ABSTRACT

HUTCHISON, KIMBERLY, N. Dissolution of Phosphate in a Phosphorus-Enriched Ultisol as Affected by Microbial Reduction.
(Under the direction of Dr. Dean L. Hesterberg).

Knowledge of the effect of reduced soil redox conditions on P dissolution is needed to better assess P mobility to surface or ground water. The objectives of our study were to determine the effect of microbial reduction on P dissolution and determine mechanisms of P release in a reduced soil. Duplicate suspensions of silt+clay from a Cape Fear sandy clay loam were reduced in a continuously-stirred redox reactor for 40 d. We studied the effects of three treatments on P dissolution: (i) 2 g dextrose kg^{-1} solids added as a microbial carbon source at time 0 d; (ii) 2 g dextrose kg^{-1} solids split into three additions at 0, 12, and 26 d; and (iii) no added dextrose. Regardless of treatment or variation in the intensity of reduction rates, dissolved reactive P (DRP) increased up to 7-fold from 1.5 to 10 mg L^{-1} and was linearly related ($R^2 = 0.79$) with dissolved organic C. Dissolved Fe and Al and pH also increased, suggesting the formation of aqueous Fe- and Al-dissolved organic matter (DOM) complexes. Separate batch experiments were performed to study the effects of increasing pH and citrate additions on PO_4 dissolution under aerobic conditions. Increasing additions of citrate increased concentrations of DRP, Fe, and Al, while increasing pH had no effect. Results indicated that increased DOM during soil reduction contributed to the increase in DRP, perhaps by competitive adsorption or by formation of aqueous ternary PO_4-Fe-DOM or PO_4-Al-DOM complexes.

Generation of DOM during microbial reduction may be an important model

parameter for more quantitatively predicting the dissolution of PO_4.

DISSOLUTION OF PHOSPHATE IN A PHOSPHORUS-ENRICHED

ULTISOL AS AFFECTED BY MICROBIAL REDUCTION
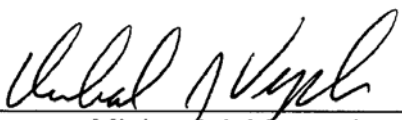
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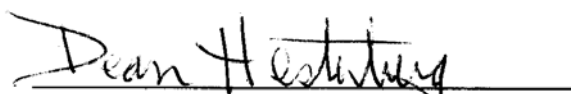Master of Science

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APPROVED BY

Michael J. Vepraskas

Philip W. Westerman

Chair of Advisory Committee
Dean L. Hesterberg

DEDICATION

I would like to dedicate this thesis to my husband Robert and our children Jessica, Elizabeth, Brian, and Michael. Each of you have given me a special gift that has helped me to succeed. Thank you - Robert for always directing me towards logical thinking, - Jessica for strength of personality, - Elizabeth for humor and kindness, - Brian for determination, and - Michael for joyful exuberance.

BIOGRAPHY

Kimberly Jane Hutchison was born August 31, 1958, in Bath, New York. In 1978, Kim married Robert A. Hutchison and over the next nine years they were blessed with the birth of their children Jessica, Elizabeth, Brian, and Michael. In 1986, Kim began her pursuit of a college education attending, part-time, Davidson County Community College in Lexington, N.C. In 1993, she received the Puterbaugh Chemist of the Year award and her Bachelors of Art degree in chemistry from the University of North Carolina at Greensboro, Greensboro, N.C. In 1995, Kim was hired as a Research Technician working with Dr. Dean Hesterberg, soil physical chemist, in the Department of Soil Science at North Carolina State University, Raleigh, NC. Kim was excited to apply her knowledge of chemistry and inorganic synthesis to practical environmental problems involving heavy metal transport in soils, as well as, learning to synthesize soil minerals used in research studies. As Kim continued her work in the Department of Soil Science, she learned how important soil science was to the environmental protection of our soil and water resources and sustainable agriculture. These realizations led Kim to become a Masters of Science candidate while continuing her present job as a Research Technician.

this time. I extend heartfelt thanks to Peggy Longmire, Daphine McKinney, and

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Finally, there are not appropriate words to acknowledge the gratitude I feel

towards my family to whom this work is dedicated. Through the course of good

and bad days, long nights and weekends, they were a constant in my life.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

<u>Rationale</u>

Long-term application of animal waste and fertilizers to agricultural land has resulted in elevated soil P concentrations. For years, fertilizers and animal waste have been applied to soils to meet the crop nitrogen (N) requirements. This has led to a 3- to 5- fold over-application of P to soils (Mikkelsen, 1997). In the United States, Europe and Canada, surface soil accumulations of P have increased to the extent that the loss of P in drainage waters and in runoff discharged to surface waters has become a management concern (Breeuwsma and Schoumans, 1987; Federico et al., 1981; Sallade and Sims, 1997a).

Phosphorus enrichment of surface waters stimulates excessive plant and algal growth, commonly referred to as eutrophication. Increased growth and decreased dissolved oxygen from P enrichment degrades water quality through increased costs of water purification, decreased aquatic species, decreased lake depths, and increased public concern over toxic and non-toxic algae (Pierzynski et al., 1994). Surface runoff of soluble P and particulate P, and P loss via subsurface flow are major pathways for P to enter surface waters (Figure 1.1) (Calvert, 1975; Hergert et al., 1981; Izuno et al., 1991). Decades of research have provided an understanding of soil P dynamics and release to runoff (Daniel et al., 1998; Sims et al., 1998). However, many of the dissolution and transport processes of soils enriched with P are not understood well enough to predict P loss quantitatively.

When P-enriched soil becomes waterlogged or particulate P is transported to surface waters the soil or soil particles encounter anoxic conditions and the oxidation-reduction (redox) potential decreases (Patrick et al., 1996).

<u>Redox Potential</u>

Soil redox potential has been positively correlated with net P dissolution (Patrick and Khalid, 1974; Holford and Patrick, 1981; Willet, 1989; Sallade and Sims, 1997b; Phillips, 1998). Poorly-drained soils and soils that have a fluctuating water table exhibit lower redox potentials. Soils with a lower redox potential (more reduced conditions) generally show enhanced P mobility. As the soil water content decreases, the redox potential will increase and likely inhibit P mobility.

The Coastal Plain region of North Carolina consists primarily of poorly-drained agricultural soils with subsurface drainage and soils with sandy surface horizons that have a low P sorption capacity. Many of these soils have elevated P concentrations due to long-term application of animal wastes (Hamm, 1999). In poorly-drained agricultural soils controlled drainage is used as a best management practice (BMP) to reduce the loss of nitrates from the soil by artificially raising the water table using a structure installed in the drain (Gilliam et al., 1997). Therefore, there is an increased risk of P mobility as these soils become saturated and the redox potential is lowered.

<u>Redox reactions</u>

Measured redox potential decreases as a soil becomes reduced. Soil reduction is usually a result of microbes oxidizing organic carbon compounds to obtain energy. The oxidation of carbon must be coupled with the reduction of an

electron acceptor in a thermodynamic sequence such as O_2, NO_3, Mn(IV), and

Fe(III). As the redox potential decreases, the following sequence of reduction

reactions typically occurs: $O_{2(g)}$ to $H_2O_{(l)}$, $NO_{3(aq)}$ to $N_2O_{(g)}$ or $N_{2(g)}$, solid-phase Mn(IV)

oxides to $Mn^{2+}_{(aq)}$, solid-phase Fe(III) hydrous oxides to $Fe^{2+}_{(aq)}$, $SO_4^{2-}_{(aq)}$ to $HS^-_{(aq)}$

or $H_2S_{(g)}$, and finally $CO_{2(g)}$ to $CH_{4(g)}$ (Pierzynski et al., 1994).

Soil reduction (lower redox potential) can affect the solid-phase speciation of

minerals, such as Fe(III) oxides (Brennan and Lindsay, 1998; Khalid et al., 1977).

Vepraskas et al. (1999) studied the development of hydric soil field indicators that

are formed by Fe(III) reduction in a created marshland. These depleted matrix

indicators appear as the soil becomes gray, a result of Fe(III) oxides being reduced

to soluble Fe(II) and leaching from the soil. Often, the soil is mottled (streaked with

orange or red), as the soluble Fe(II) re-precipitates as Fe(III) oxides when the

reduced iron encounters zones of oxygen. Brennan and Lindsay (1998) found that

the reduction of Fe(III) oxides in laboratory reactors resulted in the dissolution and

precipitation of two distinct Fe_3O_4 minerals. Khalid et al. (1977) showed differences

in the ratio of P sorption to oxalate-extractable iron (oxalate removes poorly

crystalline and amorphous iron, and organic matter-associated Fe) for separate

samples of Alfisols or Inceptisols that were reduced or oxidized for 15 days. The

isotherms for the reduced treatments showed a higher correlation between P

sorption and oxalate-extractable iron. This apparent increase in amorphous iron

mineral species under reduced conditions would increase the reactive surface area,

which could explain the greater P sorption capacity of these soils.

These results indicate a change in the crystalline phase of iron under reduced

conditions. Since P is often associated with iron (manganese) oxide minerals, the

solid-phase speciation of soil phosphorus may change as the solid-phase speciation

of the iron (manganese) mineral changes. These changes can impact processes

that control P solubility such as sorption, surface complexation, and precipitation

reactions.

Effect of redox on the dissolution of phosphate

   As the redox potential decreases changes in the solid-phase speciation of

phosphorus can occur (Roden and Edmonds, 1997; Willet, 1985) resulting in

simultaneous phosphate dissolution and removal mechanisms in the soil solution.

Figure 1.2 illustrates how the reduction of Mn(IV)- and Fe(III)-minerals could affect

the dissolution and speciation of P in soil. First, dissolution of iron oxides should

release sorbed P into solution and continued reduction should release occluded or

co-precipitated P. Roden and Edmonds (1997) showed P sorption to Fe(II)-minerals

after Fe(III)-oxide minerals underwent microbial reduction. At the same time, Fe(III)-

phosphate minerals, such as strengite ($FePO_4 \bullet 2H_2O$), could undergo reductive

dissolution (Lindsay, 1979; Willet, 1985; Miller et al., 1993). Fe(II)- and Mn(II)-

phosphate minerals have been shown to precipitate under reduced conditions.

Friedl et al. (1997) showed by electron microprobe analysis, the precipitation of

$(Fe,Mn)_3(PO_4)_2 \bullet 8 H_2O$ from MnO_2 (H^+-birnessite) in anoxic sediment. Willet (1985)

showed the precipitation of the Fe(II)-phosphate mineral, vivianite ($[Fe_3(PO_4)_2 \bullet 8$

$H_2O]$), after strengite was reduced for 11 days.

   Phosphorus can be sorbed to aluminum hydroxide and aluminosilicate

minerals that are not generally affected by reductive dissolution. Also, the

precipitation of aluminum phosphates can occur as the soil becomes reduced

(Tiessen, 1995). The concentration of dissolved aluminum in soil is higher at a lower

pH (Lindsay, 1979). As iron oxides with sorbed or occluded P undergo reductive

dissolution, the concentration of P in solution will increase. The precipitation of

aluminum phosphates can occur if the concentrations of both P and Al are high

enough (Tiessen, 1995). As the soil is reduced, the pH tends to increase and lower

the concentration of dissolved aluminum in the soil solution. Phosphate dissolution

and changes in solid-phase P species are further complicated by other reactions

involving organic matter where complexation of cations by dissolved organic matter

(DOM) with subsequent binding of PO_4 and competitive adsorption for mineral

surfaces between PO_4 and DOM (Gerke, 1992, 1993, 1997; Gerke and Hermann,

1992; Violante, 1991) can occurr.

<u>Reductive dissolution of Fe(III) oxides</u>

The reductive dissolution of Fe(III) oxides has been related to the release of P

under reducing conditions in flooded soils and sediments (Holford and Patrick, 1981;

Willett, 1989; Roden and Edmonds, 1997; Phillips, 1998; Jensen, et al., 1998;

Sallade and Sims, 1997b). Sallade and Sims (1997b) correlated total Fe-oxide

content with soluble P after soils were flooded under anoxic conditions for 21 days.

Operationaly-defined chemical fractionation of solid-phase inorganic P in sediments

(Sallade and Sims,1997b) and in soil (Maquire et al., 2000) showed Fe-associated P

and Al-associated P to be the predominant inorganic P forms. Many of the P

saturation indices that have been used to asses the relationship between excess soil

P on P desorbability and the subsequent loss of P via surface or subsurface

pathways contain variables describing relationships between Fe, Al, and P (Table 1.1; Beauchemin and Simard, 1999).

Many researchers proposed that the soil P saturation index variable would be able to better predict the risk of P loss (Sharpley 1995; Beauchemin et al., 1996; Pote et al., 1996) since the variable relates an "intensity factor" (solution P concentration) with a "capacity factor" (P sorption capacity) and is often described as linear (Kuo et al., 1988; Giroux et al., 1996; Lookman et al., 1996; Sharpley 1996). However, Sims et al. (1998) observed that soils having a degree of P saturation (DPS) > 25% tended to have higher desorbable P values. Beauchemin and Simard (1999) state that the variablity of P solubilty and P saturation index are related to the range of soils tested and the types of extractants used (e.g. Soil Test Phosphorus). The P saturation index usually explains a larger variation of P solubility than a particular single extractant, however, it has been observed that the proportion of unexplained variation in P solubility will be greater over a wider range of soils studied (Beauchemin and Simard, 1999). While there seems to be agreement on the importance of iron oxides on P sorption capacity, it does not fully explain observed differences of P solubility under reduced conditions.

For example, in laboratory studies Willet (1989) compared 1 M acetate extractable P for 18 soils that were flooded (reduced) for 4, 8, 16, or 32 days. While extractable Fe(II) [indicating reduction of Fe(III)] was positively correlated with extractable P, he could not adequately relate changes in extractable P to just one single chemical property. For example, in comparing 1 M acetate extractions for a reduced Haplumbrept (prairie soil, pH 5.2) and a Haplaquept (alluvial soil, pH 5.3)

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both soils showed similar increases in extractable Fe(II) and P. However, after

reduction of a Paleustalf (yellow, podzolic soil, pH 6.6) soil extractable Fe(II) and P

showed similar P concentrations but 8% less Fe(II) than the Haplumbrept and

Haplaquept soils. Willet (1989) speculated that differences in the amount of

extractable P were due to re-adsorption of P to mineral surfaces and interaction with

organic matter. Competing processes such as, desorption, sorption and interactions

with organic matter make it apparent that the prediction of P dissolution in reduced

soils cannot be simply correlated to an operationally-defined chemical fraction for

Fe-associated P in fractionation methods such as Mahapatra and Patrick (1971).

The study by Willet (1989) exemplifies the difficulty in trying to correlate specific

chemical properties that control P dissolution over a range of soils with different

chemical and physical properties.

Holford and Patrick (1981) studied the effects of the reduction of an acid soil

on P sorption capacity over time. They discovered that the time dependence of

competing reactions in the soil matrix is important in understanding simultaneous

dissolution and removal mechanisms that govern the net release of P.

Understanding the time dependence of these mechanisms could also help explain

results of previous studies where changes in P sorption were evaluated once at the

end of a reduction period (Khalid et al., 1977; Ponnamperuma, 1972). Suspensions

of silty loam soil were incubated under reduced conditions for 45 days (Holford and

Patrick, 1981). After 1 day of reduction there was a large increase in P sorption,

evidenced by a decrease in dissolved phosphate, and an apparent decrease in

sorption between the 2nd and 18th day. During this time, an increase in dissolved

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Fe(II) concentrations suggested an increase in the dissolution of iron hydrous oxides, possibly releasing sorbed/occluded P (Willett, 1989; Roden and Edmonds, 1997; Phillips, 1998; Jensen, et al., 1998; Sallade and Sims, 1997b). At day 27, the largest sorption of P was observed, possibly due to the precipitation of amorphous and more reactive Fe(II) hydroxide species (Arden, 1950). By 45 days, the PO_4 sorption capacity of the soil had decreased and was near to that of the untreated soil (Holford and Patrick, 1981). This study implies that P dissolution may be different for soils flooded for various lengths of time. Phillips (1998) also studied P availability and sorption under alternating oxidized and reduced conditions. During soil reduction, Phillips found the same initial sorption of P after one day (similar to Holford and Patrick, 1981), with an increase in dissolved Fe(II), Mn(II), and P solubility around day 21. Holford's experiments substantiate the validity of complex simultaneous reactions occurring in the long-term reduction of soil that lead to periods of P release and sorption during the reduction period.

Analysis of solid-phase speciation

The mobilization of P in dissolved forms from a terrestrial to aquatic ecosystem involves many complex and competing mechanisms of P dissolution and removal (Figure 1.2). Understanding these mechanisms requires that different solid-phase forms (species) of P be identified (e.g. adsorbed versus precipitated). Chemical fractionation techniques (Hedley et al., 1982; Mahapatra and Patrick, 1969; Chang and Jackson, 1957) are important for determining operational pools of P, such as, P sorbed to oxide minerals, P associated with Ca minerals, and P associated with poorly crystalline and more crystalline iron oxide minerals.

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However, these extractions may lack the ability to differentiate between related species that may affect redox behavior. For example, one extraction step in the Hedley method fractionates P associated with Fe-oxide (redox active) plus Al-oxide (non-redox active) minerals. This extraction step cannot differentiate between the Fe-oxide and Al-oxide solid-phase P species and their role in redox transformations.

X-ray absorption near edge structure (XANES) spectroscopy is a molecular-scale, physical method that can determine oxidation states and local bonding environments of a minor element in a complex matrix such as soil. Information obtained through XANES spectroscopy is important because an element is analyzed "in-situ" and is a non-destructive technique.

A typical x-ray absorption spectrum [consisting of XANES and extended x-ray absorption fine structure (EXAFS) regions] consists of a pre-edge region on the low-energy side of the absorption edge between -10 and -1 eV, an absorption edge at approximately 0 eV, a post-edge region between 2 and 30 eV, and an extended region at >30 eV (Figure 1.3). Analysis of phosphorus compounds often provide spectra that have unique features in these regions that can be used to discern information on the symmetry, electronic state and local chemical environment of P (Fendorf and Sparks, 1996). This information is obtained by comparing features of the energy positions of the white lines or absorption edges that occur as a core electron is ejected from the element of interest. For instance, Hesterberg et al. (1999) showed unique features among various crystallinities of strengite and adsorbed P on goethite (Figure 1.4a). Also, various calcium phosphate standards were compared to a soil sample, showing different spectral features in the post-edge

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region (Figure 1.4b). These distinct features might be used to identify mineral or sorbed species in a soil sample by comparing the features of standards to those of P in the soil.

Also, Hesterberg et al. 1999 showed that phosphorus K-XANES spectra for strengite ($FePO_4 \bullet 2H_2O$) and variscite ($AlPO_4 \bullet 2H_2O$) or strengite and different Al-hydroxides (Khare et al., 2004; (a) in review) contain unique features which enable one to differentiate PO_4 associated with Fe(III) minerals from PO_4 associated with Al(III) minerals. A distinct pre-edge feature is present in the spectra of Fe(III)-associated phosphates due to electron orbital configurations and electronic transitions near the x-ray absorption edge (Khare et al., (b) in review; Beauchemin et al., 2003); Hesterberg et al., 1999), and are absent in the spectra of Al-phosphates (Figure 2.5). As a result, XANES spectroscopy can differentiate between specific solid-phase chemical P species and their role in redox transformations and may be an important analytical method for redox studies.

Objectives

To quantitatively predict P dissolution and transport in reduced soils, a better understanding is needed of P release mechanisms during microbial reduction of soils. The objectives of this study were (i) to determine how the intensity of microbial reduction affects P dissolution from the surface horizon of an acidic, P-enriched Coastal Plain soil, and (ii) to determine the relative importance of various P dissolution mechanisms during the reduction of this soil.

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Table 1.1: Partial review of some P saturation indices used in different studies for acidic soils (table extracted from Beauchemin and Simard, 1999.)

References	pH, texture, and location	Indices[‡]
Beauchemin et al. (1996)	Acidic, loam Québec, Canada	$Pi_{ox}/(Al_{ox} + Fe_{ox})$
De Smet et al. (1996a,b)	Acidic to slightly alkaline, sandy loam Belgium	$P_{ox}/0.5(Al_{ox} + Fe_{ox})$
Pote et al. (1996)	Acidic, silt loam Arkansas	$P_{ox}/(Al_{ox} + Fe_{ox})$
Sharpley (1996)	Acidic to neutral, sandy to clay loams Texas and Oklahoma, USA	Strip-P/X_m
Sharpley (1995)	Mainly acidic, sandy loam to loam Oklahoma, USA	M3P/X_m
Sims et al. (1998)	Acidic, loamy sand to sandy loam Delaware, USA	$P_{ox}/(Al_{ox} + Fe_{ox})$
Van der Zee et al. (1988)	Acidic, sandy soils Netherlands	$P_{ox}/(Al_{ox} + Fe_{ox})$

[‡]P_{ox}, Al_{ox}, and Fe_{ox}: total P, Al or Fe extractable with ammonium-oxalate solution (mmol kg^{-1}); Pi_{ox}: molybdate reactive P in the non-digested extracts of ammonium oxalate (mmol kg^{-1}); X_m: P sorption maximum derived from a Langmuir isotherm (mg kg^{-1}); M3P: Mehlich-III extractable P (mg kg^{-1}); Strip-P: P extractable with Fe-oxide strips (mg kg^{-1}).
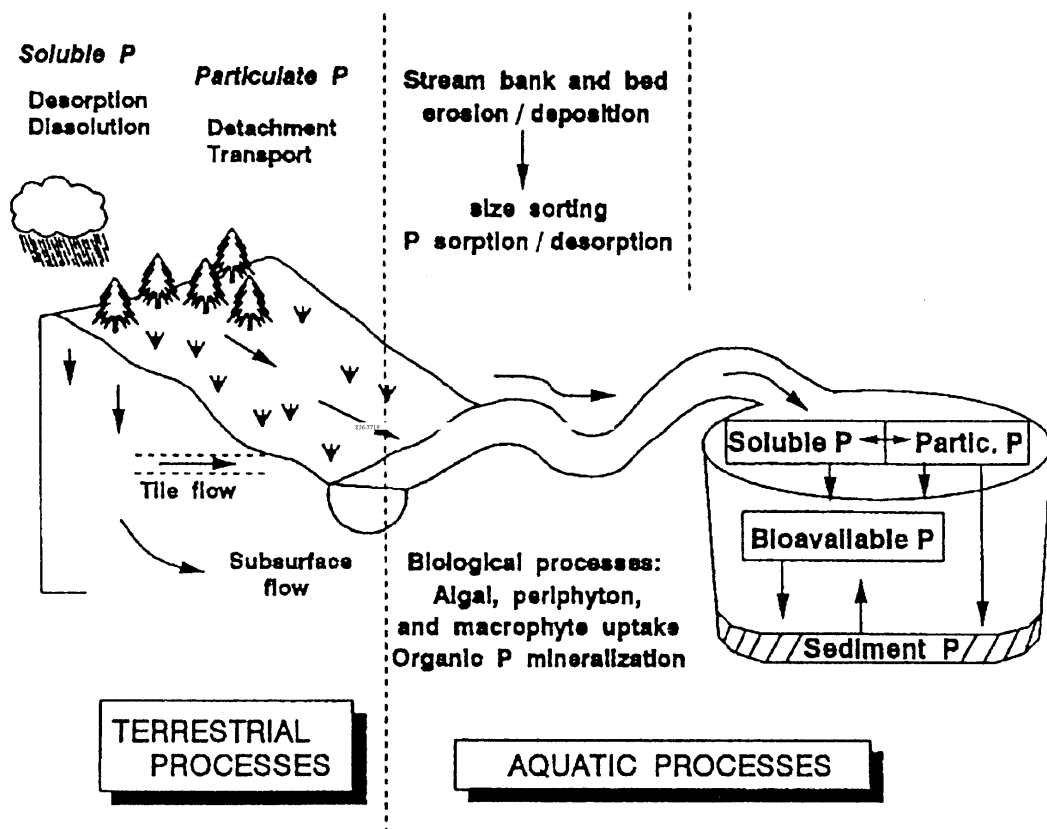
Figure 1.1: Pathways for the transfer of P from terrestrial to aquatic ecosystems (Tiessen,1995).

PHOSPHATE DISSOLUTION MECHANISMS

Dissolution of Fe(III)-oxides with adsorbed P

Dissolution of Fe(III)-oxides with co-precipitated P

Dissolution of Fe(III)-phosphates (e.g. strengite)

Mineralization of organic P

Increasing pH

PHOSPHATE REMOVAL MECHANISMS

Precipitation of Mn(II)-phosphates

Precipitation of Fe(II)-phosphates

Phosphate sorption on clay mineral surfaces

Immobilization of P (conversion to organic P)

Increasing soil reduction

Figure 1.2: Some mechanisms of phosphate dissolution and mechanisms of removal from soil solution that may occur during the reduction of an acid soil (based on Lindsay, 1979; Friedl et al., 1997; Stevenson, 1994; and Willet, 1985).

Normalized Fluorescence Yield
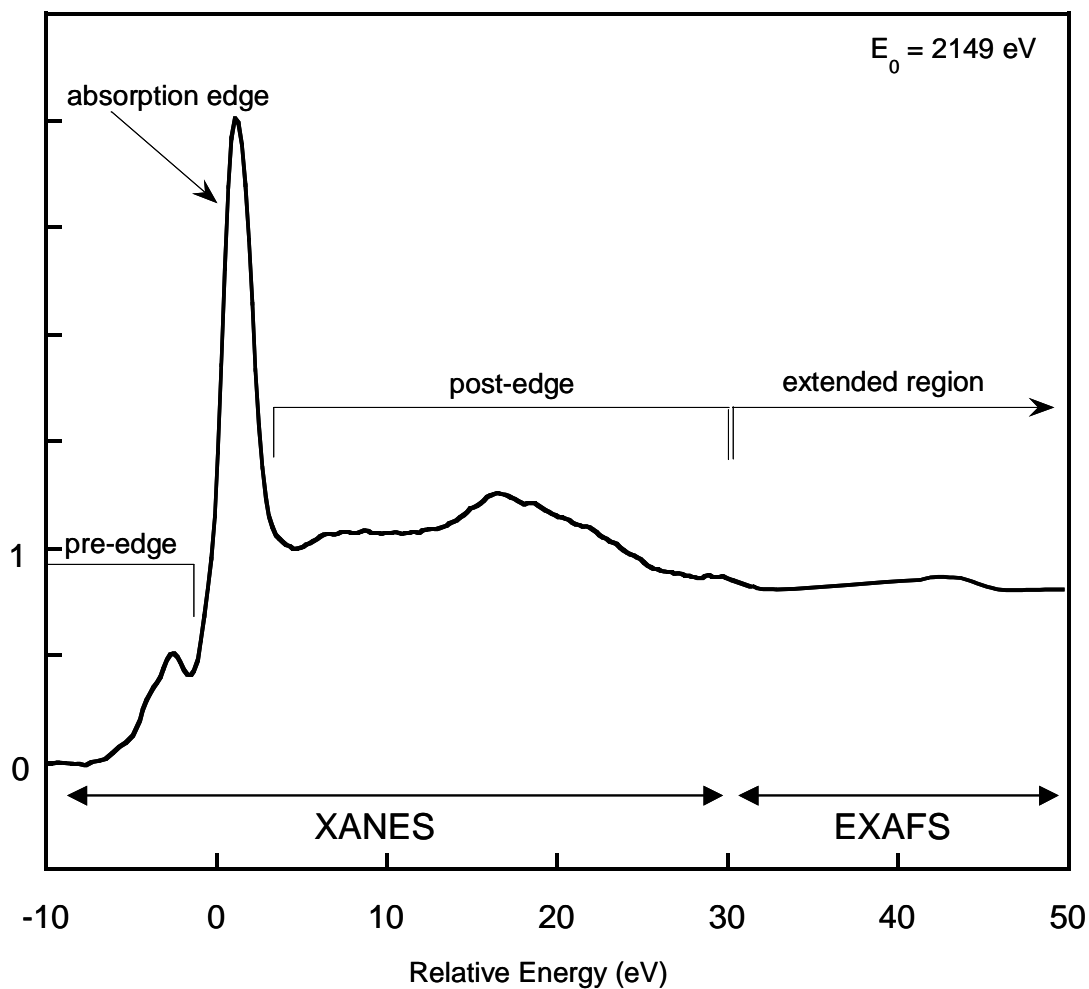
Figure 1.3: Background and baseline corrected x-ray absorption spectrum of phosphorus in crystalline $FePO_4 \bullet 2H_2O$ (strengite) showing the pre-edge, absorption edge, and extended regions of the spectra (Hesterberg et al., 1999).
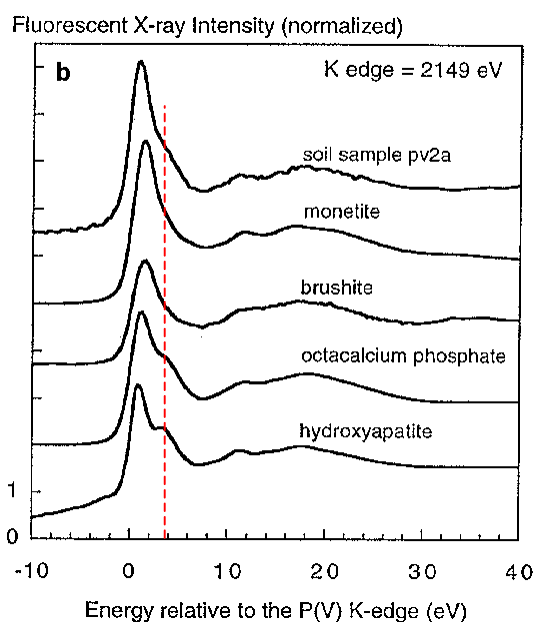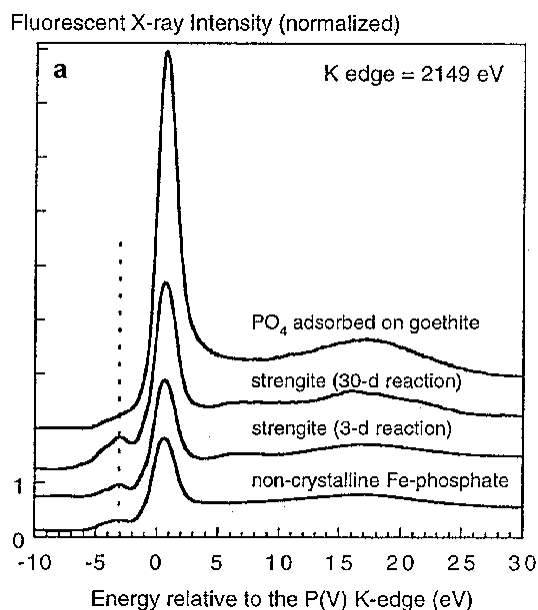
Figure 1.4: Stacked phosphorus K-XANES spectra for (a) non-crystalline iron phosphate, adsorbed phosphate, and strengite of varying crystallinity as induced by hydrothermal treatment time, and (b) for selected Ca-phosphate minerals and a soil sample (Hesterberg et al., 1999). The black-dotted line shows the pre-edge feature associated with Fe(III)-phosphate minerals (a), and the red-dotted line shows a post-edge feature associated with Ca-phosphate minerals (b).

CHAPTER 2

DISSOLUTION OF PHOSPHATE IN A PHOSPHORUS-ENRICHED

ULTISOL AS AFFECTED BY MICROBIAL REDUCTION[1]

ABSTRACT

Knowledge of the effect of reduced soil redox conditions on P dissolution is needed to better assess P mobility to surface or ground water. The objectives of our study were to determine the effect of microbial reduction on P dissolution and determine mechanisms of P release in a reduced soil. Duplicate suspensions of silt+clay from a Cape Fear sandy clay loam were reduced in a continuously-stirred redox reactor for 40 d. We studied the effects of three treatments on P dissolution: (i) 2 g dextrose kg^{-1} solids added as a microbial carbon source at time 0 d; (ii) 2 g dextrose kg^{-1} solids split into three additions at 0, 12, and 26 d; and (iii) no added dextrose. Regardless of treatment or variation in the intensity of reduction rates, dissolved reactive P (DRP) increased up to 7-fold from 1.5 to 10 mg L^{-1} and was linearly related ($R^2 = 0.79$) with dissolved organic C. Dissolved Fe and Al and pH also increased, suggesting the formation of aqueous Fe- and Al-dissolved organic matter (DOM) complexes. Separate batch experiments were performed to study the effects of increasing pH and citrate additions on PO_4 dissolution under aerobic conditions. Increasing additions of citrate increased concentrations of DRP, Fe, and Al, while increasing pH had no effect. Results indicated that increased DOM during soil reduction contributed to the increase in DRP, perhaps by competitive adsorption or by formation of aqueous ternary PO_4-Fe-DOM or PO_4-Al-DOM complexes. Generation of DOM during microbial reduction may be an important model parameter for more quantitatively predicting the dissolution of PO_4.

INTRODUCTION

Long-term land application of animal waste and fertilizers to agricultural land has resulted in a 3- to 5-fold over-application of P (Mikkelsen, 1997), and thus, elevated soil P concentrations. Accumulation of P in surface soils has increased to the extent that the loss of P in drainage waters and in runoff discharged to surface waters has become a management concern (Beauchemin et al., 1998; Breeuwsma and Schoumans, 1987; Federico et al., 1981; Sallade and Sims, 1997a) due to P contributing to the eutrophication of surface waters (Pierzynski et al., 1994).

Many of the dissolution and transport processes in soils enriched with P are not understood well enough to predict P loss quantitatively. Soil oxidation-reduction (redox) potential has been correlated with P dissolution (Patrick and Khalid, 1974; Holford and Patrick, 1981; Willet, 1989; Vadas and Sims, 1998; Phillips, 1998), where soils with lower redox potentials (more reduced conditions) generally show enhanced P mobility. Therefore, there is a concern for increased risk of P loss from poorly-drained (more reduced) soils with elevated P concentrations.

The reductive dissolution of $Fe(III)$-oxides has been related to the dissolution of P under reducing conditions in flooded soils and sediments (Holford and Patrick, 1981; Jensen et al., 1998; Maguire et al., 2000; Phillips, 1998; Sallade and Sims, 1997b; Willett, 1989). Sallade and Sims (1997b) correlated total Fe-oxide content with dissolved P after sediments were flooded under anoxic conditions for 21 days. Operationally-defined chemical fractionations of solid-phase inorganic P in sediments (Sallade and Sims, 1997b) and in soil (Maguire et al., 2000) suggested

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that P associated with Fe and Al were the predominant forms of P. Therefore,

changes in Fe mineral equilibrium or the relative distribution of PO_4 between Fe

(redox active) and Al (non-redox active) minerals could influence PO_4 dissolution

during reduction.

Phosphate associated with Fe(III) minerals can be differentiated from PO_4

associated with Al(III) minerals using x-ray absorption near-edge structure (XANES)

spectroscopy, as phosphorus K-XANES spectra for strengite ($FePO_4 \bullet 2H_2O$) and

variscite ($AlPO_4 \bullet 2H_2O$) contain unique features (Hesterberg et al., 1999). A distinct

pre-edge feature is present in the spectra of Fe(III)-associated phosphates due to

electron orbital configurations and electronic transitions near the x-ray absorption

edge (Khare et al., (b) in review); Beauchemin et al., 2003; Hesterberg et al., 1999),

and these features are absent in the spectra of Al-phosphates. Determination of the

solid-phase speciation of P using XANES spectroscopy could indicate if phosphate

is associated with redox or non-redox active minerals.

While there seems to be agreement on the importance of iron oxides on P

sorption capacity of soils, it seems to be at variance with increased P dissolution

during soil reduction. In laboratory studies, Holford and Patrick (1981) and Young

and Ross (2001) observed fluctuations in the dissolution of PO_4 and Fe over a 45 d,

or 60 to 90 d reduction period, respectively. These studies showed that the net

dissolution of Fe and PO_4 can vary over time when soils are anoxically incubated.

The rate of microbial reduction of a soil is a function of the rate of oxidation of

organic carbon by soil microbes and is influenced by factors such as temperature,

carbon source, presence and type of electron acceptors and the microbial population

present in the soil (Coyne, 1999). These factors would be different for aerobic soils, poorly-drained soils (under long-term anoxic conditions), and soils that are rapidly flooded by irrigation water, receive animal waste effluent, or are subjected to controlled drainage practices (Gilliam et al., 1997), and could result in changes in the intensity of soil reduction (Eh), soil pH, generation of DOM, and availability of P sorption sites on mineral surfaces. As a result, changes in the solid-phase speciation of phosphorus may occur during reduction, resulting in different PO_4 dissolution mechanisms (Roden and Edmonds, 1997; Willet, 1985) (Figure 1.2) and perhaps a variation in the intensity and rate of PO_4 dissolution among different soils.

Given that pH and DOM may increase during microbial reduction of soils, proposed P dissolution mechanisms include (i) reductive dissolution of Fe(III) minerals with associated PO_4, (ii) competitive adsorption for mineral surfaces between DOM and PO_4 by a ligand exchange mechanism, (iii) DOM-enhanced dissolution of surface Fe or Al with concomitant release of PO_4, (iv) the formation of aqueous ternary PO_4-Fe-DOM or PO_4-Al-DOM complexes, and (v) decreased PO_4 sorption with increasing pH.

The reductive dissolution of Fe(III)-associated PO_4 has been postulated, and includes the reductive dissolution of Fe(III) oxides (Roden and Edmonds, 1997), with adsorbed, occluded or co-precipitated P, and dissolution of Fe(III) phosphates such as strengite ($FePO_4 \bullet 2H_2O$) (Lindsay, 1979; Willet, 1985; Miller et al., 1993).

It is documented that carboxylate anions (a common functional group of many plant root exudates and soil organic matter), compete with P for sorption sites on soil minerals (Earl et al., 1979; Lopez-Hernandez et al., 1986; Violante et al., 1991).

Carboxylate anions can also solubilize Fe and Al from mineral surfaces (Gerke, 1993). As Fe or Al is released from mineral surfaces through reductive dissolution or complexation with organic acids, aqueous DOM-Fe and DOM-Al complexes can form (Gerke, 1992, 1997). These complexes may enhance the dissolution of phosphate by forming ternary, aqueous complexes of phosphate bound to DOM through Fe and Al bridges (Gerke and Hermann, 1992). For soils receiving inputs of metabolizable carbon, e.g., from animal waste amendments, mechanisms (iii) and (iv) may be important for the dissolution of PO_4 as these soils are reduced.

Soil pH typically rises during reduction and may increase after amendments with alkaline wastes, such as poultry litter (Vadas and Sims, 1998). Phosphate sorption on oxide minerals decreases with increasing pH due to increasing negative charge on the mineral surfaces (Oh et al., 1999). Also, DOM typically increases with increasing pH due to deprotonation of acidic functional groups (Swift, 1996), which may increase the dissolution of phosphate as described above.

To quantitatively predict P dissolution and transport in reduced soils such as those amended with high organic carbon inputs (e.g., from livestock waste), a better understanding is needed of P release mechanisms during microbial reduction of soils. The objectives of this study were (i) to determine how the intensity of microbial reduction affects P dissolution from the surface horizon of an acidic, P-enriched Coastal Plain soil, and (ii) to determine the relative importance of various P dissolution mechanisms during the reduction of this soil.

MATERIALS AND METHODS

Soil collection and handling

Samples were collected from the Ap horizon (0-7 cm) of a Cape Fear sandy clay loam (fine, mixed, semi-active, thermic Typic Umbraquult) in a cattle-grazed pasture on the NCDA Tidewater Research Station near Plymouth, North Carolina. The pasture had received past applications of swine lagoon effluent (exact history unknown). The soil samples were double-bagged in low-density polyethylene bags (LDPE), placed in 4-L pyrex glass jars, and transported to the lab on ice. To reduce the possibility of photochemical redox reactions, all sample handling in the laboratory was done in a glove box under a $N_{2(g)}$ atmosphere and in the absence of ultraviolet radiation (using a red-filtered safe light with an emission spectrum of 760 to 630 nm wavelength). The moist soil samples were sieved to <2 mm through a stainless steel sieve, combined, and thoroughly mixed in an LDPE bucket. The homogenized soil sample was stored in the dark at 4 °C in 4-L pyrex glass jars.

Soil Characterization

Total P was determined on samples of the whole soil, silt+clay fraction (each 0.25 g dry weight), and sand fraction (1.5 g dry weight) using the acid digestion method of Kuo (1996) with the following exceptions. A total of 3 mL of HF was added and unreacted F^- was complexed with saturated boric acid. Mehlich 3-P and inorganic forms of P such as, NH_4Cl-P, NH_4F-P, and NaOH-P were determined on the whole soil and silt+clay fractions by the procedures of Pierzynski (2000).

Dissolved reactive P was determined in filtrates by the molybdenum blue method (Olsen and Sommers, 1982).

Acid ammonium-oxalate (dark) extractable Fe and Al (Fe_{ox}, Al_{ox}) and citrate-bicarbonate dithionite (CBD) extractable Fe and Al (Fe_{CBD}, Al_{CBD}) were determined on the whole soil (0.25 and 0.5 g dry weight, respectively) and silt+clay fraction (0.25 g dry weight) according to Jackson et al. (1986). All digests and extracts were filtered through 0.2 μm Isopore polycarbonate membranes (Millipore Corp., Bedford, MA). Iron and Al were measured in the filtrates using flame atomic absorption spectrometry (FAAS).

Soil pH was measured using a 1:5 soil to water ratio (Thomas, 1996). To separate the silt+clay fraction and determine particle size distribution, the sand was wet-sieved through a 53 μm sieve. A subsample of the silt+clay fraction was further separated by centrifugation and oven-dried at 110 °C before weighing (Dixon and White, 1999). Moisture contents of the whole soil and silt+clay fractions were determined by drying triplicate samples for 24 h at 110 °C and then weighing. Results of chemical analyses were calculated on a dry mass basis.

Reduction experiment

Suspension preparation

A preliminary experiment showed that continuously stirring a suspension of whole soil led to abrasion of the magnet and glass vessel by sand particles. Therefore, we used only the silt+clay fraction of the soil in the reduction experiments. The silt+clay fraction was separated by sonicating soil in degassed, deionized water

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for 10 min intervals in an LDPE bucket, while purging the headspace with $N_{2(g)}$. Two

sequential sonications were done with a 4:1 and 2:1 water:soil ratio, respectively.

The sand and silt+clay fractions were separated as described above, and brought

into a stock suspension with a solids concentration of 65 g silt+clay kg^{-1} using

degassed, deionized water. Stock suspensions were stored for up to 5 days at 4 °C

prior to the start of each reduction experiment.

Reactor design

Reduction experiments were conducted in a 1.5 L Cytostir$^{®}$ glass bioreactor

equipped with a suspended magnetic stirring device. The overall reactor system was

designed, in part, based on the batch reactor system of Patrick et al. (1973) (Figure

A1). Upstream from the reactor, flowing $N_{2(g)}$ (0.2 or 0.5 L min^{-1}) was split, and half

was flowed through 200 mL of a standardized 0.005 mol L^{-1} NaOH solution acting as

a $CO_{2(g)}$ trap. The other half flowed through deionized water, to water saturate, then

into the reactor through a gas-dispersion tube. Effluent gas from the reactor was

also flowed through a $CO_{2(g)}$ trap (0.005 mol L^{-1} NaOH).

The reactor vessel cap was modified to accommodate a gas dispersion tube

as an inlet for $N_{2(g)}$, a sampling tube, and a port for a pH or Eh electrode. Periodic

measurements of pH and Eh were taken directly in the silt+clay suspensions using

calibrated combination pH and redox electrodes. Proper working condition of the

redox electrode was ensured by measuring the potentials of pH 4.0 and 7.0 buffer

solutions containing 30 mg of quinhydrone (Microelectrodes, Inc., Bedford, NH).

Measurements of Eh for the Ag/AgCl reference electrode were corrected to the standard hydrogen electrode (+199 mV) (Patrick et al., 1996).

Suspension treatments and sampling method

For a typical experiment, duplicate reactors containing 600 mL (control treatment) or 1200 mL of soil silt+clay suspensions (65 g kg^{-1} solids) were simultaneously reacted for 40 d while purging with $N_{2(g)}$. We studied the effects of three treatments: (1) no added dextrose (control treatment), (2) addition of 2 g dextrose kg^{-1} solids (D-glucose, anhydrous; ACS grade, Fisher Scientific, Pittsburgh, PA) at time 0 d (0-d treatment), and (3) three additions of 0.67 g dextrose kg^{-1} solids (at 0, 12, and 26 d) to yield a total addition of 2 g dextrose kg^{-1} solids (spiked-addition treatment). Suspensions were sampled periodically (over 40 d) and the total volume of suspension did not decrease by more than 20% before the final sample was removed at the termination of the experiment. The following sampling method was used to prevent oxidation of Fe(II) (Figure A2). Approximately 40 mL of suspension were removed from the reactor as four 10-mL subsamples using a glass syringe. Each of the four subsamples of suspension was transferred anoxically into four 15 mL, evacuated pyrex test tubes fitted with rubber stoppers and then centrifuged at 27,000 x g for 10 min.

To prevent exposure to oxygen, supernatant solutions from all four centrifuged test tubes were filtered through 0.2 μm polycarbonate membranes under a $N_{2(g)}$ atmosphere, by drawing a vacuum at the receiver flask (beneath the filter) of a Millipore polycarbonate, 47 mm filter holder while flowing $N_{2(g)}$ through the cover (above the filter). The filtered samples were combined into a single 40-mL sample.

All filtering was done in a $N_{2(g)}$-purged glovebox. A 2.5 mL subsample of filtrate was removed with a macropipetter and the Fe(II) in the sample was immediately complexed by adding the reagents for colorimetric analysis of Fe(II): 0.1 mL of concentrated HCl, 1 mL of 0.1% 1,10-phenanthroline, 0.5 mL of ammonium acetate buffer solution, and 1 mL of degassed, deionized water (Clesceri et al., 1989; Olson and Ellis, 1982). The remaining filtrate was acidified with HCl, and stored at 4 °C for analysis.

Chemical analysis of aqueous reactor samples

Within 24 h of complexing, Fe(II) was measured colorimetrically (λ=510 nm) in duplicate subsamples using a UV-visible spectrophotometer. Duplicate or triplicate filtrate samples were also analyzed colorimetrically (λ=840 nm) for DRP using the molybdenum blue method (Olsen and Sommers, 1982). Measurements for DRP are assumed to include PO_4 bound to Fe- or Al-DOM complexes as indicated by Gerke (1992) as a result of acid hydrolysis of Fe and Al associated with DOM and phosphate during the colorimetric process. To avoid interference of absorbance readings due to varying concentrations of DOC, blanks of each filtrate sample were prepared by replacing the coloring reagents (i.e., 1,10-phenanthroline for Fe(II) analysis and molybdate "reagent B" for PO_4 analysis) with an equal amount of deionized water and subtracting absorbance readings for the blanks from sample absorbance readings.

Sample filtrates were analyzed for DOC using a TOC analyzer. Total dissolved Fe and Al were measured on acidified samples using FAAS. The

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concentration of $CO_{2(g)}$ trapped in NaOH solutions was determined by titrating the NaOH solution for unreacted alkali with standardized HCl solution after the addition of 1 mL of 1.5 mol L^{-1} $BaCl_2$ to precipitate CO_3^{-2} as $BaCO_3$ (Anderson,1982). Reactor $CO_{2(g)}$ (mmol) titrated downstream from the reactor was corrected by subtracting the concentration of $CO_{2(g)}$ in the blank upstream of the reactor. Results of titrated $CO_{2(g)}$ (mmol) should be considered relative as the $CO_{2(g)}$ traps may not have been 100% efficient.

X-ray absorption near-edge structure (XANES) spectroscopy

XANES analysis was used to qualitatively determine whether Fe(III)-associated P was present in the silt+clay sample used in the reduction experiments. A moist sample taken at time 0 d was analyzed at Beamline X19A (National Synchrotron Light Source, Upton, NY) following methods described by Khare et al. (a) (in review). XANES data were normalized according to Khare et al. (a) (in review) and compared with data for standards of PO_4 sorbed to ferrihydrite or non-crystalline Al-hydroxide collected in that study.

pH experiment

A separate 48 h batch experiment was conducted to determine the effect of pH on DOC, and dissolved PO_4, Fe, and Al in silt+clay suspensions. Aliquots of a stirred, aqueous stock suspension of silt+clay were weighed into tared, 250 mL polycarbonate centrifuge bottles to yield 65 g kg^{-1} solids in the final 60 g sample. While stirring, duplicate samples were adjusted in random chronological order to pH 5.0, 5.5, 6.0, 6.5, 7.0, or 7.5 using standardized 0.5 mol L^{-1} KOH or HCl. The samples were brought to the final mass with deionized water and 0.5 mol L^{-1} KCl (to

yield a 5 mmol L^{-1} K^+ background), and equilibrated for 48 h by shaking at 25 °C in a temperature-controlled water bath. Periodically, throughout the equilibration period, sample pH was adjusted and K^+ concentration corrected. Redox potential was measured on each sample at time 0 and 48 h. After 48 h, suspension pH was measured and samples were centrifuged at 16,000 x g for 20 min. Filtered (0.2 μm) supernatant solutions were analyzed as described above for reactors.

Citric acid experiment

A separate 19 h batch experiment was conducted to determine the effect of a complexing organic acid (citrate) on dissolved PO_4, Fe, and Al in the silt+clay suspension. Aliquots of a stirred, aqueous stock suspension of silt+clay were weighed into tared, 30 mL polycarbonate centrifuge tubes to yield 10 g kg^{-1} solids in the final 30 g sample. While stirring, a 0.25 mol L^{-1} citric acid monohydrate ($H_3C_6H_5O_7 \bullet H_2O$) solution neutralized with 0.75 mol L^{-1} KOH was added to each duplicate sample in random chronological order, yielding final input citrate concentrations of 0, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, and 500 mmol citrate kg^{-1} solids. Samples were adjusted to pH 7.0 with 0.25 mol L^{-1} KOH or HCl (after the addition of deionized water, two times the volume of input citrate solution), brought to final mass by the addition of 0.25 mol L^{-1} KCl, and equilibrated for 19 h by shaking at 25 °C in a temperature-controlled waterbath. Periodically, suspension pH was adjusted. After the 19 h equilibration period, suspension pH was measured and samples were centrifuged at 22,000 x g for 20 min. Redox potential was measured

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on each sample at 0 and 19 h. Filtered (0.2 μm) supernatant solutions were
analyzed as described above for reactors.

RESULTS

Soil characteristics

Selected properties for the whole soil and silt+clay fraction used in the
reduction experiments are shown in Table 1. Both samples were slightly to
moderately acidic (pH 5.9 and 6.4). While the silt+clay fraction constituted 51% of
the soil mass, chemical extraction results (Table 1) indicate that the silt+clay fraction
accounted for about 86%, 71%, 100%, 85%, and 83% of the total P, Fe_{ox}, Fe_{CBD}, Al_{ox}
and Al_{CBD}, respectively. The Fe_{ox} to Fe_{CBD} ratio for the silt+clay was 0.6, consistent
with soils containing elevated amounts of poorly crystalline Fe-oxides due to active
redox processes (Schwertmann, 1985).

Plant available P and selected inorganic P parameters are shown in Table 2.2
for the whole soil and silt+clay fraction. Mehlich 3-P (related to plant available P) is
7-fold greater than what is considered to be optimum for plant growth and crop
yields (Pierzynski, 2000) indicating that the soil has excess P loading perhaps due to
past applications of swine lagoon effluent. Chemical extraction results (Table 2.2)
targeting "labile P" (NH_4Cl-P), "Al-associated P" (NH_4F-P), and "Fe-associated P"
(NaOH-P) for the whole soil and silt+clay fraction show extractable Al-associated P
to be 1.8 and 1.9 times greater, respectively, than extractable Fe-associated P which
is consistent with North Carolina Coastal Plain soils that typically have greater

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extractable Al-associated P than Fe-associated P (Novais and Kamprath, 1978). Extractions typically dissolved only part of the total P. For example, the sum of extractable fractions from the total soil were only 56% of the total P measured on the whole soil. Total P for the silt+clay fraction accounted for 86% of the total P in the whole soil (Table 2.1), indicating that these phosphorus extractions were less effective for the whole soil than for the silt+clay fraction.

Reduction experiments

Figure 2.1 shows changes in Eh, $CO_{2(g)}$ trapped in 0.005 mol L^{-1} NaOH , and pH for duplicate redox reactor solutions subjected to the three dextrose treatments. The redox potential (Eh) decreased over time for all treatments, however, the rate of reduction for the 0-d treatment was much faster than for the control and spiked-addition treatment. Evolution of $CO_{2(g)}$ occurred during all treatments and indicated that the Eh declined as a result of microbial oxidation of organic carbon (Coyne, 1999). This is particularly evident in the 0-d dextrose treatment (Figure 2.1B) where a rapid decline in Eh from 340 mV at 0d to 99 mV at 7d corresponded with the greatest incremental trapping of $CO_{2(g)}$. The Eh then stabilized at ~70 mV for the remainder of the 40-d reduction period. The Eh of the spiked-addition and control treatment decreased at about the same overall rate, between 0 and 40 d. The Eh appeared to drop more rapidly after the dextrose additions in the spiked-addition treatment, particularly after 26 d. For the control and spiked-addition treatments, the Eh exhibited a downward trend at the end of the 40-d incubation period. Overall, the average Eh for all treatments decreased significantly (p<0.01) from 410 $\pm$ 30 (initial)

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to 100 ± 30 mV (40 d), while the cumulative, trapped $CO_{2(g)}$ was 2.0 ± 0.3 mmol. With declining Eh over time, the pH increased significantly (p<0.05) towards neutrality (from 5.9 ± 0.2 to 6.7 ± 0.4), as has been observed when acidic soils are reduced (Patrick et al., 1996).

Figures 2.2 and 2.3 show changes in the concentrations of DRP, DOC, Fe(II), total Fe (Fe_T), and Al in duplicate redox reactor suspensions subjected to the three dextrose treatments. Regardless of the rate of microbial reduction (Figure 2.1), DRP increased significantly (p<0.01) by up to 7-fold, with a grand mean ranging from 1.5 ± 0.2 mg L^{-1} at 0 d to 10 ± 2 mg L^{-1} at 40 d for all three treatments. Similarly, DOC increased significantly (p<0.01) up to 5-fold ranging from 34 ± 9 mg L^{-1} at 0 d to 155 ± 45 mg L^{-1} at 40 d. When data for all three treatments were combined, a significant (p<0.01) linear relationship was found between DRP and DOC ($R^2 = 0.79$, n = 34) (Figure 2.4). For all three treatments, DRP and DOC increased at a rate consistent with the rate of decreasing Eh and increasing trapped $CO_{2(g)}$ (Figure 2.1), implying that microbial respiration led to the increases in these constituents. These trends are particularly evident for the 0-d dextrose treatment between 0 and 15 d, where a sharp increase in DRP and DOC (Figure 2.2B) corresponded with a sharp decrease in Eh (Figure 2.1). Dissolved reactive P and DOC then stabilized for the remainder of the 40-d reduction period for this treatment.

For all three treatments, the concentration of dissolved Fe(II) increased with decreasing Eh and increasing amount of trapped $CO_{2(g)}$ (Figure 2.3). When data (mmol) for all three treatments were taken together, significant (p<0.01) linear

relationships were found between DOC and dissolved Fe(II), Al_T, or Fe_T with R^2 values of 0.60, 0.30, and 0.23, respectively (correlation plots not shown). Also, significant linear relationships (p<0.01) were found between DRP and Fe(II), and Al_T, and Fe_T, having R^2 values of 0.51, 0.41, and 0.36, respectively (correlation plots not shown).

XANES spectroscopy

Figure 1.3 shows the overall features of a typical normalized and background corrected x-ray absorption spectrum. Figure 2.5 is a plot of the pre-edge region only (-6 to -1 eV) of P K-XANES spectra for PO_4 sorbed on ferrihydrite, non-crystalline Al-hydroxide, and the initial silt+clay sample. The distinct pre-edge feature can be seen for the PO_4 sorbed on ferrihydrite, whereas, no pre-edge feature is evident for the non-crystalline Al-hydroxide. The spectrum for a silt+clay sample shows a pre-edge feature intermediate between those of the standards, indicating that PO_4 was associated in part with Fe(III) and possibly with Al(III), as was also suggested by the chemical fractionation data in Table 2. The fact that the pre-edge feature in the soil XANES spectrum was weak, implies that PO_4 was more likely adsorbed on Fe-oxide minerals, rather than occurring as an Fe(III)-phosphate mineral (Hesterberg et al., 1999).

pH Experiment

Results of the experiment on chemical changes in aqueous silt+clay suspensions as a function of pH (with minimal microbial respiration) is shown in Figure 2.6. Dissolved Fe_T and Al_T increased slightly as pH increased, while DOC and DRP exhibited a minimum at pH 6.5. Dissolved organic carbon increased

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significantly (p<0.05) from 35.8 ± 0.4 mg L^{-1} to 50 ± 2 mg L^{-1}. Redox potential (Eh) indicated oxidizing conditions (Fe(II) not measured), with minimal change, over the 48 h equilibration period (grand mean = 535 ± 10 mV).

<u>Citrate Experiment</u>

Figure 2.7 shows results of a batch experiment on citrate effects on aqueous solution chemistry for silt+clay suspensions. Dissolved reactive P, Fe_T, and Al_T all increased significantly (p<0.01) with increasing additions of citrate, with DRP, Fe_T, and Al_T increasing from 0.41 ± 0.01 to 3.70 ± 0.03 mg DRP L^{-1}, 0.07 ± 0.01 to 7.9 ± 0.1 mg Fe L^{-1}, and 0.7 ± 0.3 to 11.1 ± 0.3 mg Al L^{-1}, respectively. Again, Eh remained stable (434 ± 15 mV) and conditions were oxidizing (Fe(II) not measured) during the 19 h equilibration period.

DISCUSSION

Results from redox reactor studies indicated that the intensity of microbial reduction of the silt+clay suspensions was similar for the control and spiked addition treatment but different for the 0d treatment. Regardless of the differences of the intensity of microbial reduction, concentrations of DRP after 40 d of reduction were similar for all treatments (grand mean = 10 ± 2 mg PO_4 L^{-1}). However, the maximum dissolution of PO_4 occurred most rapidly (by 15d) for the 0-d treatment, while dissolved PO_4 did not reach a maximum until after 30d for the control and spiked-addition treatment indicating that the addition of an easily metabolizable carbon source increased the rate of the net dissolution of PO_4. Increased dissolution of

organic carbon, Fe(III) [calculated as $(Fe_{(T)}-Fe_{(II)})$], Fe(II), and Al during reduction was observed for all treatments except for Fe(III) in the spiked-addition treatment.

Aqueous Fe(II) was measured in reactor supernatant solutions (for all treatments) when the Eh measurements were between 120 and 150 mV (pH 6.5 $\pm$ 0.4) indicating that the Fe(III)/Fe(II) redox couple was reached (Patrick et al., 1996). The grand mean Eh measured at 40d after reduction commenced was 100 ± 30 mV. Because both XANES analysis (Figure 2.5) and chemical extraction data (Table 2.2) indicated that the silt+clay fraction of soil contained Fe(III)-associated P, reductive dissolution of Fe(III) oxides and associated PO_4 is one possible mechanism contributing to P dissolution in our reactor experiments. This reductive dissolution mechanism has often been cited for reduced soils (Holford and Patrick, 1981; Jensen et al., 1998; Maguire et al., 2000; Phillips, 1998; Sallade and Sims, 1997b; Willett, 1989). However, Fe(II) was less significantly correlated with DRP (R^2=0.51, $p < 0.01$) than was DRP and DOC (R^2=0.79, p<0.01).

If reductive dissolution of Fe(III) from Fe-oxides and concomitant release of associated PO_4 was the only PO_4 dissolution mechanism, and no competing Fe(II) or PO_4 uptake mechanisms are important, then one would expect the ratio of dissolved Fe(II)/PO_4 to be greater than or equal to one. This hypothesis includes the possibilities that PO_4 may be sorbed as either monodentate (Persson et al., 1996) or bidentate (Goldberg and Sposito, 1985) surface complexes, and that surface Fe(III) other than those binding PO_4 may also be reductively dissolved. Our observed ratios of dissolved Fe(II)/PO_4 ranged from 0.1 to 0.3, suggesting that reductive

dissolution of Fe(III) and associated PO_4 was not the only operative mechanism.

The positive linear relationship between DOC and PO_4 (Figure 2.4) is consistent with mechanisms of competitive adsorption for mineral surfaces between DOM and PO_4 and the formation of aqueous ternary PO_4-Fe-DOM or PO_4-Al-DOM complexes. The latter mechanism is consistent with the observed higher molar ratios of $[(Fe_T + Al_T)/PO_4]$ of 3.0 and 3.2 for the 0-d and control treatment, respectively. However, for the spiked-addition treatment the molar ratio of $[(Fe_T + Al_T)/PO_4]$ was only 0.3 suggesting that dissolution of PO_4 by the formation of aqueous ternary PO_4-Fe-DOM or PO_4-Al-DOM complexes was less important for this treatment. Because increases in dissolved Fe_T and Al_T for the spiked-addition treatment were small compared to increases in dissolved Fe_T and Al_T for the 0-d and control treatments, this suggests that the mechanism of competitive adsorption between PO_4 and DOC (e.g., ligand exchange) may better explain the increase in DRP for the spiked-addition treatment. The $[(Fe_T + Al_T)/PO_4]$ ratio for the 0-d treatment was calculated from data taken at 15d while data from 40d was used for the control and spiked-addition treatment. Note that while the comparison cannot be made at 40d based on the lack of Al_T data, data from 15d may represent a maximum point of dissolution of Al_T as DOC, DRP, Fe_T, and Fe(II) all reached a maximum at 15 d and then remained fairly consistent throughout the rest of the experiment.

Dissolved organic matter is often composed of organic acids from root extracts, microbial extracts, and fulvic/humic macromolecules that contain an abundance of carboxylic acid functional groups (Swift, 1996). These carboxylate anions are known to compete with PO_4 for surface binding sites (Bolan et al., 1994;

Violante et al., 1991) and chelate Fe and Al (Berrow et al., 1982; Duff et al., 1963; Hue et al., 1986). Fox et al. (1990), showed that extraction of 10 g of a Spodosol soil (Bh horizon) with 100 mL of 1.0 mmol L^{-1} oxalate resulted in the dissolution of 3.5% of the total inorganic soil P (2.1 mmol P kg^{-1}). Berrow et al. (1982) showed that the microbial by-product, 2-ketogluconic acid derived from glucose media, was capable of chelating 17 mmol Fe kg^{-1} from a poorly-drained soil containing 646 mmol Fe kg^{-1}. Solutions of 0.1 mol L^{-1} ketogluconic acid, pH 7.0, were added to soil in a 5:1 ratio and shaken for 1 h. Duff et al. (1963) extracted 13% of Fe from β-ferric oxide, 0.8% Fe from goethite, and 2% of aluminum from aluminum hydroxides in laboratory innoculation experiments where 5 to 10 mL of medium containing glucose and ketogluconic-acid producing bacteria and 5 to 500 mg of mineral were incubated for 8 to 14 d at 25 °C. Therefore, it is possible that increases in DOM caused dissolution of PO_4, Fe, and Al in our reduced reactor solutions.

An increase in DOC may have resulted from microbial metabolism of organic C (Japenga et al., 1992) or from increasing pH as the suspensions were reduced. Dissolved PO_4, organic carbon, Fe_T, and Al_T at pH 7 from the batch experiment (Figure 2.6), were 9-, 4-, 65-, and 155-fold lower, respectively, than dissolved concentrations of these constituents at the end of the reduction experiments. These results indicated that the increase in pH during microbial reduction, in itself, had a minimal effect on increasing DOC or the dissolution of PO_4, Fe_T, or Al_T. Therefore, increases in concentrations of DOC, DRP, Fe(III), and Al_T in the reduced reactor solutions were more likely a result of increasing DOM generated by microbial

reduction rather than increasing pH.

Experiments with citrate provided insights on DOM effects on PO_4 dissolution. Citrate serves as a surrogate for soil DOM because it is often found in soil solutions (excreted by plant roots), has carboxylate functional groups, and log k values for complexation of Fe(III) and Al(III) with citrate [log k = 11.5 (Martell and Smith, 1977) and 10.92 (Gerke, 1997) for 1:1 complexes, respectively] are more comparable to those for metal-humic acid complexes than other simple organic acids such as tartrate or malate (Martell and Smith, 1977; Stevenson, 1994). Figure 2.7 shows that increasing additions of DOC as citrate resulted in increased concentrations of DRP, Fe_T, and Al_T. At the maximum citrate input of 360 mg DOC L^{-1}, the concentrations of DRP, Fe_T, and Al_T were significantly higher (p<0.01) than concentrations of DRP, Fe_T, and Al_T in the pH batch experiment. The changes in DRP, Fe_T, and Al_T in Figure 2.7 may be due to competitive ligand-exchange of citrate for PO_4 or citrate-dissolution of soil minerals. Similar mechanisms could have contributed to enhanced PO_4 dissolution with increased microbial generation of DOM during reduction of the soil material studied.

Increased dissolution of PO_4 by competitive adsorption with di- and tricarboxylic acids has been shown to occur (Violante et al., 1991; Earl et al., 1979; Lopez-Hernandez et al., 1986). Also, removal of Fe from Fe-humate complexes, and enhanced dissolution of poorly ordered Fe-oxides, humic-Fe-oxide mixtures, and surface Fe or Al by organic acids can also increase concentrations of dissolved Fe and Al (Gerke, 1993). Once PO_4, Fe, and Al are in solution, aqueous complexes of DOM-Fe or DOM-Al can bind phosphate (Bloom, 1981; White and Thomas, 1981;

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Gerke and Hermann, 1992; Gerke, 1993) and maintain higher levels of dissolved P.

CONCLUSIONS

Microbial reduction of the silt+clay fraction of a Cape Fear soil sample for

40 d using three different dextrose treatments increased DRP by 7-fold. The

intensity of reduction was similar for the control and spiked-addition treatments but

more intense for the 0-d treatment. Regardless, the maximum overall concentration

of DRP (10 ± 2 mg PO_4 L^{-1}) was similar for all treatments. However, the maximum

dissolution of PO_4 occurred after 15d of reduction for the 0-d treatment and after 30d

for the control and spiked-addition treatments. Although, reductive dissolution of

Fe(III)-oxide minerals may have contributed to DRP during microbial reduction, a

strong correlation between DRP and DOC ($R^2 = 0.79$, n = 34) and molar ratios of

Fe(II):PO_4 ranging from 0.1 to 0.3 indicated that P dissolution mechanisms involving

increasing DOM produced during microbial reduction were more important. Possible

mechanisms for enhanced P dissolution, which could not be differentiated from our

experiments, include increased ligand exchange of DOM for mineral adsorbed PO_4;

DOM-induced dissolution of Fe(III)- and Al(III)-oxide minerals with concomitant

release of sorbed PO_4, and formation of ternary aqueous PO_4-Fe(III)-DOM or

PO_4-Al(III)-DOM complexes.

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Table 2.1: Selected properties of whole soil samples from the Cape Fear soil, and the silt+clay fraction used in this study.[†]

Fraction	pH	Total Carbon	Total Nitrogen	Sand	Silt	Clay	Total[§] [P]	Fe_{ox}[‡]	Fe_{CBD}[‡]	Al_{ox}[‡]	Al_{CBD}[‡]
		$g\ kg^{-1}$		$g\ g^{-1}$			$mmol\ kg^{-1}$				
Whole soil	6.4 ± 0.1	44 ± 3	3.3 ± 0.2	49	24	27	31 ± 3	31.0 ± 0.3	34 ± 2	84 ± 8	78.6 ± 0.3
Silt+clay	5.9 ± 0.2	--	--	--	--	--	52 ± 2	43 ± 1	67 ± 7	140 ± 9	127 ± 4

[†]Reported concentrations are the mean of triplicate measurements, except particle fractionation, which was performed on a single sample.
[‡]Ammonium oxalate and citrate bicarbonate dithionite extractable Fe and Al performed according to Jackson et al. (1986).
[§]Total P performed according to Kuo (1996).

Table 2.2: Phosphorus parameters of the whole soil and silt+clay samples from the Cape Fear soil used in this study.[†]

Fraction	P parameters[‡]			
	Mehlich 3-P	NH_4Cl-P	NH_4F-P	NaOH-P
	mg kg^{-1}			
	(mmol kg^{-1})			
Whole soil	340 ± 2	9 ± 2	340 ± 11	190 ± 6
	(10.97 ± 0.06)	(0.29 ± 0.06)	(10.97 ± 0.35)	(6.1 ± 0.2)
Silt+clay	420 ± 11	11.7 ± 0.5	1341 ± 20	690 ± 44
	(13.6 ± 0.4)	(0.38 ± 0.02)	(43.3 ± 0.6)	(22 ± 1)

[†]Reported concentrations are the means $\pm$ standard deviations of triplicate measurements.
[‡]Chemically extractable P using different reagents according to methods in Pierzynski (2000).
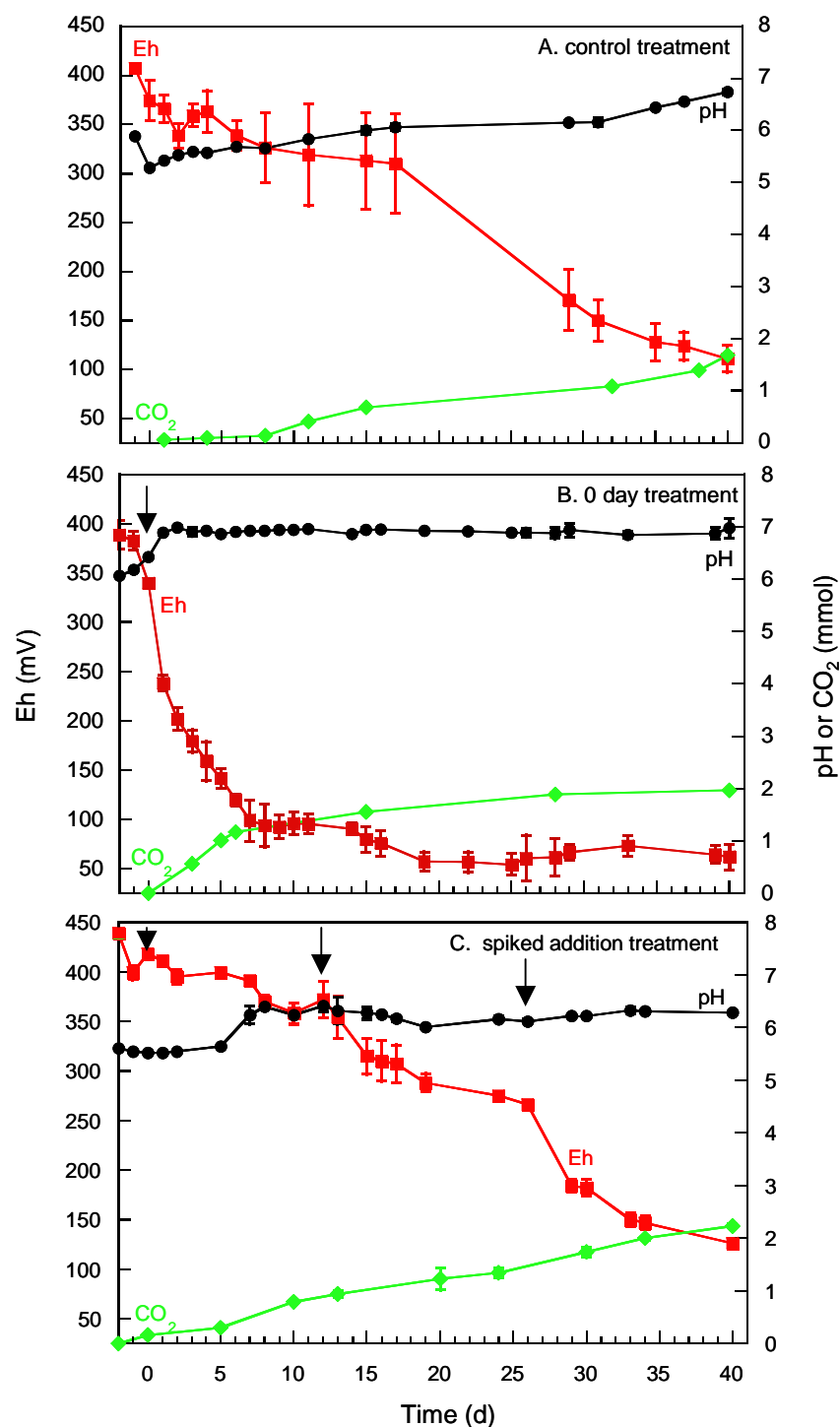
Figure 2.1: Trends in redox potential (Eh), pH, and cumulative evolved $CO_{2(g)}$ over a 40-d anaerobic reduction period of the silt+clay suspension for the control, 0-d, and spiked-addition treatments. Arrows denote times of dextrose addition. Error bars represent the standard deviation in measurements between duplicate reactors for each treatment.
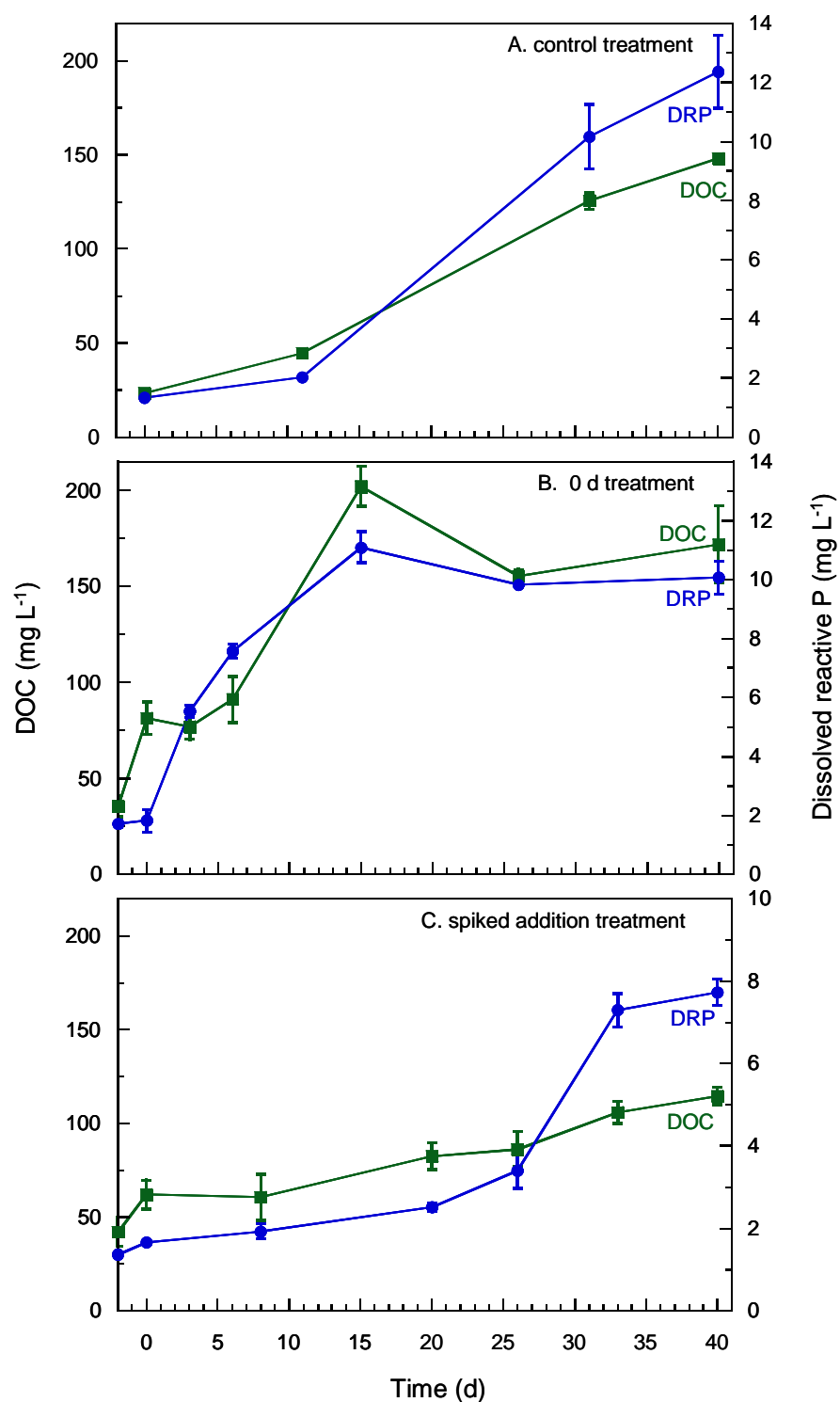
Figure 2.2: Trends in dissolved organic carbon (DOC) and dissolved reactive P (DRP) over a 40-d anaerobic incubation period of the silt+clay suspension for the control, 0-d, and spiked-addition treatments. Error bars represent the standard deviation in measurements between duplicate reactors for each treatment.
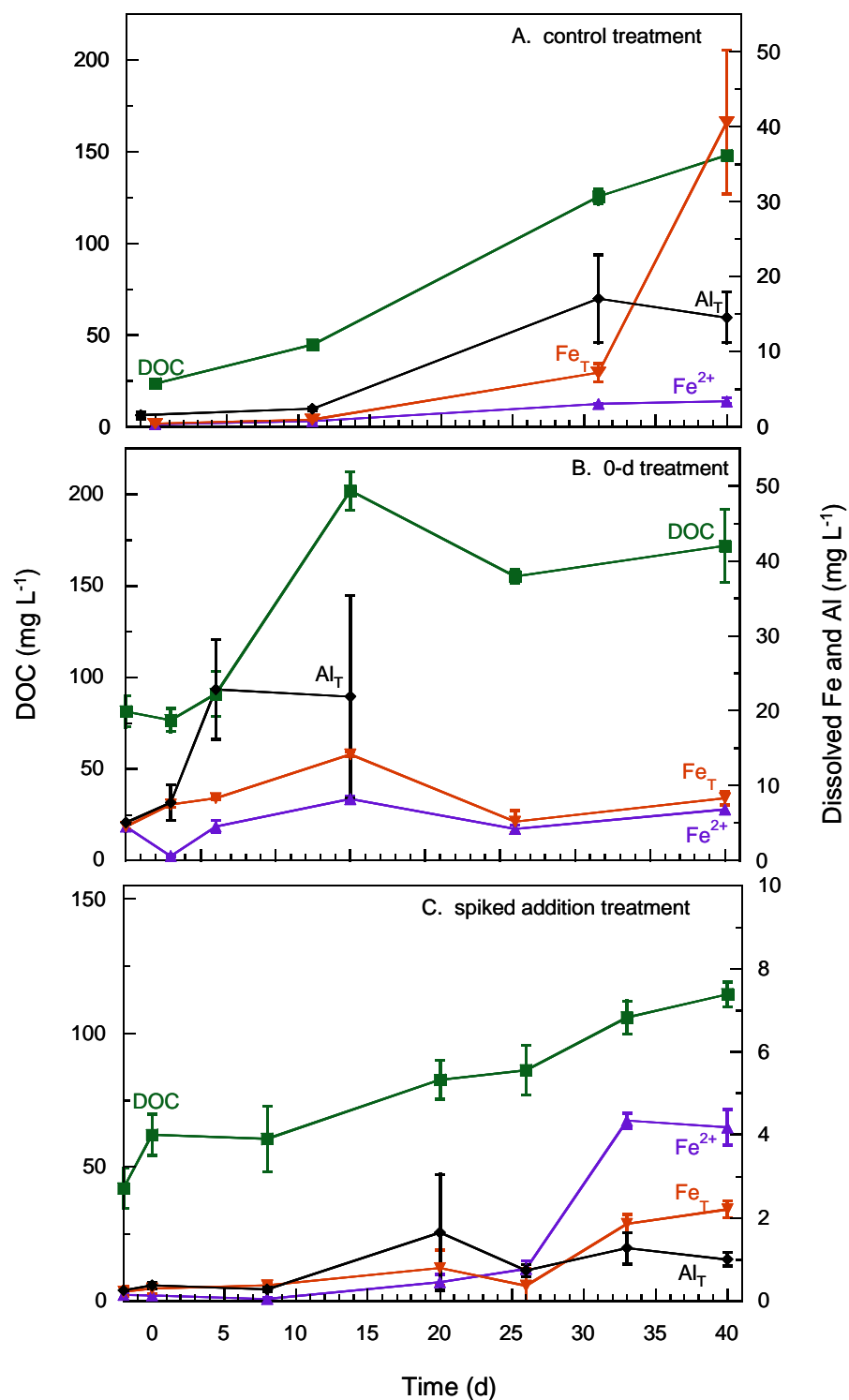
Figure 2.3: Trends in dissolved organic carbon (DOC), Fe(II), total Fe, and Al over a 40-d anaerobic incubation period of the silt+clay suspension for the control, 0-d, and spiked-treatments. Error bars represent the standard deviation in measurements between duplicate reactors for each treatment.
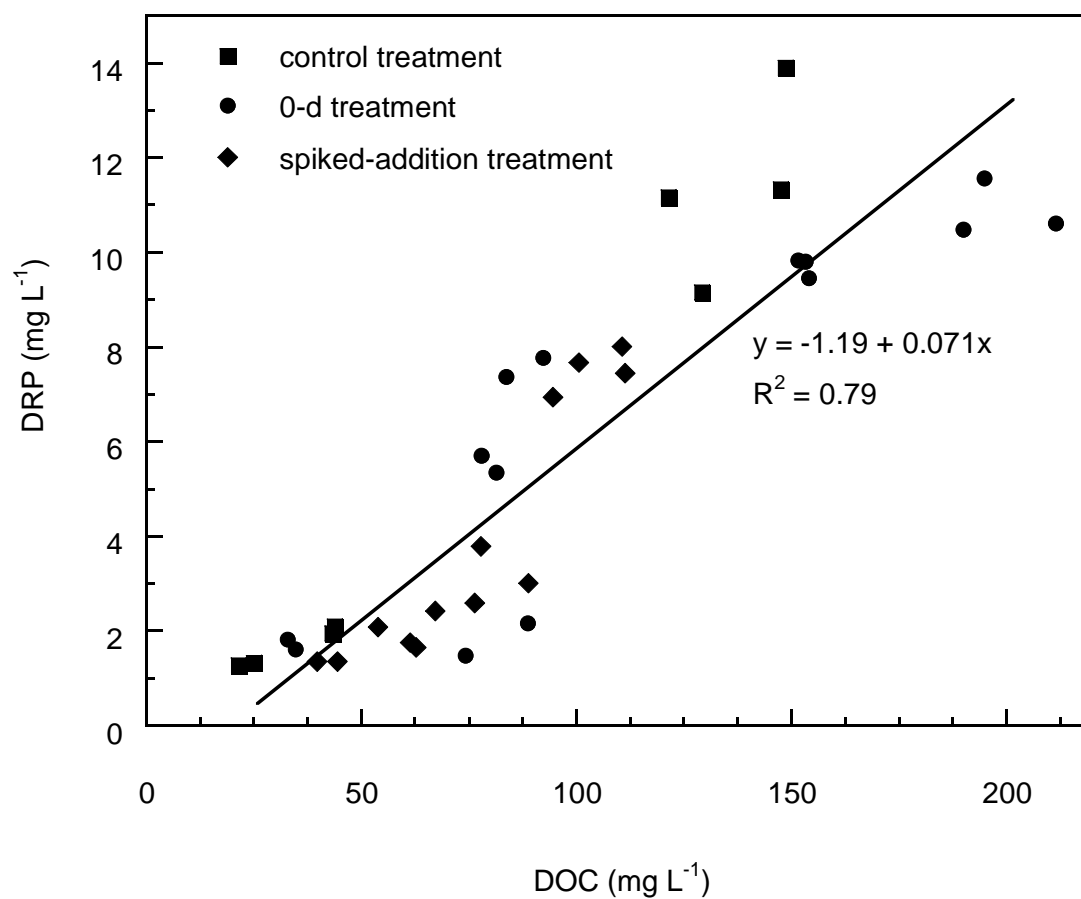
Figure 2.4: Correlation of dissolved reactive P (DRP) and dissolved organic carbon (DOC) (mg L^{-1}) for silt+clay suspensions undergoing a 40-d anaerobic incubation period with three treatments (control, 0-d, and spiked-addition).
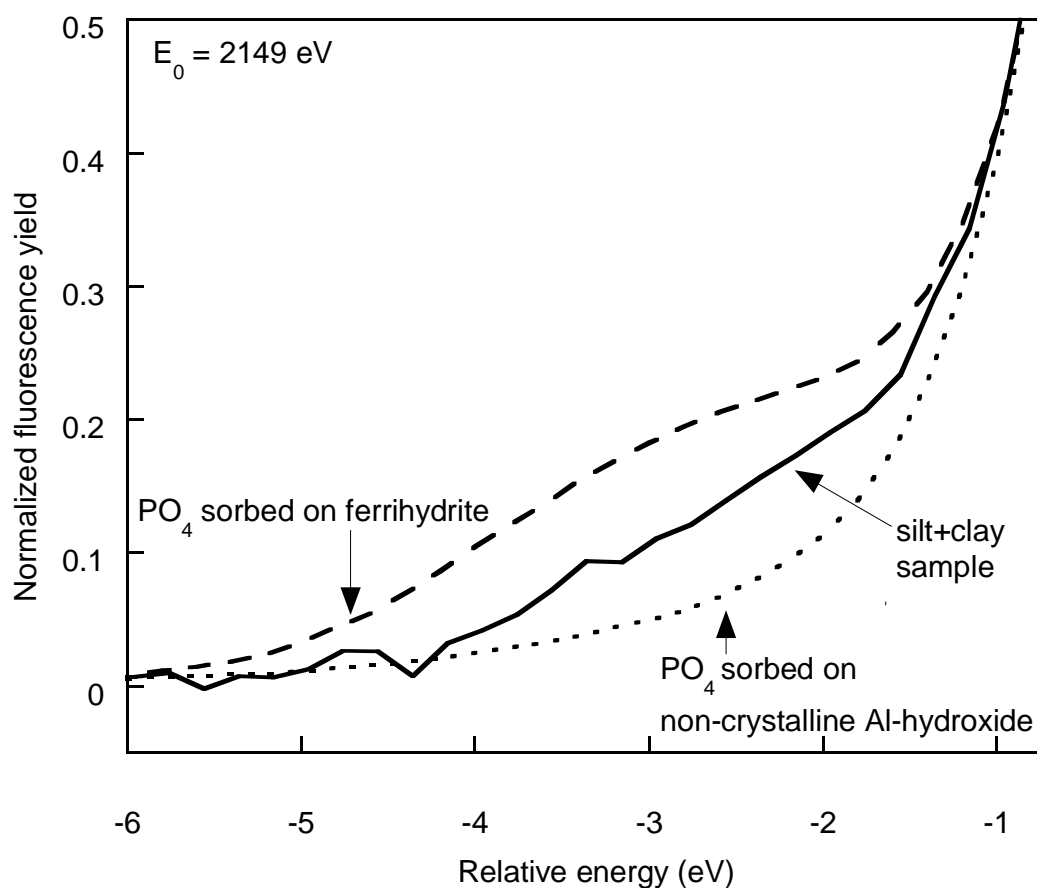
Figure 2.5: Edge-normalized XANES spectra comparing the pre-edge region for the silt+clay sample to the pre-edge region for the PO₄ sorbed to ferrihydrite and non-crystalline Al-hydroxide mineral standards (Khare et al., (a) in review).
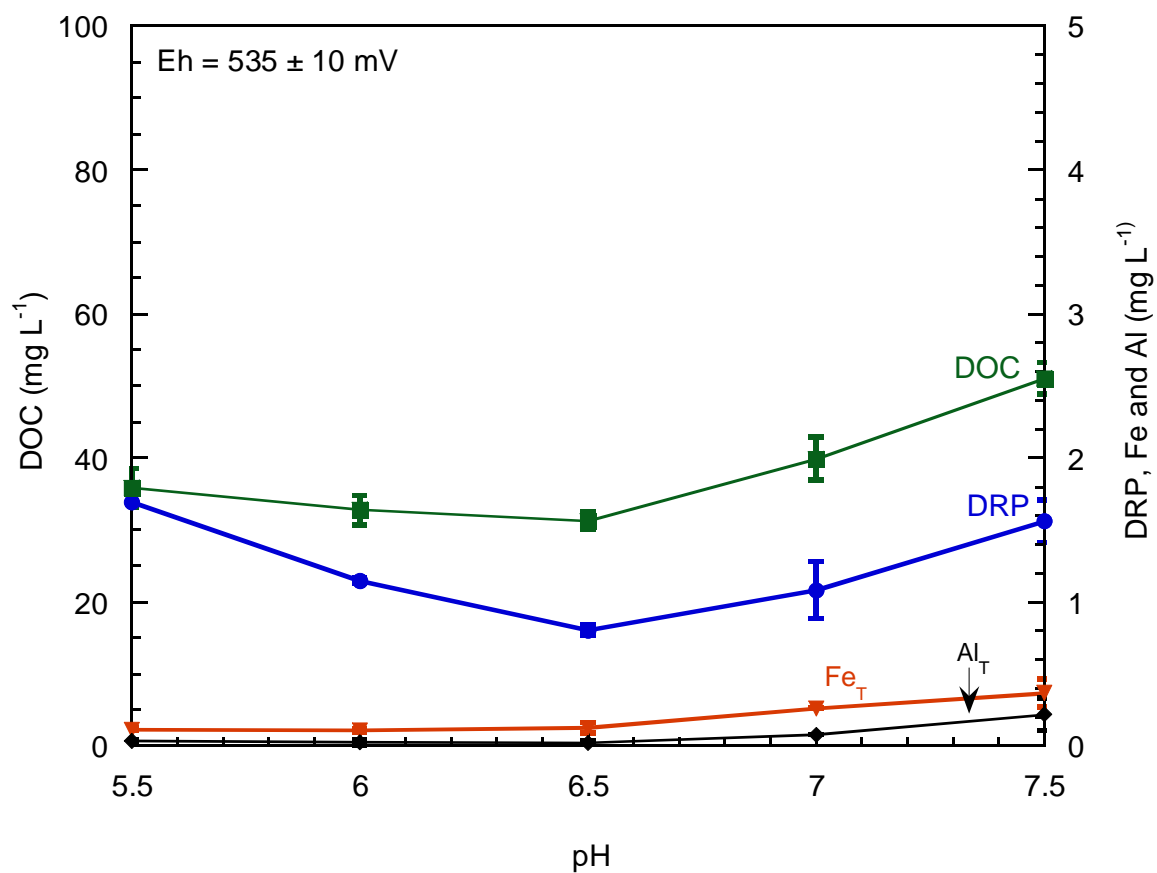
Figure 2.6: Dissolved reactive P (DRP), DOC, total Fe and Al as affected by pH for the silt+clay suspension reacted in a batch experiment for 48 h. Average Eh of the silt+clay suspensions was 535 ± 10 mV.
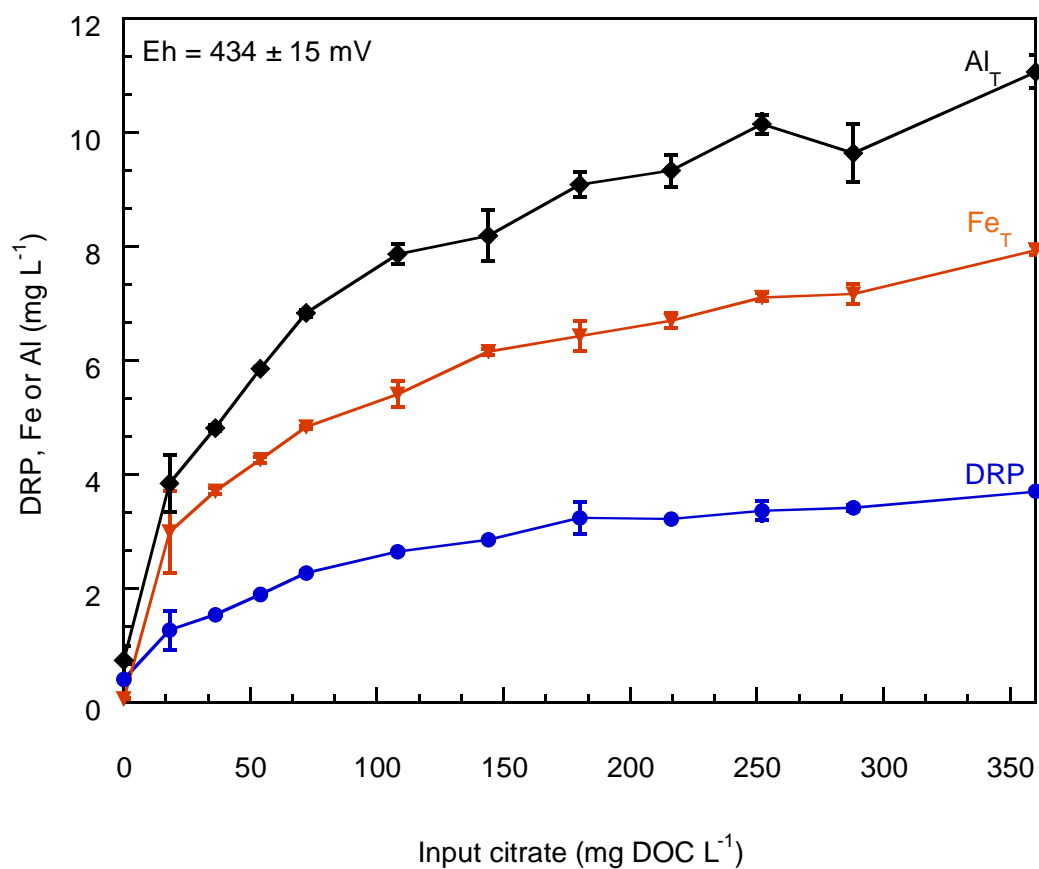
Figure 2.7: Dissolved reactive P (DRP), total Fe and Al as affected by increasing concentration of dissolved organic carbon for silt+clay suspensions reacted with different inputs of citrate for 19 h at pH 6.93 $\pm$ 0.06. Average Eh of the silt+clay suspensions was 454 $\pm$ 15 mV.

APPENDIX A

Supplementary figures

Figure A1: Reduction reactor system consisting of (a) flow meters, (b) and (g) series of 250 mL Erlenmeyer flasks for trapping of condensate and $CO_{2(g)}$ upstream and downstream from the reactor, (c) beaker of water to submerse end of tubing, (d)1000 mL Erlenmeyer flask with water to water-saturate $N_{2(g)}$, (e) reactor consisting of (e_1) a gas dispersion tube, (e_2) suspended stirring magnet, (e_3) glass sampling tube fitted with a stopcock, (f) magnetic stir plate.
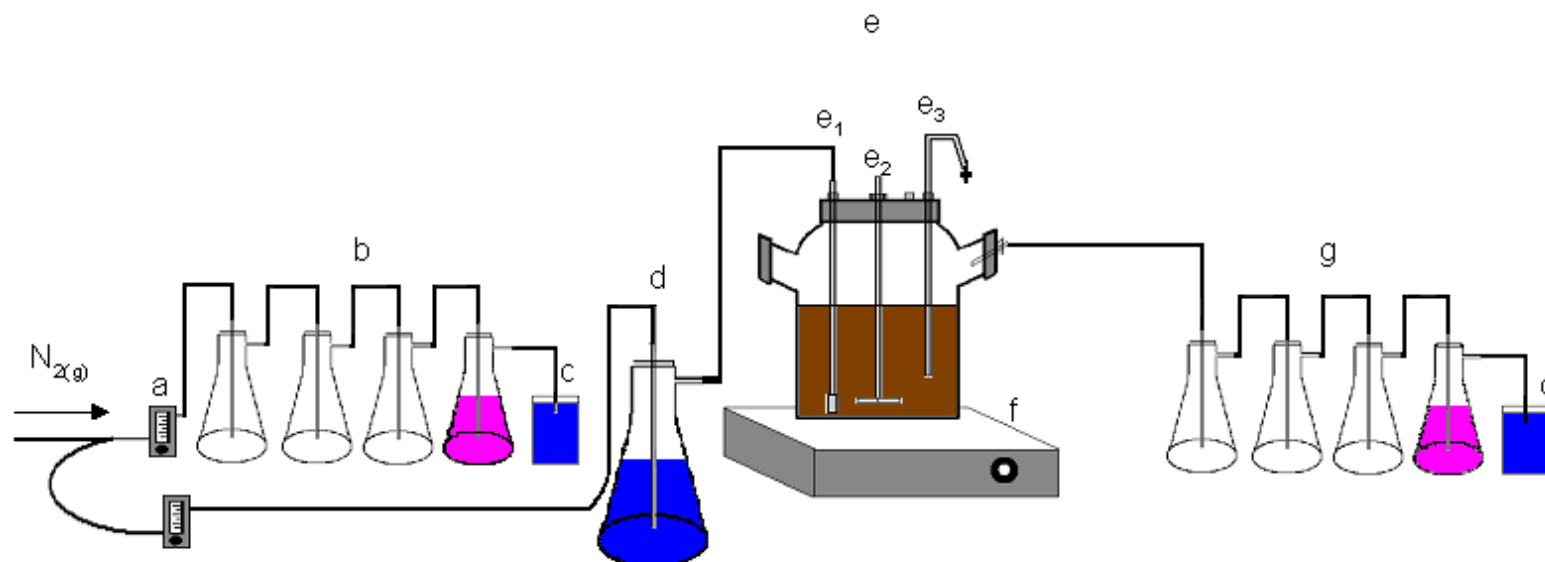
Figure A2: Schematic of sampling procedure for the reduced silt+clay suspension showing a) the reduction reactor with sampling syringe at the sampling tube, b) transfer of the silt+clay suspension to an evacuated pyrex glass centrifuge tube, c) the centrifuged suspension and supernatant solution, d) supernatant removal, and e) injection of the supernatant into a $N_{2(g)}$ filled filter flask under vacuum.
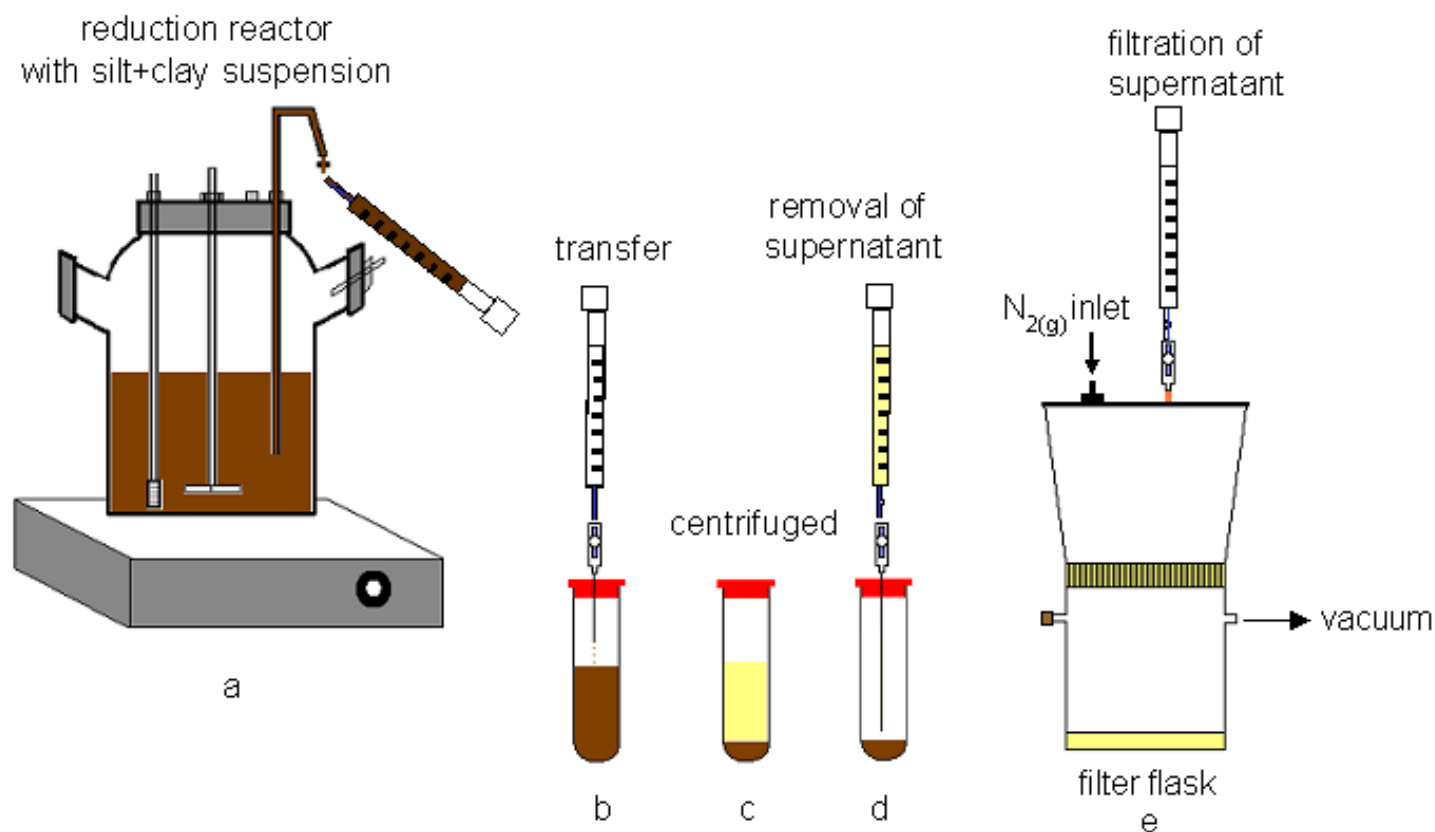
APPENDIX B

Discussion of solid-phase speciation analysis by X-ray absorption near edge structure (XANES) spectroscopy

<u>Purpose</u>

X-ray absorption near edge structure (XANES) spectroscopy was used to try to differentiate the solid-phase speciation of P for reduced silt+clay samples, and to provide molecular scale evidence of PO_4 binding to Fe-humate complexes which would lend support to our proposed mechanism of the formation of dissolved aqueous PO_4-Fe-DOM complexes.

<u>XANES spectra of silt+clay samples</u>

The ability to determine the solid-phase speciation of P in the reduced silt+clay samples over time would have been an invaluable aid towards understanding which mechanisms of phosphate dissolution were important for this acidic, Coastal Plain soil.

In the early stages of this work samples from a preliminary reduction reactor (data not reported) and from the 0-d treatment (Chapter 2) (each treated with 2 g dextrose kg^{-1}solids added at 0d) were analyzed at X19A, Brookhaven National Laboratory, Upton, NY (data aquisition, see Khare et al., 2004); note, data were baseline corrected between -40 and -5 eV and edge-normalized at 17 eV). After the initial evaluation of the spectra it appeared that XANES spectroscopy was not going to be sensitive enough to qualitatively determine differences in the solid-phase P species for these samples, possibly due to the moderately low concentration of P (31 to 62 mmol P kg^{-1}), noisy baselines, and spectral artifacts from P contamination of the mylar film that covered the samples.

After the completion of research by Khare et al. (2004; a-in review), I renormalized the data (according to Khare et al., 2004) and evaluated if the different normalization and background correction methods would show any trends in the pre-edge regions of the spectra which could indicate a shift of PO_4 associated with Fe(III)-oxide to Al-oxide minerals. Qualitative information based on the trends in pre-edge features could be assessed since all samples have the same artifacts from the mylar film.

The phosphorus K-XANES spectra for the 0-d treatment (0d and 40d of reduction for reactor 1 and 2) and the preliminary experiment (0d, 5d, 18d, and 37d of reduction) are shown in Figure B1 and B3, respectively. These plots show that individual spectra in each experiment were very similar to each other and showed no distinct spectral features in the pre-edge or post-edge region (Hesterberg et al., 1999; Beachemin et al., 2003; Khare et al., 2004).

XANES spectrum of a 0d silt+clay sample was compared with spectra of prepared standards of PO_4 sorbed on ferrihydrite or non-crystalline Al-hydroxide (Figure 2.5, Chapter 2) and showed that PO_4 was associated in part with Fe(III)-oxide minerals, probably as a sorbed phase. As the 0d sample underwent reduction one might expect to see a trend in decreasing pre-edge feature indicating that there is less PO_4 to Fe-oxide bonding character.

The pre-edge region of the phosphorus K-XANES spectra for the 0d treatment and the preliminary experiment are shown in Figures B2 and B4, respectively. The spectra in the pre-edge region for the 0-d treatment (Figure B2) and the preliminary experiment (Figure B4) show no consistent trend after 40 or 37d

of reduction, respectively. The spectra for the 0-d treatment (Figure B2) are very noisy and poor quality. While the spectra for the preliminary experiment (Figure B4) are not as noisy, they show no consistent trend and display minimal differences in the pre-edge feature. As a result, even after the spectra were renormalized and background corrected according to Khare et al. (2004), it is not possible to make any conclusions concerning changes in the solid-phase P speciation of the silt+clay samples after 40d of reduction.

Spectra for soil samples are typically noisier than for pure minerals. Improvements in data acquisition have already occurred since the time these samples were analyzed. Future redox studies for soils with a higher P concentration could improve XANES data quality. Future studies that are able to correlate dissolution data with XANES spectroscopy will lend insight to mechanisms of reductive dissolution of phosphate in soils.

PO_4 sorbed to Fe-humic acid complexes

Sample Preparation

A freeze-dried humic acid sample was dissolved according to the method described in Hutchison et al. (2001) having a 0.1 mol L^{-1} $NaNO_3$ background. Ten mL of 0.01 mol L^{-1} $Fe(NO_3)_3$ $9H_2O$ solution was added dropwise to the dissolved humic acid stock suspension. The pH was adjusted to 6.0 using 0.1 mol L^{-1} NaOH and brought to a final mass of 60 g with 0.1 mol L-1 $NaNO_3$. The humic acid/Fe stock suspension was equilibrated for 24h on a reciprocating floor shaker and again adjusted to pH 6.0. A 0.01 mol L^{-1} NaH_2PO_4 solution was slowly added to 20 g of

vigorously stirring humic acid/Fe suspension yielding a final concentration of 400 mmol P kg^{-1} humic acid. The samples was adjusted to pH 6.0 and brought to a final mass of 30 g. Periodically, the pH was adjusted to 6.0 during a 40h equilibration time at room temperature while shaking. After 40h of equilibration, the suspension was filtered through a 0.25 μm mixed-cellulose ester membrane. The filter was carefully removed and secured on a polyacrylic X19A sample holder. XANES data acquisition and normalization were performed as described earlier.

The standard mineral of PO$_4$ sorbed to ferrihydrite is described in Khare et al. (2004). The preparation of the aqueous 50 mM H$_3$PO$_4$ and 0 mM Fe solution is described in Khare et al. (b, in review).

XANES spectra of PO$_4$ sorbed to humic acid/Fe complexes

Plots of phosphorus K-XANES spectra for PO$_4$ sorbed to humic acid/Fe complexes, PO$_4$ sorbed to ferrihydrite, and aqueous 50 mM H$_3$PO$_4$ and 0 mM Fe solution are shown in Figures B5(-7 to 20 eV) and B6 (pre-edge region). The pre-edge region in each plot show the increasing pre-edge feature as the PO$_4$:Fe ratio increases. As previously discussed, Hesterberg et al. 1999, Beauchemin et al., 2003, and Khare et al. (c) (in review) show a distinct pre-edge feature present in the spectra of Fe(III)-associated phosphates due to electron orbital configurations and electronic transitions near the x-ray absorption edge. Khare et al. (c) (in review) ascribes the pre-edge feature to an electronic transition from a P 1s into a Fe(4p)-O(2p) antibonding orbital.

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The ratio of added Fe to reactive carboxyl groups was very low (0.16) so I assumed that all the Fe should be bound to the humic acid. Phosphate was added at 80% of the amount of Fe, assuming that PO_4 would bind 1:1 with the Fe bound to humic acid. If the above assumptions are true, then the spectra in Figures B5 and B6 suggest that the PO_4 is sorbed to the Fe as an Fe/humate complex. However, one cannot determine from these spectra if any of the PO_4 was bound as an aqueous $FePO_4$ complex because the spectra would look the similar. Further investigation of prepared standards of varying concentrations of PO_4 sorbed to Fe/humate complexes and aqueous PO_4/Fe complexes could be investigated using extended x-ray absorption fine structure (EXAFS) spectroscopy at the Fe edge to determine nearest neighboring atoms and coordination numbers.

Figure B1: Normalized phosphorus K-XANES spectra for moist silt+clay samples taken at time 0d, and at 40d for duplicate reactors (labeled 1 and 2) receiving 2 g dextrose kg^{-1} solids added at time 0d (0-d treatment, Chapter 2).
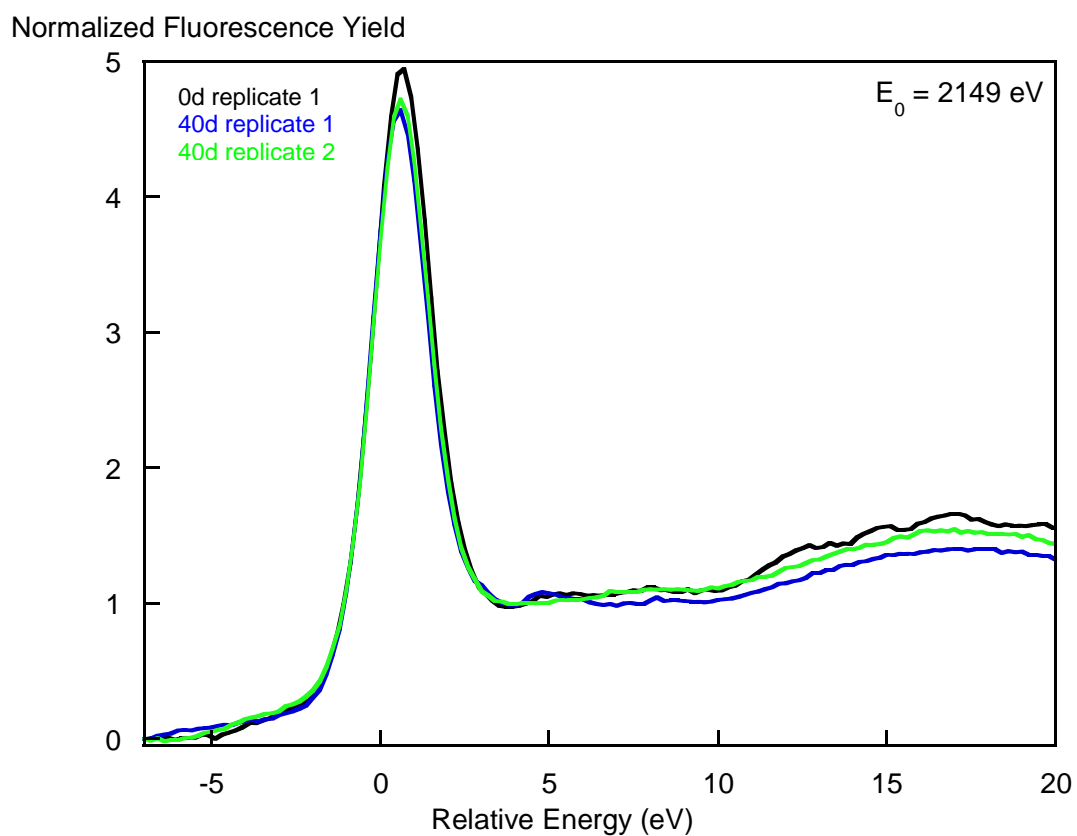
Normalized Fluorescence Yield

0d replicate 1
40d replicate 1
40d replicate 2

E_0 = 2149 eV

Relative Energy (eV)

Figure B2: Pre-edge region of the normalized phosphorus K-XANES spectra for moist silt+clay samples taken at time 0d (replicate 1), and at 40d for duplicate reactors (replicate 1 and 2) receiving 2 g dextrose kg^{-1} solids added at time 0d (0-d treatment, Chapter 2).
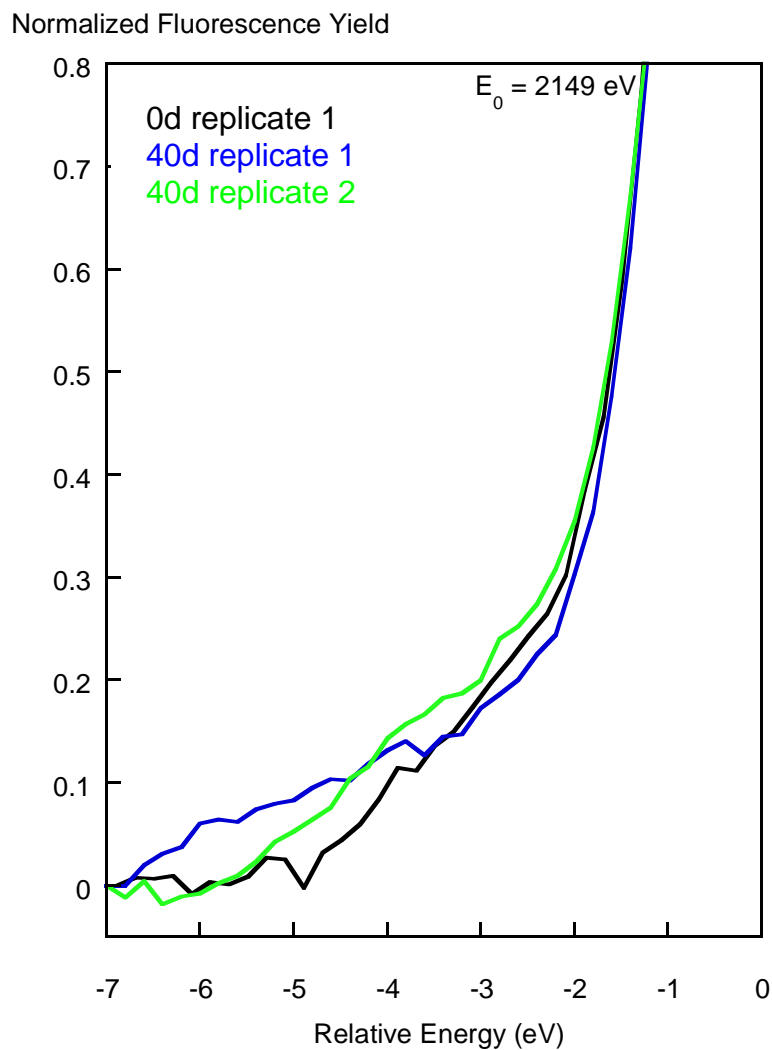
Figure B3: Normalized phosphorus K-XANES spectra for silt+clay samples taken at time 0d (t0 d), 5d (t5 d), 18d (t18 d), and 37d (t37 d) during a preliminary experiment treated with 2 g dextrose kg^{-1} solids added at time 0d.
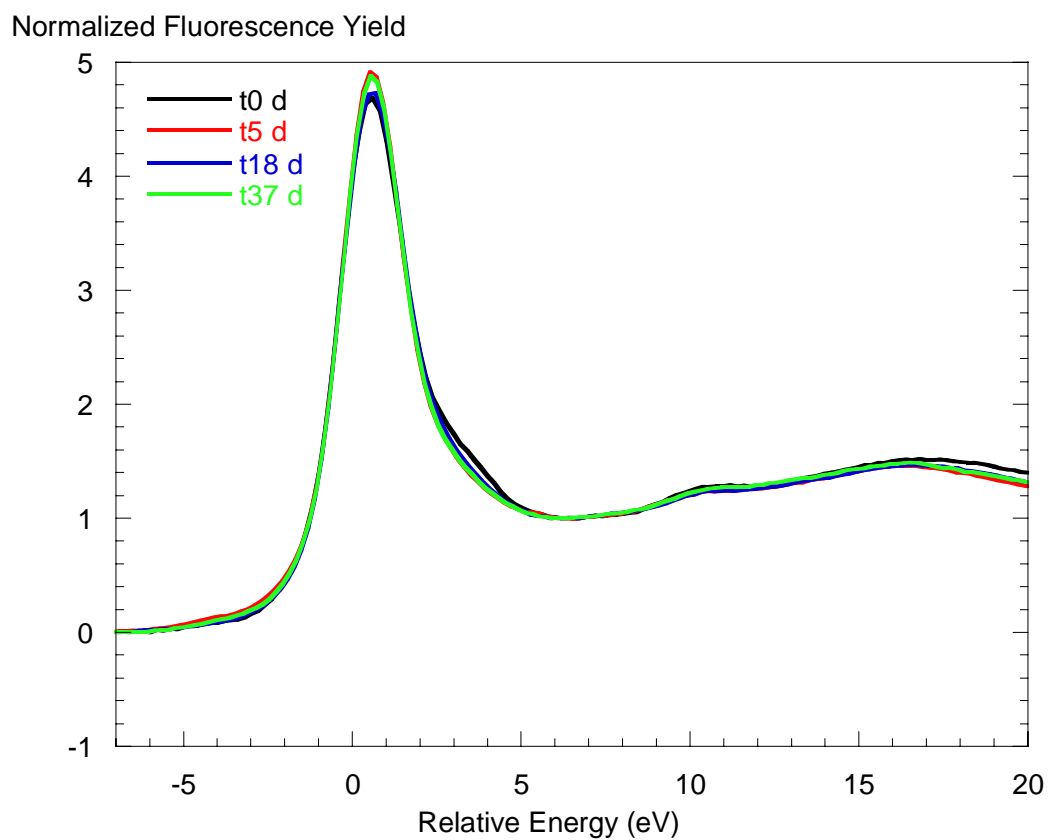
Normalized Fluorescence Yield

Figure B4: Pre-edge region of the normalized phosphorus K-XANES spectra for silt+clay samples taken at time 0 d (t0 d), 5 d (t5 d), 18 d (t18 d), and 37 d (t37 d) during a preliminary experiment treated with 2 g dextrose kg^{-1} solids added at 0d.
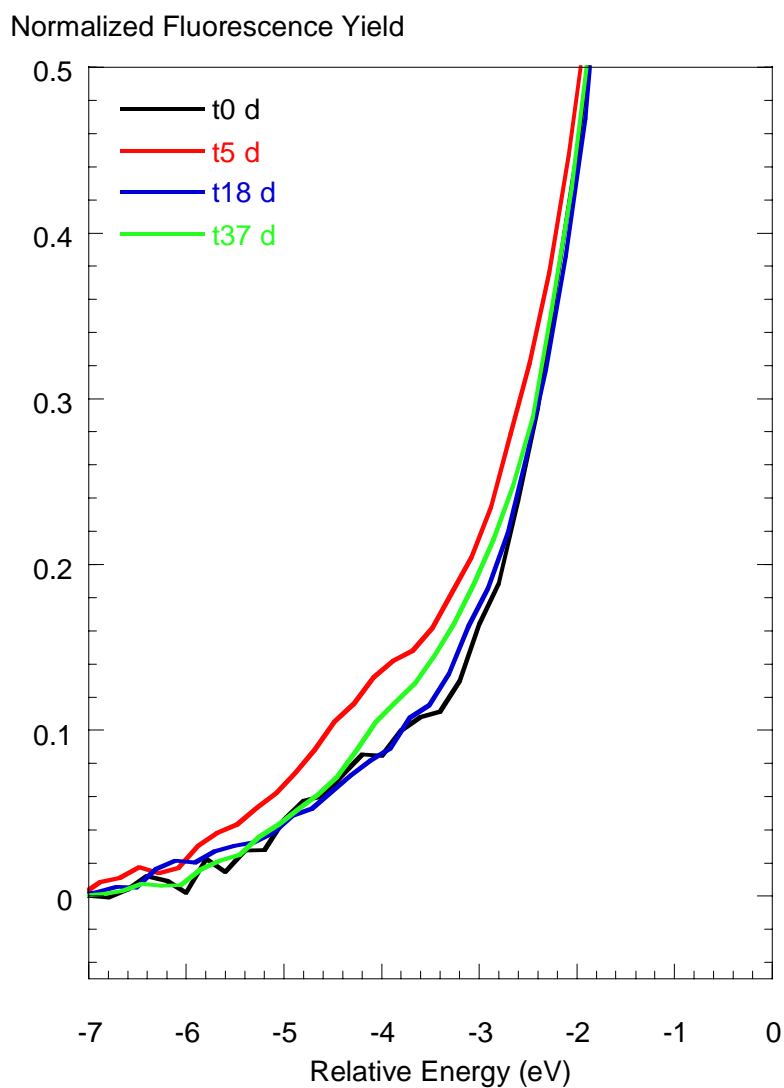

Normalized Fluorescence Yield

Relative Energy (eV)

Figure B5: Normalized phosphorus K-XANES spectra for 400 mM P + 500 mM Fe kg^{-1} HA, PO$_4$ sorbed to ferrihydrite, and 50 mM H$_3$PO$_4$ and 0 mM Fe aqueous solution.
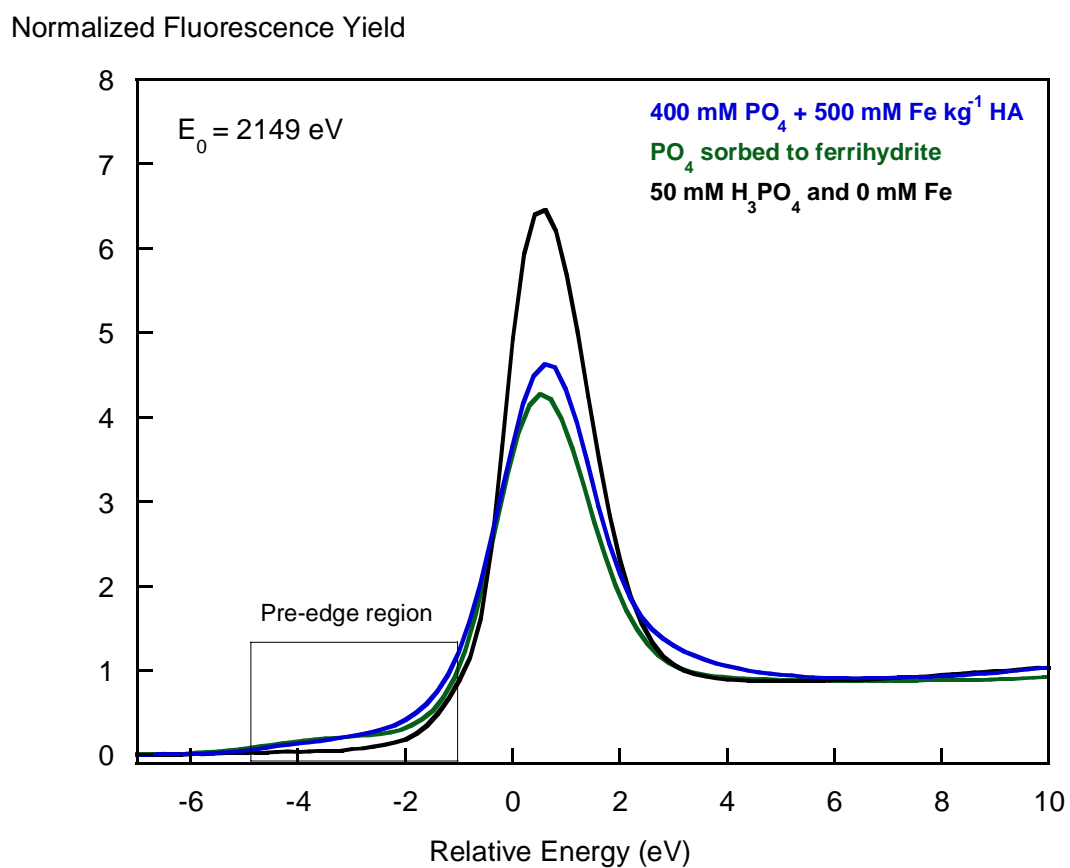
Normalized Fluorescence Yield

Figure B6: Pre-edge region of the normalized phosphorus K-XANES spectra for 400 mM P + 500 mM Fe kg^{-1} HA, PO_4 sorbed to ferrihydrite, and 50 mM H_3PO_4 and 0 mM Fe aqueous solution.

Normalized Fluorescence Yield