO_4) (top) and 1.5 mL P (bottom) added (n=3). Standard error of mean for all points was < 2.1 mg kg^{-1}.

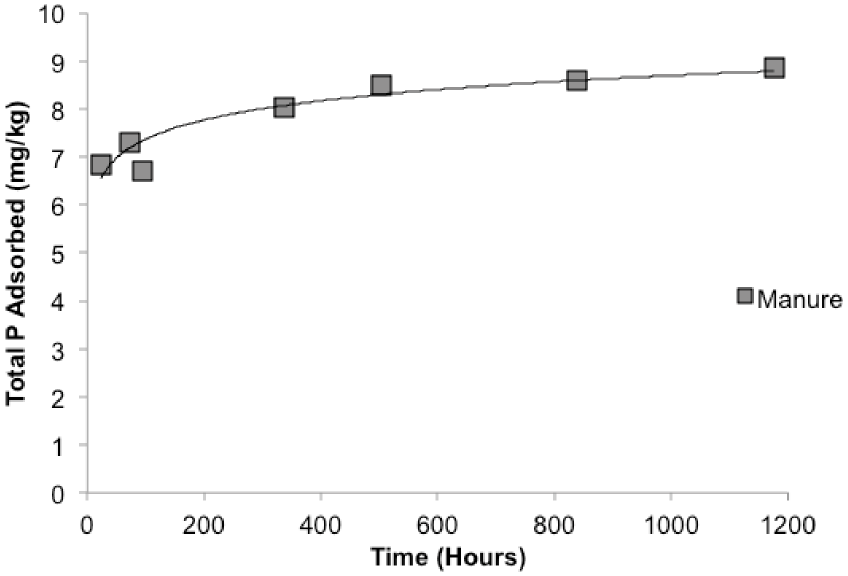

Figure 3.2 - Total phosphorus adsorbed on calcite over time in 1% manure solution (no added phosphorus) (n=3). Standard error of mean for all points was < 0.14 mg kg^{-1}**.**

Because the manure extract added P to the initial solution, to compare the effect of time on P adsorption in the manure extract and deionized water system, the amount adsorbed was calculated on a percent removed from solution basis. In the 0.5 mL added P samples, a significantly greater percent of P removal occurred in the water solution compared to the manure extract (P< 7.11e-05) (Figure 3.3). The 1.5 mL added P samples also had a greater percent of removal in the deionized water solution compared to the manure solution (P< 0.0116) (Figure 3.2). In the samples where all P present was provided by the manure solution (no added P), 50-69% of the P was adsorbed during the incubation period (Figure 3.4), with 29% of the total adsorption occurring at time periods longer than 1 day. In both the 0.5 mL and 1.5 mL added P samples, less than 10% of the adsorption occurred after the one day incubation period (Figure 3.3)

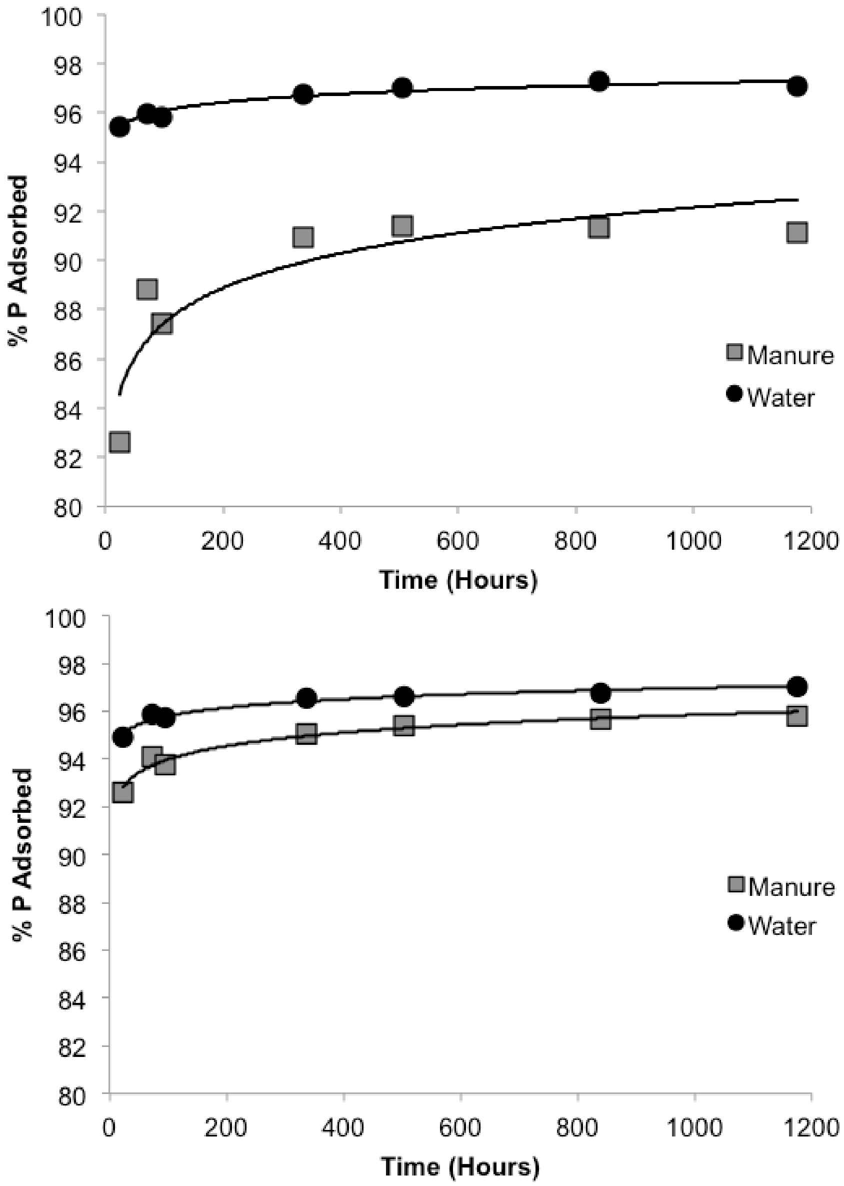

Figure 3.3 - Comparison of percent phosphorus adsorbed on calcite over time between deionized water and 1% manure solution, with 0.5 mL of 10,000 mg L^{-1} P (NaH_2PO_4) added ($P<7.11e-05$) (top), and with 1.5 mL of 10,000 mg L^{-1} P (NaH_2PO_4) added ($P<0.0116$) (bottom) (n=3). Standard error of mean for all points was ≤ 0.56 %.

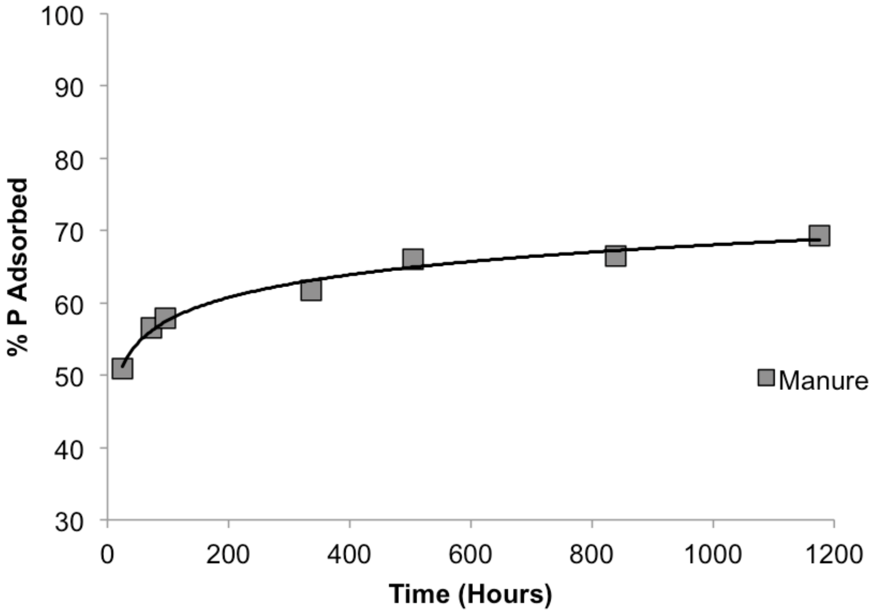

Figure 3.4 - Percent phosphorus adsorbed on calcite over time for 1% manure solution with no added phosphorus (n=3). Standard error of mean for all points was ≤ 1.1.

3.3.2 Ten Percent Manure Extract Solution

The 10% manure solutions prepared for each incubation contributed slightly greater concentrations of P (14.14 – 18.33 mg L^{-1}) compared to the 1% manure solution incubation (Appendix B, Table 3). However, it was not ten times greater, suggesting that the concentration of P in solution in the manure extract is limited by either precipitation of a P-mineral phase, or adsorption of the P on a solid in the manure. In the 10% manure extract solution created for the 24 hour incubation the total P concentration was greater than the samples incubated for 120 and 216 hours (18 mg L^{-1} compared to 14 mg L^{-1}). This likely occurred from natural variation in the manure subsamples collected to create the solutions because it is a natural material with some variation in composition. By using the initial concentration, accurate amounts of P adsorbed were calculated, and natural variability was accounted for. The average total organic C in the 10% manure solution was 416.4 mg L^{-1} (Table B.5).

The deionized water solution samples had greater total adsorption of P than the manure solution samples (Figure 3.5). When the only P supplied to solution was from the manure, adsorption was similar at all incubation times (Figure 3.6).

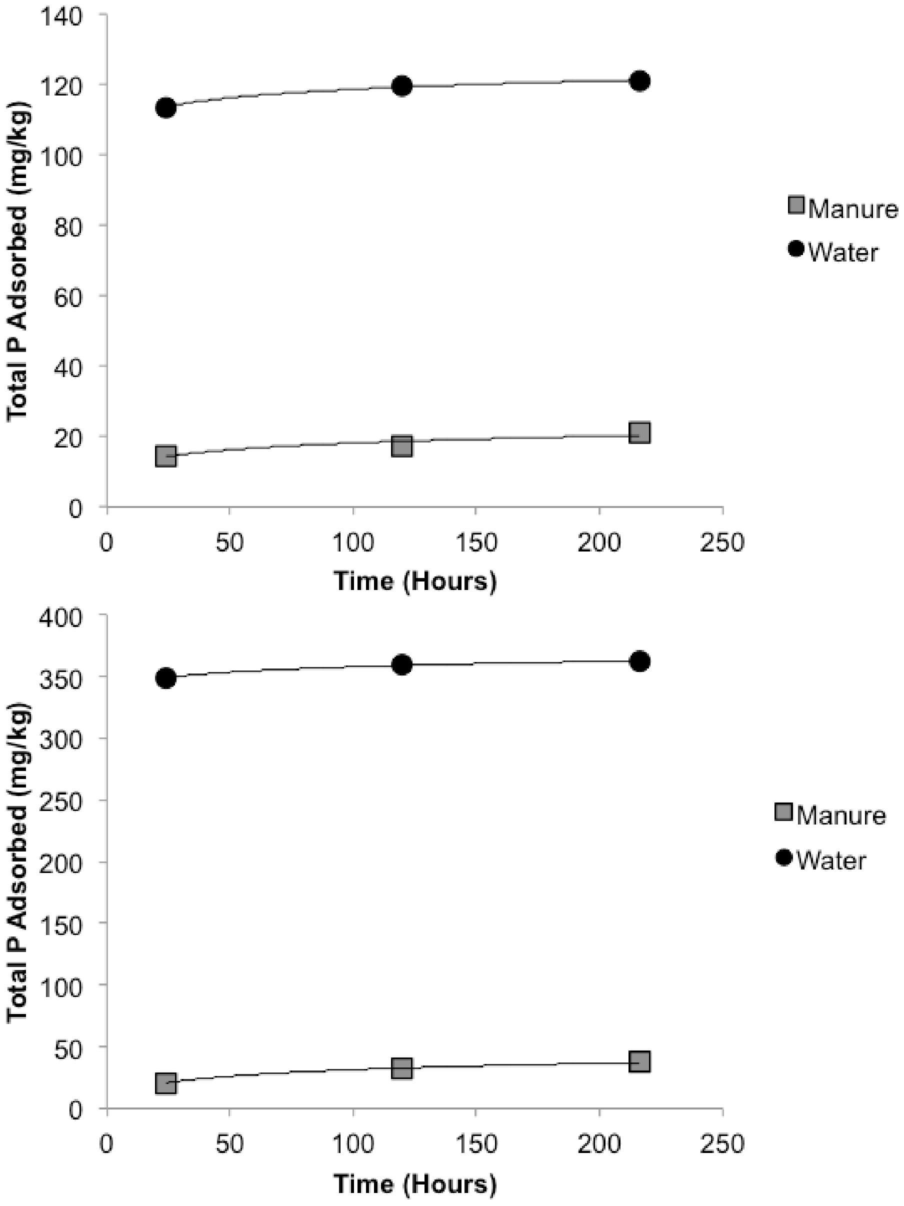

Figure 3.5 - Comparison of total phosphorus adsorbed on calcite over time between deionized water and 10% manure solution, with 0.5 mL (top) and 1.5 mL (bottom) of 10,000 mg L^{-1} P (NaH_2PO_4) added (n=2). Standard error of mean for all points was < 5.0 mg kg^{-1}.

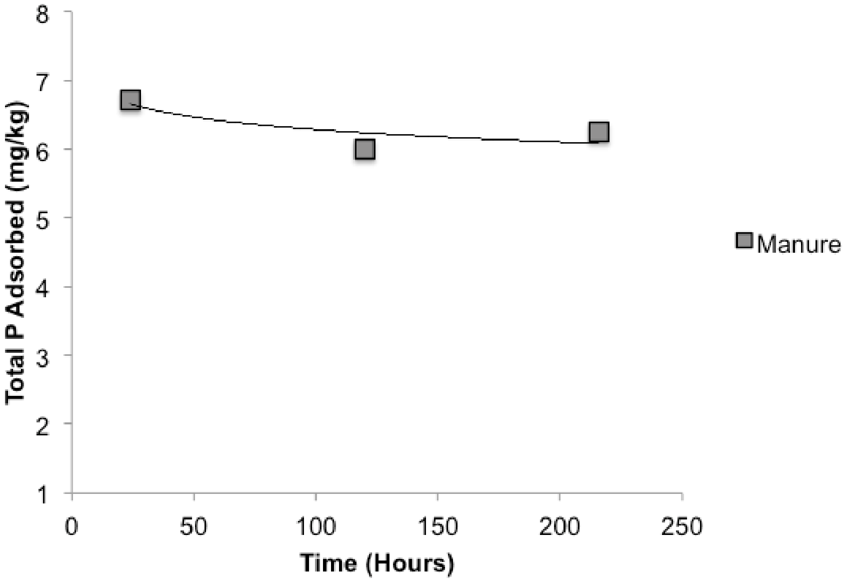

Figure 3.6 - Total phosphorus adsorbed on calcite over time in 10% manure solution, with no added phosphorus (n=2). Standard error of mean for all points was < 0.04 mg kg^{-1}**.**

When the manure solution concentration increased to 10%, there was a greater difference between the deionized water and manure solution incubations in both total adsorbed and percent adsorbed P (Figure 3.5, Figure 3.7). In the 1.5 ml and 0.5 mL added P samples, the difference in the percent of P adsorbed in the manure solution compared to deionized water was significant for all incubation times (5 – 15% vs. 91 – 97%) (Figure 3.7). In the samples with no added P, 36-44% of the P provided by the manure solution was adsorbed during incubation (Figure 3.8).

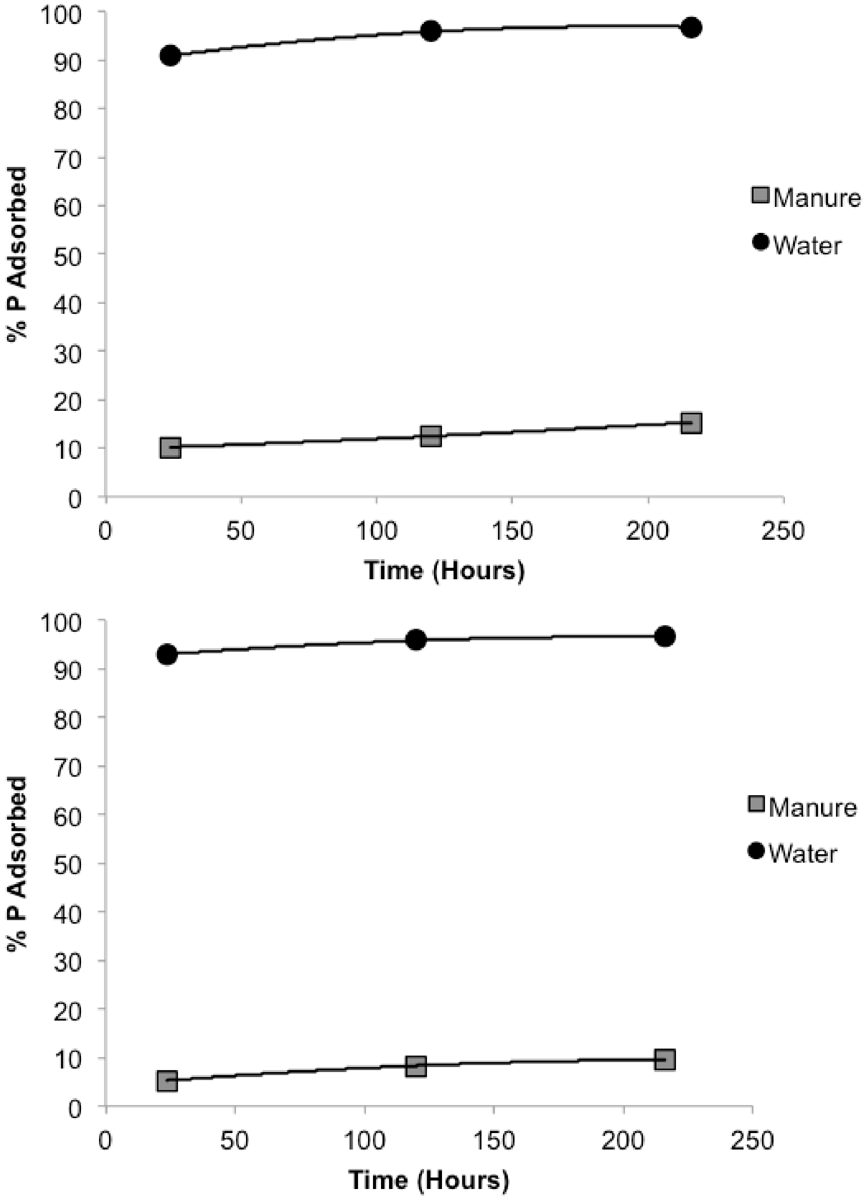

Figure 3.7 - Comparison of percent phosphorus adsorbed on calcite over time between deionized water and 10% manure solution, with 0.5 mL of 10,000 mg L^{-1} P (NaH_2PO_4) added (top) ($p<4.16e-06$), and 1.5 mL of 10,000 mg L^{-1} P (NaH_2PO_4) added (bottom) ($P<8.38e-07$) (n=2). Standard error of mean for all points was < 4.5%.

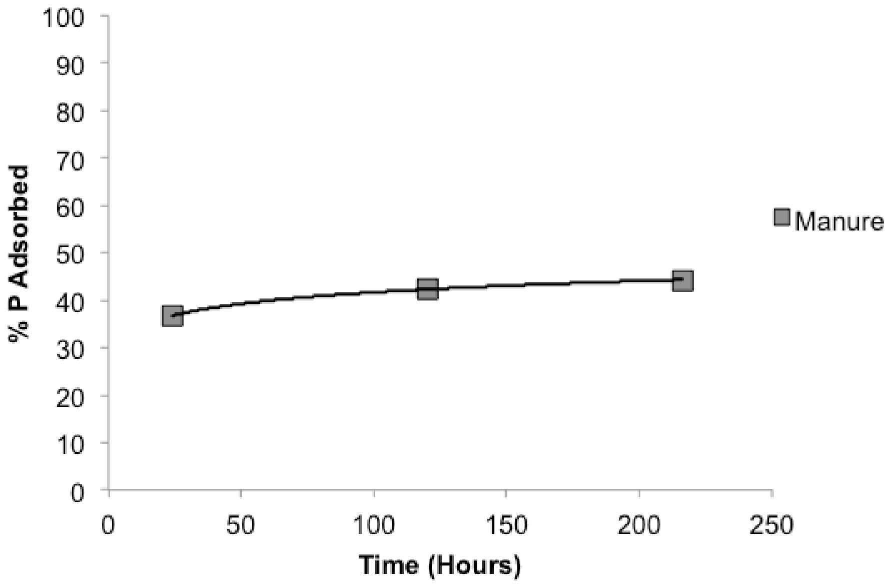

Figure 3.8 - Comparison of percent phosphorus adsorbed on calcite over time between deionized water and 10% manure solution, with no added phosphorus (n=2). Standard error of mean for all points was < 0.04%.

3.3.3 XANES Analysis

3.3.3.1 One Percent Manure Extract Solution

XANES spectra for the 1% manure solution show similar features among all incubations and both solution concentrations (Figure 3.7; Figure 3.8). All spectra collected show a distinct feature at 2154 eV (Figure 3.9; Figure 3.10).

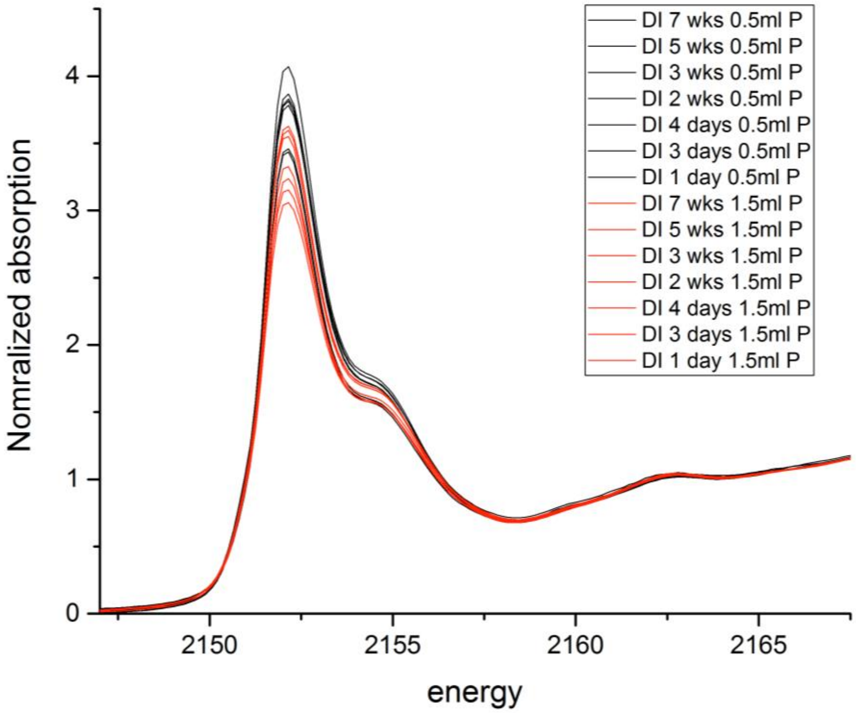

Figure 3.9 - XANES Spectra collected at SSRL for P adsorbed on calcite in deionized water, with either 0.5 or 1.5 mL of 10,000 mg/L P (NaH_2PO_4) added and incubated for 24 – 1176 hours.

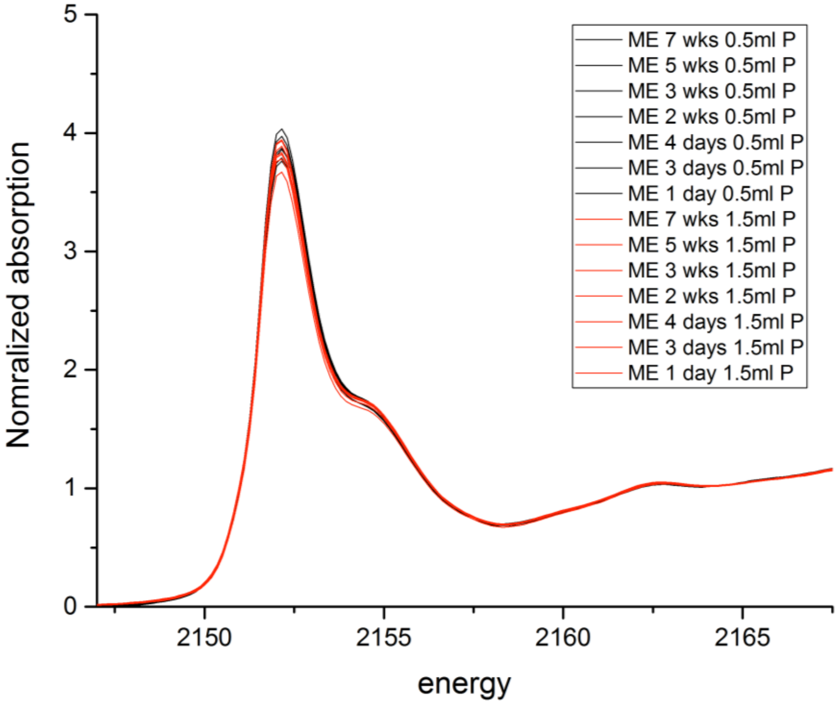

Figure 3.10 - XANES Spectra collected at SSRL for P adsorbed on calcite in 1% manure solution, with either 0.5 or 1.5 mL of 10,000 mg L^{-1} P (NaH_2PO_4) added and incubated for 24 – 1175 hours.

Comparing the collected spectra to the spectra of various P standards shows that the calcite P K-edge XANES are similar to the hydroxyapatite spectrum (Figure 3.11). Differences in white line intensity are due to self-absorption, and do not affect qualitative species interpretation.

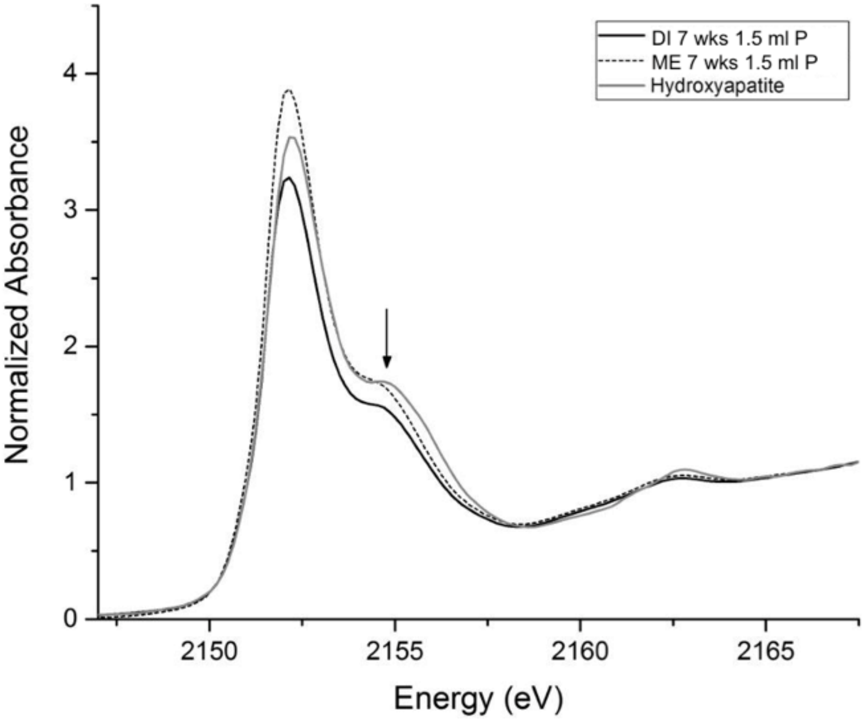

Figure 3.11 - Comparison of XANES spectra for deionized water and 1% manure solution, showing similarity of spectra features to hydroxyapatite standard**.**

3.3.3.1 Ten Percent Manure Solution

XANES spectra for the 10% manure solution show distinct differences in features between the two solution types (Figure 3.12; Figure 3.13). The deionized water solution incubations have the same distinct edge feature at 2154 eV that exist in the 1% manure solution incubation samples (Figure 3.10; Figure 3.11). The calcite samples that were in the 10% manure solution lack this feature (Figure 3.13). However, in the 1.5 mL P addition and manure extract samples, a slight shoulder at 2154 eV does occur, suggesting some hydroxyapatite formation, although less than in the DI systems.

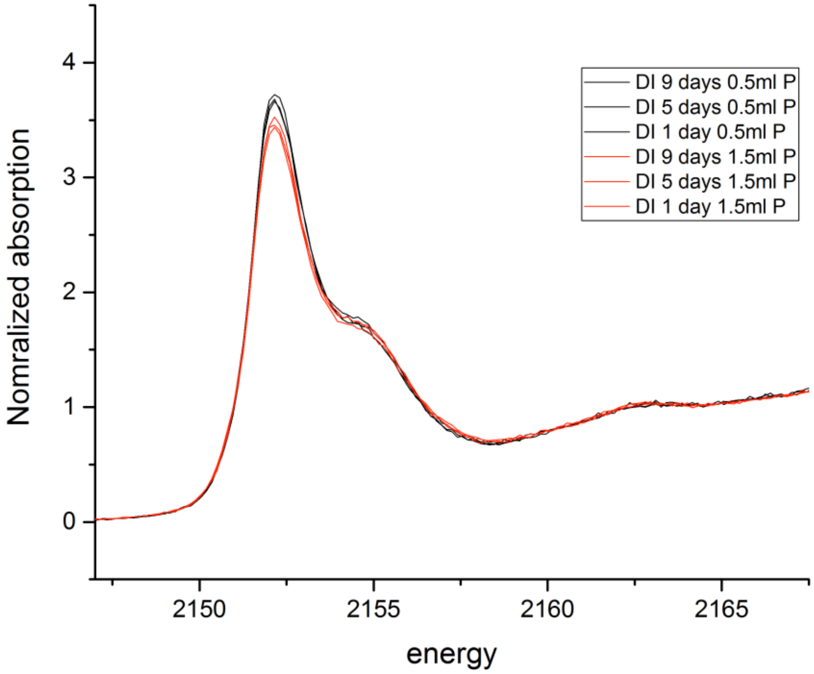

Figure 3.12 - XANES Spectra collected at CLS for P adsorbed on calcite in deionized water, with either 0.5 or 1.5 mL of 10,000 mg L^{-1} P (NaH_2PO_4) added and incubated for 24 – 216 hours**.**

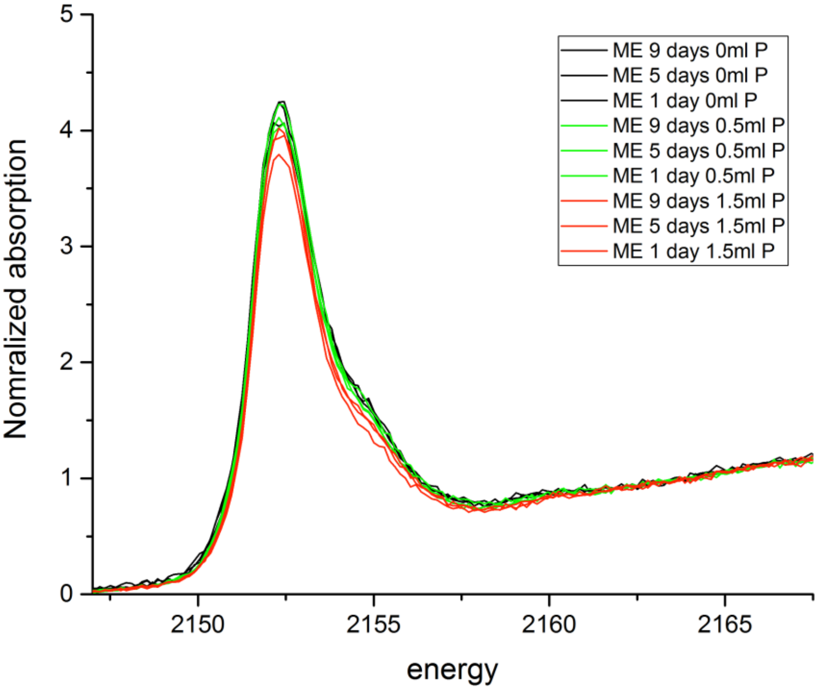

Figure 3.13 - XANES Spectra collected at CLS for P adsorbed on calcite in 10% manure solution, with 0 mL, 0.5, or 1.5 mL of 10,000 mg L^{-1} P (NaH_2PO_4) added and incubated for 24 – 216 hours**.**

Comparing the manure solution samples to apatite show the distinct differences in spectra features in the calcite incubated in 10% manure solution versus the 1% solution, suggesting a strong presence of hydroxyapatite in the 1% solution, but not the 10% manure solution samples (Figure 3.14).

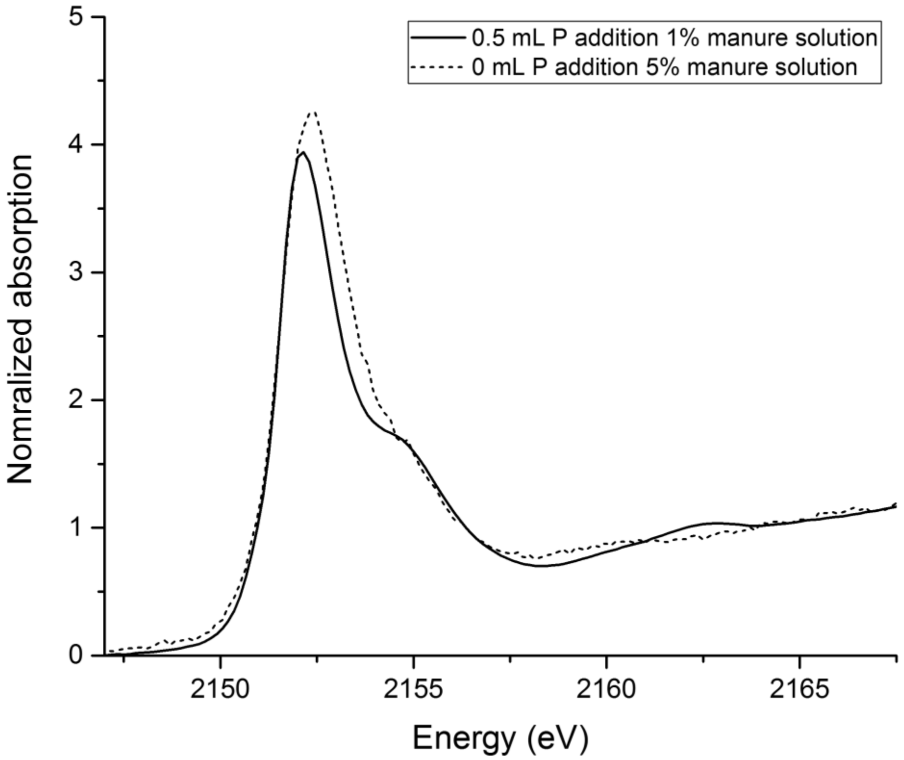

Figure 3.14 - Comparison of XANES spectra for 1% and 10% manure solution, showing similarities and differences in spectra features.

3.3.4 Organic Carbon Analysis

Results of the carbon analysis showed a significant increase in the amount of organic carbon present in solution in the 10% manure concentration compared to the 1% manure concentration (Table 3.3).

Table 3.3 – Comparison of the average organic carbon present in 1% and 10% manure solution.

Solution Type	P	Organic C
	mg L^{-1}	
1% Manure	11.85	43.59
10% Manure	13.60	416.40

3.4 Discussion

The adsorption curves show a clear difference in the amount of P adsorbed when manure is present in solution (Figure 3.3; Figure 3.5). Likely, the organic acids present in the manure extract solution are outcompeting P for sorption sites on the calcite, resulting in a higher P concentration remaining in solution (Table 3.3). These results are similar to research investigating effects of organic matter (i.e., humic acids) on P adsorption on soil minerals (Inskeep and Silvertooth, 1998; Nziguheba et al., 1998; Perassi and Borgnino, 2014; Sindelar et al., 2015). Perassi and Borgnino (2014) batch adsorption experiments to show that the negatively charged carboxyl groups in humic acid adsorb to the positively charged sites on calcite, reducing the adsorption sites available for P. This is the likely mechanism driving the reduced overall adsorption observed in our own study.

Significant differences in the total amount of P adsorbed in the 1% and 10% manure solution systems indicate a strong correlation between the amount of organic matter present and the ability of P to sorb to the calcite. In the 1% manure solution incubation, the difference in total adsorbed P between the manure and water samples is significant, with the manure samples adsorbing a lower amount overall (Figure 3.1). The effect of the manure solution is even more pronounced when the manure:solution ratio increases to 10% (Figure 3.5). The increase to 10% manure solution only added an additional 2.55 – 6.74 mg L^{-1} of phosphorus to the total initial concentrations compared to the 1% manure solutions (Appendix Table B.1; Table B.3). However, the differences in total adsorption between the 10% manure solution and the deionized water samples increased to 99 – 102 mg kg^{-1} (0.5 mL added P) and 325 – 328 mg kg^{-1} (1.5 mL added) (Figure 3.5). The decline in P adsorption with an increase in manure concentration shows that manure inhibits P adsorption and precipitation with calcite.

The XANES results confirm that organic matter in the manure solution impact P speciation. In the 1% manure solution, the organic acids are not present in high enough concentrations to change P speciation (Figure 3.10), which is hydroxyapatite in all samples. However, in the 10% manure solution, observable difference in the spectra existed, suggesting that the manure extract inhibited the formation of hydroxyapatite (Figure 3.13; Figure 3.14). Grossl and Inskeep (1992) used crystal growth experiments to calculate precipitation rates, and found that the presence of organic acids block active crystal growth sites on octacalcium phosphate seed crystals, preventing highly crystalline P forms (such as hydroxyapatite) from forming. Surface adsorption is the first stage in precipitation of mineral forms, so Grossl and Inskeep (1992) proposed that the initial blocking of sites is preventing the formation of crystalline forms.

3.5 Conclusion

P adsorption kinetic experiments on calcite shows that soluble organic matter from manure impedes P adsorption and, at high manure solution ratios inhibits apatite formation. This suggests that if enough organic matter accumulated in calcareous soils from manure applications, P solubility will increase because P adsorption and mineral precipitation would be inhibited. Future studies should quantify how soluble organic matter changes in manure-amended soils so that predictions on their effects on P speciation in soils can be made. Measures of soluble organic matter may prove useful for managing manure application to soils to limit P solubility and contamination of surface waters.

3.6 References

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Chapter 4: Use of Manure as a Crop Fertilizer, and Implications for Agriculture and the Environment

4.1 Introduction

Agriculture is a dominant economic driver in southern Idaho, and plays a vital role in producing food for the increasing global population. It is important to balance national and global food needs with continued environmental protection, and maintaining the ecological quality of our natural systems. Regulating anthropogenic effects on the phosphorus (P) cycle provides a unique challenge, as it is often a limiting nutrient for crop production, but occurs in surface waters in concentrations that decrease water quality (Ulrich et al., 2016). Agricultural activities are a major non-point source of P to surface waters, with long-term negative impacts to water quality. Phosphorus is both a limiting resource and a pollutant, thus managing its use and movement through the environment is particularly challenging.

4.2 Managing Eutrophication

Throughout the world, coastal and freshwater eutrophication continues to be a major environmental problem (Rabalais et al., 2010). Recently in the United States, 51% of the freshwater systems surveyed by the USGS were found to have nutrient levels too high to safely provide basic services including fishing and recreational activities (Dubrovsky et al., 2010). A dominant source of nutrients is agricultural activity (Dubrovsky et al., 2010; Foley et al., 2011). The input of nutrients from agricultural land to freshwater systems remains particularly difficult to regulate due to the high land mass involved, and the diffuse nature of nutrient application (Rabalais et al., 2010).

Eutrophication has long been recognized as a significant threat to water quality (Carpenter et al., 1998). The Natural Resources Conservation Service (NRCS) introduced the

P Index in 1999 to its 590 Nutrient Management Standard to attempt to control P loading to freshwater systems. However, regulations vary drastically between the states, and the standard methods for establishing the P Index may not accurately capture the true risk of a site for P loading to waters (Sharpley et al., 2012). Soil test P (STP) is the typical method used to measure P levels in soils. However, some argue that measuring the degree of P sorption (DPS) would produce a more accurate valuation of risk, as soils with a higher rate of P adsorption would carry a greater concentration of P in the eroded sediments that are transported to surface waters (Sharpley et al., 2012). Regional research has also addressed the difficulty in using standard soil test P methods to accurately model P loading to surface waters via runoff (Turner et al., 2004). Turner et al. (2004) compared the P concentrations from different soil chemical extractions to assess their ability to determine P loading from surface runoff on a catchment scale. A more thorough understanding of how chemical properties within the soil relate to P solubility and accumulation rates can help to more accurately predict P loading to surface waters (Turner et al., 2004).

In addition to properly quantifying P loading risks, numerous barriers exist to enacting best management practices aimed at environmental protection. Conservation tilling and no-till systems are known to reduce erosion rates on farms, but may result in higher costs to farmers (Sedorovich et al., 2007). Other issues further complicate efforts to reduce nutrient emissions from farms, including farmer resistance to new regulations, increased costs to farms to enact policies, and a lack of guidance for those seeking to pursue best practices (Collins et al., 2016).

Better nutrient recycling through improved agricultural practices is one potential way to protect the environment and national P supplies (Metson et al., 2016). Use of livestock manure as fertilizer for crop production would significantly reduce reliance on mined sources of P. Metson et al. (2016) estimate that just 37% of all recycled sources of P could fertilize all corn production in the United States, which is responsible for 54% of all crop production nationally. More efficient application rates could further reduce this demand, allowing US agriculture to reduce its dependency on mined P from domestic and foreign sources.

4.3 Rock Phosphate Costs and Manure Use

Several studies in recent years point to growing costs of using mined sources of P in agriculture (Mew, 2016; Withers et al., 2014). Demand for P has steadily increased over the decades, and prices have risen from under $20/ton in the 1960's to the current price of $120/ton (Mew, 2016). Since current minable P sources were developed on a geologic time scale, rock phosphate is a finite resource that needs to be carefully utilized in order to preserve long-term future availability. This further incentivizes the need to limit P losses along the supply-use chain, beyond the obvious environmental considerations (Mew, 2016).

While the use of manure as a replacement for mined phosphate may help reduce agricultural inputs to aquatic systems through tighter nutrient cycling, long-term applications of manure to the soil may impact P plant availability and transport in a significant way. There is abundant research indicating that continuous application of animal manures as a fertilizer changes the soil characteristics, altering how P moves through the environment (Abdala et al., 2015; Peak et al., 2012; Sharpley et al., 2004). Our research has found further evidence supporting this concept. In order to minimize P losses from the soil, it is important to understand how these amendments may impact the soils they are being applied to.

4.4 Southern Idaho and Phosphorus Management

In the state of Idaho, agriculture is an important economic sector of the economy, and continues to be a major producer of the nation's food supply, and a predominant source of revenue for the state. Representing 30% of the agricultural production in Idaho, dairy companies generated over $6.6 billion dollars in sales in 2012, and provided 23,000 jobs ("Idaho Dairymen's Association," 2016). These economic benefits provide a clear incentive to maintain dairy production at its current high rates for the foreseeable future. However, this would also result in the continued production of high volumes of animal waste in need of proper management and disposal. Use on agricultural fields as a source of vital crop nutrients is a natural solution to this problem.

In recent years, methods have been developed to accurately track P soil losses and manage nutrient loading to surface waters. Vadas et al. (2012) tested the Annual P Loss Estimator (APLE) model, and found it to be a useful tool for farmers to track P movement through the soil. APLE's ability to model P movement relies on input of well defined soil characteristics, including organic carbon and clay content (Vadas et al., 2012). While the quantification of organic carbon does partially account for manure amendments to the soil, it may neglect the full effect manure has on P transformations within the soil. In particular, the influence of organic acids on adsorption and mineralization of P needs to be directly accounted for when predicting P reactions and movement to manure-amended soils. Past research has found that organic acids directly impact P adsorption and mineral development. Our research has confirmed this effect for manure amendments. One output of APLE's model is the rate of P leaching from the topsoil. Our calcite modeling indicates that the organic acids in manure may decrease P adsorption, increasing overall solubility of P in soils.

It is therefore possible that APLE, or other future P loss models may underestimate P losses via leaching if this organic acid-P interaction is not incorporated into the modeling scheme.

The high concentration of calcium in the soils of southern Idaho present an added challenge to P management. The standard method of measuring P (Olsen extractable-P) only captures a relatively small segment of the total P present in the soil. Our research shows significant increases in the non-labile pools of P when manure is applied annually, which is not typically captured in Olsen-P extractions. Sharpley et al. (2012) argue that DPS is a more accurate method for quantifying potential P loss, and the highly calcareous nature of southern Idaho soils means that the potential for P sorption in the soil is incredibly high. Therefore, the potential for P loading may be much greater than what is being captured by standard monitoring techniques, and the true risk may be underestimated. Where possible, utilizing a more in-depth method of soil P quantification (such as the sequential extraction procedure used in this research) may help regulators better assess and predict P inputs to the Snake River; and subsequently develop more accurate management tools.

4.5 Conclusion

As the world population continues to grow, innovative solutions are required to produce the food needed to feed the world while also limiting environmental impacts. Using manure from livestock operations as a nutrient source for crop production provides an opportunity to minimize outside inputs of P via rock phosphate imports and application. It also provides a destination for livestock operations to send their animal waste, potentially lowering the strain of storage and disposal on livestock farms. While it may be economically beneficial to utilize manure in this method, it is important to have a complete understanding of full effects of manure on soils in order to anticipate, manage, and prevent additional environmental degradation. The unique nature of the calcareous soils of southern Idaho compared to typical agricultural soils in the Midwest and Plains states requires continued research to fully capture the regional impacts of manure use as a crop fertilizer. An accurate understanding of the movement of P from soils to surface waters is vital for protecting not only regional water quality, but agricultural productivity as well. The challenge facing both the agricultural industry and environmental regulators in the coming years is to continue to use current research to inform best management practices, balancing agricultural productivity and environmental integrity.

4.6 References

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Appendix A: Supplemental Tables and Figures for Chapter 2

Table A.1 – Average phosphorus concentrations (± standard deviation) determined for each extraction for the quality control soil samples (control plots 206 and 407, year 2013, 0-30 cm depth), collected from Kimberly, Idaho. Extracts are operationally defined to be Extract A = labile P; Extract B = Fe- and Al-bound P; Extract C = Ca-bound P.

Plot	Extraction Year	Extract A	Extract B	Extract C
		mg kg^{-1}		
Control Plot 206 (2013)	**2013**	14.16 (±0.88)	91.66 (±4.18)	621.2 (±16.06)
	2014	3.19 (±0.16)	91.15 (±8.15)	649.3 (±11.14)
	2015	4.28 (±1.26)	75.29 (±1.14)	655.3 (±10.52)
Control Plot 407 (2013)	**2013**	18.97 (±1.27)	101.7 (±4.76)	589.9 (±38.56)
	2014	10.37 (±1.18)	103.64 (±3.43)	679.2 (±18.92)
	2015	10.02 (±1.18)	98.24 (±0.58)	657.8 (±1.16)

Table A.2 - Standards used for PCA and LCF processing of XANES spectra, and the location collected or name of provider.

Standard Type	**Source**
Amorphous calcium phosphate	Dec 2014 SSRL
Carbonate apatite	Dec 2014 SSRL
Dicalcium phosphate dihydrate	Dec 2014 SSRL
Fluoroapatite	Dec 2014 SSRL
Hydroxyapatite (Fluka)	Dec 2014 SSRL
Hydroxyapatite	Dec 2014 SSRL
Octacalcium phosphate (Clarkson)	Dec 2014 SSRL
Tetracalcium phosphate	Dec 2014 SSRL
Tricalcium phosphate	Dec 2014 SSRL
Goethtite	May 2015 SSRL
Hydroxyapatite (TVA)	May 2015 SSRL
HAP diluted (10%)	Sept 2015 CLS
HAP diluted (20%)	Sept 2015 CLS
HAP diluted (5%)	Sept 2015 CLS
Pure hydroxyapatite	Sept 2015 CLS
Zinc phosphate	Sept 2015 CLS
Aluminum phosphate	Werner & Prietzel
Iron phosphate	Werner & Prietzel
Hydroxyapatite	Werner & Prietzel
Phytic acid	Werner & Prietzel
Dicalcium phosphate dihydrate	Derek Peak
Adsorbed P - pH 6.5	Derek Peak
Amorphous Calcium Phosphate	Derek Peak
Apatite	Derek Peak
Brushite	Derek Peak
Monetite	Derek Peak
Ferric Phosphate	Derek Peak
Struvite	Derek Peak
Newberyite	Derek Peak
Whitlockite	Derek Peak
Phytic Acid	Derek Peak

Table A.3 - Fit data of phosphorus speciation determined by ^{31}P-NMR spectroscopy for soil samples from Kimberly, Idaho; samples are from 0-30 cm profile.

Plot	Amendment Type	Year & Depth	Ext P mg/kg	Orthophosphate %	Po %	TotPoly %	IHP %	myo:other %	Monoester P %	Diester P %	M:D %	Cmono %	Cdiest %
104-1	17 Mg/ha	Year 1; 0-30cm	248.7	77.9	22.1	1.4	7.3	1.2	20.0	1.4	14.3	13.0	8.4
104-2	17 Mg/ha	Year 2; 0-30cm	202.3	81.8	18.2	2.1	6.7	0.7	16.1	1.4	11.5	11.5	6.1
106-1	52 Mg/ha	Year 1; 0-30cm	250.0	78.6	21.4	1.4	7.9	0.6	19.3	1.4	13.8	12.5	8.2
106-2	52 Mg/ha	Year 2; 0-30cm	310.1	79.3	20.7	1.4	5.2	1.5	17.9	2.1	8.5	10.3	9.7
107-1	Control	Year 1; 0-30cm	215.0	71.1	28.9	2.1	10.6	0.7	25.4	2.8	9.1	18.0	10.2
107-2	Control	Year 2; 0-30cm	217.3	73.2	26.8	0.7	10.6	0.7	24.7	1.4	17.6	17.7	8.5
108-1	Fertilizer	Year 1; 0-30cm	215.2	74.8	25.2	1.4	9.8	0.4	22.4	2.1	10.7	15.8	8.8
108-2	Fertilizer	Year 2; 0-30cm	225.2	74.2	25.8	1.4	9.6	0.5	23.7	1.4	16.9	16.7	8.5

Table A.4 - Concentrations of elements found in freeze-dried samples of NMR extract.

Soil Sample	Year and Depth	Dried Sample Wt (g)	Extract Al mg/L	Extract Ca mg/L	Extract Fe mg/L	Extract Mg mg/L	Extract Mn mg/L	Extract P mg/L	Extract P mg/kg
Plot 104 (17 Mg/ha)	Year 1; 0-30 cm	0.692	17.4	1409.0	0.322	32.9	0.675	19.9	248.7
Plot 104 (17 Mg/ha)	Year 2; 0-30 cm	0.695	17.7	1334.1	0.258	34.4	0.598	16.2	202.3
Plot 106 (52 Mg/ha)	Year 1; 0-30 cm	0.695	16.4	1437.8	0.288	30.7	0.651	20.0	250.0
Plot 106 (52 Mg/ha)	Year 2; 0-30 cm	0.760	14.8	1434.6	0.307	34.1	0.720	24.8	310.1
Plot 107 (Control plot)	Year 1; 0-30 cm	0.757	18.7	1236.2	0.345	34.0	0.699	17.2	215.0
Plot 107 (Control plot)	Year 2; 0-30 cm	0.688	18.5	1237.0	0.293	35.9	0.648	17.4	217.3
Plot 108 (Fertilizer)	Year 1; 0-30 cm	0.747	19.3	1129.0	0.323	34.1	0.657	17.2	215.2
Plot 108 (Fertilizer)	Year 2; 0-30 cm	0.877	19.3	1117.6	0.340	36.6	0.663	18.0	225.2

Appendix B: Supplemental Tables and Figures for Chapter 3

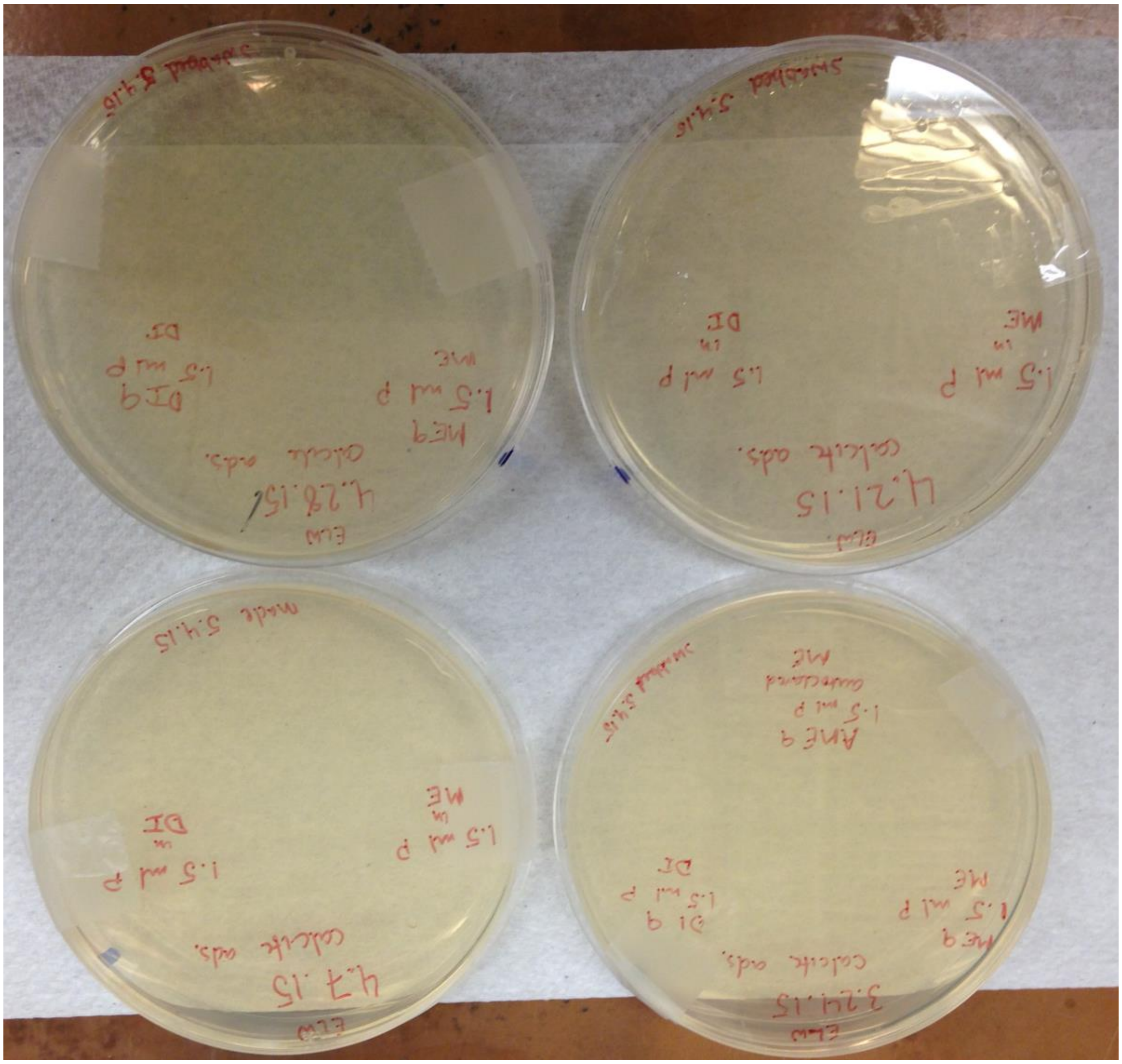

Figure B.1 - Photo of agar plates used to demonstrate successful sterilization of calcite incubation solutions. A subset of samples were swiped on agar plates and allowed to incubate for 72 hours.

Table B.1 - Phosphorus concentrations in the manure solutions prepared for each incubation in the 1% manure solution experiment. Colorimetric data was not collected for 24, 336, and 1176 hour incubations. Organic P determined by subtracting Inorganic P from Total P.

Time (Hours)	Total P	Inorganic P (ppm)	Organic P
24	13.45	-	-
72	12.93	12.40	0.53
96	11.59	10.95	0.64
336	13.00	-	-
504	12.86	13.84	-
840	12.95	13.07	-
1176	12.79	-	-

Table B.2 - Composition of inorganic and organic phosphorus in 1% manure solution following incubation with calcite (24 – 1176 hours). Inorganic P was determined colorimetrically, total P determined using ICP-AES, and organic P found by subtracting Total P – Inorganic P.

Incubation Time (Hours)	Added P (mL)	Total P	Inorganic P (mg/L)	Organic P
24	0	6.6	1.5	5.1
	0.5	24.0	24.9	-
	1.5	28.7	33.9	-
72	0	5.6	1.2	4.4
	0.5	15.5	13.7	1.7
	1.5	22.8	22.0	0.9
96	0	4.9	1.0	3.8
	0.5	17.1	15.3	1.9
	1.5	24.0	23.4	0.6
336	0	5.0	1.1	3.9
	0.5	12.5	11.1	1.3
	1.5	19.3	18.0	1.2
504	0	4.4	0.9	3.5
	0.5	11.9	10.6	1.3
	1.5	17.8	16.5	1.3
840	0	4.3	0.9	3.5
	0.5	11.9	10.1	1.8
	1.5	16.8	14.7	2.1
1176	0	3.9	0.9	3.1
	0.5	12.2	10.7	1.6
	1.5	16.2	15.9	0.3

Table B.3 - Phosphorus concentrations in the manure solutions prepared for each incubation in the 10% manure solution experiment. Organic P determined by subtracting Inorganic P from Total P.

Time (Hours)	Total P	Inorganic P (mg/L)	Organic P
24	18.33	15.78	2.55
120	14.14	9.79	4.35
216	14.16	9.99	4.17

Table B.4 - Composition of inorganic and organic phosphorus in 10% manure solution following incubation with calcite (24 – 216 hours). Inorganic P was determined colorimetrically, total P determined using ICP-AES, and organic P found by subtracting Total P – Inorganic P.

Manure Solution (10%)				
Incubation Time (Hours)	Added P (ml)	Total Solution P	Inorganic P (mg/L)	Organic P
24	0	12	11	1
	0.5	129	115	13
	1.5	373	192	181
120	0	8	6	2
	0.5	122	108	14
	1.5	357	191	166
216	0	8	6	2
	0.5	118	93	25
	1.5	352	165	187