

DAIRY MANURE-COMPONENT EFFECTS ON PHOSPHORUS RELEASE FROM
SANDY SOILS

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2007

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To my parents

ACKNOWLEDGMENTS

With all humility and sincerity, I bow before the Almighty for His benevolence and blessings for the completion of my dissertation.

It is my privilege to express my deep sense of gratitude to Dr. V. D. Nair, Research Associate Professor, Environmental Chemistry, Soil and Water Science Department, University of Florida for excellent guidance, invaluable suggestions and perceptive enthusiasm without which this work would not have its present shape. Her association and moral encouragement and approach to see life perspectives as they are, throughout this academic pursuit, would be an invaluable experience of an everlasting value.

My thanks go to my supervisory committee Willie G. Harris, George O'Connor, Roy D. Rhue, Tom A. Obreza, Luisa A. Dempere, for valuable suggestions and positive criticism during the course of investigation.

I express my sincere thanks to Willie G. Harris for providing excellent laboratory conditions, and abundant supply of peanuts.

I am grateful to my wife Syliva Lang-Josan for her untiring and apt help in reading my manuscript. I am immensely grateful to my parents, brothers and sisters Gagan and Nimar. Their inspiration, sacrifice, helpful blessing, encouragement, support, and loving emotions sustained me. I am thankful to Don Mitchell and Ilona Lang for their nurturing support, and love.

My father is a farmer, has never had a chance to go school. He put all his efforts in me to fulfill his life's ambitions. He provided me the freedom and confidence that I

needed to succeed in my life. So this degree is a commemoration to his dedications and sacrifices that he made in his life.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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May 2007

Chair: Vimala D. Nair
Major: Soil and Water Science

Phosphorus (P) in heavily manure-amended soils can be labile even years or decades after manure input cease. Knowledge of manure-derived components and their associations with P is pertinent to nutrient management for sandy soils with minimum P sorbing capacity. The overall objective of this research was to understand the effects of manure-derived components such as Mg, Si, and dissolved organic carbon (DOC) on P solubility in manure-amended sandy soils. Soil samples (0-25 cm) from manure-impacted areas were collected from the Suwannee and Okeechobee Basins of Florida. The soil release of P, Ca and Mg was studied using repeated water extractions and 1.0 M NH₄Cl extractions. Columns of the soils were leached with deionized water and leachate speciation was modeled using MINTEQ. Solid state assessments of dairy manure and manure-amended soils were done using x-ray diffraction, scanning electron microscopy, and elemental microanalysis. The inhibitory effects of Mg, Si, and manure-derived DOC on Ca-P crystallization were studied by incubating solutions with and without clay-sized solids for 20 weeks. Repeated water and ammonium chloride extractions and speciation of column leachates confirm that sparingly-soluble phases of P associated with Mg and

Ca control P release from the manure-amended soils and maintain elevated P concentrations in soil solutions even years after abandonment of the dairies. Solid state assessments suggested Mg-P and Ca-P associations in dairy manure and manure-amended soils. Formation of the most stable Ca-P mineral, hydroxyapatite, was inhibited by Mg and/or DOC, but not Si, in dairy manure amended soils. Mg-P associations in manure and manure-amended soils could maintain elevated P solubility, and Mg in soil solution could inhibit formation of stable forms of Ca-P. Therefore, consideration of Mg and Ca is necessary to explain the nature of P in manure-amended soils. Preemptive dietary controls to maximize Ca-P and minimize Mg-P in manure would be a strategy to reduce P loss from these soils in the future. Application of Al-based water treatment residuals could minimize the release of P from the manure-amended soils. DOC inhibition of Ca-P precipitation and competitive effects on P sorption reduce prospects for stabilizing P reactions in heavily manure-amended soils.

CHAPTER 1 INTRODUCTION

Continuous release of phosphorus (P) in dairy manure-amended sandy soils even years after manure addition ceases (dairy abandonment) poses both a scientific mystery and an environmental problem (Nair et al., 1995). The USEPA (1996) identified P-induced eutrophication as the most extensive cause of water quality impairment in the USA, and the USGS identified agriculture as a major source of P to surface waters (United States Geological Survey, 1999). Dairy manure accumulation in soils can increase the potential for P loss to surface waters either via erosion (Sharpley & Smith, 1983) or subsurface drainage (Mansell et al., 1991). The P enrichment can cause both surface and sub-surface water pollution (Whalen & Chang, 2001). Many soils effectively retain P, but some sandy soils can be exceptions due to a paucity of P-retaining minerals (Neller et al., 1951; Ozanne et al., 1961; Gillman, 1973; Burgoa, 1991; Mansell et al., 1991; Harris et al., 1996; Nair et al., 1998; Novak et al., 2003). Thus, the stability of manure-derived forms of P is an especially relevant environmental concern in sandy soils.

Soil environmental factors such as pH, the presence of dissolved complexing species and the kind of phosphate mineral present determine the phosphate activity in solution (Lindsay, 1979). Dairy manure-amended sandy soils typically contain large amounts of Ca and P in both solid and solution phases, with accompanying moderately higher pH (Nair et al., 2003) than the native, non-impacted soils. The conditions thermodynamically favor the formation of relatively stable Ca-P minerals (Lindsay,

1979), but the release of P from these soils can be greater than predicted from the solubility of the minerals (Wang et al., 1995). High Mg concentrations in soil solution of heavily manure-amended soils suggest that Mg, in addition to Ca, could control the release of P via a sparingly soluble Mg-P phase (Nair et al., 1995; Josan et al., 2005). Alternatively, Mg can act as an inhibiting cation for Ca-P crystallization on calcium carbonate by masking adsorption sites, in the presence of high P concentrations (Yadav et al., 1984). If the latter were the case, abandoned dairy manure-amended soils should exhibit some Ca-P stabilization after soluble salts (e.g. $MgCl_2$, $CaCl_2$) are leached (Harris et al., 1994). Active dairy manure-amended soils are those that currently receive dairy manure, whereas abandoned dairy manure-amended soils are those soils where dairy activities have ceased for at least 10 years.

Minerals such as vaterite, whitlockite, monetite and struvite in poultry and pig manure possibly control solution P and the majority of Ca and Mg in the soil solutions of soils amended with poultry manure are complexed by dissolved organic matter (Bril & Salmons, 1990). Density separation of the clay fraction of heavily P fertilized loamy soils yielded P-rich particles associated with Fe, Al and Ca (Piersenycki et al., 1990a). Scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS) of poultry manure suggested that sparingly soluble Ca and Mg -phosphate minerals controlled soil solution P concentrations (Cooperband & Good, 2002), the presence of discrete P forms/minerals in dairy manures and dairy manure-amended soils is lacking (Harris et al., 1995; Cooperband & Good, 2002). X-ray absorption near-edge spectroscopy (XANES), a non-destructive chemical speciation technique, was used to assess P minerals and P speciation in “phosphorus-enriched agricultural soils”

(Beauchemin et al., 2003). The authors estimated that most of P was present as octacalcium phosphate (OCP) (45% of total P) and hydroxyapatite (HAP) (11% of total P).

Rationale and Significance

Phosphorus in intensively manure-amended soils can be labile years or decades after abandonment (Nair et al., 1995), and cause P leakage at environmentally-unacceptable rates. Heavy loading of dairy manure to soils, leads to continuous release of P, Ca, and Mg both in active and abandoned dairy manure-amended soils (Nair et al., 1995). If a sparingly soluble Mg-P phase is responsible for the continued release of P from the manure-amended soils, other amendment (e.g. water treatment residuals) applications may be the only way to stabilize the P (O'Connor & Elliott, 2000) because all Mg-P phases are relatively soluble (Lindsay, 1979). Alternatively, reduction of P via dietary management could be a viable strategy to reduce P solubility in the manure (Dou et al., 2003; Cerosaletti et al., 2004). Additionally, if a Ca-Mg-P phase controls the release of P from the soils, it could be subject to dissolution. Ruminant feed contains both Mg and Ca and the Mg and Ca doses are typically exceeding recommended levels (National Research Council, 2001). During heat stress, increased diet potassium and magnesium levels are recommended (Beede & Shearer, 1991).

It is important to document if Ca or Mg or both are initially associated with the high release of P from dairy manure-amended sandy soils. A finding that Mg-P is prevalent would diminish the prospect of stabilization via slow crystallization and transformation of P to more stable Ca-P forms. Also, loss of more soluble forms of Ca after abandonment would diminish the prospect of P released from a Mg-P phase being precipitated as Ca-P.

Knowledge of manure-derived components and their associations with P is pertinent to nutrient management, particularly for sandy soils with minimum P sorbing components. This research probed the forms of Ca-P and Mg-P in dairy manure and dairy manure-amended soils in hopes of identifying phases responsible for long-term P release and developing mitigation approaches to reduce P loss. The overall objective was to understand the role of manure-derived components, specifically Mg, Si, and dissolved organic carbon (DOC), in maintaining high P solubility in active and abandoned dairy manure-amended sandy soils. Specific hypotheses and objectives are given below:

Hypotheses

1. Active and abandoned dairy manure-amended soils release comparable amounts of P because solution P is controlled by sparingly-soluble Mg and/or Ca phosphate phases that require many years for depletion.
2. Concentrations of P, Ca, and Mg are spatially correlated in solid manure and manure-amended soil samples.
3. Activities of DOC, Mg and Si in soil solution of manure-amended soils are sufficient, jointly or separately, to inhibit crystallization of stable Ca-P forms, thus leading toward high P release from the soils.
4. Noncrystalline Si forms (including biogenic Si in dairy manure) can retain P at circumneutral pH and high Ca activity because Ca serves as a bridge between the silicate surface and P.

Specific Objectives

1. Assess the release of P, Ca and Mg in soil solutions of dairy manure-amended soils.
2. Study the associations of P, Ca, and Mg in dairy manure and manure-amended soils using solid state assessments.
3. Study the effects of Mg, Si and DOC on Ca-P crystallization using average concentrations of the species found in manure-amended soil leachates.
4. Study the role of “low-density clay” ($<2 \text{ Mg m}^{-3}$) and “high-density clay” ($\geq 2 \text{ Mg m}^{-3}$) from manure-amended soils on Ca-P crystallization.

Chapter 2 discusses previous researches on P release, geochemical modeling, and solid state assessments in relation to soil and manure environments, and prospective inhibitors of Ca-P crystallization in soil science and other fields. The soil and manure sample collection and characterization procedures are discussed in Chapter 3. The first study (Chapter 4) assesses the release of P, Ca and Mg in soil solution and tested the hypothesis that abandoned and active dairy manure-amended soils release comparable amounts of P because solution P is controlled by a sparingly-soluble Mg-P phase or Ca-Mg-P phase that requires many years for depletion. Testing included repeated water extractions and a column leaching experiment. A selective dissolution study (Chapter 5) was done on manure-amended soils. Various inorganic and organic forms of P were measured and the relationships to dissolved Ca, Mg, Fe, and Al were documented. Chapter 6 describes the associations of P with Ca and Mg in minimally altered samples of dairy manure and manure-amended soils, using x-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and electron probe microanalyses (EPMA). The study was conducted to confirm the associations of P, Ca and Mg observed in solution P chemistry. Results tested the hypothesis that Ca-P, Mg-P or Ca-Mg-P phases exist in dairy manure and manure-amended soils, and are spatially associated at the microscopic level. The results of the column leaching experiment (Chapter 4) and x-ray diffraction studies (Chapter 6) suggested that the dairy manure-derived components can inhibit the formation of stable Ca-P mineral phases. Therefore, the inhibitory effects of dairy manure-derived components on the Ca-P stabilization were investigated using an incubation study (Chapter 7). The final chapter (Chapter 8) summarizes the four individual studies and how they relate to each other.

CHAPTER 2 REVIEW OF LITERATURE

Manure Application

In intensive dairy production areas, including the Okeechobee basin in Florida (FL), more dairy manure is generated than needed to meet crop nitrogen (N) requirements of available crop land. The long-term application of dairy manure at N-based rates in such areas has increased levels of P in soils above the crop needs (Sharpley et al., 1996; Kleinman et al., 2000), and values frequently approach or exceed environmental P thresholds. Lawsuits have been filed in some animal production area, such as in Euchaspavinaw Basin of Ozarks (DeLaune et al., 2006) to restrict the land applications of animal wastes thus leaving surplus at the farm. Sims et al. (2000) recommended the integration of soil P tests into environmentally based agricultural management practices so that optimum levels of manures can be applied to a piece of agricultural land.

Release of P Using Various Extractants

The release of P from manure-amended soils with successive extractions has been studied by several researchers, using different soil extractants. Sharpley (1996) observed that the release of P with 0.01 M CaCl₂ using Fe strips as the adsorbent decreased exponentially with successive extractions. A sequential extraction before and after 15 strip P extractions revealed that majority of P released (46%) was inorganic. Nair et al. (1995) repeatedly extracting manure-amended soils with 1.0 M NH₄Cl solution, and found that \approx 80% of total P in the surface horizons of these soils was labile P (readily soluble P) and associated with Ca and Mg-P forms. Authors speculated that P was loosely

bound with Ca and Mg, probably by some weak adsorption mechanism or as poorly crystalline solids, and as available for sustained leaching under suitable conditions. The classical use of soil P fractionation of Chang & Jackson (1956) use 1.0 M NH₄Cl to effectively remove water soluble and loosely bound P during the initial extractions. The Hedley et al. (1982) P fractionation procedure is broadly adopted to differentiate various organic and inorganic pools of P. Additionally, NH₄Cl extractable P has been defined as loosely adsorbed or easily available P (Psenner & Pucsko, 1988) or easily soluble P (Williams et al., 1967). Cooperband & Good (2002) suggested that “sparingly-soluble” Ca and Mg- P minerals (more soluble than apatite) controlled solution P concentrations in soils amended with poultry manure but were unable to directly identify the forms. He et al. (2004) sequentially extracted 0.25 g of dairy manure in 25 mL of deionized water for 2 hours, and this water extractable P was the largest fraction of total-P. Most of the estimated P was in the inorganic form (12 to 44% of manure total P), and water extractable P was better correlated ($r^2 = 0.62$) to total P than organic P ($r^2 = 0.24$). There have been few efforts to study the associated cations like Ca, Mg, Fe and Al that are released with P in manure-amended soils (Sharpley et al., 2004; Josan et al., 2005; Silveira et al., 2006).

Geochemical Models: Forms and Solubility of Phosphorus

Various computer geochemical speciation models can assist in understanding the forms and P-speciation in waste waters and soil solutions. The most commonly used models are MINTEQ (Felmy et al., 1984), MINTEQA2 (Allison et al., 1990), GEOCHEM (Sposito & Mattigod, 1980), and V-MINTEQ (Department of Land and Water Resources Engineering, 2006, an updated version of MINTEQA2).

All the models assume equilibrium among dissolved species. The assumptions for the equilibrium for aqueous (dissolved) speciation reactions are likely appropriate, because most of the interactions occur very rapidly (Pankow & Morgan, 1981). Most of the ion exchange and adsorption/desorption reactions in well stirred soils systems attain equilibrium within several hours (Mattigod, 1995). These models are called equilibria or solution speciation models and incorporate corrections for activity coefficients and solution complexation reactions and, thus, evaluate the saturation status of the solution with respect to thermodynamically stable solid phases. Many models assume activity coefficients depend only on the ionic strength and ignore specific ion effects. Ion interactions become important above ionic strengths of 0.5 mol L^{-1} , and must be considered (Mattigod, 1995). Some models, like MINTEQ (Felmy et al., 1984) and GEOCHEM (Sposito & Mattigod, 1980), also assume that the solution is in equilibrium with the thermodynamically predicted solid phases. Such models are predictive in nature and are called solution-solid equilibria models. The models modify the solution composition, assuming that only the most stable phase (or phases) can occur and that the solution is always in equilibrium with the solids. Predictive models can also be used as solution speciation models by disabling the solid phase reactions.

Zhang et al. (2001) used MINTEQA2 to predict the nature of aluminum and iron-P fractions in sandy soils of Florida. Wavelite, crandallite, variscite and strengite were predicted to stable in fertilized acid soils, whereas at higher soil pH values, Ca-P minerals were predicted to control P activities in soil solutions. Sharpley et al. (2004) studied the effect of long term manure applications on soil Ca-P forms using MINTEQA2. Ion activity products of soil solutions were calculated using ion activities determined in

extracts of soil reactions for 16 h with 0.01 M CaCl₂ at 1:5 soil/solution ratio. Long term (10-15 y) manure-amended soils were dominated by the more soluble crystalline Ca-P forms tricalcium phosphate and octacalcium phosphates than the less HAP. Shanker & Bloom (2004) cautioned that geochemical modeling results should be supported by solid state assessment techniques. They also observed that

Oversaturation alone is merely a prerequisite for mineral formations; non-equilibrium is the common state in soils and kinetics of precipitation would be an important factor to dictate whether and to what extent various Ca-, Fe-, or Al-P would accumulate in the soil.

Hutchison & Hesterberg (2004) studied the effects of dissolved organic carbon (DOC) as citrate on P dissolution on the soil amended with swine lagoon. With increasing rates of citrate concentrations, dissolved reactive P (DRP) and total Fe and total Al concentrations were increased. Geochemical modeling (V-MINTEQ) predicted 69 to 99% of aqueous Fe(III) complexed as Fe(citrate)⁰, and 87 to 100% of Al(III) complexed as Al(citrate)⁰ and Al(citrate)³⁻. The authors concluded that Fe and Al can be complexed by dissolved organic matter as Al-DOM or Fe-DOM complexes in the system. Silveira et al. (2006) studied P solubility characteristics in a dairy manure-amended sandy soil under same conditions. Using V-MINTEQ as a speciation model for leachates, they observed HPO₄²⁻ (~50% of total soluble P), and Mg-P [MgHPO_{4(aq)}] and Ca-P (CaPO₄⁻) complexes (~30 and 13% of total soluble P, respectively) as the major chemical P species and thus concluded that both Ca-P and Mg-P mineral phases can be a major factor in controlling long term P release in manure-amended soils.

Geochemical models can be valuable tools in describing P speciation in various kinds of soil environments. However, model predictions must be validated with solid state assessments to confirm the presence of a particular mineral phase or a phase

association of P with other metals at micro scale (Mackay et al., 1986; Brennan & Lindsay, 1998).

Solid State Assessments and P Associations

Solid state assessments are less destructive than chemical or solution extractions and can be helpful in providing better description of relationships of P with metals. X-ray diffraction (XRD) produces constructive interference of coherently scattered x-rays, and produces diffraction peaks related to spacing of atomic planes in samples and wavelength of x-rays (Amonette, 2002). X-ray diffraction needs minimal soil preparation; however, the identification of poorly ordered/short range materials is impossible (Harris et al., 1994). Scanning electron microscopy (SEM) provides large depth of fields and requires minimal sample preparation, and particles can be seen at very high resolution (Goldstein et al., 2003). Pierzynski et al. (1990a) successfully quantified the P minerals formed under excessively fertilized soils where total P concentrations ranged from 540 mg kg⁻¹ to 8340 mg kg⁻¹. Researches used XRD, scanning electron microscopy (SEM) equipped with energy dispersive spectroscopy (EDS), and Fourier-transformed infrared spectroscopy to identify P minerals in concentrated clay size fractions obtained from excessively fertilized soils. The use of XRD was unsuccessful in identifying P bearing minerals in the soils. However, SEM identified P-rich particles in the soils with ‘detectable quantities’ of Al, Si, Ca and Fe that were associated with P. Huang & Shenker (2004) studied the solid state speciation of P in stabilized sewage sludge using XRD, SEM, and energy dispersive x-ray spectroscopy (EDXS). The XRD patterns confirmed the formation of brushite, ferrian variscite, calcite and dolomite in ferrous sulfate stabilized sludge. The SEM images also showed an elemental spatial correlation of P and Ca, P and Fe and confirmed the presence of minerals detected in XRD analyses. Using

transmission electron microscopy, Jager et al. (2006) observed the presence of HAP (with significantly broadened XRD peaks), which was confirmed by the solid state nuclear magnetic resonance (NMR) technique. The Ca/P ratio of 1.52 estimated by the NMR studies agreed with Ca/P ratios obtained from chemical analyses of nanocrystalline hydroxyapatite.

Prospective Inhibitors on Ca-P Crystallization

Magnesium is a biologically essential element and reduces heat stress in ruminants (Beede & Shearer, 1991). In the presence of high concentrations of grass K, the animal absorbs less Mg in the rumen and suffers hypomagnesemia (Littledike et al., 1983). As a result, there is a tendency to feed more Mg as feed supplements. Mg affects the crystallinity of synthetic apatite (LeGeros et al., 1989; Bigi et al., 1993) and also inhibits the formation of apatite (LeGeros et al., 1989; Abbona & Franchini-Angela, 1990). An amorphous phosphate phase, magnesium-whitlockite, was observed in the presence of Mg (LeGeros & LeGeros, 1984). Martin & Brown (1997) investigated the effect of Mg on the formation of Ca-deficient HAP [$\text{Ca}_9\text{HPO}_4(\text{PO}_4)_5\text{OH}$, CDHAP]. The progress of the reaction was determined by isothermal calorimetry. They observed two heat peaks during the formation of CDHAP in water at 37.4°C with 10 mM of Mg concentrations. High Mg ion concentrations (3.16 M) resulted in the formation of Newberyite ($\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}$), but no Ca-P mineral phase was observed. Researches concluded that Mg-P complexes are more likely to inhibit the Ca-P formation than magnesium chloride complexes. Mg delays (or prevents) the conversion of the amorphous Ca-P phase into a crystalline P phase (LeGeros et al., 1976). Sahai (2005) modeled the apatite nucleation using crystallography, and NMR and suggested that an outersphere complex of Mg with P formed faster than Ca-P inner-sphere complexes, which inhibited the Ca-P interactions

at the active silanol sites. The behavior of Mg was attributed to a greater charge density for Mg than Ca, which favors greater electrostatic attractions at the active sites. The effect of manure-derived silica on Ca-P interactions has not been studied. In a dental study, Damen & Cate (1992) observed decreased induction time of Ca-P precipitation in the presence of SiO₂, which resulted in spontaneous precipitation of calcium phosphate with a wide range of Ca:P ratios from supersaturated solutions. Addition of organic amendments to calcareous soils increased P solubility with time more than a single addition of inorganic phosphate (O'Connor et al., 1986). Inskeep & Silvertooth (1988) observed that organic acids common to soil environments inhibited HAP precipitation and concluded that organic acids adsorbed on to crystal seeds acting as nuclei for crystal growth. Lindsay et al. (1989) suggested that adsorption regulates P retention at low P concentrations, whereas mineral precipitation controls solubility at high P concentrations. However, Kim et al. (2005) observed that formation of HAP from amorphous calcium phosphate is an internal rearrangement process rather than a dissolution-precipitation process. Long term addition of dairy manure to land not only increases P concentrations but Ca, Mg, Si and DOC also. The effect of these manure-derived components on Ca-P interactions needs to be addressed.

CHAPTER 3 SOIL AND MANURE CHARACTERIZATION

Soil Sampling

Soil samples were collected from manure-amended soils at four active (ACS-1 to ACS-4) and five abandoned dairies (ABS-1 to ABS-5), and four minimally-impacted soils (MIS-1 to MIS-4) from the Suwannee River Basin and Lake Okeechobee Basin of Florida. The active dairy manure-amended soils currently receive dairy manure, whereas the abandoned dairy manure-amended soils no longer receive high levels of dairy manure daily. Abandoned dairy sites were formerly heavily manure-amended and received high manure loads similar to the active dairy sites. Years of abandonment ranged from 12-32 years. Soils were collected by tile spade to a depth of 25 cm, or to the bottom of the Ap horizon (whichever was shallower), from representative locations within the high-intensity areas. Composited Ap horizon soil samples were also taken at each site using a 4.0 cm diameter soil auger for bulk density calculations. Representative soil profiles (to a two-meter depth) were sampled to include Bt/Bh-horizons (when present) for classification and characterization purposes. Parent materials for all soils were sandy marine sediments. Slopes were < 2% and drainage classes were estimated to be poorly- to somewhat poorly drained. Active sites were bare or nearly bare, whereas abandoned sites were grassed. Two of the active sites were on Spodosols and two were on Ultisols. All abandoned sites were on Spodosols. One active site (ACS-1) and one abandoned site (ABS-1) had fill material greater than 25-cm thick, which obviated classification for the purposes of this study. Samples from one active (ACS-4) and one abandoned dairy site

(ABS-2) were supplied by other researchers. Soil profiles descriptions were not confirmed for the two sites, but both sites were located in areas dominated by Spodosols. Soil samples were collected to approximate depths of 25 cm for active and 15 cm for abandoned dairies. All samples were either dried shortly after collection or stored moist under refrigeration. Soils were air dried and crushed to pass 2-mm sieve before use. It also helped in potential screening out CaCO_3 material applied as fill material. All minimally-impacted (MIS 1- 4) soil samples were obtained from other researchers (Graetz et al., 1999).

Physicochemical Properties of Soils (Soil Characterization)

The soil pH, which is an indicator of soil reaction, was measured with a glass calomel electrode assembly using 1:2 soil water suspensions. Soil electrical conductivity (EC, dS m^{-1}) was determined in 1:2 soil:water ratio with the help of a conductivity bridge. Total P and metals i.e. Ca, Mg, Fe, Al, Na, and K in the soils were determined by the procedure outlined by Anderson (1974). One gram of soil was weighed into a 50 mL glass beaker and placed in a muffle furnace at 350°C for an hour. The furnace temperature was raised to 550°C and soil was ignited at this temperature for 2 h. The furnace was allowed to cool for overnight. Few water drops were used to moist the ash and 20 ml of 6.0 M HCl was added with a graduated cylinder and allowed the solution to evaporate slowly on a hot plate (80°C). Additionally 2.25 ml of 6.0 M HCl was added to the digested sample to dislodge the residue and solution was transferred quantitatively (Whatman # 41 filter paper) in to a 50 ml volumetric flask by washing the beaker several times with smaller amounts of DI water. The filtrate was collected in the same flask each along with rinsing the sides of the filter paper into the flask. Phosphorus was analyzed by

ascorbic acid colorimetry (Murphy & Riley, 1962) (U.S. EPA, 1993; method 365.1). All metals were analyzed by atomic absorption spectrophotometry.

Particle Size Fractionation and Mineralogical Analysis

Air-dried soil samples (50 g each) were treated with bleach (10% sodium hypochlorite, adjusted to pH 9.5), at a 1:20 soil:bleach ratio, overnight to oxidize organic matter (Lavkulich & Wiens, 1970). The supernatants were siphoned off and the soils transferred to 250-mL centrifuge bottles for washing (3 times) with 1.0 M NaCl to remove entrained bleach. Samples were then washed with deionized (DI) water (3-4 times) to remove salt, until the supernatant appeared turbid. Deionized water adjusted to pH \approx 9.5 with Na₂CO₃ was added to promote dispersion. Sand was collected by sieving, and clay and silt by centrifugation (Whittig & Allardice, 1986). Oriented mounts for clay were prepared for X-ray diffraction (XRD) by depositing 250 mg of clay as a suspension onto a porous ceramic tile under suction. Clay was saturated on the tiles with Mg and solvated with glycerol following an initial XRD scan. Silt was mounted as an oriented dry powder on a low-background quartz crystal mount.

Manure Sampling

Fresh manure samples (~ 20% solids) were collected from four dairies at different locations in Florida using 5 gallons polyvinyl buckets. The manure samples were dried at 65°C and then ground to pass a 2mm stainless steel sieve.

Manure Characterization

The manure pH, EC, total P and total metals i.e. Ca, Mg, Fe and Al were determined as outlined in the soil characterization section.

Mineralogical Analysis

Manure samples were treated with 10% hypochlorite adjusted to pH 9.5 using large plastic beakers. The manure to bleach ratio was 1:50 (manure-amended soil:bleach ratio was 1:20), as manure contains more organic material than the soils. After treatment to reduce organics the sample were wet-sieved (45 μ M) to separate the sand from silt and clay size fractions. Silt and clay fractions were separated using centrifugation (Whittig & Allardice, 1986). Resulted materials i.e. both silt and clay fractions were scanned for mineral characterization as explained in the soil characterization section.

QA/QC for Analyses

To assure data quality for P and metal analyses for soil and manure samples proper QA/QC procedures were adopted and included 1 blank, 1 replicate, 1 spike, and 1 certified standard after every 15 samples run. All extractions were performed on triplicate samples.

Statistical Analyses

Pair wise comparison by ANOVA of P and metal concentrations of active, abandoned and minimally-impacted soils were done. Mean separations were done by the Waller-Duncan procedure at 5% level of significance. Computations were performed using SAS Institute software (SAS, 2001).

Results and Discussion

Soil Chemical Characterization

Active dairy soils had higher pH values (7.1 – 7.9) than the pH of abandoned dairy manure-amended soils (6.0 – 7.2) (Table 3-1). Higher pH values in manure-amended soils are due to high inputs of Ca and Mg from dairy manure (Kingery et al., 1994; Nair et al., 1995; Iyamuremye et al., 1996; Eghball, 2002) and the buffering effects of added

bicarbonates, organic acids with carboxyl and phenolic hydroxyl groups (Sharpley & Moyer, 2000; Whalen et al., 2000). The minimally-impacted soils had significantly ($p < 0.05$) lower pH values (3.8 – 5.7) than manure-amended soils. Manure-amended soils had significantly greater EC values than minimally-impacted soils (Table 3-1) attributable to the large amounts of Na, Ca, Mg and other salts in the added manure. Average Ca and Mg concentrations for active (Ca = 7086 mg kg⁻¹, Mg = 1292 mg kg⁻¹) and abandoned dairy (Ca = 11082 mg kg⁻¹, Mg = 603 mg kg⁻¹) soils were exceeded than the average Ca and Mg concentrations of minimally-impacted soils (Ca = 301 mg kg⁻¹, Mg = 25 mg kg⁻¹). One of the abandoned dairy soils (ABS-1) had an extremely high total Ca concentration (>35,000 mg kg⁻¹), probably as a result of lime added as a fill material to the soil. Total P concentrations were similar for active and abandoned dairy soils (average ~ 2000 mg kg⁻¹), but much greater than minimally-impacted soils (average ~ 100 mg kg⁻¹). Manure-amended soils and minimally-impacted soils had similar total Al and Fe concentrations, which suggested that the long term addition of dairy manure did not change Al and Fe concentrations significantly ($p > 0.05$) because both Al and Fe are not a major dietary constituent of dairy animals (NRC, 2001).

Soil Mineralogy

Quartz was the dominant mineral in the sand, silt and clay size fractions of all soils except for the one abandoned dairy sample (ABS-1) presumably influenced by fill material (Figures 3-1, and 3-2), in which calcite (CaCO₃) was dominant in the silt and clay. The silt fraction of an active dairy site, also influenced by fill, had high calcite as well. Calcite was detected in all samples and was probably derived from either manure or amendments used to stabilize the soil for heavy animal traffic.

Other minerals present in minor to moderate amounts included kaolinite (two abandoned dairies and one active dairy), smectite (one abandoned dairy), and hydroxyl-interlayered minerals (three active dairies). No phosphate minerals were directly identified in the samples analyzed via XRD. Pierzynski et al. (1990) and Harris et al. (1994) were also unable to identify distinct P minerals in excessively fertilized and dairy manure-impacted soils, respectively. Either the phosphate phases are noncrystalline or mineral concentrations are too low for detection (<1%) without further preconcentration (e.g., selective dissolution, density separation, etc.), or both. The presence of a broad “amorphous hump” on clay XRD plots, observed between 16-20 2θ (Figure 3-1), suggested the presence of appreciable noncrystalline material, probably biogenic silica (Harris et al., 1994) derived from plant phytoliths used as forage to dairy animals. Minimally-impacted soils exhibited the same XRD peaks observed in manure-amended soils, except the presence of hump related to amorphous biogenic Si (Figure 3-3).

Manure Characterization

Manures pH values (Table 3-2) exceeded soil pH (Table 3-1) values, ranging from 8.2 to 8.6. Manure total P concentrations ranged from 5965 to 6137 mg kg^{-1} on a dry weight basis. Kleinman et al. (2005) surveyed the composition of 68 dairy manure samples and reported average total P concentration of 6900 mg kg^{-1} . Manure samples used in this study contained similar averaged (5965 mg kg^{-1}) total P concentrations. The Ca and Mg concentrations ranged from 9899 to 13503 mg kg^{-1} and 2808 to 4281 mg kg^{-1} , respectively and represent significance of P, Ca and Mg. Both Al and Fe concentrations in manure were minimal. On average, manure samples contained 77% organic matter on a dry weight basis, mainly from plant phytoliths used as fodder. Thus, long-term

applications of dairy-manure not only buildup P concentrations in soils, but also result in accumulation of Ca, Mg, and very fine digested plant materials.

Manure Mineralogy

The XRD patterns of clays of four dairy manures from four locations yielded similar results. Irrespective of the management practices, the same kind of mineralogical components were observed (Figure 3-4). All samples dominated by a hump (16-20, 2 θ), which is believed to be biogenic silica derived from plant phytoliths. No P-bearing minerals were detected in the manure samples. Quartz was detected in manure samples, likely due to using sand as bedding material. Calcite was also observed in dairy manures.

Summary and Conclusions

Dairy manures contain higher amounts of P, Ca and Mg than dairy manure-amended soils. The long term addition of dairy manure significantly altered soil chemical properties and nutrient concentrations compared to minimally-impacted (native) soils. Both active and abandoned manure-amended soils had higher pH values than native soils, accompanied by higher electrical conductivity values. There was a significant P buildup in manure-amended soils. The native P retention capacity of sandy soils is low (Mansell et al., 1991), and is almost certainly exceeded by the levels of P loading characteristic of high-intensity areas near dairy barns (Nair et al., 1995 and 1998). Calcium phosphate minerals are predicted to be stable under these conditions using chemical equilibrium modeling (Wang et al., 1995), where as no P-bearing minerals were detected in manure-amended soils. Pre-concentrations of clay size fractions and minimally alteration of soil samples can be helpful in identifying P-mineral phases associated in the soil.

Table 3-1. Characteristics of active and abandoned manure-amended soils, and minimally manure-impacted soils.

Sample ID	pH	^f EC	Total	Total	Total	Total	Total
		dS m ⁻¹	P	Ca	Mg	Fe	Al
		-----mg kg ⁻¹ -----					
^a ACS 1	7.9	0.61	1752	8786	629	845	3184
ACS 2	7.1	0.68	3117	5909	2166	1231	4014
ACS 3	7.5	0.63	3251	8452	1600	1161	2691
ACS 4	7.6	0.62	1215	5197	774	435	311
Mean	7.5a ^e	0.64 a	2334 a	7086 a	1292a	918a	2550a
SD	0.3	0.03	1007	1799	723	363	1590
^b ABS 1	6.8	0.53	2796	35549	1063	869	1860
ABS 2	7.0	0.34	1944	4000	584	820	1237
ABS 3	6.4	0.35	2485	6708	374	194	1184
ABS 4	6.0	0.44	1001	3256	271	358	1528
ABS 5	7.2	0.82	2230	5897	722	347	308
Mean	6.7a	0.50a	2091a	11082a	603b	518a	1223a
SD	0.5	0.20	686	13748	312	306	578
^c MIS 1	5.7	0.21	58	52	23	1055	2854
MIS 2	6.6	0.20	63	642	41	194	136
MIS 3	3.8	0.11	193	446	25	153	122
MIS 4	5.0	0.07	101	62	15	318	1439
Mean	5.3b	0.15b	104b	301b	25c	430a	1138a
^d SD	1.2	0.07	63	292	11	423	1300

^aACS = active dairy manure-amended soil

^bABS = abandoned dairy manure-amended soil

^cMIS = minimally-impacted soil.

^dSD= standard deviation

^eMean values of soil parameters within active, abandoned and minimally impacted soils followed by the same letter in a column are not significantly different ($p > 0.05$).

^fElectrical conductivity (EC) 1:2 soil: water ratio

Table 3-2. Characteristics of dairy manures collected from four locations in Florida.

Sample	pH	Organic Matter ^a	EC ^b	Total P	Total Ca	Total Mg	Total Fe	Total Al
		%	dS m ⁻¹	-----mg kg ⁻¹ -----				
Manure-1	8.2	62	0.92	6071	13052	2808	203	118
Manure-2	8.5	83	0.98	6137	11310	4281	268	667
Manure-3	8.6	84	0.91	5670	13503	3888	147	347
Manure-4	8.5	79	0.87	5983	9899	3054	125	399
Mean	8.5	77	0.92	5965	11941	3508	186	383
^c SD	0.2	10	0.05	207	1658	693	64	225

^aorganic matter determined by loss on ignition

^bEC = Electrical conductivity; 1:2 manure: water ratio

^cSD = standard deviation

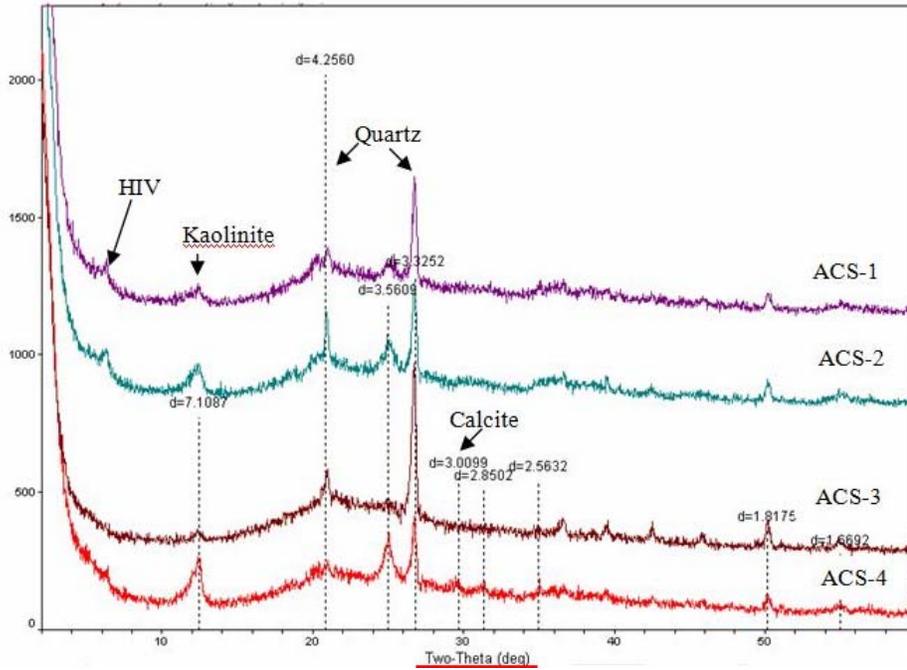


Figure 3-1. X-ray diffraction patterns of clays obtained from active dairy (ACS) manure-amended soils. HIV = hydroxyinterlayered vermiculite.

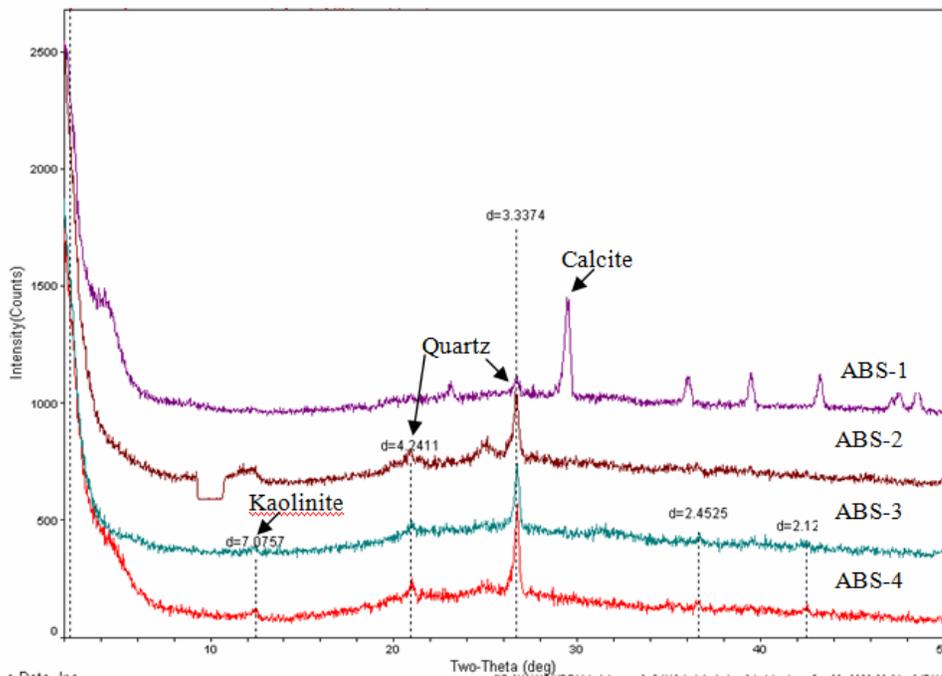


Figure 3-2. X-ray diffraction patterns of clays obtained from abandoned dairy (ABS) manure-amended soils.

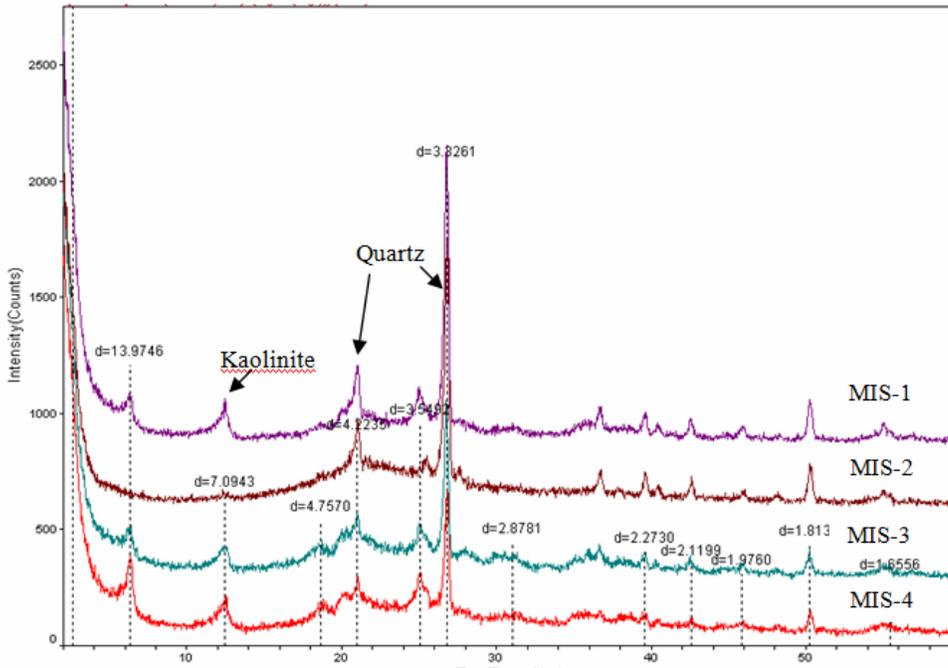


Figure 3-3. X-ray diffraction patterns of clays obtained from minimally-impacted (MIS) soils.

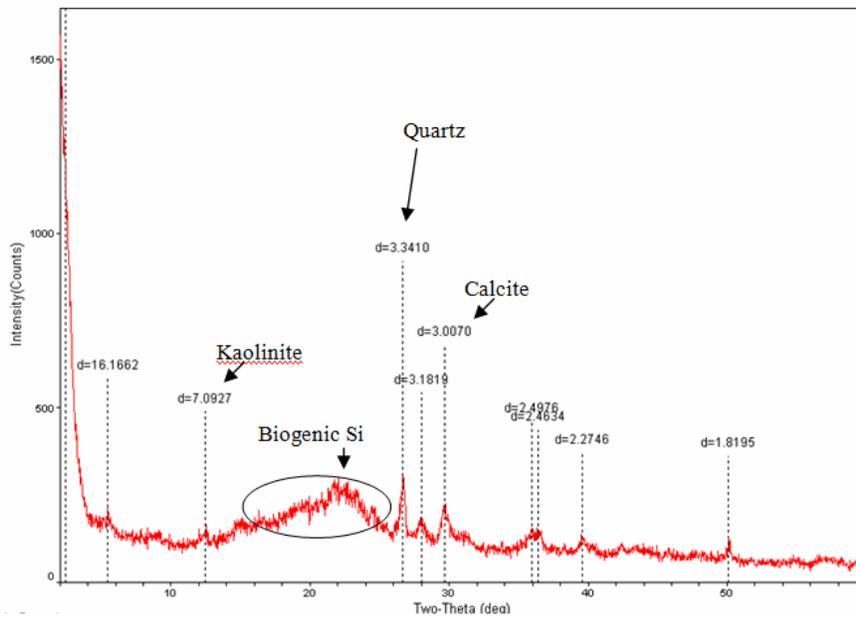


Figure 3-4. X-ray diffraction pattern of clay obtained from an oven dried dairy manure.

CHAPTER 4
ASSOCIATED RELEASES OF PHOSPHORUS, CALCIUM AND MAGNESIUM IN
SOIL SOLUTIONS FROM DAIRY MANURE-AMENDED SOILS

Introduction

Continuous release of P from dairy manure-amended soils enriches adjacent water bodies with P (Graetz & Nair, 1995). Long term addition of manure to soils can alter the chemical and physical characteristics (Nair et al., 1995; Eghball, 2002; Josan et al., 2005; Silveira et al., 2006). Generally, the fate of P in soils is controlled by the inherent soil components; however, excessive manure applications can alter the nature and fate of P forms by the dairy manure-derived components. Several studies of P in dairy manure-amended soils have been conducted. Nair et al. (1995) studied the forms of P in manure-amended soils of south Florida and found that 70% of total P in surface soil was associated with Ca-Mg. Results presented earlier (Chapter 3) showed that the addition of dairy manure in soils increased total Ca, and total Mg concentrations in soils. Therefore, we hypothesized that abandoned and active dairy manure-amended soils release comparable amounts of P because solution P is controlled by a sparingly-soluble Mg-P and/or Ca-Mg-P phase that requires many years for depletion. The hypothesis was tested in two studies. The first study consisted of repeated water extractions (wide soil:solution ratio and minimum soluble salt). The second study was a column leaching experiment, implemented to provide a narrower soil:solution ratio as compared to sequential extractions. The narrower soil:solution ratio was expected to more closely approximate

equilibrium conditions and, therefore, cation and anion concentrations useful to chemical modeling of P speciation in the leachates.

Methods and Materials

Repeated Water Extractions and Chemical Analyses

Soil (20 g) was repeatedly extracted with 200-mL of deionized water (DI) eight times. The soil suspensions were initially shaken for 5 min, and repeated for progressively longer intervals (0, 0.5, 3, 6, 12, 24, 36 and 48 h), for a total of 8, in successive extractions. After each extraction, the samples were centrifuged at 738 x g for 5 min. The supernatants were collected by decanting and filtered 0.45 μm filter. All extractions were conducted at room temperature (25°C). The collected filtrates were analyzed for pH, electrical conductivity (EC), soluble reactive phosphorus (SRP), and total dissolved Ca, Mg, Na, K, Fe and Al.

All P determinations were carried out on a UV-visible recording spectrophotometer at 880 nm wave-length via a molybdate-blue colorimetric procedure (Murphy & Riley, 1962) (U.S. EPA, 1993; method 365.1). The filtrates were analyzed for metals by atomic absorption spectroscopy. Total inorganic carbon and total carbon was determined in all the extracted solutions using a carbon analyzer (TOC-5050A, Shimadzu) (method 5310A, 1992). Total organic carbon in the solutions was determined by difference.

Soil Leaching Characterization and Chemical Equilibrium Modeling

Leachates from surface samples of four active and four abandoned dairy-manure impacted soils were collected using a column approach (Figure 4-1). Ten acrylic columns were constructed with a stopper at one end fitted with glass tube and glass wool. Air-dried forms of the 10 soils (4 active dairy and 4 abandoned dairy manure-amended soils, including two replications from each soil type) were packed in columns to a height of 30

cm and a density of 1.2 g cm^{-3} . The internal diameter of the columns was 5 cm, so the volume occupied by the soil to a depth of 30 cm was $(3.1416 * 2.5 * 2.5 * 30) = 589 \text{ cm}^3$. Therefore the weight of soil in each column was $589 * 1.2 = 707 \text{ g}$. Soil was adjusted to a moisture content of 25% (air dry basis) by very slowly adding 283 mL of DI water. This amount was calculated as $x - 707 \text{ g} = 0.25 x$, where “x” = final weight after water added sufficient to equal 25% of the final soil weight. This amount of water would constitute about 88 % of the pore volume. Columns were subsequently leached by slowly adding 283 mL of DI water over a 2-hour period, 2.35 mL min^{-1} , and allowed to drain for at least 16 hours (overnight). Leachates were collected in 500-mL beakers, using plastic wrap to keep out dust, and transferred to scintillation vials for chemical analyses. A portion of leachates was transferred to 20 mL plastic vials and kept frozen for backup analyses. Leachates were analyzed for metals (Ca, Mg, Na, K, Fe, Si and Al) by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using EPA method 200.7. Soluble reactive phosphorus (SRP) concentrations were measured using the ascorbic acid colorimetry (U.S. EPA, 1993; method 365.1). Chlorides were determined using EPA method 325.2, nitrates by automated colorimetry with the use of ALPKEM Auto-analyzer (U.S. EPA, 1993; method 353.2), ammonium by the semi-automated colorimetry method (U.S. EPA, 1993; method 350.1), and sulfate by ion chromatography with separator AS-14 (DIONEX) (U.S. EPA, 1993; method 300.0). The pH of leachates was determined and the ionic strength (μ) calculated from EC measurements (Griffin & Jurinak, 1973):

$$\mu = 0.013 \times EC \quad (1)$$

Dissolved organic carbon was determined by TOC-5050A, Shimadzu (method 5310A, 1992). Interferences from the inorganic carbon in leachates were first removed by sparging with CO₂-free gas after acidification of the sample (Sharp & Peltzer, 1993).

Visual-MINTEQ version 2.51 (Department of Land & Water Resources Engineering, 2004) was used as a chemical equilibrium model for speciation calculations and solubility equilibrium indices for leachates. The model was chosen over other existing models because of its wide applicability in soil science, its windows-based data input (rather than DOS mode), its extensive thermodynamic database for the P species to be modeled, and its ease of addition and modification of data codes (Mattigod, 1995). The model was set to a charge balance of 30% i.e. the program would be terminated if charge balance exceeded more than 30%. The activity corrections were calculated by Davis equation (Davis, 1962) using Davis 'b' parameter 0.3. The equation is given below

$$\log \gamma_i = -AZ_i^2 \left(\frac{\mu^{1/2}}{1 + \mu^{1/2}} - 0.3\mu \right) \quad (2)$$

where γ_i is the activity coefficient of species 'i' and represents the ratio of the activity of an ion to its concentrations (c_i) (Lindsay, 1979), A is a temperature-dependent constant, with a water value = 0.509 at 25°C, Z_i is the valence of the ion 'i', and μ is the ionic strength define as

$$\mu = \frac{1}{2} \sum c_i Z_i^2 \quad (3)$$

Oversaturated solids were not allowed to precipitate, excluding the infinite solids, finite solids or possible solids. Gaussian model for dissolved organic matter (DOM) was selected to take into the account of the complexation of metals by dissolved organic matter. Both pH-dependency and competition among multiple components that bind with

DOM are considered in this model (Dobbs et al., 1989a,b). This model is easy to use, as it required dissolved organic carbon as input, as compared other two metal-humic complexation models available in MINTEQ.

Results and Discussion

Repeated Water Extractions

The EC values of repeated water extractions were greatest ($p<0.01$) for active dairy soils (Table 4-1), intermediate for abandoned dairy soils, and least for minimally-impacted soils, with no overlap (Figure 4-2). The trend reflects the salts in manure and partial depletion via leaching upon abandonment and cessation of manure loading. Calcium removed by repeated extractions followed the same trend as for EC with respect to site groupings (active>abandoned >minimally-impacted), but differed in that there was no decline with successive extractions for active dairies (Figure 4-3). Active dairy manure-amended soils had more ($p<0.01$) Ca in the eighth extractions (62.6 to 95.3 mg Ca kg⁻¹ soil) than abandoned dairy soils (32.9 to 62.0 mg Ca kg⁻¹ soil) (Table 4-1). The release of Mg and P, in contrast to Ca, was similar ($p<0.01$) for active and abandoned dairy soils; however, like Ca, there was less ($p<0.01$) Mg and P release for the minimally-impacted soils (Figures 4-4 and 4-5). The data are not consistent with the concept of a highly-soluble phase of Mg and P that is depleted with abandonment. Data are consistent with the concept that Mg and P exist mainly in sparingly-soluble form(s), which would require a long time for depletion. The correlation between Mg and P release was much stronger than the correlation between Ca and P release for both active (Figure 4-6) and abandoned dairy (Figure 4-7) soils, suggesting a Mg-P phase in the manure-amended soils. The r^2 value of Mg with P changed from 0.68 to 0.71 for the active dairies and from 0.62 to 0.75 for the abandoned dairies when the first two extracts (most heavily

influenced by soluble salts, including short equilibration time) were removed from the regression equation. Thus the P released from high intensity area dairy soils during repeated water extractions was more closely associated with Mg than Ca release. Release of Mg and P was similar in both active and abandoned dairy sites. The data are consistent with observations that old abandoned sites remain a source of P. Release of Ca was less for abandoned dairies than active dairies.

Soil Leaching and Chemical Equilibrium Modeling

Concentrations of ions column leachates were much greater than in the repeated extractions (mean SRP = 33 mg L⁻¹, Ca = 87 mg L⁻¹, and Mg = 54 mg L⁻¹ in the leachates vs SRP = 4 mg L⁻¹, Ca = 7 mg L⁻¹, and Mg = 3 mg L⁻¹ for repeated water extractions), because of the much narrower soil:solution ratio in the column study (Detailed results of column leachates are presented in Appendix A). The leachates, in contrast to repeated extractions, showed a negative relationship between SRP and Ca and Mg concentrations (Figure 4-8). The trend can be attributed to a common ion effect in the leachates arising from the high concentrations of salts (consistent with high EC in the initial leachates). The use of V-MINTEQ speciation model calculated the saturation index (SI) values of different P minerals. The index represents relative concentrations of ions, and is defined as the difference between the log of the ion activity product (IAP) and the log of the solubility product (Ksp) for a particular solid i.e.

$$SI = \log IAP - \log Ksp \quad (4)$$

Both IAP and Ksp are calculated the same way, and the only difference is that IAP is ratio of the activity of products to reactants measured in soil solutions, whereas the Ksp is the ratio of activity of products to reactants that will be present in soil solution at

equilibrium with a specific mineral (Essington, 2003). SI values were interpreted using the criteria outlined by Bohn & Bohn (1987). According to this criteria if the SI values are $-1 < SI < 0$ for a specified mineral, the leachate is considered to be saturated with respect to that P-mineral. It can also be concluded that this particular P-mineral is present and can control the solubility, provided equilibrium conditions exist. For SI values < -1 , leachate is regarded as undersaturated with respect to the mineral, and the mineral is in disequilibrium and would dissolve. For SI values > 0 , the leachate is considered as supersaturated with respect to the P mineral and if the mineral is present, it can precipitate. Chemical modeling (Table 4-2 & 4-3) indicated that all leachates were supersaturated with respect to all but the most soluble Ca-P minerals (monetite and brushite), whereas all leachates were either undersaturated or near saturation with respect to all Mg-P minerals. The data are consistent with the idea of a sparingly-soluble Mg-P and Ca-P phase controlling P release from the manure-amended soils and that the soil phases would maintain elevated P concentrations in soil solutions even years after abandonment of the dairies. Sharpley et al. (2004) conducted a study on 20 yr old manure-amended silty loam soils. Addition of dairy manure altered the Ca-P chemistry of the soils from hydroxyapatite to tricalcium phosphate and octacalcium phosphate reaction products. Sharpley et al. (2004) concluded that P release from the soils is controlled by these Ca-P forms. However, Sharpley et al. (2004) did not consider the possible role of Mg in controlling P release from the soils. Sylveira et al. (2006) used manure-amended sandy soils of the Okeechobee basin, in a small column leachate study that last for 36 weeks and resulted in ~ 35 pore volume equivalents of leaching. The research concluded that both Ca-P and Mg-P minerals control P release from these

soils. Sylveira et al. (2006) recommended using (Al or Fe-based) water treatment residuals (WTR) to minimize the release of P from the soils. The addition of WTR changes the P release behavior of the soils to adsorption-desorption mechanisms instead of simple dissolution of sparingly soluble P forms associated with Ca and Mg.

Summary and Conclusions

During repeated water extractions, release of P from high intensity area dairy manure-amended soils was more closely associated with the release of Mg than Ca. In addition, release of Mg and P in repeated water extractions was similar for both active and abandoned dairy manure-amended soil samples. Active dairy soils released more Ca than abandoned dairy soils and the EC of the extracts was also higher for active dairy soils. Column leachate data suggested that leachates obtained from this study were supersaturated with respect to the most stable Ca-P minerals and were near saturation with respect to soluble Ca-P minerals. The leachates were either undersaturated or near saturation for all Mg-P minerals considered. In effect, there appears to be no significant highly-soluble phase of Mg and P that becomes depleted with abandonment. Therefore, we concluded that the release of P from manure-amended soils is associated with the release of both Mg and Ca. The solubility of P in sandy soils amended with dairy manure likely controlled by sparingly soluble Mg-P and Ca-P phases, which are expected to continue to release P for a very long time, probably several decades. Abandoned dairies in this study were 12 to 32 years old.

Table 4-1. Cumulative average release of SRP, Ca, Mg and EC in repeated water extractions of active, abandoned and minimally-impacted soils.

Soil Type	N	^d EC (dS m ⁻¹)	^f SRP -----mg kg ⁻¹ -----	Ca	Mg
^a ACS	4	^e 1.11a	495a	691a	271a
^b ABS	5	0.47b	441a	477b	251a
^c MIS	4	0.13c	0.3b	80.1c	5.34b

^aACS = active dairy manure-impacted soil

^bABS = abandoned dairy manure-impacted soil

^cMIS = minimally-impacted soil

^dEC = electrical conductivity

^eMean values of soil parameters within active, abandoned and minimally-impacted soils followed by the same letter in a column are not significantly different using Waller-Duncan procedure at 5% level of significance ($p \leq 0.05$)

^fSRP = Soluble reactive phosphorus

Table 4-2. Saturation indices (SI) in active and abandoned dairy manure-amended soils.

Sample ID	Struvite	Farringtonite	Newberyite	Monetite	Brushite	Whitlockite	OCP ^c	HAP ^d
	-----Mg-P minerals-----			-----Ca-P minerals-----				
	-----SI= log IAP – log Ksp-----							
ACS ^a 1.1 ^b	-2.52	-3.54	-2.28	-1.31	-1.59	1.70	-0.73	10.48
ACS 1.3	-1.26	-2.95	-1.65	-0.54	-0.82	2.70	1.05	11.72
ACS 1.7	-1.70	-2.87	-1.36	-0.23	-0.51	2.87	1.52	11.73
ACS 2.1	-0.77	-1.22	-0.99	-0.38	-0.66	2.96	1.47	12.06
ACS 2.3	-0.89	-0.99	-0.51	0.11	-0.17	3.20	2.20	12.07
ACS 2.7	-1.09	-1.19	-0.54	0.15	-0.13	3.22	2.26	12.06
ACS 3.1	-0.43	-0.84	-1.28	-0.82	-1.10	2.89	0.96	12.36
ACS 3.3	-0.09	-0.18	-0.87	0.14	-0.14	3.06	2.08	11.74
ACS 3.7	-0.70	-1.04	-0.31	0.29	0.01	3.09	2.27	11.66
ACS 4.1	-0.21	-0.85	-1.09	-0.36	-0.64	3.67	2.23	13.52
ACS 4.3	-0.28	-1.03	-0.60	0.17	-0.11	3.62	2.68	12.84
ACS 4.7	-0.42	-1.39	-0.61	0.29	0.01	3.64	2.82	12.77
ABS 1.1	-1.52	-3.65	-1.85	-0.42	-0.70	2.95	1.41	12.09
ABS 1.3	-1.94	-3.65	-0.99	0.37	0.09	4.09	3.35	13.58
ABS 1.7	-1.53	-11.95	-1.02	0.40	0.12	3.46	2.74	12.28
ABS 2.1	-0.96	-3.15	-0.96	-0.12	-0.40	3.48	2.24	12.84
ABS 2.3	-0.88	-1.39	-0.37	0.55	0.27	4.00	3.44	13.22
ABS 2.7	-2.07	-1.12	-0.53	0.46	0.18	3.18	2.52	11.66
ABS 3.1	-1.27	-2.12	-1.48	-0.16	-0.45	4.60	3.32	15.12
ABS 3.3	-1.63	-1.68	-0.86	0.54	0.26	3.46	2.88	12.16
ABS 3.7	-2.26	-3.05	-9.10	0.51	0.23	2.77	2.16	10.80
ABS 4.1	-1.60	-3.45	-1.15	0.10	-0.18	2.66	1.62	10.91
ABS 4.3	-1.47	-3.82	-0.67	0.66	0.38	3.06	2.61	11.22
ABS 4.7	-1.93	-3.79	-0.85	0.49	0.21	2.59	1.96	10.45

^aACS = active dairy manure-impacted soil; and ABS = abandoned dairy manure-impacted soil

^b1.1, 1.3, and 1.7 corresponds to 1st, 3rd, and 7th leaching events of soil.

^cOCP = Octacalcium phosphate {Ca₄H(PO₄)₃.3H₂O}

^dHAP = Hydroxyapatite Ca₅(PO₄)₃(OH)

Struvite: NH₄MgPO₄·6H₂O

Farringtonite: {Mg₃(PO₄)₂}

Newberyite: (MgHPO₄·3H₂O)

Monetite: (CaHPO₄)

Brushite: (CaHPO₄·2H₂O)

Ca-whitlockite: {Ca₃(PO₄)₂} (β)

Table 4-3. Percent of observations that are undersaturated, saturated, and supersaturated for selected minerals^a based on chemical equilibrium modeling of active and abandoned dairy column leachates.

Soils/Minerals	Struvite NH ₄ MgPO ₄ ·6H ₂ O	Farringtonite Mg ₃ (PO ₄) ₂	Newberyite MgHPO ₄ ·3H ₂ O	Monetite CaHPO ₄	Brushite CaHPO ₄ ·2H ₂ O
Undersaturated					
Active dairies	32	57	32	4	11
Abandoned dairies	89	100	21	0	0
Saturated					
Active dairies	68	43	68	32	64
Abandoned dairies	11	0	79	11	21
Supersaturated					
Active dairies	0	0	0	64	25
Abandoned dairies	0	0	0	89	79

^aBased on comparison of computed saturation index (SI) from ion activity product (IAP) and solubility product (Ksp) (Bohn & Bohn, 1987).



Figure 4-1. Column set-up used for the column leaching study.

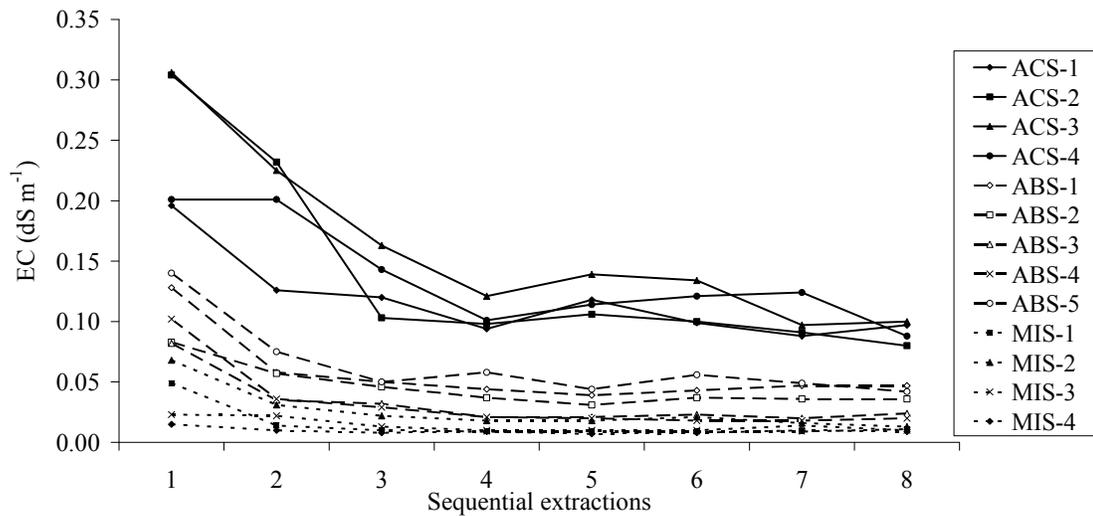


Figure 4-2. Changes in electrical conductivity (EC) (dS m^{-1}) with repeated water extractions. ACS = active dairy manure-impacted soil; ABS = abandoned dairy manure-impacted soil; MIS = minimally impacted soil.

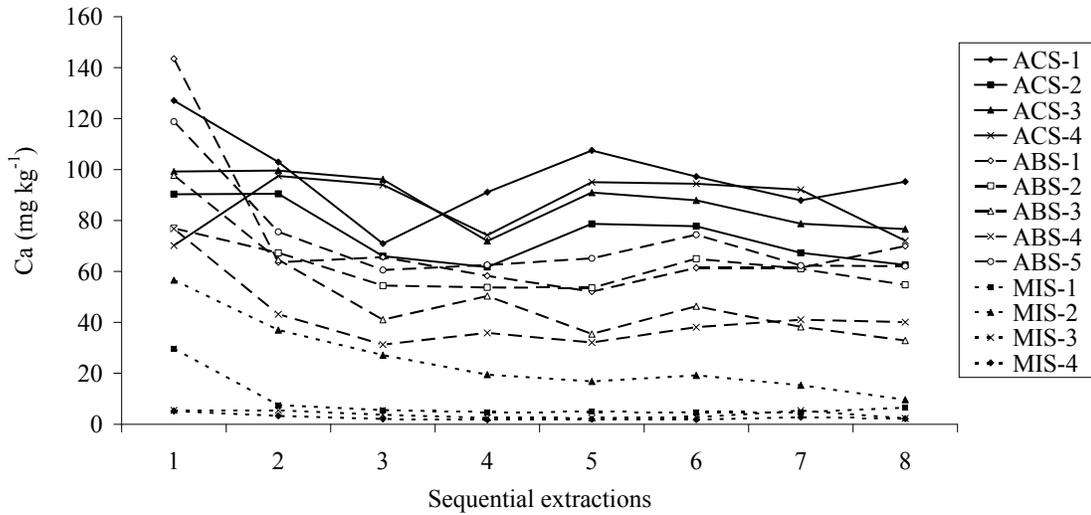


Figure 4-3. Changes in Ca concentrations (mg kg⁻¹) with repeated water extractions. ACS = active dairy manure-amended soil; ABS = abandoned dairy manure-amended soil; MIS = minimally impacted soil.

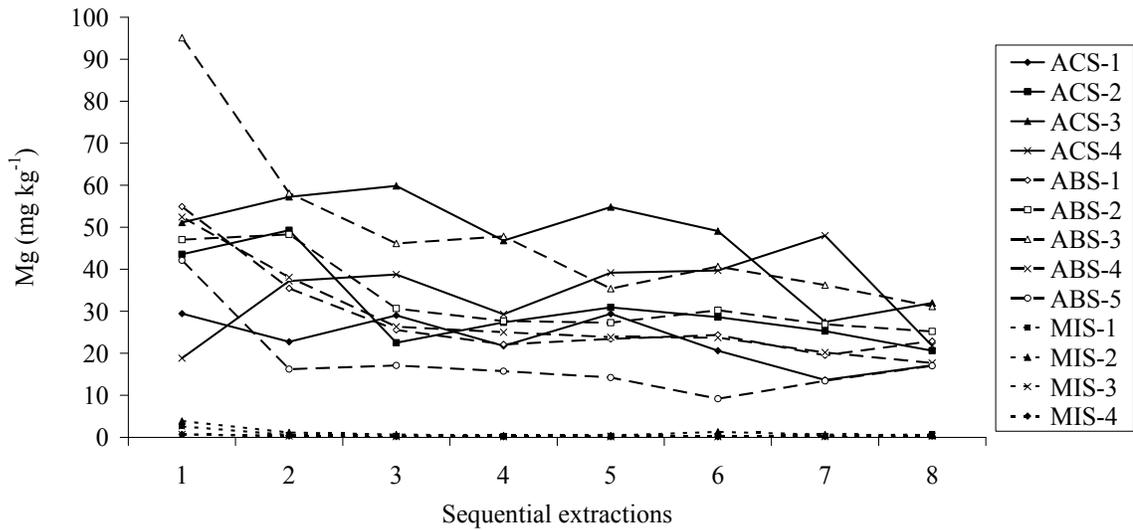


Figure 4-4. Changes in Mg concentrations (mg kg⁻¹) with repeated water extractions. ACS = active dairy manure-amended soil; ABS = abandoned dairy manure-amended soil; MIS = minimally impacted soil.

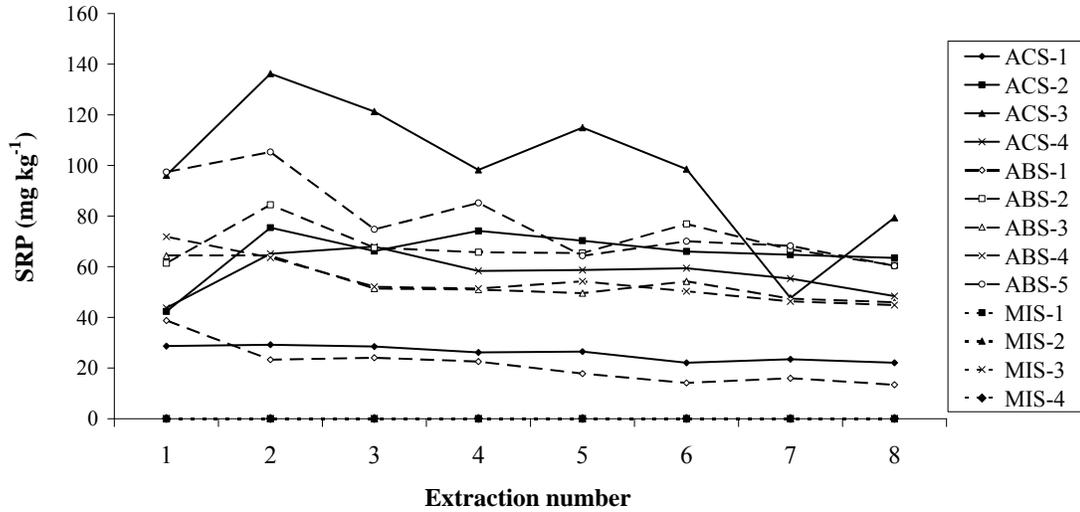


Figure 4-5. Changes in soluble reactive phosphorus (SRP) concentrations (mg kg^{-1}) with repeated water extractions. ACS = active dairy manure-amended soil; ABS = abandoned dairy manure-amended soil; MIS = minimally impacted soil.

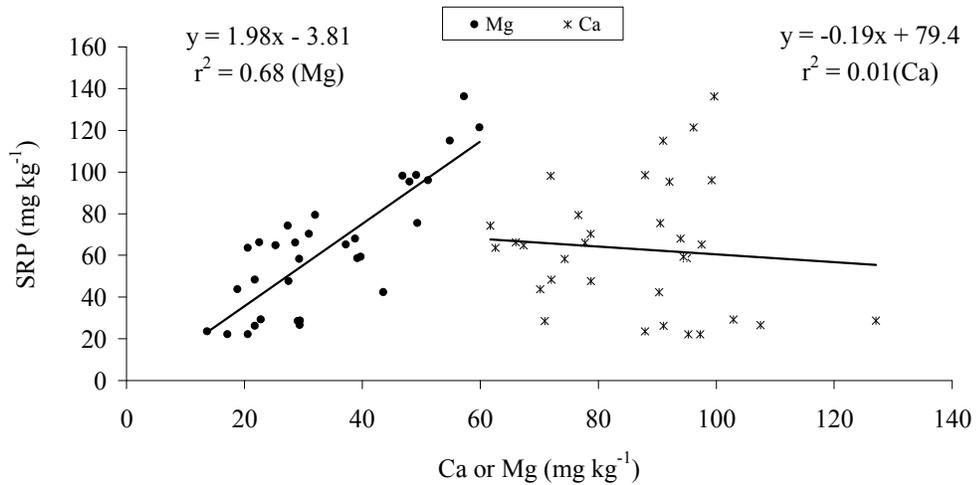


Figure 4-6. Relationships between soluble reactive phosphorus (SRP) and Mg and Ca released during repeated water extractions of active dairy manure-amended soils.

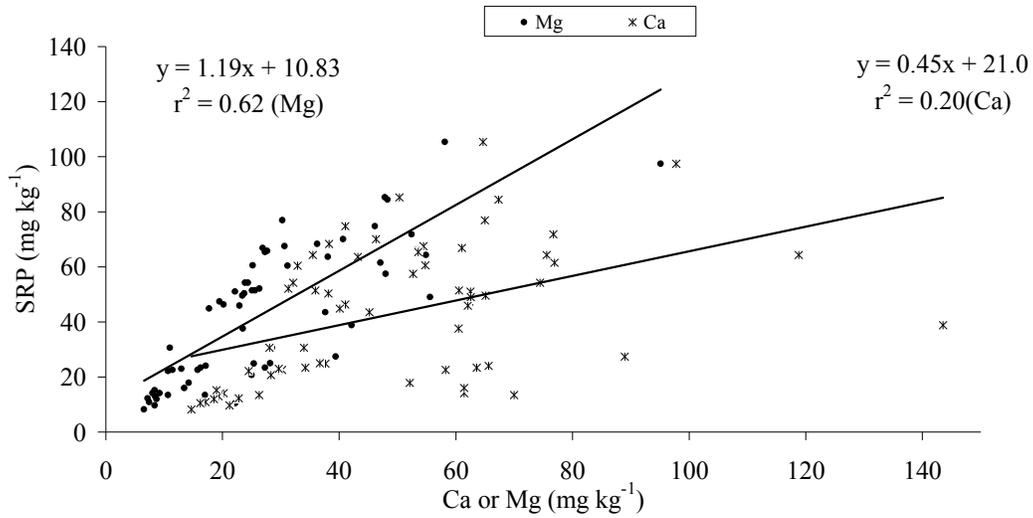


Figure 4-7. Relationships between soluble reactive phosphorus (SRP) and Mg and Ca released during repeated water extractions of abandoned dairy manure-amended soils.

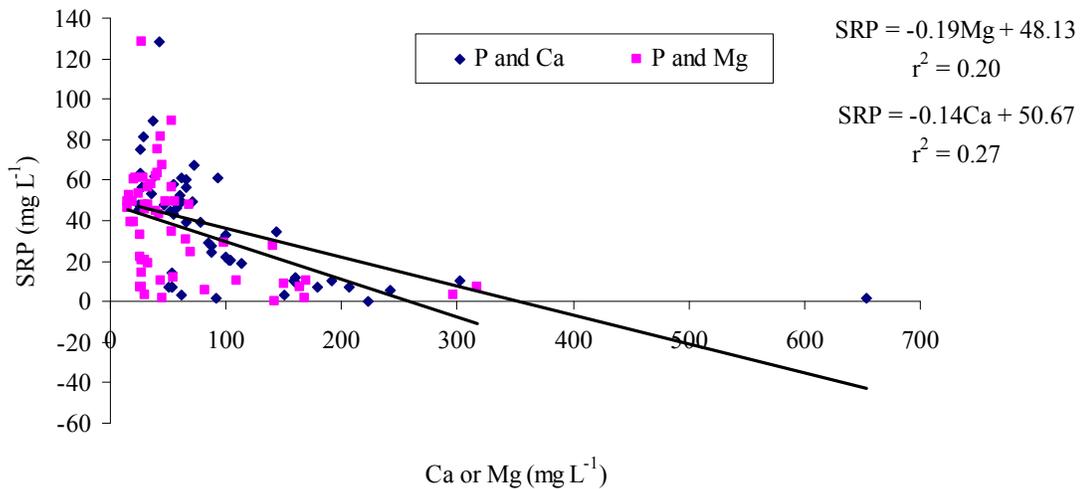


Figure 4-8. Relationships between soluble reactive phosphorus (SRP) and Mg and Ca released during the column leaching of dairy manure-amended soils.

CHAPTER 5
RELATIONSHIPS BETWEEN PHOSPHORUS, CALCIUM AND MAGNESIUM
INFERRED FROM SELECTIVE DISSOLUTION

Introduction

Repeated water extractions can provide valuable information about the release pattern of different P phases that may exist in manures or manure-amended soils. The use of 8 repeated water extractions to study the release of P from the soils was demonstrated in Chapter 4. The data suggested that water can extract P indefinitely from the dairy manure-amended soils. Graetz & Nair (1995) used 10 extractions with DI water to extract P from dairy manure-amended soils. From 2 to 18% of total soil P was extracted, and the authors concluded that this “labile-P” will solubilize in several years.

The P concentrations in the 8th repeated water extraction used in this study ranged from 20 mg kg⁻¹ to 80 mg kg⁻¹, and similar concentrations were expected to be released indefinitely. In another experiment, Silveira et al. (2006) used 40 repeated water extractions to deplete P concentrations to undetectable levels from dairy manure-amended soils. Such experiments are time consuming. Using a higher ionic strength solution, for example 1.0 M ammonium chloride (NH₄Cl) (Chang & Juo, 1963; Hieltjes & Lijklema, 1980) or 1.0 M potassium chloride (KCl) (Reddy et al., 1998), can simultaneously extract both “soluble and exchangeable P” using fewer number of extractions.

In addition to repeated extractions, sequential extraction techniques are used widely to obtain information of various P forms in soils, manures, deposited sediments (Chang &

Juo 1963; Martin et al., 1987; Magnus et al., 1988; He et al., 2005). Sequential extraction procedures use chemical reagents with varying degrees of chemical strengths. The first step in these procedures is to extract or dissolve easily soluble components or forms of nutrients relatively available to plants (Chang & Juo, 1963). Sequential extraction procedures have been used extensively to make inferences about the fate of P. The continuous release of P by sequential extractions with NH_4Cl had been demonstrated for dairy manure-amended soils (Graetz & Nair, 1995; Nair et al., 1995) and for the lake sediments (Pettersson & Istvanovics, 1988). Fewer authors have reported the concomitant release of Ca, Mg, Fe and Al along with P for different kinds of sequential extraction studies (Nair et al., 2003; Sharpley et al., 2004; Silveira et al., 2006). Generally, the first two extractions efficiently dissolve CaCO_3 during successive extractions by 1.0 M NH_4Cl (Hieltjes & Lijklema, 1980).

In this study, both repeated and sequential extractions were used to document the release of P, as well as the release of Ca, Mg, Fe and Al. This work is based on a study conducted by Nair et al. (2003). Authors used 1.0 M NH_4Cl to study P release characterization of manure and manure-amended soils. Ammonium chloride was tested for its ability to selectively extract the Mg-P phase first; however, this does not preclude NH_4Cl extraction of Ca-P particularly in the first few extractions. It was hypothesized that the repeated use of NH_4Cl can extract P and associated cations preferentially Ca and Mg.

Material and Methods

Phosphorus Fractionation: Repeated and Sequential Extractions

For this study, two soil samples from active dairies (ACS-2 & ACS-4), and two samples from abandoned dairies (ABS-3 and ABS-4) were selected (Table 3-1) as these

samples consisted relatively lower amounts of CaCO_3 . The soil phosphorus (P) fractionation scheme used 1.0 M NH_4Cl , 17 h for 0.1 M NaOH , and 24 h for 0.5 M HCl solutions for sequential P extractions (Hieltjes & Lijklema, 1980) and modified by Nair et al. (1995). A 1:10 soil/solution ratio was used for all extractions. All extractions were carried out at 298 K. In pilot study, ten repeated NH_4Cl extractions were sufficient to reduce Mg concentrations to undetectable ($< 1.0 \text{ mg L}^{-1}$). Thus soil samples were extracted with 1.0 M NH_4Cl adjusted to pH 7.0 (using 0.5 M KOH) from 1 to 10 times before being extracted with 0.1 M NaOH , and finally with 0.5 M HCl . For example, a soil sample was extracted once with NH_4Cl before being extracted by NaOH and HCl , and another was extracted twice, another three times, another four times and so forth, up to ten times with NH_4Cl , before conducting the subsequent extractions with NaOH and HCl (Figure 5-1).

The NaOH fraction is expected to extract Fe+Al associated P and the HCl fraction, Ca+Mg associated P (Chang & Jackson, 1957; Williams et al., 1967; Hieltjes & Lijklema, 1980; Hedley et al., 1982; Psenner & Pucsko, 1988). Soil samples were shaken end to end in 30 mL polypropylene centrifuge tubes for 2 h for 1 M NH_4Cl extractions, 17 h for 0.1 M NaOH and 24 h for 0.5 M HCl extractions. The suspensions were centrifuged for 15 min @ 3620 x g and then filtered (0.45 μm) filters to obtain solutions for analyses. Following the sequential extractions, soil samples were ashed and digested with 6 M HCl to obtain the residual P fraction (Andersen, 1976). Residual P fraction represents the stable P forms, and is often considered to be organically bound (Hedley et al., 1982). Entrained solution after each extraction was determined by weight and

corrections were applied during the final calculations of P, and metal concentrations in each fraction.

Analyses of P and Metals

The 1.0 M NH₄Cl, 0.1 M NaOH, and 0.5 M HCl extracts were analyzed for both inorganic-P (Pi) and total dissolved P (TDP). The TDP was determined by potassium persulfate (K₂S₂O₈) digestion method (EPA method 365.1, 1993). The potassium persulfate used in this study was a certified ACS standard (Fisher Catalog No. P282-500). The TDP of the 1.0 M NH₄Cl, 0.1 M NaOH and 0.5 M HCl extracted solutions was determined by adding 1 mL of 5 M H₂SO₄ + 0.3g of K₂S₂O₈ to 5 mL aliquots of each filtered extract in digestion tubes. Samples were digested by placing digestion tubes on a digestion block at 125-150°C for 2-3 h so that a 0.5 mL of solution remained. The digestion tubes were then covered with glass digestion caps and temperature raised to 380°C for 3-4 h. Digested samples were cooled, diluted with 10 mL of DI water and vortexed to ensure thorough mixing. The solutions were then stored in 20 mL scintillation plastic vials at room temperature for analyses. A series of P standards were also digested to obtain similar matrix effects for colorimetric determinations. Proper QA/QC protocol was adopted and included a blank, a duplicate, a certified QC after every 15 samples during each digestion run.

Inorganic-P was determined from solutions obtained by filtering (0.45µM) undigested centrifuged supernatant. Phosphorus (both TDP and Pi) was analyzed using ascorbic acid colorimetry (Murphy & Riley, 1962) (U.S. EPA, 1993; method 365.1). The difference between TDP and Pi yielded the organic-P pool (Po). Concentrations of Ca, Mg, Fe, and Al were determined in both digested and undigested samples by atomic absorption spectrophotometry.

The residual fraction obtained after the 0.5 M HCl extraction was transferred to labeled glass beakers and was digested with 6 M HCl after heating in a muffle furnace for 2 h at 623 K and then raising the temperature to 823 K for 3 h (Anderson, 1974). Both P and metals including Ca, Mg, Fe and Al were analyzed in the residual fraction of digested samples.

To assure data quality for P and metal analyses proper QA/QC procedures were adopted and included 1 blank, 1 replicate, 1 spike, and 1 certified P standard after every 15 samples run. For atomic absorption analyses 1 blank, 1 replicate, 1 spike and 1 standard were analyzed after every 15 samples. The standard curve for each metal was calibrated by resloping after 15 samples.

Results and Discussion

Release of P, Ca, and Mg in Repeated 1.0 M NH₄Cl Extractions

The use of 1.0 M NH₄Cl extracted more P, Ca and Mg compared to repeated water extractions. The release of P from active dairy soils declined more sharply with repeated NH₄Cl extractions than the abandoned dairy manure-amended soils, and then leveled off (Figure 5-2). For active dairy soils the first three extractions released an average of 65% of total summed P obtained in 10 repeated NH₄Cl extractions. In abandoned dairy soils, an average of 40% of total summed P was released. The data suggested that P in abandoned dairy soils became stabilized with time and had leached from soils following abandonment. The soils released comparable amounts of P using repeated water extractions and P concentrations did not show a declining trend (Figure 4-1). For repeated NH₄Cl extractions active dairy soils behaved differently than the abandoned dairy soils. The NH₄Cl extractable P has also been recognized as labile or loosely bound-P (Chang & Juo, 1963; Hieltjes & Lijklema, 1980; Graetz & Nair, 1995; Nair et al., 1995; Nair et al.,

2005). During repeated NH_4Cl extractions, both Ca and Mg concentrations declined for all soils. ACS-2 soil (Figure 5-3) released significantly greater amounts of Ca compared to the other three soils. Release of greater Ca concentrations in ACS-2 soil sample after 10 repeated NH_4Cl extractions was attributed to the presence of high amounts of CaCO_3 in the soil (confirmed by X-ray diffraction Figure 3-2), which was dissolved by 1.0 M NH_4Cl (Hieltjes & Lijklema, 1980). A soil with high amounts of CaCO_3 is expected to release Ca for long periods. The Mg concentrations in the first two repeated extractions declined sharply and then leveled off after five extractions for all soils (Figure 5-4).

The use of NH_4Cl exhaustively extracted both Mg and P in the initial repeated extractions of active dairy soils, which suggests a probable sparingly soluble Mg-P phase. Omitting data for the 1st extraction, the remaining data were subjected to regression analyses both for the active and abandoned dairy soils (Table 5-1).

Data suggest that the release of P was a linear function of Ca+Mg release for the active dairy soils, whereas the release of P is a logarithmic function of Ca+Mg release for the abandoned dairy soils. The correlation coefficients for soils ACS-2 and ACS-4 were 0.946 and 0.996, respectively, showing there was little unexplained variance in the regression. The release of both Ca and Mg with each other were also significantly correlated ($r = 0.855$ for ACS-2 and $r = 0.989$ for ACS-4), consistent with a specific pool of Ca-Mg-P phase(s) in the soils, responsible for a continuous P release. The suspected mineral phase could be amorphous whitlockite (a partially Mg substituted calcium orthophosphate). The presence of high amount of DOC can impede the crystal growth of whitlockite, acting as a buffer for maintaining this sparingly soluble phase in amorphous form (Inskeep & Silvertooth, 1988).

Phosphorus Concentrations in Sequential Extractions

Forms of P extracted by 1.0 M NH₄Cl (Pi and Po), 0.1 M NaOH (Pi and Po), 0.5 M HCl (Pi and Po), and in the residual P pool for the two active (Figures 5-5 and 5-6) and two abandoned dairies (Figures 5-7 and 5-8) are depicted. Both NH₄Cl-Pi and NH₄Cl-Po forms increased for all soils with repeated NH₄Cl extractions. The NH₄Cl-Pi was significantly greater than the NH₄Cl-Po in all soils, and Pi fraction dominated all extractions.

Nair et al. (1995); Gale et al. (2000); Sharpley et al. (2004); and Silveira et al. (2006) reported similar dominance of inorganic-P over organic-P in soils following longterm amendments. The cumulative increase of P with repeated NH₄Cl extractions can be attributed to the loosely bound P forms in dairy manure-amended soils, and suggests that one or two NH₄Cl extractions would not be enough to study P behavior of these soils. The percentage release of P from ACS-2 soil with repeated NH₄Cl extractions was significantly smaller than from the ACS-4 soil. For example the percentage NH₄Cl-Pi released for ACS-2 soil (Figure 5-5) during 1st, 5th and 10th extractions was 9%, 20%, and 25%, respectively, whereas for ACS-4 soil (Figure 5-6) the release was 25%, 64%, and 75%, respectively.

The relative low P release in NH₄Cl repeated extractions for ACS-2 soil can be attributed to the resorption of P by the presence of relatively high amounts of CaCO₃ applied as a fill material to stabilize land for proper dairy operations. Accordingly, the NaOH-Pi fraction for ACS-2 soil (Figure 5-5) exceeded the NaOH-Pi fraction of ACS-4 soil (Figure 5-6). In the abandoned dairy soils the percentage NH₄Cl-Pi released for ABS-3 soil (Figure 5-7) during the 1st, 5th, and 10th extractions was 6%, 27%, and 39%,

respectively and 8%, 25%, and 44% for ABS-4 soil (Figure 5-8). Both abandoned dairy soils released P more gradually than the active dairy soils.

Active dairy manure-amended soils (ACS-2 & ACS-4) had less P in NaOH fraction than the abandoned dairy manure-amended soils (ABS-3 & ABS-4). Overall, the NaOH-Po fraction of abandoned dairy soils was greater than the NaOH-Po fraction of active dairy soils, which suggested immobilization of P with time or mineralization of organic P to inorganic forms that were extracted by NH_4Cl . Nair et al. (1995) reported similar trends. The HCl-P pool appeared to be depleted in both types of dairies (Figure 5-5 to 5-8) following repeated NH_4Cl extractions. Abandoned dairy soils also had significantly greater HCl-Po pools than active dairy soils. This fraction deserves further study with similar manure application histories. He et al. (2006) demonstrated that HCl fraction extracted from animal manure contains organic-P fractions in addition to inorganic-P. Our data suggest that with abandonment, P was stabilized not only into NaOH-Po forms but also as HCl-Po forms. The residual fraction extractable P was significantly correlated with Ca, Al and Fe for active dairy soils. For abandoned dairy soils the extractable P in residual fraction was significantly associated with Ca and Al but not with Fe (Table 5-4). The residual fraction generally dominated by recalcitrant organic-P material (Hedley et al., 1982), as observed in this study, and Ca, Fe and Al can be occluded with Po.

The associated release of P and Ca, Mg, Fe and Al to NaOH and HCl fractions (Table 5-2 & 5-3) were also determined. NaOH reportedly extracts Al-Fe P (Chang & Jackson, 1957; Williams et al., 1967; Hieltjes & Lijklema, 1980; Psenner et al., 1985; Nair et al., 1995; Toor et al., 2006); however there have been no confirmatory analyses of this form in dairy manure-amended soils. Dairy manure contains large amounts of Ca and

Mg, and continuous manure application for years can alter the chemical forms of P initially associated with Fe and Al (Sharpley et al., 2004). In this study, NaOH extractable P and Ca were significantly correlated in both active and abandoned dairy manure-amended soils (Table 5-2) Correlation coefficients of P with metals for various P fractions extracted from active and abandoned dairy soils are given in Appendix B. For these heavily manure-amended soils, the strong relationship between the P and Ca of NaOH extractions might be an artifact of the 'left over' Ca after incomplete NH_4Cl extractions. The HCl-P pool is mainly associated with Ca and Mg, if determined after one NH_4Cl extraction followed by NaOH extraction, thus subject to dissolution with repeated NH_4Cl extractions. However, the dissolution of sparingly soluble Ca-P, Mg-P and/or Ca-Mg-P solids with repeated NH_4Cl extractions, the release of P in HCl extractions was significantly correlated only with Ca (Table 5-3). While working on dairy manure-amended soils Nair et al. (1995 and 2003), authors suggested that Ca-P and Mg-P associations are not stable P forms and can be released upon the onset of next rainfall event. Sharpley et al. (1996) used Fe-P strips to extract P continuously from manure-amended soils and found out that P released more rapidly from manure-amended soils than from the soils with minimal manure impact.

Summary and Conclusions

The repeated extraction of manure-amended soils with NH_4Cl suggested that readily-soluble P is associated with Mg. After depleting the Mg, further P release was highly correlated with Ca. The release of P in dairy manure-amended soils, using NH_4Cl repeated extractions, is controlled by Ca and Mg-P phases, which are sparingly soluble. Active dairy soils release more P in the first two NH_4Cl extractions than abandoned dairy soils. Most of P released in NaOH and HCl extraction was associated with Ca

concentrations. Data also suggested that repeated NH_4Cl extractions mineralized the HCl -P pools that may not be available for release with fewer numbers (8) of repeated water extractions. Phosphorus in residual fraction was almost constant during the sequential extractions, and the P appeared to be associated with Ca, Fe and Al. We conclude that the initial high release of P from dairy manure-amended soils may be due to the presence of Ca-P and Mg-P phase/s, which will take years to deplete. However, with time the release of P from these soils will likely be controlled by a relatively less soluble Ca-P phase provided there is no further manure application.

Table 5-1. Total dissolved phosphorus (TDP) as a function of Ca+Mg in repeated 1.0 M NH₄Cl extractions (Data from 1st extraction omitted).

Soil Type	Soil ID	N	Regression Equation	r ²
Active	^a ACS-2	9	TDP = 0.107(Ca+Mg) - 0.912	0.895 ^{††}
	ACS-4	9	TDP = 0.246(Ca+Mg) - 0.226	0.993 ^{††}
Abandoned	^b ABS-3	9	TDP = 0.042(Ca+Mg) + 2.3242	0.536 [†]
	ABS-4	9	TDP = 0.047(Ca+Mg) + 0.831	0.604 [†]
	ABS-3	9	TDP = 0.624Ln(Ca+Mg) + 1.488	0.703 ^{††}
	ABS-4	9	TDP = 0.421Ln(Ca+Mg) + 0.523	0.793 ^{††}

^aACS, active dairy manure–amended soil

^bABS, abandoned dairy manure–amended soil.

Regression coefficients were significant at $p \leq 0.01$ (††) and $p \leq 0.05$ (†) as determined by LSD

Table 5-2. Sequential release of total dissolved phosphorus (TDP) as a function of Ca, Mg, and Fe in 0.1 M NaOH extractions

Soil Type	N	Regression Equation	r ²
Active	20	TDP = 1.597Ca ^{††} - 2.589Mg + 0.112Al - 1.519Fe + 1.796	0.751 [†]
Abandoned	20	TDP = 1.068xCa ^{††} - 0.199Mg + 0.091Al - 0.276Fe + 8.108	0.644 [†]

Regression coefficients were significant at $p \leq 0.01$ (††) and $p \leq 0.05$ (†) as determined by LSD

Table 5-3. Sequential release of total dissolved phosphorus (TDP) as a function of Ca, Mg, Fe, and Al in 0.5 M HCl extractions

Soil Type	N	Regression Equation	r ²
Active	2	TDP = 0.217Ca [†] + 3.36Mg - 0.028Al - 3.120Fe	0.776 [†]
	0	- 0.025(CaxMg) + 1.322	
Abandoned	2	TDP = 0.752Ca ^{††} + 0.752Mg - 0.116Al +	0.954 ^{††}
	0	1.831Fe - 0.016(CaxMg) - 1.930	

Regression coefficients were significant at $p \leq 0.01$ (††) and $p \leq 0.05$ (†) as determined by LSD

Table 5-4. Sequential release of total dissolved phosphorus (TDP) as a function of Ca, Mg, Fe, and Al in residual fractions

Soil Type	N	Regression Equation	r ²
Active	2	TDP = 0.181Ca ^{††} - 0.142Mg - 0.021Al [†] -	0.937 ^{††}
	0	0.493Fe ^{††} - 0.08(CaxMg) + 0.920	
Abandoned	2	TDP = 0.604Ca ^{††} + 0.242Mg - 0.008Al +	0.982 ^{††}
	0	0.572Fe ^{††} - 0.090(CaxMg) [†] - 1.930	

Regression coefficients were significant at $p \leq 0.01$ (††) and $p \leq 0.05$ (†) as determined by LSD

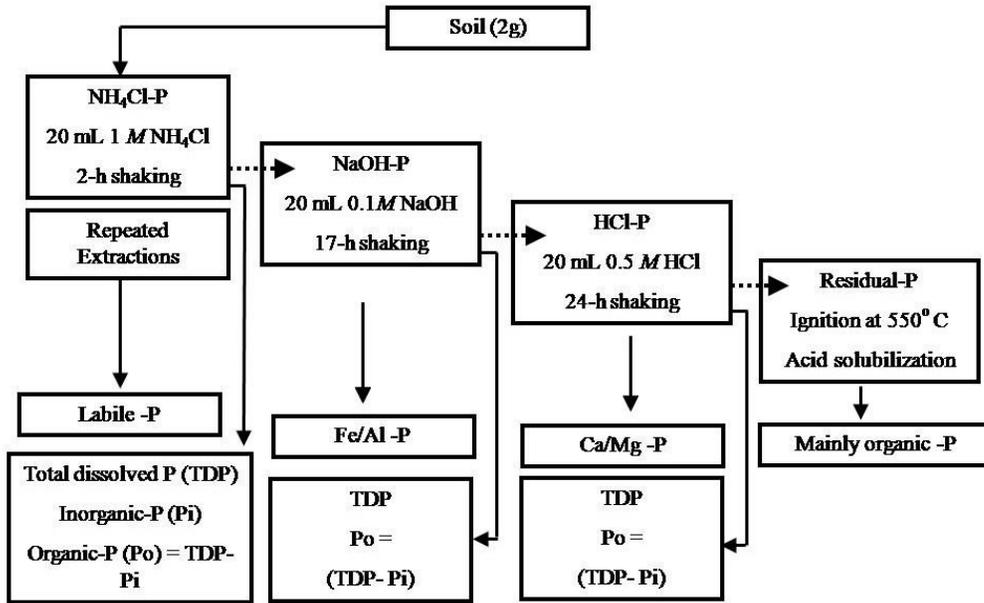


Figure 5-1. Schematic of repeated and sequential extraction procedure adapted from Nair et al. (1995).

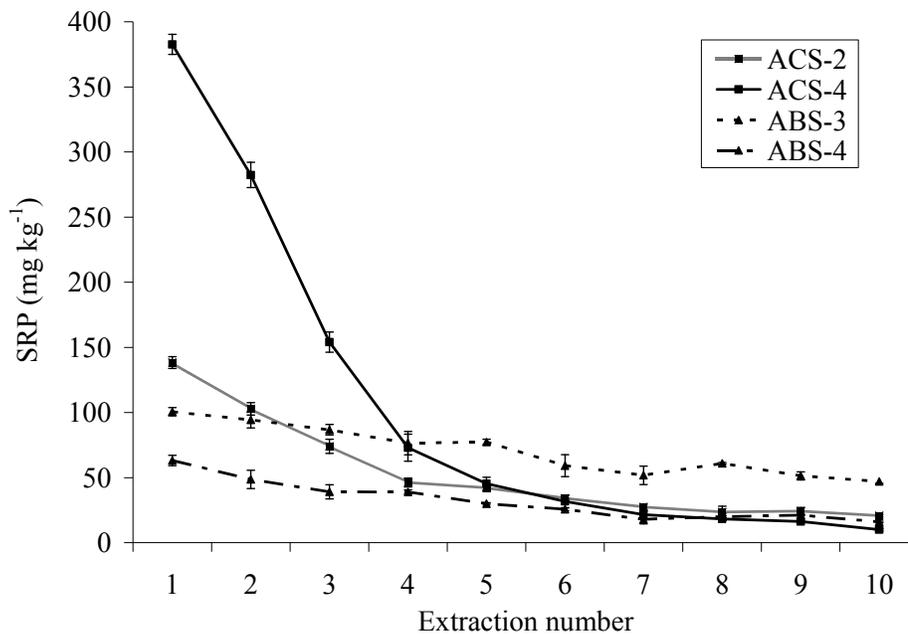


Figure 5-2. Release of phosphorus using 1.0 M NH₄Cl repeated extractions in active and abandoned dairy manure-amended soils. ACS = active dairy manure-amended soil; ABS = abandoned dairy manure-amended soil.

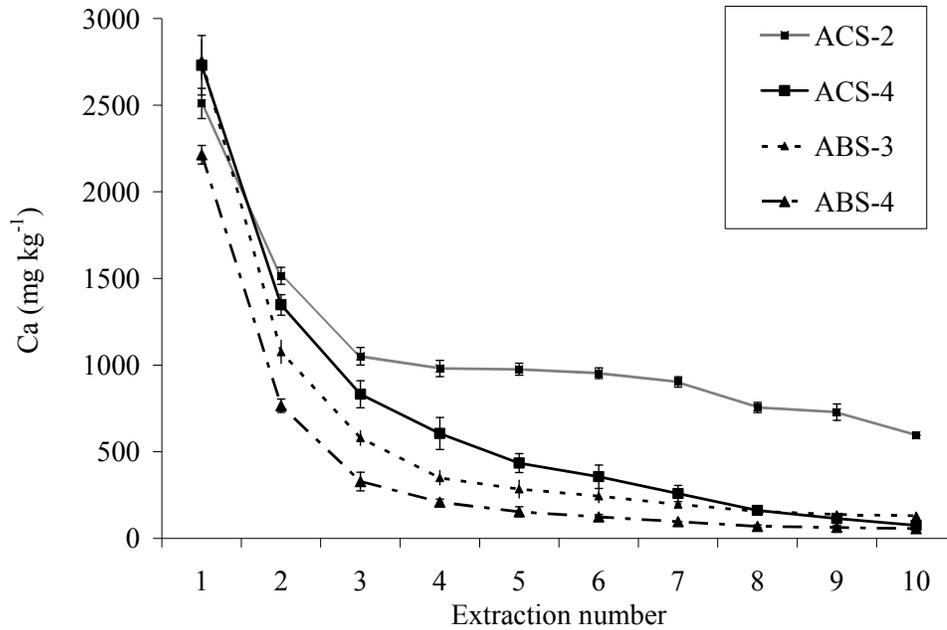


Figure 5-3. Release of calcium using 1.0 M NH_4Cl repeated extractions in active and abandoned dairy manure-amended soils. ACS = active dairy manure-amended soil; ABS = abandoned dairy manure-amended soil.

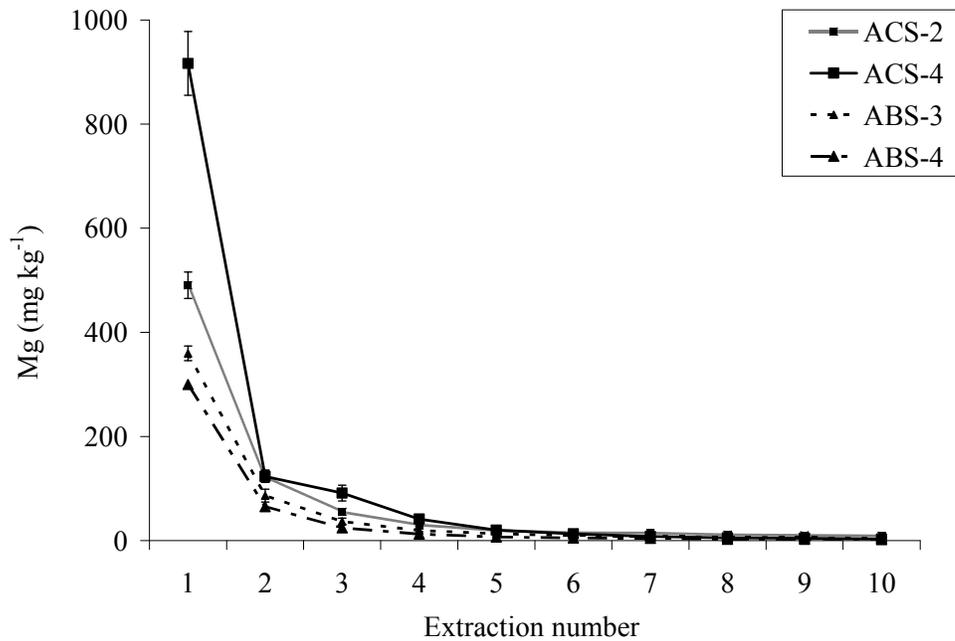


Figure 5-4. Release of magnesium using 1.0 M NH_4Cl repeated extractions in active and abandoned dairy manure-amended soils. ACS = active dairy manure-amended soil; ABS = abandoned dairy manure-amended soil.

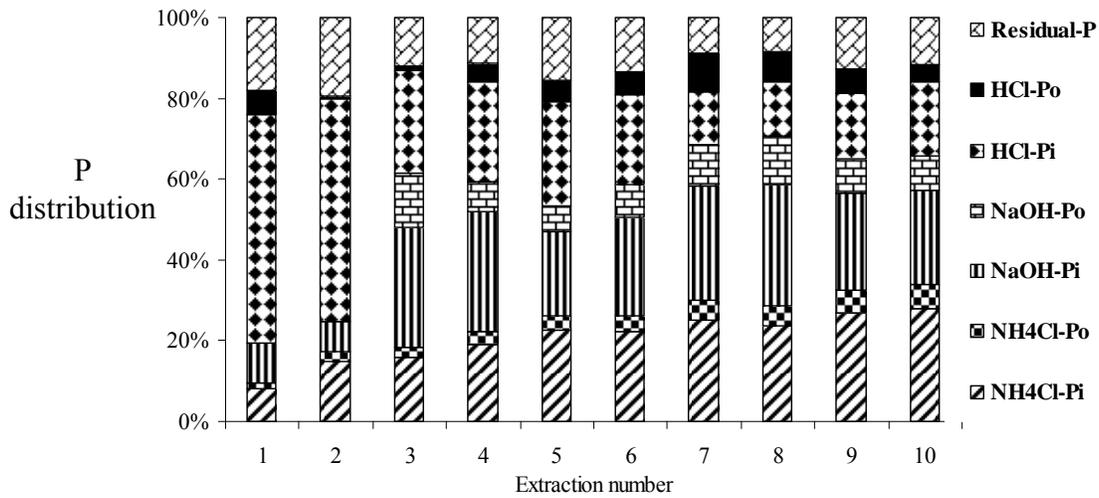


Figure 5-5. Distribution of P in 1.0 M NH_4Cl , 0.1 M NaOH, 0.5 M HCl, and residual-P fractions for an active dairy soil (ACS-2).

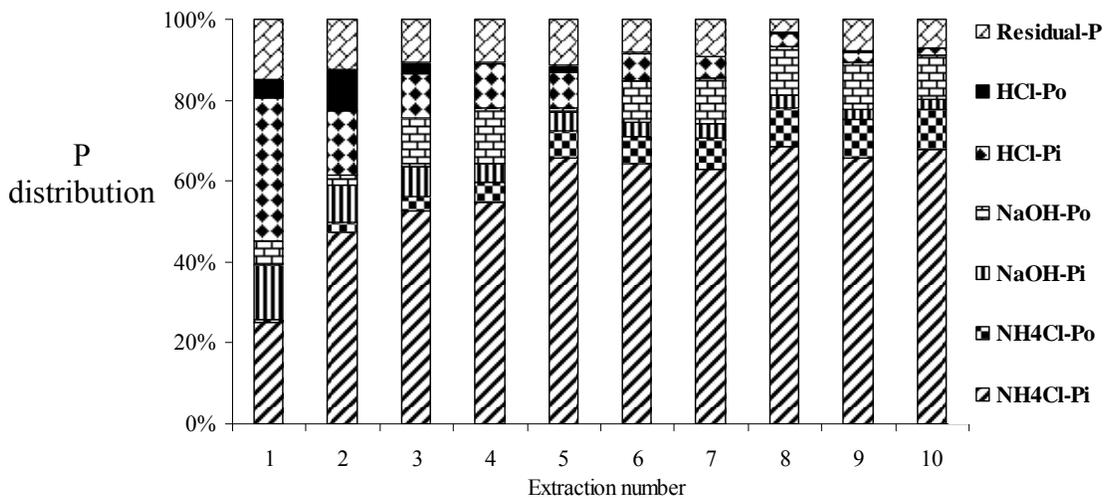


Figure 5-6. Distribution of P in 1.0 M NH_4Cl , 0.1 M NaOH, 0.5 M HCl, and residual-P fractions for an active dairy soil (ACS-4).

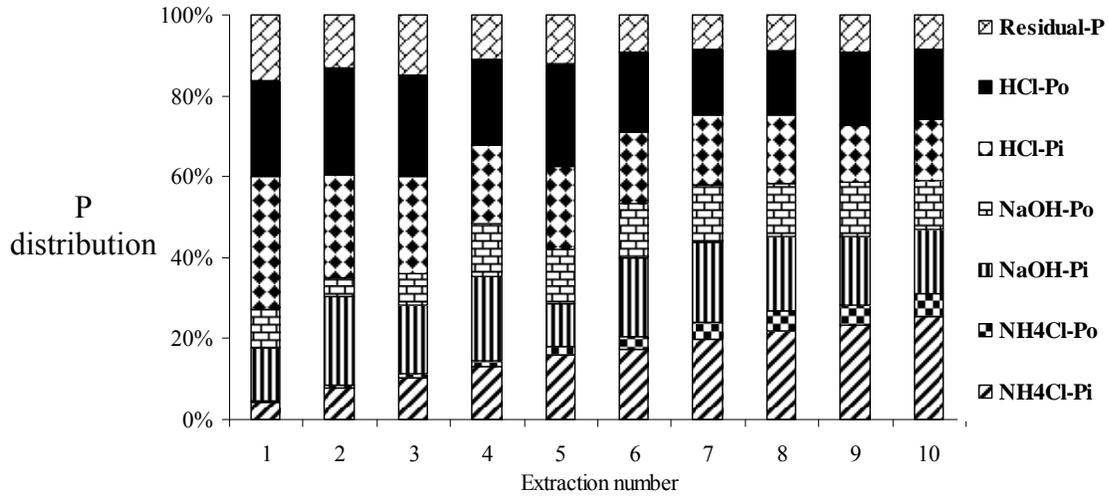


Figure 5-7. Distribution of P in 1.0 M NH_4Cl , 0.1 M NaOH, 0.5 M HCl, and residual-P fractions for an abandoned dairy soil (ABS-3).

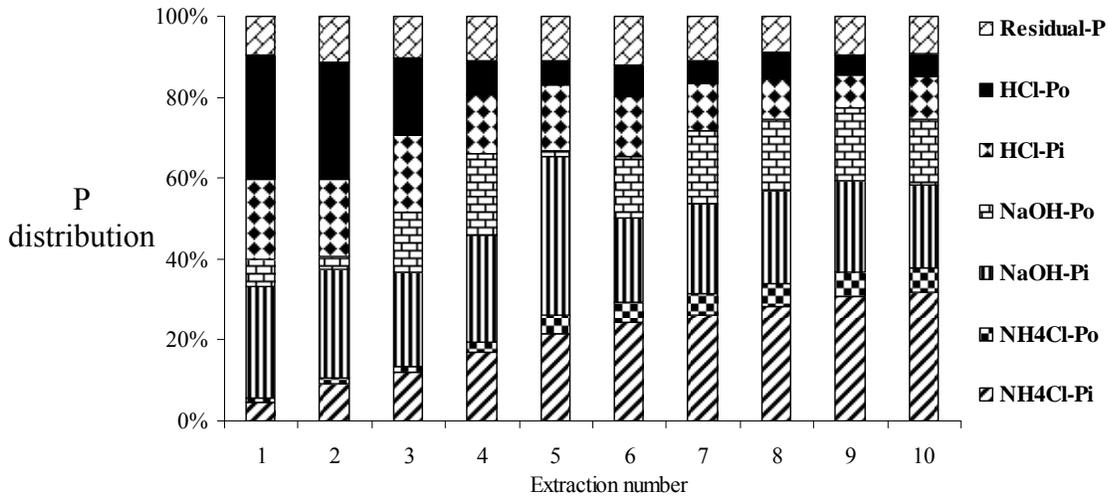


Figure 5-8. Distribution of P in 1.0 M NH_4Cl , 0.1 M NaOH, 0.5 M HCl, and residual-P fractions for an abandoned dairy soil (ABS-4).

CHAPTER 6
SOLID STATE ASSESSMENTS: CONFIRMING THE ASSOCIATIONS OF
PHOSPHORUS, CALCIUM AND MAGNESIUM

Introduction

Repeated (water and NH_4Cl) and sequential ($\text{NH}_4\text{Cl} \rightarrow \text{NaOH} \rightarrow \text{HCl}$) extractions used in previous chapters revealed information about the nature of P forms in dairy manure-amended soils and, hence, form stability under given conditions. Sequential or repeated extractions have inherent limitations, which were outlined by Harris (2002): “1) overlap in solubility of various P forms for water or other extractants, 2) time dependency, 3) loading rate (e.g., extractant/soil ratio) dependency, 4) readsorption of P forms by CaCO_3 , and consequent misallocation to P “targets” by subsequent extractions (as observed in NaOH extractions in the 5th Chapter), 5) variations in solubility of targeted forms from soil to soil, 6) presence of buffers in some soils that can reduce the effectiveness of an extractant, and 7) potential hydrolysis of organic-P forms which can lead to an overestimation of inorganic-P forms.

Therefore, associated release of P with Ca and Mg of dairy manure-amended soils does not confirm phase associations. Direct assessments using solid state analytical tools, such as X-ray diffraction (XRD), energy dispersive spectroscopy (EDS), scanning electron microscopy (SEM), and X-ray absorption near edge spectroscopy (XANES) are necessary to confirm P-solid phases. Pierzynski et al. (1990a) conducted density separation of clays obtained from excessively fertilized (inorganic) soils to concentrate P for subsequent XRD, SEM, and EDS analyses to quantify “P rich particles”. Phosphorus

was associated with Al and Si (Pierzynski et al., 1990b). Beauchemin et al. (2003) performed sequential extractions on soils that received animal manure for 25 years. Various Ca-P minerals dominated P forms in the B-horizon of an acidic loamy soil (pH 5.5) as confirmed by XANES analyses. About 45% of total P was present as octacalcium phosphate (OCP) and 11% of total P as hydroxyapatite (HAP). There has been limited solid phase assessments of P associations with Mg and Ca for dairy manure-amended soils (Cooperband & Good, 2002; He et al., 2003; Nair et al., 2003; Sharpley et al., 2004).

The objective of this work was to confirm the P associations with Ca and Mg suggested in the repeated water and NH_4Cl extraction experiments. The findings were also expected to aid in explaining the P speciation results observed in a column leaching study (Chapter 4). Four approaches were used to study the associations of P with Ca, Mg, Fe, and Al in dairy manures and dairy manure-amended soils at the microscopic levels. The first approach involved the use of XRD analyses of clays obtained from dairy manure-amended soils. The second approach specifically dealt with dairy manures, and addressed the hypothesis that a sparingly soluble phase of Ca-Mg-P present in dairy manures can be crystallized to a well defined P crystalline phase when subjected to high temperature (550°C). In the third approach, the clays obtained in the first approach and the ashed dairy manures in second approach were examined under SEM for dot map images and the spatial associations of P, Ca, and Mg were evaluated. In addition to spatial associations, semi-quantitative analyses of metals of “P-rich particles” were also performed. The fourth approach involved the use of electron probe microanalyses (EPMA) of dry sieved silt+clay fractions of dairy manure-amended soils.

Material and Methods

Approach I: X-ray Diffraction of Untreated Clays

Air-dried samples of dairy manure-amended soils (50 g each) and dairy manures (100 g each) were Na-saturated by several washings with 1M NaCl and centrifuging at 728 x g after each washing to remove supernatant. Excess salt was then removed by rinsing with DI water using the same centrifugation procedure. The final rinse was determined as the first rinse in which the supernatant appeared turbid. The Na-saturated samples were wet-sieved to separate the sand from silt and clay. Silt and clay were separated by mixing with DI water and subsequent centrifugation (Whiting & Allardice, 1986). Clay was collected in decanted supernatant, until a clear supernatant was achieved. The silt in the bottom of centrifuge bottle was dried and stored. Clay suspensions were flocculated using 1.0 M NaCl. Clay was transferred to 0.45- μ m filters and washed with 25 mL of DI water to remove salt. The salt-free clay was transferred from the filter to a glass slide using a rubber policeman, allowed to dry, and then gently crushed to a powder and stored in glass scintillation vials.

Oriented mounts for clay were prepared for XRD analyses by depositing approximately 250 mg of clay as a suspension onto a porous ceramic tile under suction. Tiles were dried in a glass desiccator and then scanned with CuK α radiation, with the tube energized at 35 kV and 20 mA current. Scans were conducted at 2°/ minute over a 2 θ range of 2° to 60°. Minerals were identified following criteria outlined by Whittig & Allardice (1986).

Approach II: Ashed and Whole Dairy Manure Analyses

Dairy manures collected from different dairies (Chapter 2) were dried and ground to 2mm with an automated grinder. Five gram samples of manure were ashed in triplicate

in a muffle furnace at 550°C for 5 h. The ashed samples were ground using a porcelain agar mortar and then transferred to glass vials and capped. Additionally, 2mm-ground whole dairy manures were passed through a 1-mm-mesh stainless steel sieve. The materials (manure and ash) were prepared for XRD by gently loading the powder into an XRD mount in manner that minimized preferred orientation (Harris et al., 1994). Mounts were scanned using the same XRD methodology as outlined in the approach I.

Approach III: SEM Imaging and EDS Analyses

For successful SEM and EDS analyses, samples need to be conductive in nature, to minimize electron accumulation at the surface of the sample. Such accumulation (“charging”) prevents optimal resolution. Clays (4 active and 4 abandoned) and ashed manures obtained in the first and second approach respectively were dispersed ultrasonically and mounted on carbon stubs. The samples were then subjected to evaporative carbon coating to enhance conductivity and minimize charging. Mounts were then subjected to a scanning electron microscope (JOEL JSM-6400) equipped with an Oxford model number 6506 EDS system for the X-ray analysis. The SEM images were obtained by operating at 15 kV beam voltage and 60 μ A probe current. Images were taken at a series of magnifications while adjusting contrast and brightness. Energy dispersive spectroscopy was performed at a beam voltage of 15 kV. This analysis was performed at three different locations on each sample to account for compositional variations. Elemental compositional analyses were performed using the x-ray analysis and SEM quantitative routine of the Oxford Link ISIS software. The software incorporates atomic number, x-ray absorption, and fluorescence (ZAF factor) correction factors while performing semi-quantitative analysis (Goldstein et al., 2003).

Approach IV: Electron Microprobe Microanalyses of Whole Silt + Clay of Dairy Manure-amended Soils

Three active (ACS-2 –ACS-4) and two abandoned (ABS 1- ABS2) dairy soil samples were subjected to electron microprobe (JEOL Superprobe-733, operating at 15 kV). Silt+clay fractions were obtained by dry sieving soils through 45 μ M sieve using a mechanical shaker for 15 min. One centimeter diameter disks (3 disks for each sample) of sieved material were prepared by subjecting these samples to a pressure of 34474 kPa (5000 PSI) using a hydraulic press. Carbon coating was performed on the silt+clay disks as explained in approach III. Microprobe analyses were done on each disk using 100 μ m beam size to quantify P, Ca, Mg, Al and Si concentrations. Standards used to calibrate the microprobe, which were served for QA/QC, were calcite for Ca, apatite for P, dolomite for Mg, and quartz for Si. Additionally Ca - PET crystal (Pentaerythritol) and P,Si,Mg,Na -TAP crystal (Thallium acid Phthalate) were used as monochromators.

The technique has analytical accuracy of \pm 1-2% by weight, but can be exploited up to 0.1 – 1% by carefully extracting characteristics x-ray peaks and considering minimum peak interferences (Goldstein et al., 2003). The relatively large beam size was selected to minimize auto correlations that can arise from local surface imperfections.

Results and Discussion

Both active and abandoned dairy clays had similar mineralogy (Figure 6-1). X-ray diffractograms of untreated clays (Figure 6-1) were dominated by quartz, with some kaolinite and traces of hydroxyinterlayered vermiculite (HIV). No P-bearing mineral was detected. An amorphous hump was observed from 22-26 2θ , which reflected the presence of biogenic Si, derived from plant phytoliths present in dairy manure. Either the P-bearing minerals were amorphous in nature or XRD was not sensitive enough to detect

crystalline phases due to small amounts. Pierzynski et al. (1990a) and Harris et al. (1994) used similar approaches and were unable to identify P bearing minerals in highly fertilized soils and in dairy manure-amended soils, respectively. Dairy manure samples (<1.0 mm) contained whewellite ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$; calcium oxalate), calcite (CaCO_3), and quartz as shown by XRD analyses (Figure 6-2). The presence of calcite means that there is a significant amount of Ca not in the form of a soluble salt or associated with P in a discrete phase. Quartz is common in dairy manure of grazing cows, probably due to ingestion of small amounts of surface soil material. In ashed dairy manure samples, both calcite and quartz were present, but no whewellite (Figure 6-3) because the latter decomposed to CO_2 and H_2O at 823 K. The Ca-Mg-P mineral whitlockite (Figure 6-3) was tentatively identified in ashed samples from XRD data. Formation could be attributed to the re-crystallization of semi- crystalline/amorphous Ca-P or Mg-P or a Ca-Mg-P phase present in dairy manures. All dairy manures, collected from three locations, showed similar possible P phase crystallization at 550°C . EDS analyses of the ashed samples showed high intensity peaks of Mg, Ca and P (Figure 6-4), consistent with a Mg-Ca-P phase, which might be amorphous in nature prior to heating, sparingly soluble, and undetectable by XRD analyses. Spatial associations of both Mg-P and Ca-P in manure (Figures 6-5 & 6-6) were also observed in SEM/EDS dot map images and spectra, confirming that Mg-P is a manure component rather than a component formed in the soil environment after the application of dairy manures.

This finding could be helpful in formulating new diets to reduce manure-P solubility by adjusting Ca:Mg ratios in addition to P for dairy animals (Daniel et al., 2006). Many authors advocate a reduction of P in dairy diets to reduce the P solubility

(Wu et al., 2001; Dou et al., 2003; Toor et al., 2005). However, P interacts within the animal gut with Mg and Ca (Herrera et al., 2006) and by decreasing the Mg to the optimal levels in dairy diets, Mg-P formation can be minimized and Ca-P interaction can be enhanced (Herrera et al., 2006). Minimized Mg-P formation could be beneficial as it would reduce P solubility and high P leaching potential (Lindsay, 1979) as all Mg-P minerals are relatively soluble than Ca-P minerals. The P-rich particles of dairy manure clays, observed under SEM (Figure 6-7), also showed the dominance of Mg, Ca and P as observed in EDS spectrum (Figure 6-8). There were 24 samples of manure were observed under SEM, and 46% of the samples showed the spatial associations of P with Ca and Mg both.

Microprobe analyses of silt+clay samples showed that active dairy silt+clay samples had greater average concentrations of P (0.38%) and Mg (0.64%) than the abandoned dairy samples (P = 0.31% and Mg = 0.25 %) (Figure 6-9). This parallel decline since abandonment suggests that both Mg and P were depleted from the soil with time, and is consistent with the elements being released via the dissolution of a common phase. Release of Mg and P was closely associated in repeated water extractions (Figures 4-6 & 4-7), and did not differ between active and abandoned dairies as did Ca, which was released to a lesser extent in abandoned dairies. Active and abandoned dairies had similar Ca concentrations ($2.3 \pm 0.5\%$), which were about an order of magnitude greater than Mg and P concentrations. Both Ca and Mg were significantly ($p < 0.01$) correlated spatially with P in abandoned dairy silt +clay fractions (Figures 6-9c and 6-9d). For active dairy samples, only the Mg and P relationship was statistically significant ($p < 0.01$). A possible

explanation for poor correlation of Ca and P in active dairy manure-amended silt+clay fractions can be due to the presence of CaCO_3 applied as a fill material.

Summary and Conclusions

Solid state techniques confirmed spatial associations of P with both Ca and Mg in dairy manure and manure-amended soils. Results were reasonably consistent with release trends for the elements observed in repeated water (Chapter 4) and NH_4Cl extractions (Chapter 5), as well as with what could be concluded about mineral equilibria from chemical speciation of column leachates (Chapter 4). Implications are that Mg is associated with P in manure and manure-amended soils to at least an equivalent extent as Ca. However, specific minerals were not identified and uncertainty remains as to crystallinity of P phases as well as to whether P is associated with Ca and Mg separately or within a common Ca-Mg-P phase (e.g., whitlockite) that was tentatively identified in ashed manure samples.

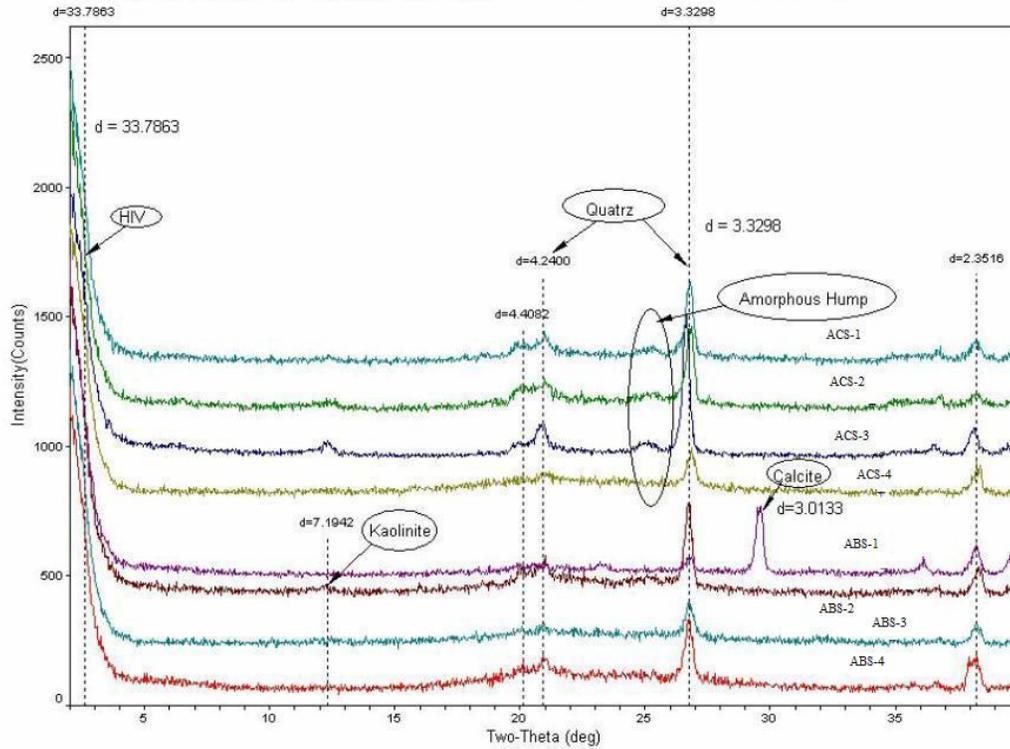


Figure 6-1. X-ray diffraction patterns of four active (ACS-1 to ACS-4) and four abandoned (ABS-1 to ABS-4) dairy manure-amended untreated clays. HIV = hydroxyinterlayered vermiculite.

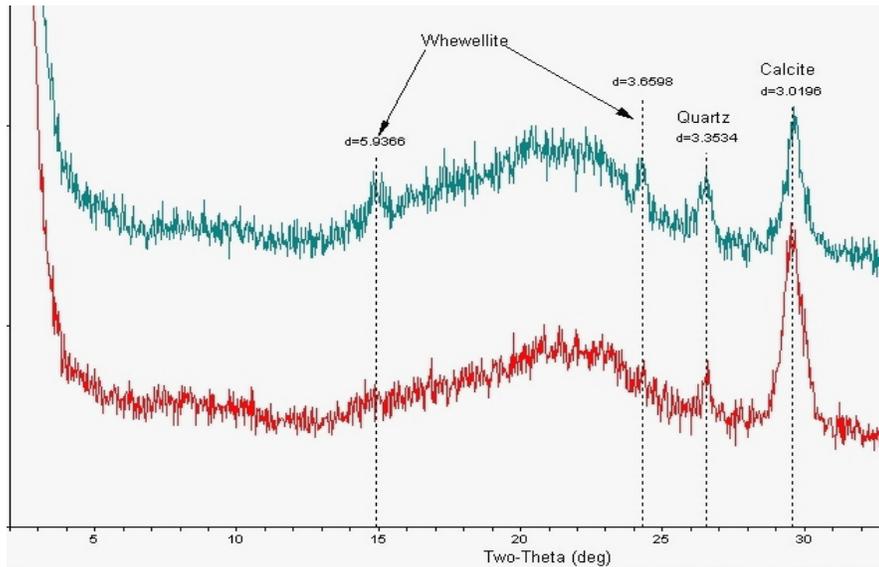


Figure 6-2. X-ray diffraction pattern of < 1.0 mm dried dairy manures showing the presence of whewellite, quartz and calcite.

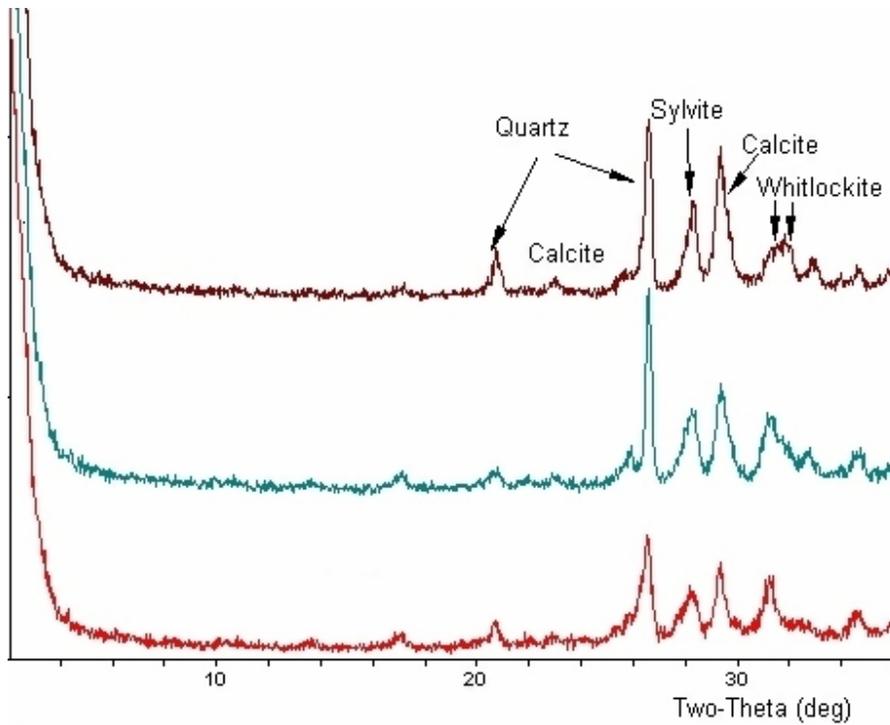


Figure 6-3. X-ray diffraction patterns of oven dried and ashed dairy manures (3) showing the presence of Mg-Ca Whitlockite (Mg-Ca phosphate).

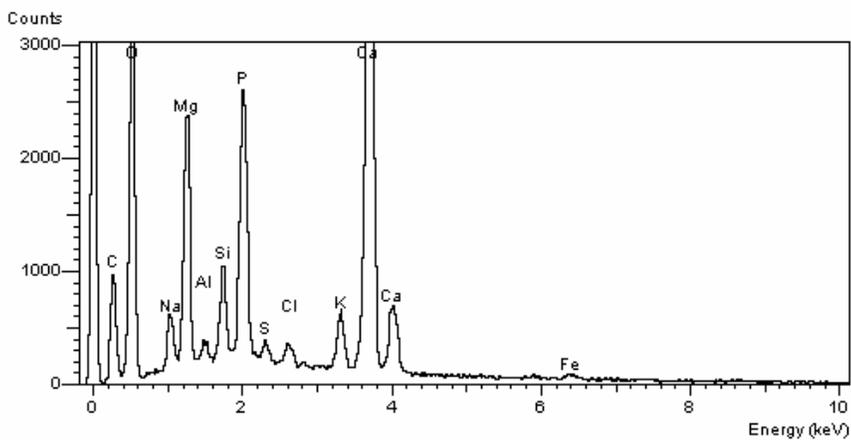


Figure 6-4. Energy dispersive spectrum of an ashed dairy manure showing the high intensity peaks of Ca, Mg and P.

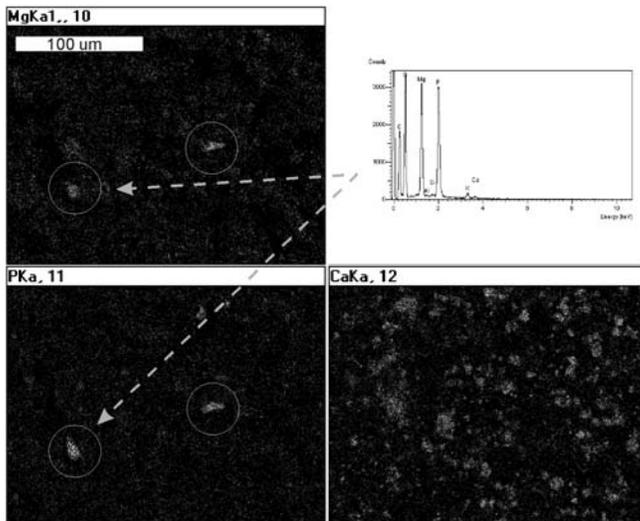


Figure 6-5. Energy dispersive dot maps of a dairy manure showing an association of Mg and P.

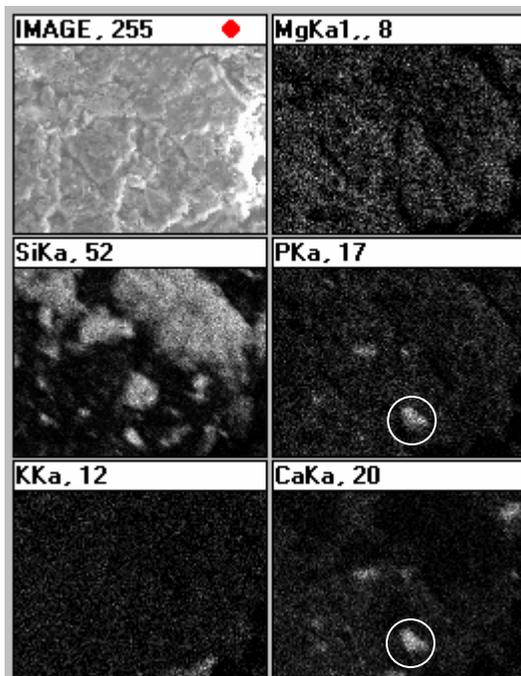


Figure 6-6. Energy dispersive dot maps (scale 20 μm) of a dairy manure-amended soil clay showing an association of P and Ca.

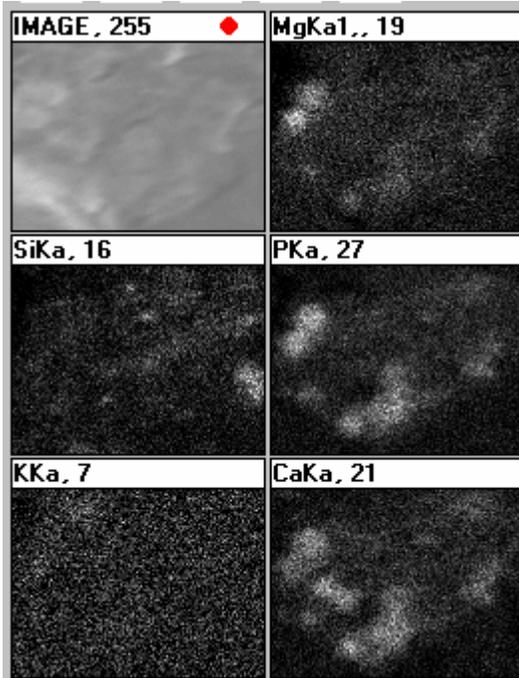


Figure 6-7. Dot image of dairy manure showing the spatial associations of Mg, P, and Ca.

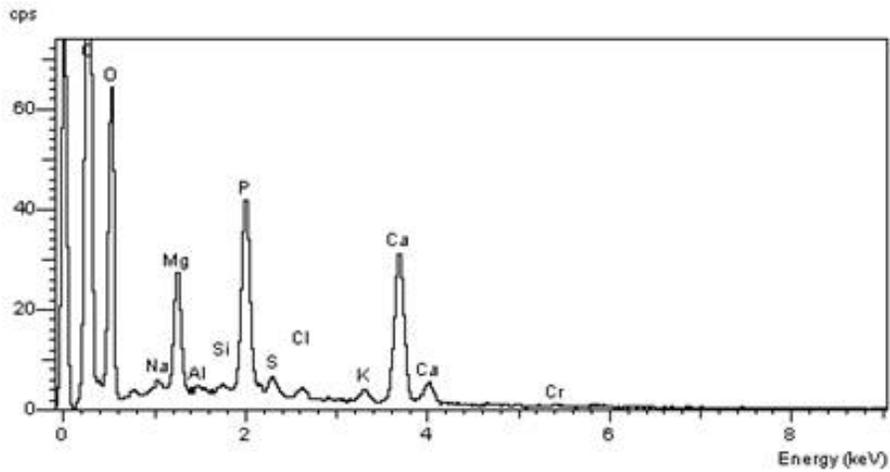


Figure 6-8. EDS spectrum of a manure P rich particle obtained at 400X magnification showing the dominance of Mg, P, and Ca.

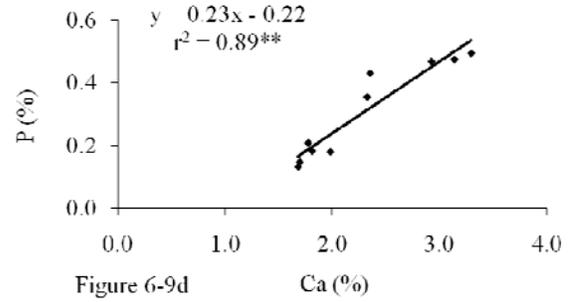
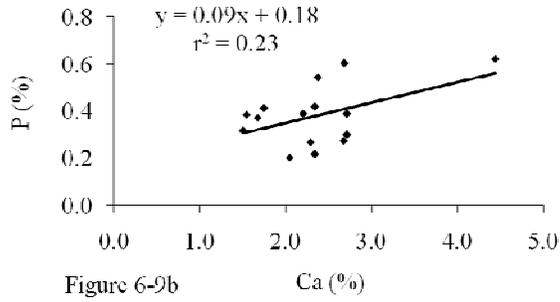
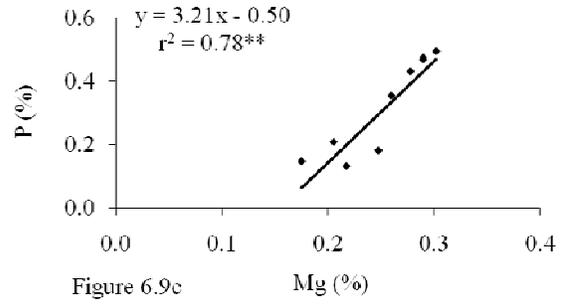
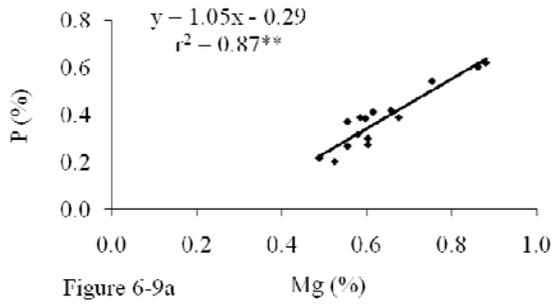


Figure 6-9. Relationships between P, Mg and Ca for active (6-9a & 6-9b) and abandoned dairy (6-9c & 6-9d) manure-amended dry sieved (45 μ m) silt+clay using the electron probe microanalyses (EPMA). **significant at $p < 0.05$.

CHAPTER 7
CALCIUM PHOSPHATE CRYSTALINITY AND DAIRY MANURE COMPONENTS

Introduction

Phosphate chemistry in dairy manure-amended sandy soils may be influenced by the presence of Mg, biogenic silica (derived from plant phytoliths), and manure-derived organic matter and its myriad of organic acids (Inskeep & Silvertooth, 1988). Manure-amended soils systems are typically in a state of disequilibrium (Silveira et al., 2006), and even the high pH accompanied by high Ca concentrations do not effectively stabilize Ca-P forms (Harris et al., 1994; Josan et al., 2005). Apatite and metastable crystalline Ca phosphates (e.g., octacalcium phosphate OCP, tricalcium phosphate) are not detectible by XRD (at least, not in the bulk clay) even after years of manure addition despite high total P concentrations (Harris et al., 1994). Density gradient centrifugation has been used successfully on soil clay fractions (Jaynes & Bigham, 1986; Pierzynski et al., 1990a) to study the different Ca-P minerals formed in the soil environment. Pierzynski et al. (1990a) partitioned clays of heavily fertilized soils into three density fractions (<2.2, 2.2 to 2.5, and > 2.5 Mg m⁻³) using non aqueous heavy density liquid containing polyvinylpyrrolidone and tetrabromoethane.

This study addressed potential inhibitory effects of manure-derived components on Ca-P crystallization and the fate of P in manure-amended soils, particularly soils with minimal native P retention capacity. Results relate to the question: Why are abandoned dairy manure-amended soils continue to leach high amounts of P after years of

abandonment under the conditions of high pH and high Ca concentrations? It was hypothesized that:

1. Activities of Mg, Si, and DOC in soil solutions of manure-amended soils are sufficient, jointly or separately, to inhibit crystallization of stable Ca-P forms, thus allowing high P release from these soils.
2. Noncrystalline Si forms (including biogenic Si in dairy manure) can retain P at circumneutral pH and high Ca activity. This hypothesis is based on the idea that Ca can serve as a bridge between the silicate surface and P.

The hypotheses were tested by the following objectives:

1. To study the effects of Mg, Si and DOC on Ca-P crystallization using average inorganic species concentrations found in manure-amended soil leachates.
2. To investigate the effects of “low-density clay” ($<2 \text{ Mg m}^{-3}$) and “high-density clay” ($\geq 2 \text{ Mg m}^{-3}$) of manure-amended soils on Ca-P crystallization.

Material and Methods

The study required isolating soil clays, treatment to remove carbonates and organic matter, and separation into two density fractions. Procedures for accomplishing the objectives are given in the following sections.

Particle Size Separations of Dairy Manure-amended Soils

The method used to separate particle sizes in soils is a slight modification of the method developed by Whittig & Allardice (1986). Air-dried samples (50 g each) were Na-saturated by several washings with 1.0 M NaCl in 250-mL centrifuge bottles. Samples were shaken for 5 min on a reciprocal shaker for each washing. The supernatant was decanted after centrifugation at $738 \times g$ (2000 rpm). Excess Na was rinsed with deionized (DI) water by centrifugation and decantation. The final rinse was determined as the first rinse that appeared turbid. The Na-saturated sample was wet-sieved ($45 \mu\text{m}$) to separate the sand from silt and clay. Silt and clay were separated by centrifugation (Whitting & Allardice, 1986), using DI water and decanting supernatant as clay, repeated until a clear

supernatant was achieved. Silt collected in the bottom of centrifuge bottle was dried and stored. Clay suspensions were flocculated using 1.0 *M* NaCl. Clay was collected from suspensions by 0.45- μm filtration and washed with at least 25 mL of double DI water to remove excess salt. Salt-free clay was transferred from the filter to a glass slide using a rubber policeman, allowed to dry, gently crushed to a powder, and stored.

Carbonate and Organic Matter Removal from the Resulting Clays

To remove carbonates, 2 g of clay was treated with 100-mL of 1 *M* sodium acetate solution, buffered at pH 5.0 with glacial acetic acid (Anderson, 1963; Jackson, 1985), and heated in a boiling water bath for 30 min with intermittent stirring. The same procedure was repeated twice more after decanting the clear supernatant. The treated clay was transferred to a 0.45- μm filter and washed with DI water. The retentate was then subjected to organic matter removal. A 5-mL aliquot of sodium acetate buffer and 10 mL (30%) of hydrogen peroxide (H_2O_2) were added to the samples in beakers and allowed to stand overnight. The following day the samples were placed on a hotplate at 85°C and gently heated for 10 min. The last step was repeated and more H_2O_2 was added to obtain a light colored solid material. The samples were transferred to 0.45- μm filters, washed with DI water using suction, dried, and stored in glass scintillation vials for further analyses.

Density Separations for Soil Clay Materials

A Na polytungstate solution with a density of 2.0 Mg m^{-3} was prepared and adjusted to pH 7.0. Half-gram samples of treated (carbonates and OM removed) clay were placed in 50-mL centrifuge tubes containing 35-mL of the Na polytungstate solution, shaken on a reciprocal shaker for 30 min, and centrifuged at 3268 \times g (4000

rpm) for 2 minutes. The supernatant was decanted, and the dense material was transferred to a labeled container. The obtained light and dense fractions were again transferred into the centrifuge tubes containing 35-mL of 2.0 Mg m^{-3} Na polytungstate solution, sonicated using an ultrasonic water bath for 2 min and then shaken on a reciprocal shaker for half an hour. The material was centrifuged again at 4000 rpm for 2 minutes, and the above process was repeated until no further separation was evident. The accumulated dense- and light-fraction suspensions were filtered through a $0.45 \text{ }\mu\text{m}$ filter, rinsed 5 times with 10-mL aliquots of DI water, transferred to a glass slide with a rubber policeman, allowed to air-dry, and transferred to labeled scintillation vials. Mineralogical analyses were performed on both light and dense fractions prior to use for incubation experiments.

Preparation of Incubating Solutions

Chemically defined solutions mimicking the average inorganic chemical composition (Table 7-1) in the leachates of manure-amended soils (Chapter 4) were prepared in double deionized (DDI) water. Four stock solutions were prepared and adjusted to a final pH of 6.8 by equilibrating with atmospheric CO_2 and by adding 0.1 M HCl or 0.1 M NaOH solutions. At this pH the precipitate of Ca-P minerals did not take place immediately; however, it was expected that Ca-P crystallization would take place in the control, as the ion activity product was supersaturated with respect to hydroxyapatite. Solution 1 served as a control (Table 7-2) and contained all the same components except Mg, Si, and DOC. Solution 2 consisted of the control solution plus Mg, but no Si and DOC. Solution 3 consisted of the control solution plus Si, but no Mg and DOC. Solutions 1, 2 and 3 were prepared from analytical grade reagents and were filtered through $0.45\text{-}\mu\text{m}$ filters prior to use. Solution 4 consisted of the control solution plus DOC but no

added Si or Mg. The DOC solution, which served as a base solution for this treatment, was prepared as follows: 100 g of each of the soil samples were mixed together and treated to remove salts and carbonates using 200-mL of the 1.0 *M* sodium acetate following the methods outlined in the carbonate removal section of this chapter. The sodium acetate treated material was washed 5 times with 1.0 *M* NaCl solution using a centrifuge and four 250-mL bottles at 738 x *g* (2000 rpm). The residual NaCl salt was rinsed with DI water via centrifugation until the solutions appeared turbid. The turbid solution was retained in the 250-mL bottles. All the 250-mL bottles were shaken for 16 h on a reciprocal shaker. The suspensions were allowed to settle for 2 h, and then filtered through a 0.45- μm filter using filtration jars to collect the DOC solution. The DOC solution was analyzed for Ca, Mg, K, Na, and Si using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (EPA method 200.7). Ammonium was analyzed by the semi-automated colorimetry method (U.S. EPA, 1993; method 350.1); nitrates by automated colorimetry with the use of an ALPKEM Auto-analyzer (U.S. EPA, 1993; method 353.2), and sulfate by ion chromatography with a separator AS-14 (DIONEX) (U.S. EPA, 1993; method 300.0). Dissolved organic carbon was determined by TOC-5050A, Shimadzu (method 5310A, 1992). Interferences from the inorganic carbon were first removed by sparging with CO₂ -free gas after acidification of the sample (Sharp & Peltzer, 1993). The DOC solution was later diluted to DOC concentrations representative of column leachate DOC concentrations.

Incubation Setup, Monitoring and Solution Analyses

The effects of Mg, Si, and DOC on Ca-P crystallization in the presence and absence of solids (clay fractions obtained from density separations), were determined

with four replications in the absence of solids. Three replications included the light-clay fraction ($\rho < 2.0 \text{ Mg m}^{-3}$) and four replications included the dense fraction ($\rho > 2.0 \text{ Mg m}^{-3}$). Throughout the discussion of the incubation study, the word ‘solids’ will refer to the clay-sized material derived from the manure-amended soils. 100 mg of solids and 50-mL of incubating solution (Table 7-2) were placed in 150-mL nalgene bottles and loosely capped. The containers were stored in the dark and maintained at room temperature (25°C) for 20 weeks. Solutions were monitored on a weekly basis for water levels, and small amounts of DDI water were added when necessary to compensate for evaporation. One mL of supernatant was withdrawn at 1, 3, 5, 10 and 20th week intervals, diluted to 8-mL with DDI water, and filtered through a 0.45- μm filter. The resulting solutions were analyzed for Ca and Mg using atomic absorption spectroscopy. Inorganic P concentrations were measured using ascorbic acid colorimetry (U.S. EPA, 1993; method 365.1). Details of incubation treatments are explained in Table 7-2.

Solid State Assessments

X-ray diffraction analyses

Low- and high-density clay fractions were subjected to x-ray analyses using a side-packed powder mount and scanned with $\text{CuK}\alpha$ radiation energized at 35 kV and 20 mA current. Scans were conducted at $2^{\circ}/\text{minute}$ over a 2θ range of 2° to 60° . The precipitates obtained after 20 weeks of incubation were collected on filter paper, spread on a low-background quartz crystal XRD mount, air dried, and scanned for x-ray analyses (Chapter 6).

Energy dispersive spectroscopy analyses

Energy dispersive spectroscopy (EDS) was performed at a beam voltage of 15 kV. The analysis was performed at three locations on each sample so that compositional

variations could be accounted for accurately. Elemental compositional analyses were performed using the x-ray analysis and SEM quantitative routine of the Link ISIS software. The software incorporates atomic number, x-ray absorption, and fluorescence (ZAF factor) correction factors and performs semi-quantitative analysis (Goldstein et al., 2003).

Statistical Analysis

To test the differences in solution concentrations of P, Ca, Mg, and DOC after the specified incubation periods, a non-parametric test (Kruskal-Wallis) was used ($p < 0.05$). Computations were performed in Minitab version 14.0 (Minitab, 2004).

Results and Discussion

Effects of Mg, Si and DOC on Ca-P Crystallization

In the absence of solids, median concentrations of P in the equilibrating solutions declined significantly, from 68 mg L^{-1} to 28 mg L^{-1} , for both the control and the Mg treatment over the 20 weeks of incubation (Figure 7-1). P concentrations were significantly lower ($p < 0.05$) for the Mg treatment than for the control from the 1st week to the 10th week of incubation; however after the 20th week, there was no significant difference between the P concentrations in the Mg treatment and the control. The sharp decline in the P concentrations in the 1st through 10th week coincided with the formation of precipitates in both the control and the Mg treatment solutions. The Mg concentrations remained almost constant ($168 \pm 5 \text{ mg L}^{-1}$) during this incubation study.

Mineralogical analyses of the precipitates revealed that the only crystalline Ca-P phase precipitate that formed were brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) in the presence of Mg (Figure 7-2), and hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$) in the control (Figure 7-3) after the 10th and 20th week of incubation. Nielsen (1984) observed that cation dehydration plays an

important role in the growth rate of crystals. Magnesium has a dehydration rate that is 3000 times slower than that of a Ca ion, and thus the incorporation of Mg into hydroxyapatite can be negligible (Martin & Brown, 1997). Neither a Ca-deficient nor a Mg incorporated non-crystalline Ca-P phase was observed; however, in the presence of Mg, a simple Ca-P crystalline structure (brushite) with Ca:P 1:1 was precipitated and detected by XRD, then a more complex structure with Ca:P 5:3 (HAP). Corresponding declines in Ca concentrations were also observed for the Mg and control treatments (Figure 7-4).

The data suggest that Mg promoted the precipitation of brushite and as a result the solution P concentrations were significantly lower ($p < 0.05$) in Mg treatments for the first 5 weeks of incubation (Figure 7-1). In the control, HAP crystallization was delayed, resulting in higher P concentrations until the 5th week of incubation. The concentrations of Mg used in this experiment were very high compared to those used by other researches. This is because most studies on the formation of Ca-P minerals pertain to the fields of dentistry and analytical chemistry, and such studies typically use lower concentrations of Mg. Using solutions with low Mg concentrations, which are continuously stirred or agitated, results in the formation of amorphous Ca-P phases (Ferguson & McCarty, 1971). In this incubation study, the solutions were made to mimic actual leachate concentrations of dairy-manure amended soils (which have comparatively higher Mg concentrations), and the solutions were not stirred; this enabled the formation of a crystalline, yet relatively soluble, Ca-P mineral phase.

The Si did not inhibit the formation of HAP. The P and Ca concentrations declined but were similar in both the control and Si treatment solution (Figure 7-5). Silicon

concentrations did not decline significantly ($p > 0.05$) during the incubation study. The role of elemental Si in the formation of HAP has been studied in dentistry by Damn & Cate (1992). Silicon behaved as a heterogeneous nucleation substrate that stabilized the formation of the calcium phosphate nuclei, and reduced the development time of stable nuclei (critical nuclei). Hidaka et al. (1993) also studied the role of Si (silicic acid) in the formation of calcium phosphate precipitates and observed no inhibition of Ca-P formation. However in soil systems, Shariatmadari & Mermut (1999) studied the phosphate sorption-desorption behavior of silicate clay-calcite systems at various Mg and Si concentrations (0-15 mg L⁻¹). The researches concluded that addition of Si decreased the P sorption of calcite, and attributed the effect to Ca-Si ion-pair formation.

In the DOC treatment, P concentrations in the equilibrating solutions after 20 weeks of incubation were significantly greater ($p < 0.05$) than in the control (Figure. 7-6). During incubation, P concentrations declined from 68 mg L⁻¹ to 58 mg L⁻¹ attributable to the immobilization of P by the DOC. Later, this was confirmed by measuring total dissolved P in stored frozen solution aliquots that had been taken during the 20 weeks of incubation. Total dissolved P (TDP) values were greater than SRP values, and TDP concentrations declined during the incubation period, which can be related to the flocculants observed at the bottom of DOC treatment bottles.

Additionally in the DOC treatment, there also was a significant decline in Ca concentrations after 20 weeks of incubation, which was most likely due to Ca-DOC complexation. This was corroborated by analyzing the same frozen aliquots taken from the incubating solutions for DOC. The decline in DOC concentrations (Figure 7-7) was also correlated with observations of increasing flocculation of DOC in the solutions

during the incubation period. Assessments of the solids in the DOC treatments, possibly from the DOC flocculation, did not reveal the formation of Ca-P mineral phases (Figure 7-3). Thus, the presence of DOC completely inhibited the formation of Ca-P mineral phases. Grossl & Inskeep (1991, 1992) documented the inhibition of both OCP and tricalcium phosphates precipitation in presence of organic acids like humic, fulvic, citric, and tannic acids. Inhibition was attributed to the blockage of adsorption sites of CaCO_3 by DOC and also stated that presence of DOC favors the formation of brushite, as opposed to more thermodynamically stable Ca-P phosphates.

MINTEQ analyses of solutions species after 20 weeks of incubation revealed high ionic activity products. The results suggest that even though the Ca and P concentrations declined significantly in the incubating solutions over the 20 weeks, the solutions remained supersaturated. Therefore, the inhibition of the Ca-P minerals formation was due to the presence of DOC and not to the decreased Ca and P solution concentrations.

Effects of Solids on Ca-P Crystallization

The presence of solids resulted in higher P concentrations in the incubated solutions than in the absence of solids (Figure 7-8). The decline of P concentrations during the incubation in the presence of solids might have been due to the P adsorption onto the clay; no mineral phases of Ca-P were detected by XRD in any of the treatments containing the solids. Phosphorus and Ca concentrations in final solutions were significantly lower ($p < 0.05$) for the low-density clay ($\rho < 2.0 \text{ Mg m}^{-3}$) than for the dense fraction ($\rho > 2.0 \text{ Mg m}^{-3}$) (Figure 7-10). The greater sorption of P and Ca by the low-density clay may relate to its greater surface reactivity arising from either noncrystalline (biogenically-derived) Si or residual organic matter not removed by H_2O_2 , and is consistent with a Ca-bridging mechanism for P retention. Dominance of Si in low-density

fraction was confirmed by EDS (Figure 7-11), and the presence of noncrystalline material was indicated by a broad XRD peak (“amorphous hump”) characteristic of noncrystalline material. Also, SEM imaging of < 50- μm material verified the presence of appreciable biogenic Si (Figure 7-12).

Summary and Conclusions

Representative concentrations of Mg in manure-amended soils inhibit the formation of HAP but not the precipitation of a more soluble Ca-P mineral (brushite). Brushite that forms locally in a soil matrix would be subject to dissolution in the next rainfall event, thus favoring sustained P leaching from these soils. In the presence of DOC, Ca-P crystallization was completely inhibited and no Ca-P mineral was detected. Si had no inhibitory effect on Ca-P stabilization; and HAP was formed. Lower P and Ca concentrations were observed for low-density clay relative to high-density clay, possibly due to greater solids reactivity (as inferred from evidence of a higher proportion of noncrystalline material) in conjunction with a Ca bridging sorption mechanism. No Ca-P crystallization took place in the presence of clay size fractions. Generally, solids act as a nucleation seed, adsorbed P thus provides a surface of Ca-P interaction. Such an effect was not observed in this study.

Table 7-1. Average leachate composition of manure-amended soils used for the incubating solutions

Chemical Species	P	Ca ²⁺	Mg ²⁺	Si ⁴⁺	DOC	Fe ²⁺		
mg L ⁻¹	68.0	312	179	23	427	0.60		
Chemical Species	Al ³⁺	K ⁺	Na ⁺	NH ₄ ⁺	Cl ⁻	NO ₃ ⁻	SO ₄ ²⁻	
mg L ⁻¹	0.30	375	144	39.0	169	257	345	

Table 7-2. Incubation treatments to study the effects of Mg, Si, and DOC on Ca-P crystallization in the presence and absence of manure-derived solids

Absence of Solids		No. of Replicates
Control	Solution I- no potential inhibitor	4
Treatment 1	Solution II-Mg as the only potential inhibitor	4
Treatment 2	Solution III-Si as the only potential inhibitor	4
Treatment 3	Solution IV-DOC as the only potential inhibitor	4
^a Presence of Light Solids		
Treatment 4	Solution I-light solids as the only potential inhibitors	3
Treatment 5	Solution II-Mg and light solids as potential inhibitors	3
Treatment 6	Solution III-Si and light solids as potential inhibitors	3
Treatment 7	Solution IV-DOC and light solids as potential inhibitors	3
Presence of Dense Solids		
Treatment 8	Solution I-dense solids as the only potential inhibitor	4
Treatment 9	Solution II-Mg and dense solids as potential inhibitors	4
Treatment 10	Solution III-Si and dense solids as potential inhibitors	4
Treatment 11	Solution IV-DOC and dense solids as potential inhibitors	4

^aLess number of replications due to low material availability

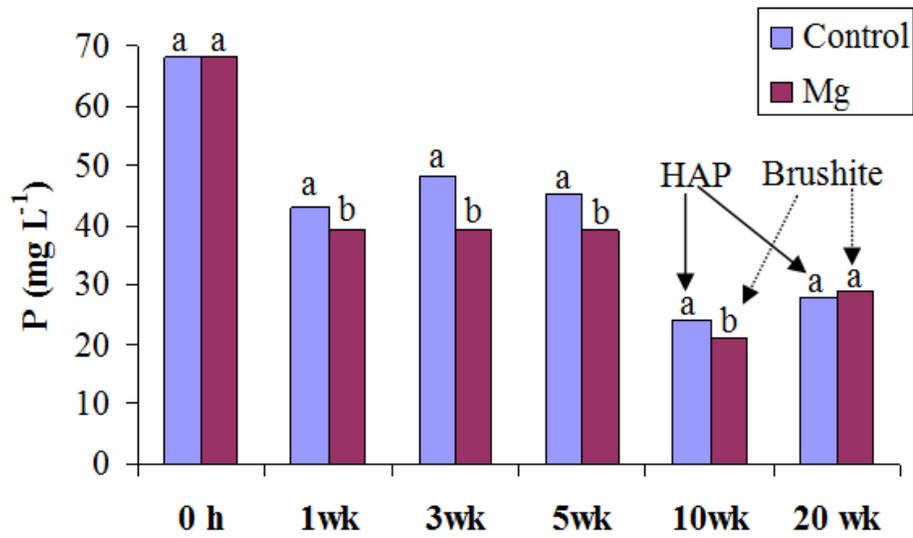


Figure 7-1. P concentrations in the presence of Mg during 20 weeks (wk) of incubation. Different letters (a and b) indicate statistically significant differences among median concentrations ($p \leq 0.05$) observed after an incubation period. HAP = Hydroxyapatite.

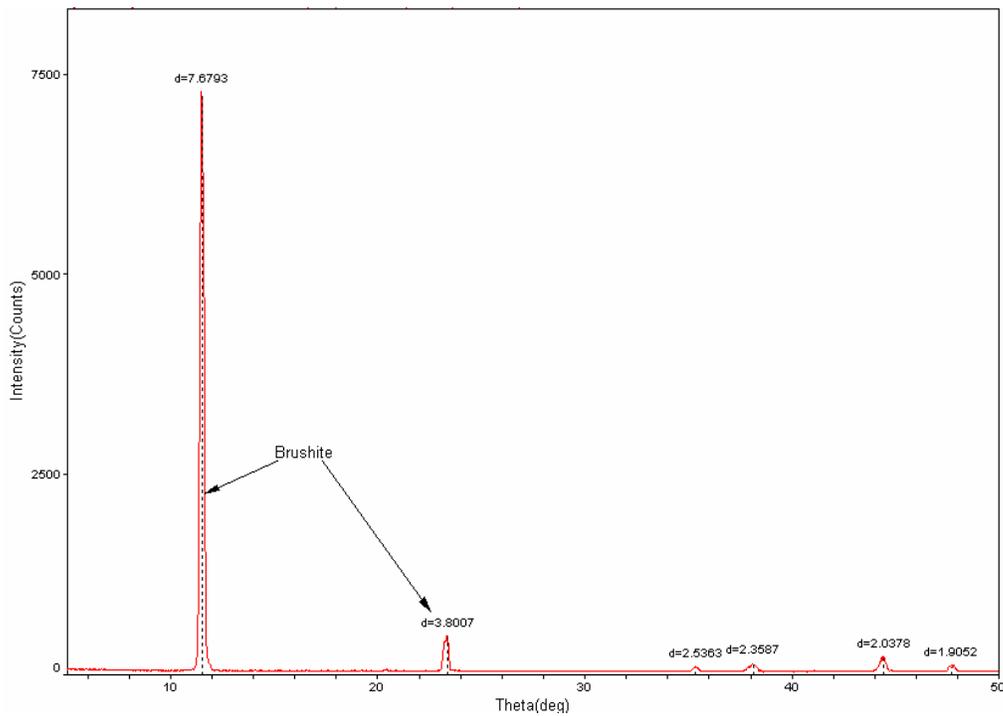


Figure 7-2. XRD pattern of the precipitate in the Mg solution after 20 weeks of incubation.

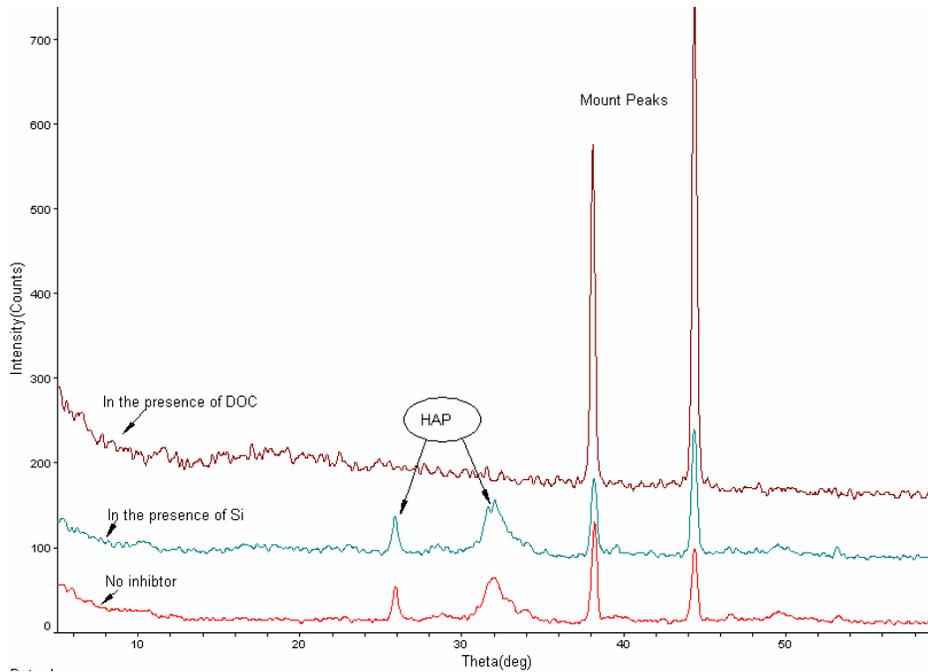


Figure 7-3. XRD patterns of precipitates observed after 20 weeks of incubation in control (no inhibitor), Si, and DOC treatments.

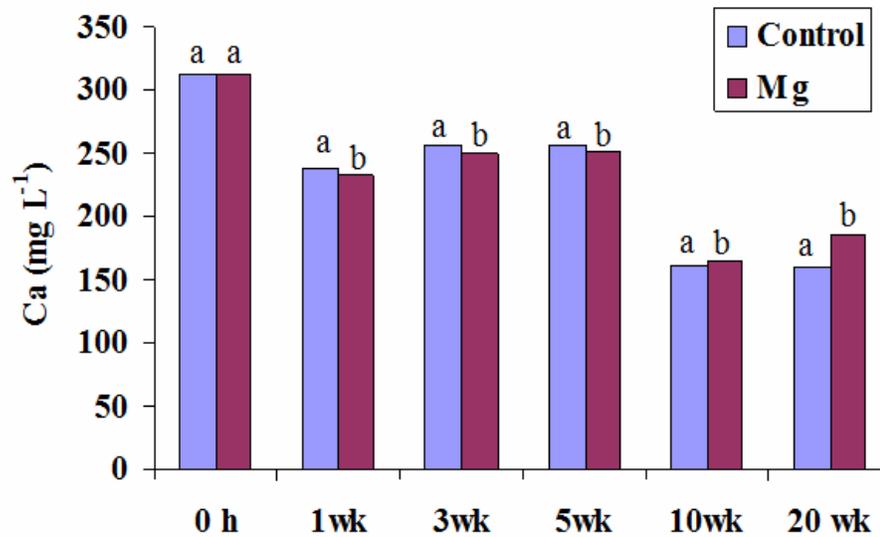


Figure 7-4. Ca concentrations for control and Mg treatment during 20 weeks of incubation. Different letters indicate statistically significant differences ($p \leq 0.05$) of median Ca concentrations after an incubation period.

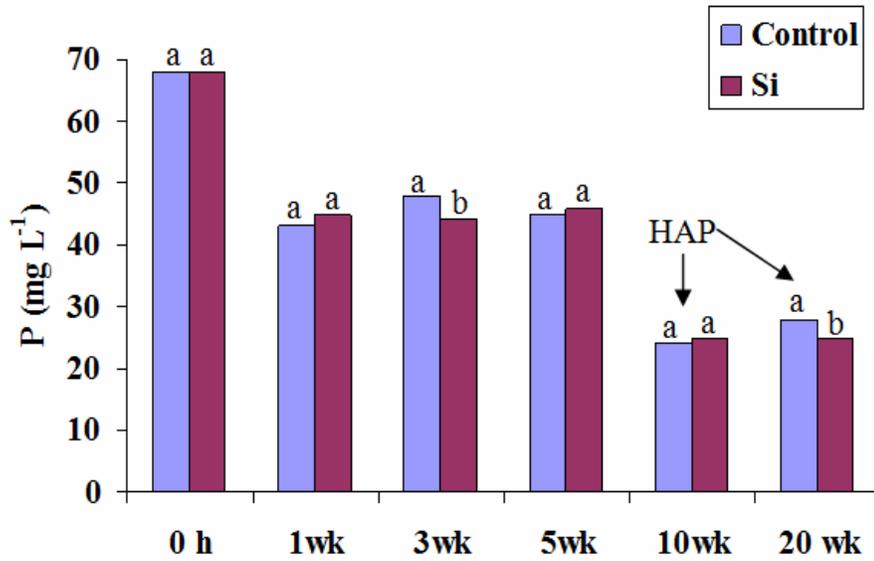


Figure 7-5. Variations in P concentrations in the presence of Si during 20 weeks of incubation. Different letters indicate statistically significant differences at $p \leq 0.05$. HAP=Hydroxyapatite.

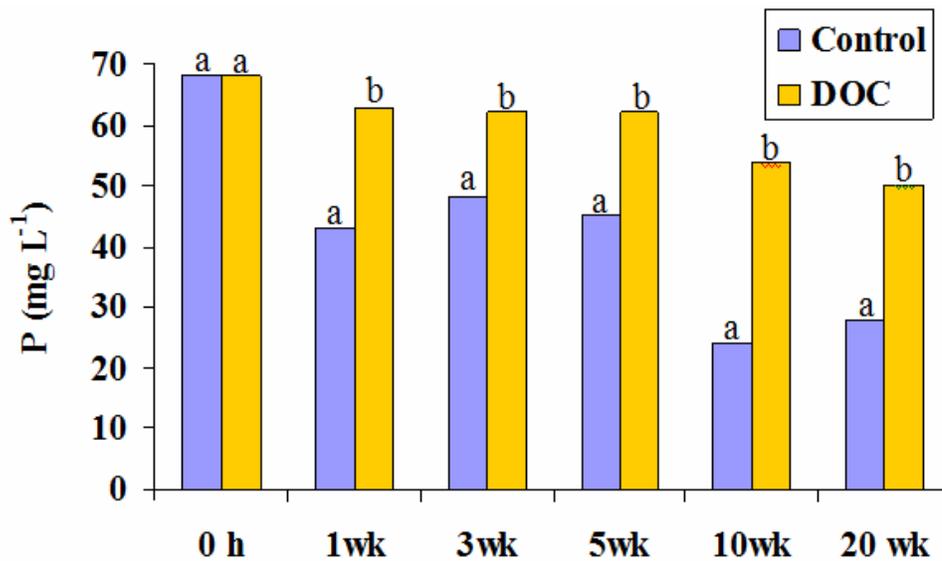


Figure 7-6. P concentrations in the presence of soil DOC during 20 weeks of incubation. Different letters indicate a significant difference ($p \leq 0.05$) after the specified incubation period.

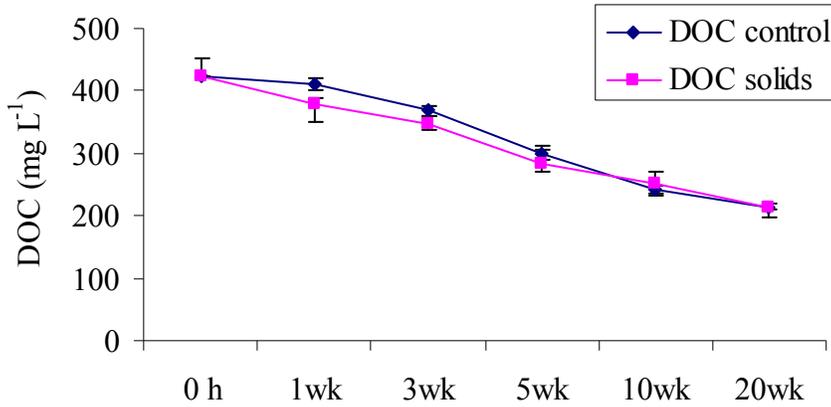


Figure 7-7. Changes in DOC concentrations in control and in the presence of solids during the 20 week (wk) incubation study.

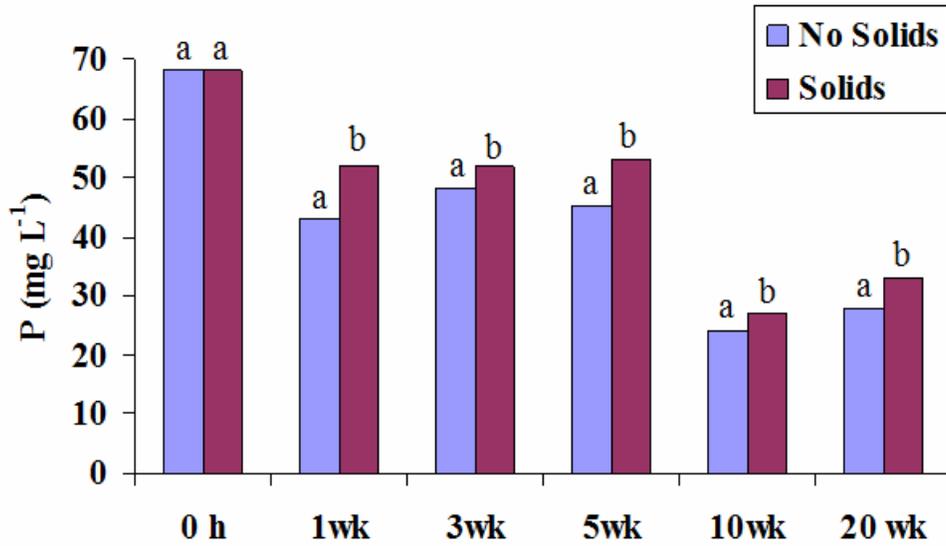


Figure 7-8. Effects of clay size fractions on P concentrations during 20 weeks of incubation. Different letters indicate statistically significant differences at $p \leq 0.05$ level.

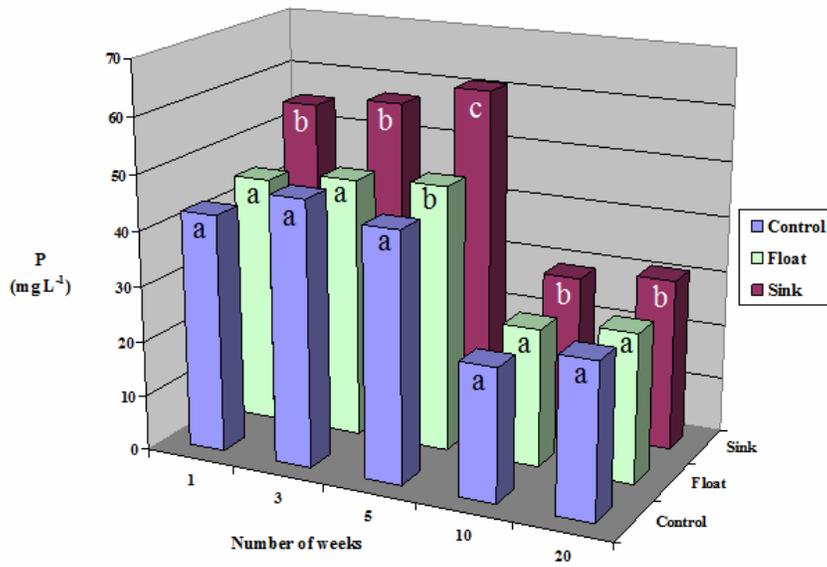


Figure 7-9. Variations in P concentrations due to the presence of low-density- (float) and high-density- (sink) clay during 20 weeks of incubation. Different letters indicate a significant difference ($p \leq 0.05$) after the specified incubation period.

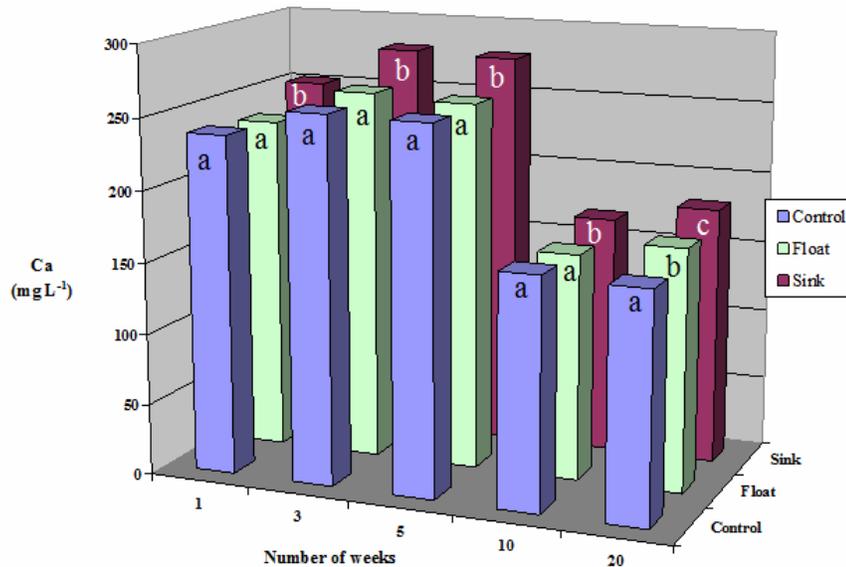


Figure 7-10. Variations of Ca concentrations due to the presence of low-density- (float) and high-density- (“sink”) clay during 20 weeks of incubation. Different letters indicate a significant difference ($p \leq 0.05$) after the specified incubation period.

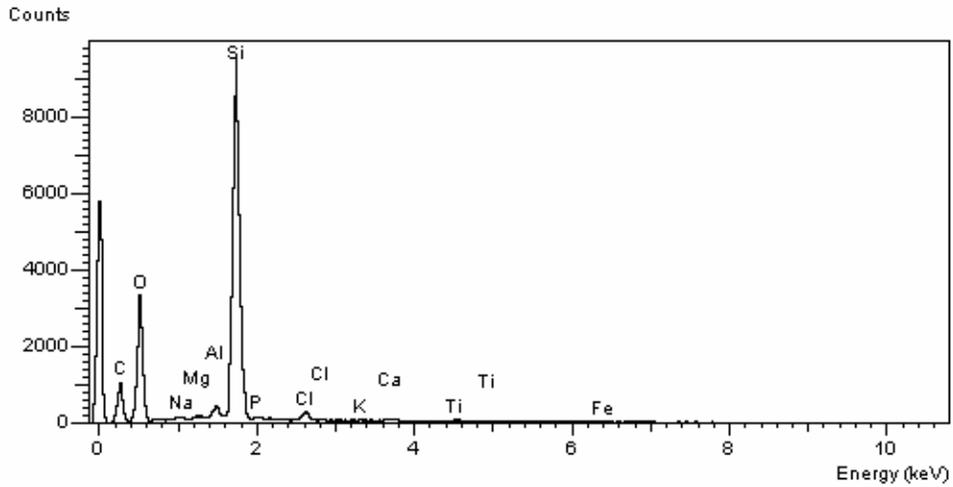


Figure 7-11. EDS spectrum of the low-density clay showing the dominance of Si.

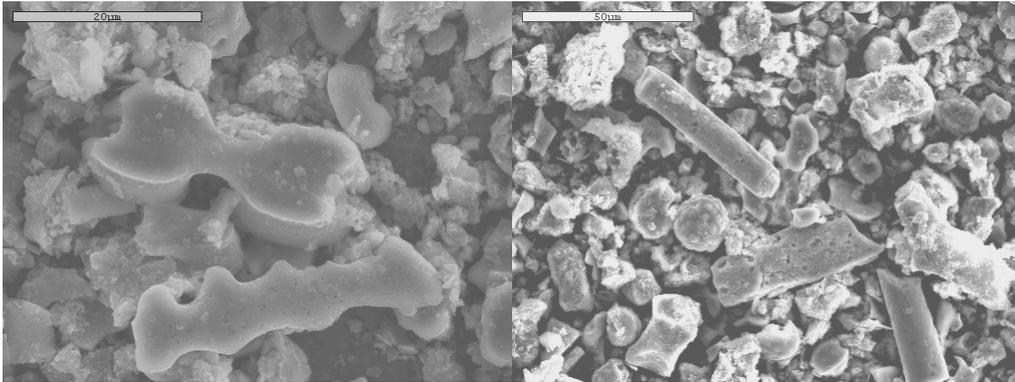


Figure 7-12. SEM imaging of low-density clay showing the presence of biogenic silica (“dumbbell” serrated shaped particles in image on left; rod shaped particles in image on right).

CHAPTER 8 SUMMARY AND CONCLUSIONS

Concentrated dairies result in accumulation of dairy manure on soils, giving rise to risks of nutrient losses that can have deleterious effects on water quality. Phosphorus in intensively-loaded soils can be labile even years or decades after site abandonment, causing leakage at environmentally-unacceptable rates. Barriers to formation of stable crystalline Ca phosphates, which soil solution data suggest would be thermodynamically favored, pose both a scientific mystery and an environmental problem. The first step in pursuit of a solution is to determine effects of critical soil components on P stability. Dairy manure provides the most reactive components for most sandy soils. If the component(s) inhibiting Ca-P crystallization could be eliminated or disabled, the result should be more P assimilation and less P loss to surface water via lateral flow to streams, etc. An understanding of reasons for continuous release of P from manure-amended soils is critical for adopting nutrient management strategies to reduce the risk of P loss. The overall objective of this dissertation was to understand the effects of dairy manure-derived components (Mg, Si and DOC) on P release and Ca-P crystallization in dairy manure-amended sandy soils. Research encompassed in this dissertation documented inhibition effects of Mg and DOC on P stabilization. It also provided multiple lines of evidence that P in dairy manure and dairy-manure-impacted soils is associated with Mg as well as Ca, even though P has been presumed to exist primarily as Ca-P in dairy manure. The association with Mg is significant because Mg-P and Ca-Mg-P are

sparingly-soluble salts that would not transform to less soluble phases and hence would tend to continue to release P over the long term.

Dairy manure-amended soils were categorized into two types: 1) active dairies, operating as dairies at the time of soil sampling; and 2) abandoned dairies, not operating for at least 10 years prior to soil sampling. Soil samples were collected from both dairy types and analyzed for basic properties. Additionally, minimally-impacted soils (never under dairy operations; low P concentrations) were acquired from previous studies (Chapter 3). Release of P and other ions from the soils was studied using repeated water extractions, column leaching (Chapter 4), and repeated NH_4Cl extractions (Chapter 5), coupled with a sequential fractionation procedure. Associated release of P, Mg, and Ca in the extraction assessments was consistent with solid phase associations of these elements that were corroborated by various solid state assessments of dairy manure and manure-amended soils (Chapter 6). An incubation study was conducted to determine the effects of manure-derived components on Ca-P crystallization (Chapter 7).

Manure-amended soils (active and abandoned) had significantly higher ($p < 0.05$) pH, and EC values, and concentrations of P, Ca, Mg and DOC than minimally-impacted soils. All soils, manure-amended and minimally-impacted, were sands and dominated by quartz in all size fractions. The clay fractions for all soils contained some secondary phyllosilicates, including kaolinite, hydroxyinterlayered vermiculite, and smectite. Dairy manure samples had high P, Ca, Mg, and DOC concentrations, but small amounts of Fe and Al. Additionally, CaCO_3 was present in all manure and manure-amended soils. Long-term addition of dairy manures increased soil pH and EC, and resulted in accumulations of P, Ca, Mg, Si, and DOC.

Release of P, Ca, and Mg from dairy manure-amended soils was evaluated by a repeated water extraction experiment (employing a wide soil: solution ratio) and a column leaching approach (narrow soil:solution ratio, to better simulate equilibrium conditions). The P released in repeated water extractions was significantly correlated ($p < 0.05$) with the release of Mg both in active and abandoned dairy soils. Release of Ca and P was not as strongly correlated as was the release of Mg and P, possibly due to the presence of other Ca compounds (CaCO_3) in addition to Ca-P. Column leachates were supersaturated with respect to the most soluble Ca-P minerals and were either undersaturated, or near saturation, for all Mg-P minerals. The data suggest that P is associated with Mg as well as Ca in manure-amended soils. Possible sparingly soluble phases of Ca-P, Mg-P, or Ca-Mg-P (the mineral whitlockite in amorphous form) control the release of P. Under natural environmental conditions, P release from sparingly soluble Mg-P or Ca-Mg-P is likely to continue for a long time.

Repeated extractions with 1.0 M NH_4Cl extracted greater amounts of P than the repeated water extractions. During the first three NH_4Cl extractions, active dairy manure-amended soils released greater amounts of P than abandoned dairy manure-amended soils. Thereafter, P concentrations stabilized and active dairy manure-amended soils behaved like the abandoned dairy soils. The release of P in repeated NH_4Cl extractions was significantly correlated with the release of both Ca and Mg, again suggesting that sparingly soluble Ca and Mg-P phases control P release. Repeated use of NH_4Cl depleted P from the HCl pool. The release of P in NaOH extractions was correlated to the release of Ca. This relationship is likely due to the residual effect of Ca-associated P that was carried over from the previous NH_4Cl extractions. The release of P in HCl extractions

was associated with Ca concentrations. The residual fractions in all soils were unaffected by sequential extractions, and residual P appeared to be associated with Ca, Fe and Al. The collective extraction data are consistent with the hypothesis that the initial high release of P from dairy manure-amended soils is due to the presence of sparingly soluble Ca-P and Mg-P phase/s, which will take years to deplete. With time (likely over a period of several decades) the release of P from the soils will be controlled by a relatively less soluble Ca-P phase, provided there is no further manure application.

Solid state techniques confirmed spatial associations of P with both Ca and Mg in dairy manure and manure-amended soils. Results were reasonably consistent with release trends for the elements observed in repeated water (Chapter 4) and NH_4Cl extractions (Chapter 5) as well as with what could be inferred about mineral equilibria from chemical speciation of column leachates (Chapter 4). The data suggest that Mg is associated with P in manure and manure-amended soils to at least an equivalent extent as Ca. Despite efforts to concentrate minerals using density and particle size fractionations in conjunction with selective dissolution specific minerals were not identified. Therefore, uncertainty remains as to crystallinity of P phases and to whether P is associated with Ca and Mg separately or within a common Ca-Mg-P phase (e.g., whitlockite) that was identified in ashed manure samples.

The incubation experiment confirmed that Mg inhibited the formation of HAP while favoring formation of a more soluble Ca-P mineral (brushite). The possible explanation for Mg inhibition can be ascribed to its smaller ionic radius (0.044nm) compared with the Ca radius (0.066nm). The smaller Mg ions can prevent development of long-range order necessary for the crystallization of HAP. Dissolved organic C and

clay-sized fractions from manure-amended soils also inhibited Ca-P crystallization, while Si had no inhibitory effect.

Results of extraction, solid-state, and incubation assessments all implicated Mg as a factor that could reduce the stability or potential stabilization of P in manure-impacted soils. There are two possible roles of Mg in preventing P stabilization: (i) the higher solubility of P phases that contain Mg and (ii) the inhibiting effect of dissolved Mg on formation of potentially stable Ca-P such as hydroxyapatite.

Results of this study indicate that risk of P release from soils (and impact on adjacent water bodies) could be reduced by minimizing Mg concentrations in manure. One option would be to maximize Ca-P interaction in animal gastro-intestinal tract by minimizing dietary Mg, without adversely impacting animal health. To date, all research has concentrated on reducing TP in the animal diet to reduce the TP in the manure, which has limitations due to dietary P requirements to maintain animal health. This study also suggests that Al-based amendments (e.g., Al-stabilized water treatment residuals) would mitigate P loss more effectively than Ca-based amendments, since the latter would likely experience the same inhibitory effects of Mg as the manure with respect to Ca-P stabilization. However, the presence of high DOC concentrations may decrease the effectiveness of WTR materials; Fe-based amendment could be evaluated as a way to sequester DOC and reduce its inhibition of P stabilization

APPENDIX A
 DETAILS OF LEACHATE CONCENTRATIONS USED FOR V-MINTEQ
 ANALYSES

Table A-1. Column leachate pH, EC and P concentrations of leachates

Soil	Leaching Events						
	1	2	3	4	5	6	7
	pH						
^a ACS-1	8.33	8.54	8.25	7.97	8.29	8.04	8.02
ACS-2	8.07	7.97	8.11	8.47	8.28	8.19	8.08
ACS-3	8.56	8.16	7.94	7.94	7.91	8.16	7.88
ACS-4	8.47	8.18	8.07	7.73	7.59	8.15	8.11
^b ABS-1	7.83	7.94	7.91	7.72	7.6	7.69	7.56
ABS-2	8.05	7.85	7.80	7.69	7.6	7.68	7.52
ABS-3	8.55	7.51	7.53	5.2	7.43	7.43	7.26
ABS-4	7.34	7.54	7.17	7.08	7.06	7.25	7.17
	EC (dS M ⁻¹)						
ACS-1	2.44	0.89	0.61	0.43	0.45	0.43	0.36
ACS-2	4.78	2.79	1.02	0.67	0.76	0.63	0.52
ACS-3	4.25	2.61	1.02	0.89	0.93	0.71	0.72
ACS-4	3.50	3.80	1.23	1.08	1.00	0.75	0.64
ABS-1	4.02	1.01	0.6	0.58	0.51	0.45	0.42
ABS-2	1.69	0.61	0.34	0.35	0.36	0.28	0.27
ABS-3	1.90	1.13	0.47	0.43	0.39	0.33	0.30
ABS-4	3.05	0.98	0.51	0.34	0.40	0.32	0.33
	P (mg L ⁻¹)						
ACS-1	0.5	1.9	3.6	7.0	7.0	13.9	7.0
ACS-2	7.0	29.2	61.7	48.0	56.6	45.5	48.0
ACS-3	3.6	27.5	48.0	89.0	81.3	63.4	75.4
ACS-4	7.0	10.4	30.9	67.7	58.3	128.3	53.1
ABS-1	1.9	10.4	19.0	20.7	32.6	22.4	20.7
ABS-2	8.7	24.1	49.7	56.6	49.7	42.9	44.6
ABS-3	5.3	12.2	39.5	39.5	52.3	46.3	49.7
ABS-4	10.4	34.4	60.8	60.8	60.0	49.7	49.7

^aACS = active dairy manure-amended soil

^bABS = abandoned dairy manure-amended soil

Table A-2. Concentrations of Ca, Mg and dissolved organic carbon (DOC) observed in column leachates.

	Leaching Events						
	1	2	3	4	5	6	7
Ca (mg L ⁻¹)							
^a ACS-1	223.1	91.4	61.5	52.8	52.7	53.9	50.9
ACS-2	205.9	85.4	37.9	30.6	27.6	24.9	24.4
ACS-3	150.4	87.5	46.6	36.7	28.1	26.2	26.0
ACS-4	178.9	190.8	97.3	72.4	54.1	41.7	35.3
^b ABS-1	654.0	159.2	113.7	104.5	100.2	99.6	102.1
ABS-2	162.0	87.7	70.8	65.3	60.9	54.9	52.3
ABS-3	241.8	159.3	78.1	66.1	59.7	57.0	55.6
ABS-4	302.7	142.9	93.1	61.8	66.3	60.2	58.8
Mg (mg L ⁻¹)							
ACS-1	142.1	45.4	30.0	26.8	26.5	27.5	26.5
ACS-2	317.5	99.0	39.8	33.0	32.9	30.2	30.0
ACS-3	296.7	141.5	68.2	52.9	43.2	41.0	41.4
ACS-4	164.0	169.7	65.5	44.9	35.2	27.6	24.3
ABS-1	167.5	44.4	32.4	29.8	26.4	25.6	27.5
ABS-2	150.2	69.1	56.6	53.0	47.7	42.7	39.3
ABS-3	81.4	54.1	20.5	17.7	16.0	15.1	15.1
ABS-4	109.5	53.9	28.7	22.5	20.9	18.5	18.6
DOC (mg L ⁻¹)							
ACS-1	469	179	119	80	61	52	40
ACS-2	398	350	265	162	123	97	80
ACS-3	497	394	295	212	142	105	86
ACS-4	711	818	603	423	299	194	139
ABS-1	463	245	166	150	109	141	75
ABS-2	251	143	104	75	64	62	46
ABS-3	170	166	140	118	84	76	61
ABS-4	334	215	144	121	87	79	70

^aACS = active dairy manure-amended soil^bABS = abandoned dairy manure-amended soil

Table A-3. Concentrations of K, Fe and Al observed in column leachates

	Leaching Events						
	1	2	3	4	5	6	7
	K (mg L ⁻¹)						
^a ACS-1	392	178	122	91	67	50	31
ACS-2	745	474	299	245	219	179	153
ACS-3	711	532	383	325	274	247	222
ACS-4	493	585	397	303	266	220	206
^b ABS-1	211	96	67	61	48	39	37
ABS-2	127	72	42	27	18	13	10
ABS-3	125	128	87	79	72	61	53
ABS-4	100	85	60	47	38	28	23
	Fe (mg L ⁻¹)						
ACS-1	0.51	0.41	0.69	0.74	0.59	0.12	0.10
ACS-2	0.29	0.51	0.58	0.28	0.34	0.38	0.39
ACS-3	0.24	0.31	0.39	0.45	0.41	0.12	0.08
ACS-4	0.78	0.96	1.58	1.67	1.32	1.04	0.27
ABS-1	0.15	0.17	0.17	0.25	0.15	0.11	0.09
ABS-2	0.08	0.07	0.06	0.06	0.03	0.05	0.06
ABS-3	0.10	0.16	0.25	0.37	0.53	0.61	0.39
ABS-4	0.25	0.25	0.18	0.12	0.14	0.17	0.13
	Al (mg L ⁻¹)						
ACS-1	0.38	0.34	0.28	0.23	0.16	0.15	0.12
ACS-2	0.25	0.49	0.54	0.40	0.33	0.23	0.20
ACS-3	0.10	0.16	0.21	0.17	0.15	0.12	0.10
ACS-4	0.25	0.34	0.46	0.41	0.29	0.22	0.15
ABS-1	0.06	0.09	0.09	0.09	0.08	0.09	0.08
ABS-2	0.10	0.10	0.09	0.10	0.10	0.10	0.11
ABS-3	0.12	0.17	0.26	0.21	0.22	0.19	0.18
ABS-4	0.22	0.32	0.35	0.27	0.31	0.27	0.27

^aACS = active dairy manure-amended soil

^bABS = abandoned dairy manure-amended soil

Table A-4. Concentrations of sulfate, chloride and ammonium observed in column leachates

	Leaching Events						
	1	2	3	4	5	6	7
Sulfate (mg L ⁻¹)							
^a ACS-1	279.3	18.5	3.3	1.5	1.4	^c nd	nd
ACS-2	408.9	85.9	7.8	3.9	2.8	nd	nd
ACS-3	476.7	127.0	11.0	4.8	2.7	nd	nd
ACS-4	175.0	250.8	16.1	3.9	1.8	nd	nd
^b ABS-1	331.9	42.2	3.9	1.7	0.9	nd	nd
ABS-2	82.3	13.1	3.3	2.2	1.4	nd	nd
ABS-3	95.7	52.5	5.6	2.0	1.2	nd	nd
ABS-4	831.7	268.2	35.2	2.5	1.1	nd	nd
Chloride (mg L ⁻¹)							
ACS-1	69.7	11.8	7.7	5.2	3.3	3.3	5.4
ACS-2	450.6	85.4	19.2	12.6	8.4	6.7	5.2
ACS-3	324.2	104.2	24.0	15.0	9.9	7.7	5.8
ACS-4	155.6	294.8	47.0	34.9	21.6	15.2	10.1
ABS-1	72.0	12.7	12.7	5.8	5.5	5.4	4.6
ABS-2	65.5	12.3	17.2	4.1	3.8	3.7	3.6
ABS-3	30.6	23.6	8.2	6.1	4.9	4.6	4.4
ABS-4	48.5	28.8	12.6	8.8	9.1	4.7	4.2
Ammonium (mg L ⁻¹)							
ACS-1	43.3	33.0	24.5	19.4	15.1	9.1	7.4
ACS-2	27.9	10.8	5.7	4.0	4.0	4.0	4.0
ACS-3	41.6	31.3	22.8	9.1	16.0	12.6	9.1
ACS-4	51.8	72.3	38.2	19.4	26.2	22.8	21.1
ABS-1	59.5	41.6	32.2	27.1	24.5	19.4	14.3
ABS-2	16.0	7.4	2.3	2.3	1.4	2.3	1.4
ABS-3	9.1	12.6	8.3	7.4	5.7	5.7	4.0
ABS-4	29.6	22.8	17.7	14.3	12.6	9.1	9.1

^aACS = active dairy manure-amended soil^bABS = abandoned dairy manure-amended soil^cnd = not detectable

Table A-5. Concentrations of nitrates, and silicic acid (H₄SiO₄) observed in column leachates

	Leaching Events						
	1	2	3	4	5	6	7
	Nitrates (mg L ⁻¹)						
^a ACS-1	3.7	2.5	0.7	0.1	6.5	0.5	nd
ACS-2	457.4	82.0	^c nd	0.2	nd	nd	nd
ACS-3	190.1	59.3	0.0	0.1	nd	0.2	0.5
ACS-4	25.2	47.9	0.1	0.2	nd	nd	nd
^b ABS-1	684.8	4.8	nd	Nd	nd	0.1	nd
ABS-2	138.9	19.6	0.2	Nd	nd	nd	nd
ABS-3	207.1	90.1	0.3	Nd	nd	nd	nd
ABS-4	93.5	8.2	0.3	Nd	nd	nd	nd
	H ₄ SiO ₄ (mg L ⁻¹)						
ACS-1	33.4	27.3	24.5	23.6	20.7	21.6	17.0
ACS-2	28.1	23.6	21.7	21.3	20.4	18.4	16.6
ACS-3	41.1	36.1	35.8	32.7	28.1	26.0	24.5
ACS-4	37.9	39.3	40.5	43.7	44.4	42.2	42.9
ABS-1	73.7	75.4	78.3	80.7	74.1	68.6	68.3
ABS-2	59.8	40.6	33.4	29.8	28.1	26.5	25.7
ABS-3	59.5	57.0	48.2	43.1	37.8	35.2	32.7
ABS-4	45.2	37.8	28.9	18.0	21.0	19.3	18.9

^aACS = active dairy manure-amended soil^bABS = abandoned dairy manure-amended soil^cnd = not detectable

APPENDIX B
CORRELATION MATRIX FOR DIFFERENT P FRACTIONS OF DAIRY MANURE-AMENDED SOILS

Table B-1. Correlation matrix for different fractions in active dairy manure-amended soils

	<i>NH₄Cl TDP</i>	<i>NH₄Cl Ca</i>	<i>NH₄Cl Mg</i>	<i>NH₄Cl DOC</i>	
NH ₄ Cl_TDP	1.000				
NH ₄ Cl_Ca	0.773	1.000			
NH ₄ Cl_Mg	0.826	0.855	1.000		
NH ₄ Cl_DOC	0.905	0.945	0.931	1.000	
	<i>NaOH TDP</i>	<i>NaOH Ca</i>	<i>NaOH Mg</i>	<i>NaOH Fe</i>	<i>NaOH Al</i>
NaOH_TDP	1.000				
NaOH_Ca	0.812	1.000			
NaOH_Mg	0.272	0.546	1.000		
NaOH_Fe	0.142	0.245	-0.107	1.000	
NaOH_Al	0.424	0.388	0.286	0.331	1
	<i>HCl P</i>	<i>HCl Ca</i>	<i>HCl Al</i>	<i>HCl Mg</i>	<i>HCl Fe</i>
HCl_P	1.000				
HCl_Ca	0.946	1.000			
HCl_Al	0.603	0.581	1.000		
HCl_Mg	0.930	0.855	0.505	1.000	
HCl_Fe	0.449	0.306	0.578	0.454	1.000
<i>Residual</i>	<i>P</i>	<i>Ca</i>	<i>Al</i>	<i>Mg</i>	<i>Fe</i>
P	1.000				
Ca	0.895	1.000			
Al	0.723	0.802	1.000		
Mg	0.838	0.914	0.950	1.000	
Fe	0.890	0.888	0.936	0.972	1.000

Table B-2. Correlation matrix for different fractions for abandoned dairy manure-amended soils

	<i>NH₄Cl TDP</i>	<i>NH₄Cl Ca</i>	<i>NH₄Cl Mg</i>	<i>NH₄Cl DOC</i>	
<i>NH₄Cl_TDP</i>	1.000				
<i>NH₄Cl_Ca</i>	0.502	1.000			
<i>NH₄Cl_Mg</i>	0.420	0.989	1.000		
<i>NH₄Cl_DOC</i>	0.745	0.924	0.892	1.000	
	<i>NaOH TDP</i>	<i>NaOH Ca</i>	<i>NaOH Mg</i>	<i>NaOH Fe</i>	<i>NaOH Al</i>
<i>NaOH_TDP</i>	1.000				
<i>NaOH_Ca</i>	0.781	1.000			
<i>NaOH_Mg</i>	0.344	0.423	1.000		
<i>NaOH_Fe</i>	-0.157	-0.243	-0.102	1.000	
<i>NaOH_Al</i>	0.309	0.178	0.232	0.289	1.000
	<i>HCl P</i>	<i>HCl Ca</i>	<i>HCl Al</i>	<i>HCl Mg</i>	<i>HCl Fe</i>
<i>HCl_P</i>	1.000				
<i>HCl_Ca</i>	0.840	1.000			
<i>HCl_Al</i>	-0.058	-0.033	1.000		
<i>HCl_Mg</i>	0.618	0.880	0.118	1.000	
<i>HCl_Fe</i>	-0.737	-0.316	0.320	0.001	1.000
<i>Residual</i>	<i>P</i>	<i>Ca</i>	<i>Al</i>	<i>Mg</i>	<i>Fe</i>
<i>P</i>	1.000				
<i>Ca</i>	0.962	1.000			
<i>Al</i>	-0.468	-0.379	1.000		
<i>Mg</i>	-0.079	-0.023	-0.039	1.000	
<i>Fe</i>	0.955	0.906	-0.499	0.056	1.000

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BIOGRAPHICAL SKETCH

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