

## ABSTRACT

BROWNFIELD, CHRISTOPHER S. Phosphorus Dissolution in Soil Material from a Carolina Bay as Affected by Reducing Conditions. (Under the direction of Dean L. Hesterberg and Michael J. Vepraskas).

The restoration of drained agricultural soils to wetlands may result in the dissolution of P from reduced soils, causing the eutrophication of nearby surface waters. Therefore, continued improvements in the ability to predict P dissolution are necessary to minimize its potential environmental hazard. The objectives of this project were to determine if P dissolution would occur when soil material from a drained Carolina bay wetland was reduced, and to hypothesize explanations of differences in P dissolution between soils based on aqueous solution chemistry. Suspensions ( $15 \text{ g kg}^{-1}$ ) of  $< 53 \text{ }\mu\text{m}$  separates from surface samples (0-10 cm) of six poorly drained soils were subjected to microbial reduction for 25 d in a continuously stirred reactor. In a separate experiment, saturated whole soil samples ( $2.5 \text{ g H}_2\text{O g}^{-1}$  solids) were incubated under  $\text{O}_2$ -free conditions for 62 d. In addition to total P, Fe, Mn, and Al; dissolved reactive phosphate (DRP), Fe(II), and dissolved organic carbon (DOC) were measured in filtrate samples from both experiments. A net increase in P dissolution (two-fold increase, up to  $1.2 \text{ mg L}^{-1}$ ) was observed for only one of six suspensions (Ponzer 1) in the continuously stirred reactor experiment. In that suspension the molar P:Fe(II) suggested that reductive dissolution of Fe(III)-bound P could not fully account for the P that dissolved, and DOC was highly correlated with P dissolution. For reactor suspensions in which no net P dissolution occurred, oxalate-extractable Al was negatively correlated ( $p < 0.05$ ) with final [DRP], and DOC concentrations were approximately 2-fold lower than in the Ponzer 1 suspension. In the static incubation experiment, P dissolution occurred in all four samples, and the highest concentration was seen in the Ponzer 1 sample

(three-fold increase, up to 2.2 mg DRP L<sup>-1</sup>). Dissolved organic carbon concentrations were between 2 and 4 fold higher in the static incubation experiment than the highest concentration observed in the stirred reactor experiment, and offer a qualitative explanation for the additional P dissolution that occurred in static incubation experiment. The results of these experiments suggest that P concentrations in soils of the restored wetland will increase upon reduction to levels that are environmentally threatening, and that interaction of DOC with PO<sub>4</sub> or minerals that bind PO<sub>4</sub> plays an important role in the release of P.

**PHOSPHORUS DISSOLUTION IN SOIL MATERIAL FROM A  
CAROLINA BAY AS AFFECTED BY REDUCING CONDITIONS**

By

**CHRISTOPHER SCOTT BROWNFIELD**

A thesis submitted to the Graduate Faculty of  
North Carolina State University  
In partial fulfillment of the  
Requirements for the Degree of  
Master of Science

**SOIL SCIENCE**

Raleigh, NC

2007

**APPROVED BY:**

---

---

Dr. Dean Hesterberg  
Co-Chair of Advisory Committee

---

---

Dr. Michael Vepraskas  
Co-chair of Advisory Committee

---

---

Dr. Daniel Richter  
Member of Advisory Committee

---

---

Dr. Suzanne Beauchemin  
External Member of Advisory  
Committee

## BIOGRAPHY

Chris Brownfield was born in Raleigh, North Carolina in June, 1982. He earned a B.S. in Chemical Engineering and a B.A. in Chemistry, both from North Carolina State University. Interests in environmental chemistry brought him to the Soil Science Department. In his spare time, he enjoys playing sports and thinking about soil chemistry.

## ACKNOWLEDGEMENTS

I would like to thank Dean Hesterberg, Mike Vepraskas, Dan Richter, and Suzanne Beauchemin. Their work ethic, curiosity, and intelligence constantly commanded my best academic efforts. It is a privilege to work and study under scientists of such high quality, and I anticipate that I will enjoy the benefits of my experiences at N.C. State for years to come.

Over the last three years, Kim Hutchison helped me to refine my laboratory techniques, helped me develop and find appropriate analytical procedures, and offered consistent encouragement. My fellow lab-mates and graduate students, Chris Murray, Daphine McKinney and Amanda Zelasko, helped me to stay motivated, also by providing encouragement, and by displaying positive attitudes and setting good examples. Dr. Wei Shi and Chris Niewoehner provided skilled advice that helped me to design the reactor and static incubation systems. Chris Niewoehner also completed the particle size analysis with the help of Tripp Cox. Guillermo Ramirez and Lisa Lentz were patient, knowledgeable, and thorough in providing other sample analysis services. The efficient efforts of Jon Zehr and Jacob Majikes, two undergraduates working in our lab, saved me time and effort when I had very little to spare. I would also like to thank several organizations that funded my work. Basic funding for this project was provided by North Carolina Department of Transportation Grant No. HWY-2004-19, by USA-NRI Grant No. 2005-35107-16253, and by The North Carolina Agricultural Foundation. Additional funding was provided by N.C. Beautiful through the Moore Fellow program, which also offered me the opportunity to network with professionals interested in North Carolina and the stewardship of its natural heritage.

Finally, I would like to thank my entire family for their encouragement, patience, and support; and for their stimulating interest in my labors.

# TABLE OF CONTENTS

|   |           |
|---|-----------|
| LIST OF FIGURES .....   | vi        |
| LIST OF TABLES .....  | vii       |
| <b>CHAPTER ONE: POTENTIAL IMPACTS OF PHOSPHORUS ON WATER QUALITY<br/>DURING WETLAND RESTORATION .....</b> | <b>1</b>  |
| INTRODUCTION .....  | 2         |
| The Phosphorus Cycle in Soils .....   | 6         |
| Aqueous Phosphate .....   | 8         |
| Organic P: Forms and Solubility .....   | 9         |
| Inorganic P: Forms and Solubility .....   | 10        |
| Organically Complexed Inorganic P: Forms and Solubility .....   | 12        |
| The Effect of Reduction on P Dissolution .....  | 13        |
| REFERENCES .....  | 17        |
| <b>CHAPTER TWO: REDUCTIVE DISSOLUTION OF SOIL P UNDER ANAEROBIC<br/>CONDITIONS .....</b>                  | <b>22</b> |
| INTRODUCTION .....  | 23        |
| MATERIALS AND METHODS .....   | 26        |
| Site History .....  | 26        |
| Sampling .....  | 26        |
| Continuously Stirred Reactor (CSR) Experiment .....   | 28        |
| Static Incubation Experiment .....  | 31        |
| Aqueous Solution Analyses .....   | 32        |
| Solids Characterization .....   | 33        |
| Statistical Analysis .....  | 34        |
| RESULTS .....   | 35        |
| Soil Characteristics .....  | 35        |
| CSR Experiment .....  | 38        |
| Static Incubation Experiment .....  | 49        |
| DISCUSSION .....  | 52        |
| The Meaning of Static Incubation and CSR Results .....  | 52        |
| Implications for Wetland Restoration at Juniper Bay .....   | 54        |
| CONCLUSIONS .....   | 55        |
| REFERENCES .....  | 56        |
| <b>APPENDICES .....</b>   | <b>60</b> |
| APPENDIX A: THE ORIGIN OF CAROLINA BAYS AND THE PROCESS OF<br>RECLAIMING THEM .....                       | 61        |
| APPENDIX B: P <sub>T</sub> SURVEY OF SOILS AT JUNIPER BAY .....   | 64        |

|  |    |
|--|----|
| APPENDIX C: GLUCOSE REDUCTION IN RELATION TO OTHER REDOX<br>COUPLES..... | 65 |
| APPENDIX D: pH AND Eh DATA.....  | 66 |
| APPENDIX E: TOTAL P, Fe, Al, Mn DATA .....                               | 68 |
| APPENDIX F: REDOX-AFFECTED AQUEOUS COMPONENT<br>CONCENTRATIONS .....     | 70 |

## LIST OF FIGURES

|  |    |
|--|----|
| Figure 1.1. Generalized P cycle in soils.....  | 6  |
| Figure 1.2. Examples of dissolved PO <sub>4</sub> species.....   | 8  |
| Figure 1.3. Phytic acid, or myo-inositol hexaphosphate.....  | 9  |
| Figure 2.1. Location of sampling points at Juniper Bay .....   | 27 |
| Figure 2.2. Extractable forms of Fe (a) and Al (b) (mg kg <sup>-1</sup> ) in the < 53 μm solids .....  | 37 |
| Figure 2.3. Temporal trends in Eh (mV).....  | 38 |
| Figure 2.4. Total CO <sub>2</sub> evolved (mmol) from reduced and oxidized suspensions.....  | 39 |
| Figure 2.5. Temporal trends in pH for selected replicates of all suspensions .....   | 40 |
| Figure 2.6. Fe(II) dissolution in reduced suspensions .....  | 41 |
| Figures 2.7a and 2.7b. Dissolved reactive phosphorus (DRP) concentrations in reduced (b)<br>and oxidized (a) suspensions of < 53 μm organic soil separates. .... | 42 |
| Figures 2.8a and 2.8b. Dissolved reactive phosphorus (DRP) concentrations in reduced (a)<br>and oxidized (b) suspensions of < 53 μm mineral soil separates.....  | 43 |
| Figure 2.9. Scatter plots of DRP and Fe(II) in the Ponzer 1 sample.....  | 43 |
| Figure 2.10. Concentrations of P and Fe(II) (μmol L <sup>-1</sup> ) in the reactor solutions at t = 20d...   | 45 |
| Figure 2.11a and 2.11b. Dissolved organic carbon (DOC) during 25 d in reduced (a) and<br>oxidized (b) suspensions. ....  | 46 |
| Figure 2.12a and 2.12b. Dissolved organic carbon (DOC) during 25 d in reduced (a) and<br>oxidized (b) suspensions.....   | 46 |
| Figures 2.13a and 2.13b. Correlations of DRP and DOC in organic (a) and mineral (b)<br>suspensions .....   | 47 |
| Figure 2.14. Final [DRP] as predicted by Al <sub>ox</sub> concentrations in the Ponzer 1 sample. ....  | 48 |
| Figures 2.15a, 2.15b, and 2.15c. Concentrations of DRP (a), dissolved Fe(II) (b), and DOC<br>(c) after 2 and 62 d of incubation at 25 °C.....                    | 50 |
| Figure B.1. P <sub>tot</sub> concentrations (mg kg <sup>-1</sup> ) in air-dried surface soil samples (0-10 cm) from<br>Juniper Bay .....                         | 64 |
| Figure F.1. Eh (mV) and concentrations (mg L <sup>-1</sup> ) of redox-active species for a suspension of<br>mineral and organic soil material.....               | 70 |

## LIST OF TABLES

|   |    |
|---|----|
| Table 2.1. Characterization of whole soil and < 53 $\mu\text{m}$ separates used in 62 d static incubation and 25 d continuously stirred reactor experiments ..... | 36 |
| Table 2.2. Changes in pH, Eh, DRP, Fe(II) and DOC concentrations of incubated soil samples after 62 d of anaerobic incubation at 25°C. ....                       | 50 |

**CHAPTER ONE:**  
POTENTIAL IMPACTS OF PHOSPHORUS ON WATER QUALITY  
DURING WETLAND RESTORATION

## INTRODUCTION

Phosphorus is an essential nutrient for life, and chemical reactions inherent to wetland soils can increase the availability of P for biological uptake (Patrick and Khalid, 1974; Ponnampereuma, 1972). While P is non-toxic, excessive dissolved P concentrations in surface waters can lead to eutrophication, an over-enrichment of a water body with biological nutrients that results in excessive biological production (Correll, 1998). As recently as 1998, the United States Environmental Protection Agency (US-EPA) had not established criteria for P impacts on water quality (Parry, 1998), but as little as 10-20  $\mu\text{g P/L}$  is suspected to cause eutrophication in P-limited freshwaters of the southeastern USA (Sawyer, 1947; Vollenweider, 1968; Correll, 1998). In contrast, agricultural crops grown in upland soils typically demand 100-200  $\mu\text{g P/L}$  in soil solution for optimal growth (Fox and Kamprath, 1970). Because wetlands are commonly positioned between extensively fertilized agricultural lands and nutrient-sensitive surface waters (Reddy et al., 1999), their function as a sink or source of P is critically important to water quality.

Wetland reclamation includes the drainage or filling of wetland soils to increase the separation of a soil's surface from the water table. For centuries, wetlands have been reclaimed for agriculture and forestry (Ewing et al., 2004), and for at least the last 50 years their reclamation has been followed by inputs of P in the form of commercial fertilizers (Lilly, 2003). In North Carolina, reclamation efforts started in the early 1700's around Lake Mattamuskeet in Hyde County and continued on a much grander scale one-half century later near Lake Phelps in Washington County (Lilly, 2003). Planters at Lake Mattamuskeet and Lake Phelps relied on organic matter decay to supply P since commercial fertilizers were not readily available or well understood (Lilly, 2003). Since the initial efforts near Lake Phelps

and Lake Mattamuskeet, the formation of drainage districts has facilitated the conversion of wetlands throughout the eastern U.S. (Meyer, 1994), especially after World War II (Ewing et al., 2004). Rates of wetland loss reached a national peak at over half a million acres per year between the 1950's and 1970's, (Meyer, 1994), a period in which the use of P fertilizers increased five-fold (USDA-ERS, 2007), and water quality declined rapidly on a national scale (Richardson and Vepraskas, 2001). At this time there was no formal protection for wetlands against drainage and development, and the federal government even subsidized their conversion (Ewing et al., 2004).

In 1972 wetland reclamation was partially regulated by the Federal Water Pollution Control (Clean Water) Act, Section 404, which charges the U.S. Army Corps of Engineers (Corps) to permit the discharge of dredged or fill materials in wetlands. Five years later Congress amended the Act to require mitigation, or replacement, of wetland functions eliminated by Section 404 permits. The Farm Bills of 1985 and 1990 significantly penalized wetland drainage for agricultural use (Voteller and Muir, 2002). Commonly termed the *Swampbuster* program, these bills penalized landowners that drained wetlands by revoking their right to participate in many agricultural subsidy programs (Ewing et al., 2004; Voteller and Muir, 2002). The Farm Bill of 1990 also established the Wetland Reserve Program, which offers buy-out incentives and cost-share programs to private landowners who establish wetland easements or restore reclaimed wetlands.

Today, 74% of the nation's wetlands are owned as private lands (Voteller and Muir, 2002), underscoring their potential to change in land use. The Corps annually reviews about 90,000 Section 404 permits and mandates the mitigation of about 45,000 wetland acres (Rea, 2001). Since the hydrologic restoration of drained, agricultural lands is often perceived to be

straight-forward, they make common restoration targets (Ewing, 2004). Scientists are concerned, however, that reducing redox conditions established in soils during restoration of wetlands from high-P agricultural soils may result in excessive P discharge to nutrient-sensitive surface waters (Sallade and Sims, 1997; Shenker et al., 2004; Young and Ross, 2001). This concern is especially important for North Carolina, a state that once had more natural wetland acreage than all but five others (Mitch and Grosselink, 2000) and also has an abundance of high-P agricultural soils (Cahoon and Ensign, 2004).

My research is concerned with chemical processes that control P dissolution in soils at Juniper Bay, a Carolina bay wetland in Robeson County, NC undergoing restoration.<sup>1</sup> Minor drainage efforts at Juniper Bay started about 100 years ago (Ewing, 2003). Between 1905 and 1909, trees were harvested and drainage ditches were constructed along a narrow corridor within the bay as part of the Raleigh and Charleston shortline railroad (Ewing, 2003). Canal Industries purchased the property in the 1960's and harvested timber a few years later. Mr. Robert Freeman of Robeson County bought the timbered bay from Canal Industries in 1975 and he supervised major drainage efforts during the dry summer of 1979 (Ewing, 2003). Mr. Freeman and his son grew common Robeson County crops in the bay from 1980 until the 1990's, including corn, soybeans, tobacco, cotton, wheat, oats, millet, okra, and lettuce (Ewing, 2003).

In 1999 the North Carolina Department of Transportation (NC-DOT) purchased Juniper Bay to mitigate the destruction of nearby wetlands due to highway construction (Ewing, 2003). Shortly thereafter, the DOT commissioned wetland soil scientists at North Carolina State University (NCSU) to study the feasibility and success of their restoration

---

<sup>1</sup> For a brief summary of the formation of Carolina bays and the process of reclaiming them, the reader is referred to Appendix A.

efforts. Prior to hydrologic restoration, Ewing (2003) reported that Mehlich III extractable P (M3P) concentrations in the top 100 cm of mineral soils from Juniper Bay were about 60 mg/kg higher than in nearby, unaltered reference bays, and Kreiser (2003) reported that P concentrations in the main surface outflow at Juniper Bay increased 4.5 fold (up to 90  $\mu\text{g P L}^{-1}$ ) during rainfall events  $> 5$  cm. Preliminary batch incubation experiments conducted in the laboratory on soil material from Juniper Bay showed that dissolved reactive phosphorus (DRP) concentrations increased threefold (up to 350  $\mu\text{g/L}$ ) as the soils underwent reduction (Moll, 2004).

The goal of this research is to determine if P will dissolve from soils at Juniper Bay after wetland hydrology is restored, and to understand the processes that control P dissolution in organic-rich soils such as those at Juniper Bay. Knowledge of the chemical processes controlling P dissolution in soils at Juniper Bay would be useful in predicting the success of other wetland restoration efforts throughout the southeastern United States. In preface to a more detailed study of phosphate chemistry in soil material from Juniper Bay, it is instructive to review the current knowledge about the behavior of P in soils.

# LITERATURE REVIEW

## The Phosphorus Cycle in Soils

In its most generalized form, the P cycle in soils (Fig 1.1) is an exchange of between pools of P in soil solids by way of the soil solution.

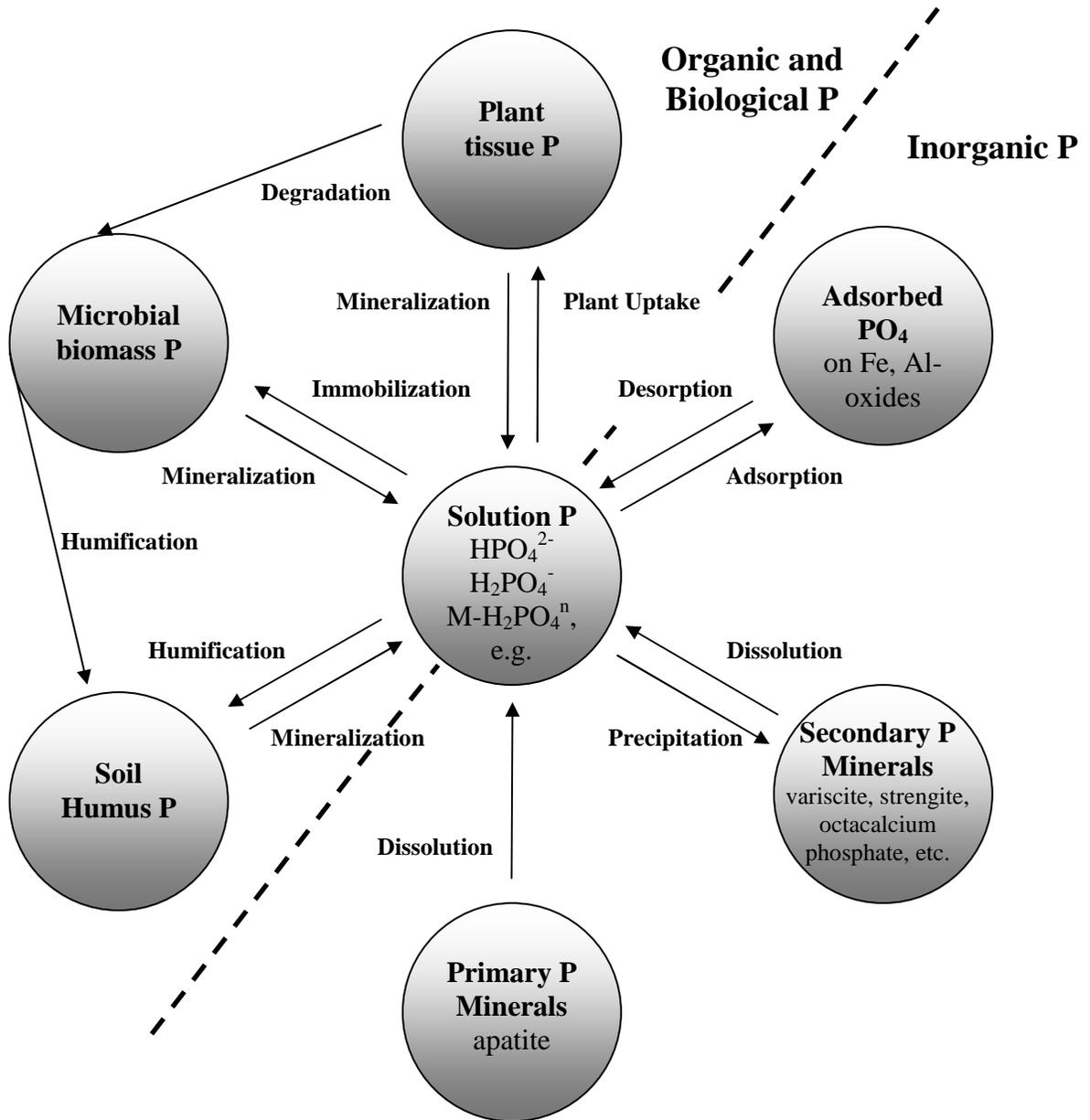


Figure 1.1. Generalized P cycle in soils.

Orthophosphate ( $\text{PO}_4$ ) is a common molecular component of all types of soil P, and the dissolved orthophosphate ions ( $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$ ) play a central role in the soil P cycle (Fig.1.1). These ions are taken up by plant roots, immobilized by microbes, and react strongly with dissolved metals and metal oxide surfaces (Reddy et al., 1999). Reddy et al. (1999) reported estimates of the diffusive mass transfer coefficient for P in the range of  $0.01 - 0.1 \text{ m d}^{-1}$  owing to its very low diffusivity in water ( $D^0 = 8.74 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ ; Edwards and Huffman, 1959). A combination of these factors results in very low concentrations of orthophosphate in soil solution, typically in the range of  $1 \mu\text{g P L}^{-1} - 1 \text{ mg P L}^{-1}$  (Brady and Weil, 2004). In contrast, the total P concentration in soils is on average about  $600 \text{ mg P kg}^{-1}$  (Lindsay, 1979), so greater than 99.9% of P is typically in the solid phase (Brady and Weil, 2004).

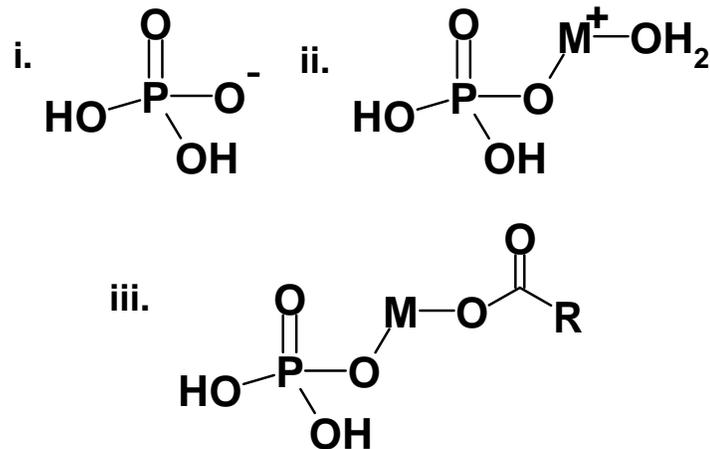
Fig. 1.1 shows the two most general forms of P in soil solids: organic ( $\text{P}_o$ ) and inorganic P ( $\text{P}_i$ ). Organic and inorganic P are similar in that both include modified forms of  $\text{PO}_4$ , but they differ in the chemical constituents to which  $\text{PO}_4$  is bonded. Organic P is predominantly bonded to carbon-containing remains of soil biota (Stevenson, 1994), while  $\text{P}_i$  can be bound on particle surfaces or within minerals that contain Fe, Al, Ca, K, Mg, and Mn (Lindsay, 1979). The differences in the surrounding molecules are principally responsible for the solubility of P, and it is important to consider the distribution of P in each solid-phase pool when estimating the potential for phosphorus dissolution.

Various indexes have been used to quantify the saturation of soil solids with phosphorus (Beauchemin and Simard, 1999). Generally, these indexes are operationally-defined by chemical extractions and involve some ratio or correlation between *reactive* or *plant available* phosphorus (*sensu* Haygarth and Sharpley, 2000) and extractable iron and

aluminum. Although these indexes are useful for P management, a higher level of understanding would be achieved by understanding the fundamental chemistry of soil P, enhancing the ability to predict P dissolution during wetland restoration.

### Aqueous Phosphate

Aqueous-phase  $\text{PO}_4$  includes orthophosphate ions (predominantly  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$  at typical soil pH levels), complexes of the orthophosphate ion with metal cations such as  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Al}^{3+}$ , and  $\text{Ca}^{2+}$  (Lindsay, 1979), and dissolved phosphate-metal-organic matter complexes (Lindsay, 1979). The pKa values of dissolved acid species are 2.15 ( $\text{H}_3\text{PO}_4$  -  $\text{H}_2\text{PO}_4^-$ ), 7.20 ( $\text{H}_2\text{PO}_4^-$  -  $\text{HPO}_4^{2-}$ ), and 12.35 ( $\text{HPO}_4^{2-}$  -  $\text{PO}_4^{3-}$ ). Between pH 4 and pH 6.5, the pH range of common acid soils of the Atlantic Coastal Plain, orthophosphate is predominantly diprotonated (Lindsay, 1979), as shown in Figure 1.2 (i).



**Figure 1.2. Examples of dissolved  $\text{PO}_4$  species (Tjedor-Tjedor and Anderson, 1990).**

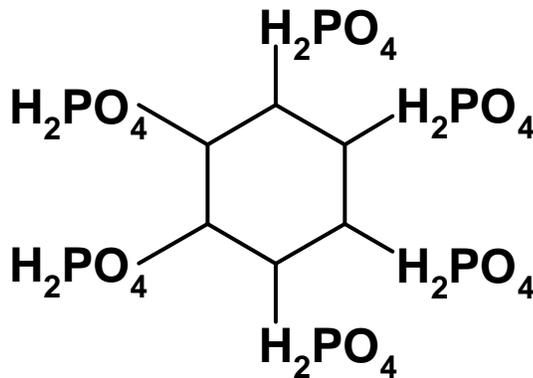
Figure 1.2 also shows an inner sphere aqueous complex of orthophosphate with a generic multivalent metal cation (ii) and a model  $\text{PO}_4$ -metal-humic acid aqueous complex (iii). As shown, both of these complexes are dissolved and serve to decrease the activity of free ionic orthophosphate in soil solution, although they may increase the overall bioavailability of P (Lindsay, 1979).

### Organic P: Forms and Solubility

Organic P typically accounts for 15-80% of the total P in soils, with the upper limit found in wetland organic soils (Stevenson, 1994). Organic P can be classified into two broad categories (modified from Stevenson, 1994):

- organic compounds of the soil humus and microbial biomass
- nano-precipitated inorganic compounds bound by the soil humus

Because it is so heterogeneous, only about half of all  $\text{P}_o$  can be accounted for in known compounds (Stevenson, 2004). Essington (2004) reported that < 1 - 60% of the organic P in soils is in the form of inositol phosphates, one recalcitrant structure of which is shown in Fig.1.3.



**Figure 1.3. Phytic acid, or myo-inositol hexaphosphate.**

Other forms of  $P_o$  include phospholipids and nucleic acids, which are more short-lived in soil environments than phytic acid (Stevenson, 1994), and typically compose less than 10% of the total  $P_o$  in soils (Essington, 2004; Stevenson, 1994). Among the most labile of all forms of organic P are phosphoproteins used in energy transfer, such as adenosine triphosphate (ATP) and its mono- and di-phosphorus metabolites. Because they are so labile, however, only traces of phosphoproteins are normally found in soils (Stevenson, 1994).

Organic P compounds associated with the soil humus and microbial biomass are essentially immiscible with water when polymerized with other non-polar humus constituents (Essington, 2004). Therefore mineralization is a major pathway for  $P_o$  dissolution and, ultimately, transformation of  $P_o$  into  $P_i$  (Harrison, 1987). Mineralization is typically mediated by biologically produced enzymes such as phosphatases, nucleases, and phytase (Quiquamquiox and Mousain, 2006), although abiotic hydrolysis can also contribute (Baldwin et al., 2005). While gross P mineralization is a significant pathway for the conversion of  $P_o$  to  $P_i$ , immobilization of mineralized P can actually deplete soil solution P during periods of rapid biotic growth (Oberson and Joner, 2005).

### **Inorganic P: Forms and Solubility**

Although much of the P in wetland soils can be in the organic form, the pool of  $P_i$  has remarkable influence on P solubility, especially under reduced redox conditions (Willet, 1989). Lindsay (1979) compiled thermodynamic data on the solubility of pure phosphate minerals, and in the case of Fe(III)- and Al(III)-phosphates in soils, all respective mineral solubilities increase with increasing pH.

In highly weathered soils, Fe(III)- and Al(III)-oxide and hydroxide mineral surfaces are major sites for P sorption (Kamprath, 1998; Gerke, 1993; Violante and Pigna, 2002; Welp et al., 1983). These sites are thought to control P dissolution through adsorption reactions at low phosphate concentrations and through precipitation reactions at high phosphate concentrations (Lindsay et al., 1989). Scheidegger and Sparks (1996) explains that, at least for metal cations, a continuum exists between adsorption (surface complexation) and surface precipitation. Low surface coverages by a cation result in a dominance of bonding by adsorption. As surface coverage increases, nucleation results in the formation of distinct clusters (Scheidegger and Sparks, 1996). As surface coverage increases even further, a surface precipitate develops and eventually dominates (Scheidegger and Sparks, 1996). With regard to  $\text{PO}_4$ , data show that non-crystalline Al-phosphate precipitates when a high concentration of  $\text{PO}_4$  is reacted with hydroxide (Nanzyo, 1984), and that the transition from adsorption to precipitation occurs at dissolved  $\text{PO}_4$  concentrations much lower than those calculated for phosphate in equilibrium with goethite and iron phosphate (Li and Stanforth, 2000). Mineral interactions inhibited precipitation of P in binary suspensions of ferrihydrite and non-crystalline Al-hydroxides (Khare et al., 2005).

Kreller et al. (2003) investigated competitive inhibition of  $\text{PO}_4$  adsorption on hydrous iron oxides by gallic acid, tannic acid, and peat derived humic material. Using a chemical force microscopic technique, they found evidence of phosphate adsorption on surfaces dominated by A-type (non-bridging) Fe-OH functional groups (at pHs between 4 and 8). When the surfaces contained high concentrations of adsorbed natural organic acids, however, the strong adsorption interactions between orthophosphate and the Fe(III)-hydroxide surface were eliminated. Kreller et al. (2003) concluded that adsorption of  $\text{PO}_4$  to Fe(III) and

Al(III)-hydroxide surfaces is inhibited by competitive adsorption of DOM to those same surfaces.

Various extractants have been developed to determine the relative abundance and crystallinity of Fe(III) and Al(III) (hydr)oxides, and consequently can provide information about P sorption capacities of soils. The crystallinity of Fe(III) and Al(III) minerals affects the P sorption capacities of soils by modifying reactive surface area and site density, with minerals of greater crystallinity having lower surface area and lower PO<sub>4</sub> adsorption capacity (Essington, 2003, Stumm and Morgan, 1996). An extractant that supposedly targets organically complexed Fe(III) and Al(III) is sodium pyrophosphate (Jackson et al., 1986). Ammonium oxalate (pH 3, dark) is used to extract Fe and Al from poorly crystalline minerals, non-crystalline solids, and organic matter (Schwertmann, 1964; Schwertmann, 1973), while citrate-bicarbonate-dithionite (CBD) reagent (Jackson et al., 1986) is used to reductively dissolve and extract Fe(III) from crystalline oxides like goethite and hematite in addition to non-crystalline forms (Jackson et al., 1986; Parfitt and Childs, 1988).

### **Organically Complexed Inorganic P: Forms and Solubility**

The nature of the interaction of dissolved organic matter with mineral surfaces has a profound effect on the P binding characteristics of a soil, but is not well understood. In some cases DOM has been shown to reduce P sorption by competitively adsorbing to mineral surfaces (Kreller et al., 2003), while on the other hand it is understood that dissolved humic substances containing chelated Fe(III) increase the PO<sub>4</sub> sorption capacity of soils (Gerke, 1993).

Research has consistently shown that  $\text{PO}_4$  is mainly bound to humic substances via Fe- and Al- bridges (Hermann and Gerke, 1992; Levesque and Schnitzer, 1967; White and Thomas, 1981), which have an extraordinary capacity to bind P. Gerke and Hermann (1992) reported that the bound P:Fe ratio of humic-Fe complexes was ten times higher than that of poorly crystalline iron oxide. Evidence suggests that P sorption is also increased by DOM in more complex systems. Gerke (1993) investigated the effect of adding humic substances on P adsorption to poorly ordered Fe-oxides. Gerke (1993) found an increase in P sorption during 56 d of reaction in mixed humic acid and Fe-oxide suspensions and attributed the increase to a combination of the inhibition of Fe-oxide crystallization and organic complexing of Fe(III) by humic substances.

### **The Effect of Reduction on P Dissolution**

Reduction has been shown to increase  $\text{PO}_4$  in soil solution, especially in soils high in organic matter (Hutchison and Hesterberg, 2004; Young and Ross, 2001). Many different mechanisms of  $\text{PO}_4$  dissolution can occur in reduced soils (Hutchison and Hesterberg, 2004), but one of the most common is thought to occur through Fe(III) reduction (Patrick and Khalid, 1974). In anaerobic soils, Fe(III) is reduced to more soluble Fe(II) and bound P dissolves also (Bartlett and James, 1993; Lindsay, 1979; McBride, 1994; Patrick and Khalid, 1974; Ponnampuruma, 1972; Stumm and Morgan, 1996). Limnologists have long since recognized this mechanism as a major P input to lakes (Mortimer, 1941). In paddy soils it is relied upon to supply P for rice production (Patrick and Mahapatra, 1968; Willet, 1989). More recently, Shenker et al. (2004) studied reductive dissolution of Fe(III) hydroxides in

restored wetland peat soils from Israel, and reported that it was mainly responsible for P release in 120 d incubation experiments.

Ryan and Gschwend (1992) indicated that Fe(III) reduction can mobilize organic colloids that use Fe(III) as a cementing agent. Thompson et al. (2006) dispersed mineral and organic colloids during reduction cycles that also reduced Fe(III), but suggested that redox-induced pH changes controlled colloid dispersion more than Fe(III) reduction.

Aluminum(III), unlike Fe(III), does not undergo redox transformations in soils, so in a strict sense Al(III) bound P is not subject to dissolution by reduction of the surrounding mineral matrix. Nevertheless, pH increases brought about by the reduction of acid soils can result in the dissolution of Fe(III) and Al(III) phosphates (Ponnamperuma, 1972). In large part, however, Al(III) may serve to diminish P dissolution during reduction cycles, as the following research illuminates.

Chacon et al. (2006) saw a decrease in aqueous  $\text{PO}_4$  concentrations after an initial increase when a seasonally flooded forest soil from Venezuela was subjected to anaerobic conditions. They attributed this decrease to secondary reactions of soluble P with other non-redox sensitive soil elements, specifically humic-Al(III) complexes. In a laboratory incubation study, Murray and Hesterberg (2006) studied the reductive dissolution of an Fe-oxide and sorbed orthophosphate in the presence of an Al-(hydr)oxide (boehmite) mineral. They reported a net dissolution of P in the absence of boehmite but little to no net P dissolution when boehmite was added, and concluded that Al from boehmite sorbed to the Fe-oxide surface and blocked electron transfer necessary for Fe(III) reduction.

Hogan et al. (2004) investigated P sorption capacities in soils from three restored and three adjacent natural wetlands in Maryland. They found that pyrophosphate extractable Al

concentrations were an order of magnitude greater in soils from natural wetlands than from restored wetlands, although P sorption was higher in the latter. They correlated P sorption in restored wetlands with non-extractable and oxalate-extractable Al; oxalate-, pyrophosphate-, and HCl- extractable Fe; and clay content. In the natural wetlands P sorption was correlated only with pyrophosphate extractable Al, suggesting that P sorption by Fe(III) in restored wetlands may be transitory.

Richardson (1985) and Darke and Walbridge (2000) reported that the P adsorption capacity in wetland ecosystems can be predicted solely from the acid ammonium oxalate extractable Al content. Richardson (1985) determined the P sorption capacities of three wetland soils from NC, two wetland soils from Michigan, and one wetland soil from Maryland. The P-sorption capacities of the Ponzer (Terric Medisaprist), Dare (Typic Medisaprist), and Arapahoe (Typic Humaquept) samples from North Carolina were 700, 800, and 1400 mg P kg<sup>-1</sup>, respectively, up to four times less than the P sorption capacities reported for samples from wetland soils in Maryland and Michigan.

Some studies suggest that DOM produced by reduction affects Al(III) (and Fe(III)) solubility (Gerke, 1993; Nierop et al., 2002), and therefore reduction may have another indirect effect on PO<sub>4</sub> dissolution. Jansen et al. (2002) reported that Fe(III) bound more strongly to DOM than did Al(III), but Al(III) bound more strongly than did Fe(II), suggesting that in reduced systems, humic substances might preferentially bind to Al(III) over dissolved Fe(II). Preferential complexation of aqueous Al<sup>3+</sup> or its hydrolysis products could then increase the dissolution of Al(III) minerals by producing undersaturated conditions. Hutchison and Hesterberg (2004) studied the rates and mechanisms of P dissolution from the surface horizon of a Cape Fear sandy clay loam (Typic Umbraquult) from Plymouth, NC. They

recorded up to sevenfold increases in DRP (from 1.5 up to 10 mg/L) after 40 days of microbial reduction in a continuously-stirred redox reactor. In a separate batch experiment, Hutchison and Hesterberg (2004) studied effects of increasing pH and citrate additions on phosphate dissolution under aerobic conditions. Their results indicated that DOM produced during soil reduction contributed to the increase in DRP, suggesting competitive adsorption between phosphate and DOM for iron and aluminum oxide minerals or to the formation of ternary DOM-Fe-PO<sub>4</sub> or DOM-Al-PO<sub>4</sub> complexes.

Long-term P retention in wetlands can also be affected by transformations in Fe(III) and Al(III) crystallinity. Despite recent findings by Thompson et al. (2006), observations of Fe-crystallinity in soils indicate that redox cycling preserves the poorly-ordered nature of Fe(III) and Al(III) oxides (Kuo and Mikkelsen, 1978). Thus, redox cycling could decrease PO<sub>4</sub> sorption not only by the dissolution of Fe(III)-bound P, but also by the gradual reduction in P-reactive surface area through increased ordering of Fe(III) minerals.

## REFERENCES

- Baldwin, D.S., J.A. Howitt, and J.K. Beattie. 2005. Abiotic degradation of organic phosphorus compounds in the environment. *In* B.L. Turner, E. Frossard, and D.S. Baldwin (ed.) *Organic phosphorus in the environment*. CAB International, Wallingford, Oxfordshire, U.K.
- Bartlett, R.J., and B.R. James. 1993. Redox chemistry of soils. *Adv. Agron.* 50:151-208.
- Beauchemin, S., and R.R. Simard. 1999. Soil phosphorus saturation degree: review of some indices and their suitability for P management in Quebec, Canada. *Can. J. Soil Sci.* 79:615-625.
- Brady, N.C., and R.R. Weil. 2004. *Elements of the nature and properties of soils*. 2<sup>nd</sup> Edition. Pearson, Upper Saddle River, NJ.
- Cahoon, L.B. and S.H. Ensign. 2004. Spatial and temporal variability in excessive soil phosphorus levels in eastern North Carolina. *Nutrient Cycling In Agroecosystems*. 69(2): 111-125.
- Chacon, N., S. Flores, and A. Gonzalez. 2006. Implications of iron solubilization on soil phosphorus release in seasonally flooded forests of the lower Orinoco River, Venezuela. *Soil Bio. Biochem.* 38:1494-1499.
- Correll, D. 1998. The role of phosphorus in the eutrophication of receiving waters: a review. *J. Environ. Qual.* 27:261-266.
- Darke, A., and M.R. Walbridge. 2000. Al and Fe biogeochemistry in a floodplain forest: implications for P retention. *Biogeochemistry*. 51:1-32.
- Edwards, O.W., and E.O. Huffman. 1959. Diffusion of aqueous solutions of phosphoric acid at 25 degrees. *J. Phys. Chem.* 63:1830-1833.
- Essington, M.E. 2004. *Soil and water chemistry: an integrative approach*. CRC Press, Boca Raton, FL.
- Ewing, J.M. 2003. Characteristics of soils in a drained Carolina bay wetland prior to restoration. Ph.D. diss. North Carolina State Univ., Raleigh.
- Ewing, J.M., M.J. Vepraskas, and C.W. Zanner. 2004. Using historical records of land use to improve wetland mitigation. *Southeast. Geog.* 45:25-43.
- Fox, R.L., and E.J. Kamprath. 1970. Phosphorus sorption isotherms for evaluating the phosphate requirement of soils. *Soil Sci. Soc. Am. Proc.* 34:902-907.

Gerke, J. 1993. Phosphate adsorption by humic/Fe-oxide mixtures aged at pH 4 and 7 and by poorly ordered Fe-oxide. *Geoderma*. 59:279-288.

Gerke, J., and R. Hermann. 1992. Adsorption of orthophosphate to humic-Fe-complexes and to amorphous Fe-oxide. *Z Pflanzenernaehr. Bodenkd.* 160:427-432.

Harrison, A.F. 1987. Soil organic phosphorus: a review of world literature. CAB International, Wallingford, Oxfordshire, U.K.

Haygarth, P.M., and A.N. Sharpley. 2000. Terminology for phosphorus transfer. *J. Environ. Qual.* 29:10-15.

Hermann, R., and J. Gerke. 1992. Complexation of iron (III) to humic substances of a humic podzol at pH 2.5-6.4 – quantification of the organically complexed iron by pyrophosphate extraction. *Z. Pflanzernahr. Bodenkd.* 155:229-232.

Hogan, D.M., T.E. Jordan, and M.R. Walbridge. 2004. Phosphorus retention and soil organic carbon in restored and natural freshwater wetlands. *Wetlands*. 24(3):573-585.

Hutchison, K.N., and D. Hesterberg. 2004. Dissolution of phosphate in a phosphorus-enriched ultisol as affected by microbial reduction. *J. Environ. Qual.* 33:1793-1802.

Jackson, M.L., C.H. Lim, and L.W. Zelazny. 1986. Oxides, hydroxides, and aluminosilicates. *In* A. Klute (Ed.) *Methods of Soil Analysis Part 1: Physical and mineralogical methods*, 2<sup>nd</sup> ed. Soil Science Society of America, Inc. Madison, Wisconsin.

Jansen, B., K.G.J. Nierop, and J.M. Verstraten. 2002. Influence of pH and metal/carbon ratios on soluble organic complexation of Fe(II), Fe(III) and Al(III) in soil solutions determined by diffusive gradients in thin films. *Anal. Chim. Acta.* 454(2):259-270.

Kamprath, E.J. 1998. Fate of phosphorus in North Carolina soils. *Soil Sci. North Carolina Proc.* 41:15-17.

Khare, N., D. Hesterberg, S. Beauchemin, and S. Wang. 2004. XANES determination of adsorbed phosphate distribution between ferrihydrite and boehmite in mixtures. *Soil Sci. Soc. Am. J.* 68:460-469.

Kreiser, G. 2003. A wetland restoration project: water budget and nutrient analysis of a drained Carolina Bay. M.S. thesis. North Carolina State Univ., Raleigh.

Kreller, D.I., G. Gibson, W. Novak, G.W. van Loon, J.H. Horton. 2003. Competitive adsorption of phosphate and carboxylate with natural organic matter on hydrous iron oxides as investigated by chemical force microscopy. *Colloids and Surfaces A: Physicochem. Eng. Aspects.* 212:249-264.

- Kuo, S., and D.S. Mikkelsen. 1978. Distribution of iron and phosphorus in flooded and unflooded soil profiles and their relation to phosphorus adsorption. *Soil Sci.* 127/1:18-25.
- Levesque, M. and M. Schnitzer. 1967. Organo-metallic interactions in soils: 6. Preparation and properties of fulvic acid-metal phosphates. *Soil Sci.* 103:183-190.
- Lilly, J.P. 2003. Organic soils. In Papers commemorating a century of soil science. Soil Science Society of North Carolina. (Available online at <http://agronomy.agr.state.nc.us/sssnc/index.htm>) (Verified 24 Feb. 2007).
- Lindsay, W.L. 1979. Chemical equilibria in soils. John Wiley & Sons, New York.
- Lindsay, W.L., P.L.G. Vlek, and S.H. Chien. 1989. Phosphate minerals. P. 1089-1130. *In* J.B. Dixon and S.B. Weed (ed.) Minerals in soil environments. 2<sup>nd</sup> edition. SSSA Book Ser. 1. SSSA, Madison, WI.
- McBride, M.B. 1994. Environmental chemistry of soils. Oxford University Press, New York.
- Meyer, W.B. 1994. When dismal swamps became priceless wetlands. *Am. Herit.* 45:108-117.
- Mitch, W.J., and J.G. Gosselink. 2000. Wetlands. John Wiley and Sons, New York.
- Moll, L. 2004. Time course of P and Fe dissolution during microbial reduction of soil samples from Juniper Bay. Poster Presentation at NCSU Undergraduate Research Symposium. 22 April 2004.
- Mortimer, C.H. 1941. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.* 29:280-329.
- Murray, G.C., and D. Hesterberg. 2006. Iron and phosphate dissolution during abiotic reduction of ferrihydrite-boehmite mixtures. 70:1318-1327.
- Nanzyo, M. 1984. Diffuse reflectance infrared-spectra of phosphate sorbed on alumina gel. *J. Soil Sci.* 35(1):63-69.
- Nierop, K.G.J, B. Jansen, and J.M. Verstraten. 2002. Dissolved organic matter, aluminium and iron interactions: precipitation induced by metal/carbon ratio, pH, and competition. *Sci. Tot. Environ.* 300:201-211.
- Oberson, A., and E.J. Joner. 2005. Microbial turnover of phosphorus in soil. *In* B.L. Turner, E. Frossard, and D.S. Baldwin (ed.) Organic phosphorus in the environment. CAB International, Wallingford, Oxfordshire, U.K.

- Parfitt, R.L. and C. W. Childs. 1988. Estimation of forms of Fe and Al: A review, and analysis of contrasting soils by dissolution and Moessbauer methods. *Aust. J. Soil Res.* 26:121-144.
- Parry, R. 1998. Agricultural phosphorus and water quality: a U.S. Environmental Protection Agency perspective. *J. Environ. Qual.* 27:258-261.
- Patrick, W.H., and I.C. Mahapatra. 1968. Transformations and availability to rice of nitrogen and phosphorus in waterlogged soils. *Adv. Agron.* 20:323-360.
- Patrick, W.H., and R.A. Khalid. 1974. Phosphate release and sorption by soils and sediments: effect of aerobic and anaerobic conditions. *Science.* 186:53-55.
- Ponnamperuma, F.N. 1972. The chemistry of submerged soils. *Adv. Agron.* 24:29-96.
- Quiquampiox, H., and D. Mousain. 2005. Enzymatic hydrolysis of organic phosphorus. In B.L. Turner, E. Frossard, and D.S. Baldwin (ed.) *Organic phosphorus in the environment.* CAB International, Wallingford, Oxfordshire, U.K.
- Rea, K. 2001. Corps Regulators have large, difficult mission. In *USACE Missions: Regulatory Program, Protecting our Water Resources.* Engineer Update Vol. 25 No.7. U.S. Army Corps of Engin., Washington, D.C.
- Richardson, C.J. 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science.* 228:1424-1427.
- Richardson, J.L., and M.J. Vepraskas (ed.). 2001. *Wetland soils: genesis, hydrology, landscapes, and classification.* CRC Press, Boca Raton, FL.
- Ryan, J.N., and P.M. Gschwend. 1992. Effect of iron diagenesis on the transport of colloidal clay in an unconfined sand aquifer. *Geochim. Cosmochim. Acta.* 56:1507-1521.
- Reddy, K.R., R.H. Kadlec, E. Flaig, and P.M. Gale. 1999. Phosphorus retention in streams and wetlands: a review. *Crit. Rev. in Environ. Sci. Tech.* 29(1): 83-146.
- Sallade, Y.E., and J.T. Sims. 1997. Phosphorus transformations in the sediments of Delaware's agricultural drainageways: I. Effect of reducing conditions on phosphorus release. *J. Environ. Qual.* 26:1579-1588.
- Sawyer, C.N. 1947. Fertilization of lakes by agricultural and urban drainage. *J. New England Water Works Assoc.* 69:109-127.
- Shenker, M., S. Seitelbach, S. Brand, A. Haim, and M.I. Litaor. 2004. Redox reactions and phosphorus release in re-flooded soils of an altered wetland. *Eur. J. Soil Sci.* 56(4):515-525.

- Scheidegger, A.M., and D.L. Sparks. 1996. A critical assessment of sorption-desorption mechanisms at the soil mineral/water interface. *Soil Sci.* 161(12):813-831.
- Schwertmann, U. 1964. Differenzierung der Eisenoxide des Bodens durch Extraktion mit Ammoniumoxalat-Lösung. *Z. Pflanzenernähr. Bodenk.* 105:194-202.
- Schwertmann, U. 1973. Use of oxalate for Fe extraction from soils. *Can. J. Soil Sci.* 53:244-246.
- Stevenson, F.J. 1994. *Humus Chemistry*. John Wiley and Sons, New York.
- Stumm, W., and J.J. Morgan. 1996. *Aquatic chemistry: chemical equilibria and rates in natural waters*. 3<sup>rd</sup> Edition. John Wiley and Sons, New York.
- Thompson, A., O.A. Chadwick, D.G. Rancourt and J. Chorover. 2006. Iron-oxide crystallinity increases during soil redox oscillations. *Geochim. Cosmochim. Acta.* 70:1710-1727.
- United States Department of Agriculture Economics Research Service. 2007. U.S. consumption of selected phosphate and potash fertilizers, 1960-2005 [Online]. Available at: <http://www.ers.usda.gov/Data/FertilizerUse/> (verified 15 April, 2007).
- United States Congress. 1972. Federal water pollution control act (Clean water act). Public Law 92-500,33. 1251.
- Violante, A. and M. Pigna. 2002. Competitive sorption of arsenate and phosphate on different clay minerals and soils. *Soil Sci. Soc. Am. J.* 66:1788-1796.
- Voteller, T.H. and T.A. Muir. 2002. Wetland protection legislation. In *National water summary on water resources*. WSP 2425. U.S. Geol. Surv. Washington, D.C.
- Vollenweider, R.A. 1976. Advances in defining critical loading levels of phosphorus in lake eutrophication. *Mem. 1<sup>st</sup>. Ital. Idrobiol.* 33:53-83.
- Welp, G., U. Herms, and G. Bruemmer. 1983. Influence of soil reaction, redox conditions, and organic-matter on the phosphate content of soil solutions. *Z. Pflanzenernähr. Bodenk.* 146:38-52.
- White, R.E. and G.W. Thomas. 1981. Hydrolysis of aluminum on weakly acidic organic exchanges: implications for phosphate adsorption. *Fert. Res.* 2:159-167.
- Willet, I.R. 1989. Causes and reduction of changes in extractable phosphorus during flooding. *Aust. J. Soil Res.* 27:45-54.
- Young, E.O., and D.S. Ross. 2001. Phosphate release from seasonally flooded soils: A laboratory microcosm study. *J. Environ. Qual.* 30:91-101.

**CHAPTER TWO:**  
REDUCTIVE DISSOLUTION OF SOIL P UNDER ANAEROBIC  
CONDITIONS

## INTRODUCTION

Drained agricultural lands often make convenient sites for wetland restoration projects (Ewing et al., 2004). Because chemical reactions that occur in wetland soils can increase P dissolution (Patrick et al., 1974; Ponnampetuma, 1972), the potential for P to dissolve in restored soils is critically important to water quality (Sallade and Sims, 1997; Shenker et al., 2004; Young and Ross, 2001).

To understand the potential for P to dissolve when high-P agricultural soils are converted to wetlands,  $\text{PO}_4$  chemistry under reducing conditions should be understood in greater detail. For decades the reductive dissolution of iron-(hydr)oxide minerals and associated P has been recognized as a major source of P in lakes (Mortimer, 1941) and in paddy soils used for rice production (Patrick and Mahapatra, 1968; Willet, 1989). More recently, Shenker et al. (2004) attributed P dissolution in restored wetland peat soils mainly to the reductive dissolution of Fe(III) during 120 d of incubation under anaerobic conditions. However, the simple understanding of adsorbed P dissolution from Fe(III) mineral surfaces as a consequence of reduction should be challenged for two reasons.

Firstly, P also binds to Al(III) in soils, which is not redox active. Studies suggest that long term P sorption is linked to the presence of Al(III) minerals (Darke and Walbridge; 2000; Richardson, 1985) and Al(III)-humic complexes (Hogan et al., 2004). Under reducing conditions, Al (III) can decrease P dissolution by sorbing P released during reduction (Chacon et al., 2006) and retarding Fe(III) dissolution, probably by blocking electron transfer to Fe(III) mineral surfaces (Murray and Hesterberg, 2006). Long-term P retention in wetlands can also be affected by Fe(III) and Al(III) crystallinity, the poorly ordered nature of which is thought to be preserved by redox cycling (Kuo and Mikkelsen, 1978).

Secondly, organic matter interacts with Fe(III) and Al (III) mineral surfaces, affecting a soils ability to bind P. Research has consistently suggested that P binds to humic substances via Fe- and Al- bridges (Hermann and Gerke, 1992; Levesque and Schnitzer, 1967; White and Thomas, 1981), which may enhance the ability of a soil to bind P (Gerke, 1993; Gerke and Hermann, 1992). Under reducing conditions, however, P has been shown to dissolve from soils that are high in organic matter (Hutchison and Hesterberg, 2004; Young and Ross, 2001). One explanation for this phenomenon is redox-dependent (Ryan et al., 1992) or redox-associated (Thompson et al, 2006) mobilization of Fe(III)-cemented organic colloids, which can expose organic and occluded Fe(III)-P to hydrolysis and reductive dissolution processes, respectively. Mobilization of organic colloids can also result in competition of DOM with P for adsorption sites on Al(III) and Fe(III) mineral surfaces (Kreller et al., 2003), and in the alteration of Al(III) and Fe(III) solubility through the formation of DOM-metal complexes (Gerke, 1993; Nierop et al., 2002). Jansen et al. (2002) reported that Fe(III) bound more strongly to DOM than did Al(III), but Al(III) bound more strongly than did Fe(II), suggesting that in reduced systems increased complexation of Al(III) by DOM could enhance the dissolution of Al(III)-bound P.

Thus, more research is needed to assess the fate of P in reduced soils that are rich in organic matter. The goal of this research is to determine the potential for organic and organic-rich soils from Robeson County, NC to dissolve P under reducing conditions, increasing the risk for transport of P to nutrient-sensitive surface waters. Our objectives are to compare the reductive dissolution of P among soil samples that vary in organic matter content, and to measure auxiliary redox-related chemical parameters that would imply possible mechanisms responsible for variations in P dissolution. Knowledge of the chemical

processes affecting P dissolution in soils from Robeson County, NC is essential to developing possible remediation strategies there, and is also useful in predicting the success of related wetland restorations in the future.

## MATERIALS AND METHODS

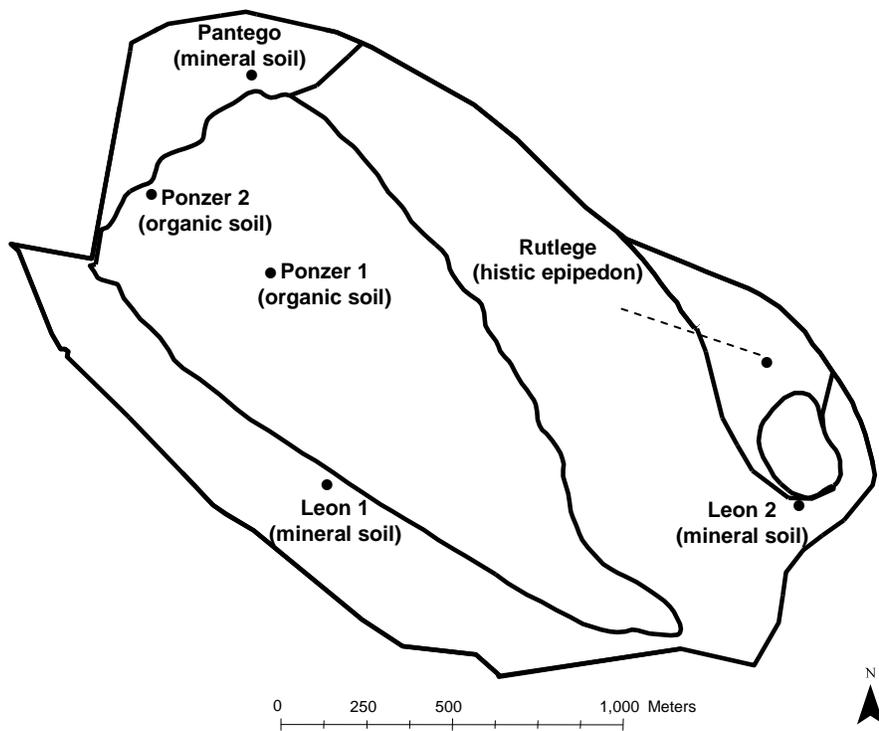
### Site History

Soil samples used for laboratory experiments came from Juniper Bay, a drained, 254 ha Carolina bay in Robeson County, NC, USA (34°30'21" N, 79°01'22" W). Carolina bays are elliptical shaped wetlands that occur on the Atlantic Coastal Plain, but predominate near the NC-SC political boundary. Juniper Bay was timbered and extensively drained for agricultural use beginning in the 1970's. Corn, cotton, millet, oats, soybeans, tobacco, and wheat were among the crops grown there until the property was purchased by the NC DOT in 1997 for wetland mitigation. Hydrologic restoration of Juniper Bay started in 2006 and involves raising the water table by filling in key drainage ditches with borrow material from the site. Restoration has resulted in shallow (< 1 m) ponds that cover approximately 10 % of the bay year-around, predominantly near locations from which surface material was borrowed.

### Sampling

Concentrations of total P ( $P_{tot}$ ) in surface soil samples (0 to 10 cm) from 19 locations at Juniper Bay were determined by the ignition method (Kuo, 1996) using a Garmin GPS III Plus (Olathe, KS) (Appendix B). From the initial evaluation of the nineteen locations at Juniper Bay, six sites were selected that had high  $P_{tot}$  concentrations and varying amounts of organic carbon as determined by hand-texturing and color (Figure 2.1). In July, 2005, approximately 8 L of soil were collected from each of the surface horizons (0 to 10 cm) at the six selected locations, which were mapped as Ponzer (Terriic Haplosaprist), Leon (Aeric Alaquod), Pantego (Umbric Paleaquult), or Rutlege (Typic Humaquept) soils. The samples

were packed directly into glass jars in the field and transported within 6 h to a refrigerator, where they were stored at 4 °C for < 14 d before being passed through a 2-mm stainless steel (SS) sieve. Once sieved, samples were mixed by hand for ~ 10 min and subdivided among 1 L glass mason jars, then frozen in the dark for up to 14 mo. before each sub-sample was thawed for experimentation. Particle size analysis was performed by the pipette method (Gee and Bauder, 1986) after removing organic matter by combustion at 450°C for 24 h. Bulk densities were determined using the core method (Blake and Hartge, 1986) and triplicate cores obtained within a 1 m radius of each sampling site.



**Figure 2.1. Location of sampling points at Juniper Bay where soil samples were removed from the surface horizon (0 to 10 cm) for laboratory experiments. Soil samples were named according to mapped soil series (solid lines). The outer-most solid line also delineates the property boundary.**

## Continuously Stirred Reactor (CSR) Experiment

### *Reactor Setup*

The effect of microbial reduction on  $\text{PO}_4$  dissolution from Juniper Bay soil samples was determined using continuously-stirred soil suspensions under oxygen-free conditions. The general reactor setup is described by Hutchison and Hesterberg (2004) and is based on redox reactors developed by Patrick et al. (1973). Trends in phosphate dissolution were monitored in duplicate suspensions during to 25 d of reduction. Aerobic (control) reactors were also monitored to ensure P dissolution was caused by reduction. Either two or four reactors were run concurrently, and samples from all six locations in Figure 2.1 were used, resulting in a total of 18 reactor experimental units.

One liter of frozen soil material was allowed to thaw for 2-4 h as needed at room temperature ( $\sim 22^\circ\text{C}$ ). The soil material was mixed thoroughly ( $\sim 10$  min) by hand with a SS spatula in a 4 L low density polyethylene (LDPE) bucket and soil moisture contents were determined by oven-drying subsamples at  $105^\circ\text{C}$  for 24 h. The thawed soil material was re-packed in glass jars and stored at  $3^\circ\text{C}$  while soil moisture contents were determined. After determining moisture content, the samples were mixed again and each sample was divided evenly between two, tared, 2 L high density polyethylene (HDPE) bottles. De-ionized water was added at 2:1 water:soil by mass. The bottles and their contents were thoroughly mixed by shaking for 1 h at  $200\text{ cycles min}^{-1}$  to disrupt any aggregates.

Preliminary experiments showed that continuously stirring suspensions of whole soil material resulted in abrasion of the reactor vessel. Therefore, only the silt and clay fractions of the samples were used in stirred reactor experiments. To separate the silt and clay fractions, the shaken suspensions were wet sieved through a  $< 53\ \mu\text{m}$  sieve (No. 270) and the

silt and clay fractions were mixed into 4L glass jars. The suspended solids concentrations of the silt and clay suspensions were measured by oven-drying subsamples of the well mixed suspensions at 105 °C for 24 h, and were stored for 3 to 5 d at 3 °C before beginning a stirred reactor experiment.

After returning the suspensions to ambient temperature, a portion of suspension containing 22.5 g of solids was siphoned from the middle of the continuously stirring suspensions into a tared, LDPE beaker. This suspension was poured directly into a glass reactor vessel (Kimble-Kontes, Vineland, NJ), and settled solids were rinsed into the reactor vessel using precisely enough de-ionized water to result in a final solids concentration of 15 g kg<sup>-1</sup> in 1.5 kg of suspension. Suspensions were stirred continuously with a magnetic stirrer at 500 rpm and 25 °C during each redox incubation experiment.

Standardized pH and redox (Eh) electrodes were sealed into rubber-stoppered ports in the lid of the reactor vessel. Each set of Eh and pH electrodes were connected to individual potentiometer and a computer to automatically record measurements at 15-minute intervals, which were later averaged on an hourly basis. A Pt-tip combination redox electrode with a Ag/AgCl reference electrode was used for Eh measurements and all measurements were corrected to the standard hydrogen potential (+ 199 mV) (Patrick et al., 1996). Redox electrodes were cleaned with de-ionized water and tested for functioning in solutions of hydroquinone in pH 4 and 7 buffers at least every 6 days. The pH electrodes were cleaned and re-standardized (if necessary) at the same time. Aberrant Eh readings for about 1 d following re-immersion of the electrodes into the suspension were omitted.

Carbon dioxide-free air (National Welders Supply Company, Charlotte, NC) was bubbled through each suspension at a rate of 5 to 10 mL min<sup>-1</sup> for 3-5 d to achieve a

consistent Eh before reduction was initiated. Gas bubblers (Kimble-Kontes, Vineland, NJ) containing 0.1 M or 0.5 M NaOH solution were placed in the gas flow stream before and after the reactor vessel to trap and measure microbial CO<sub>2(g)</sub> production during the incubation experiment (Hutchison and Hesterberg, 2004).

Based on preliminary batch-reduction experiments, a dextrose spike of 2 g kg<sup>-1</sup> solids was added as a 1 mL aliquot of aqueous solution at t = 0 d to stimulate microbial reduction. Immediately after adding dextrose, CO<sub>2</sub>-free air was replaced by 99.99% pure N<sub>2(g)</sub> flowing at 20 mL min<sup>-1</sup> to induce O<sub>2</sub>-free (anaerobic) conditions. In control (oxidized) reactor experiments, CO<sub>2</sub>-free air was not replaced by N<sub>2(g)</sub>, but a dextrose spike was added.

### ***Reactor Sampling***

Sub samples of the continuously-stirred suspensions were removed without exposure to oxygen after 0, 1, 2, 3, 4, 6, 8, 10, 12, 15, 18, and 25 d of incubation. A 60 cm<sup>3</sup>, N<sub>2(g)</sub>-purged glass syringe and stopcock was connected to the sample port installed in the reactor cap, and approximately 40 mL of suspension were withdrawn from the reactor into the syringe. A needle was attached to the syringe and the suspension was injected into three evacuated (-65 cm Hg), 15-mL borosilicate glass sample tubes fitted with rubber septa. These samples were centrifuged at 8100 x g for 10 min, and the supernatant solutions were filtered through a 0.2 µm Isopore polycarbonate filter (Millipore, Billerica, MA) and subdivided for aqueous component analysis. All filtering and subdivision of filtrate samples was done in a glove box under an N<sub>2(g)</sub> using a red-filtered safe light to prevent exposure to oxygen and ultraviolet radiation (Hutchison, 2003). Cumulative sampling during the entire experiment removed less than 40 % of the original suspension mass.

## **Static Incubation Experiment**

### ***Incubation Set up***

In order to investigate the effect of reduction on P dissolution over longer time periods, a separate experiment was conducted to assess the PO<sub>4</sub> dissolution potential of reduced whole soil material. Because the static incubation experiment was conducted after the CSR experiment, there remained only enough soil material from four of the six locations sampled in July, 2005. Triplicate, 50 g (oven-dry basis) sub-samples from those four locations at Juniper Bay were saturated (2.5 g H<sub>2</sub>O g<sup>-1</sup> solids) and incubated in sealed, 500 mL glass mason jars for 2 and 62 d, and concentrations of DRP, Fe(II), and DOC were measured in each sample. One hundred twenty five grams of de-ionized, degassed- water were used to saturate each sample, resulting in a solids concentration of 285 g kg<sup>-1</sup>. The samples were mixed vigorously with a glass stir rod to remove entrapped air bubbles and to homogenize. The headspace of each jar was flushed with N<sub>2(g)</sub> in an N<sub>2(g)</sub>-filled glove box and air-tight caps were applied. The samples were randomly positioned in an incubator at 25 ± 0.5°C. Jars were allowed to equilibrate for 2 d in the incubator before the initial sampling. To prevent excessive pressure build-up within jars, their caps were quickly opened twice during the incubation (at 17 and 47 d) in an N<sub>2(g)</sub>-filled glove box, then samples were returned promptly to the incubator.

### ***Incubation Sampling***

Jars were removed from the incubator at 2 d and at 62 d for destructive sampling in an N<sub>2(g)</sub>-filled glove box. In the glove box, each jar was shaken by hand about 5 to 7 times to mix its contents. The pH and the Eh of the soil suspensions in each jar were measured by

placing the electrode in the suspension while flushing the headspace with N<sub>2</sub> gas and lightly swirling the jar's contents. The jars were then re-capped and shaken by hand to suspend all solids. Solids were allowed to settle for 30 seconds, and a portion of the unsettled suspension was transferred to a 50 mL, polycarbonate (PC) centrifuge tube with Teflon taped threads. The sub-samples were capped, removed from the glove box, and centrifuged at 8,100 x g for 10 min. The supernatant was filtered under N<sub>2</sub> gas using 0.2 µm Isopore filters and subdivided for DRP and Fe(II) analyses by the same methods as those used in the continuously stirred reactor experiments. The remaining filtrate was transferred to LDPE (2 d samples) or evacuated, borosilicate glass (62 d samples) bottles and refrigerated at 3 °C for 60 d before measuring TOC, P, Fe, Al, and Mn by the methods described below.

### **Aqueous Solution Analyses**

Dissolved reactive phosphorus and Fe(II) were measured colorimetrically in filtrate samples using the modified ascorbic acid method (Kuo, 1996) and the 1,10 phenanthroline method (Loeppert and Inskeep, 1996), respectively. To account for interference from dissolved organic carbon in the Fe(II) analysis, a blank from each filtrate sample was prepared by replacing the phenanthroline reagent with an equal volume of DI water and subtracting absorbance readings of the blanks from those of the samples. Absorbance readings at 840 nm (for DRP) or 510 nm (for Fe(II)) were determined with a UV-210PC UV-Visible Spectrophotometer (Shimadzu Corporation, Kyoto, Japan) within 24 h and 15 d, respectively. If not analyzed immediately, both samples were stored at ambient temperature (~ 22 °C) in the dark. Dissolved organic carbon (DOC) was measured in the filtrate samples using a TOC-5050 total organic carbon analyzer (Shimadzu Corporation, Kyoto, Japan).

Prior to analysis, DOC samples were stored for up to 120 d in the dark at 3 °C. Total dissolved concentrations of P, Fe, Al and Mn ( $P_T$ ,  $Fe_T$ ,  $Al_T$ , and  $Mn_T$ ) were measured by inductively coupled plasma (ICP) emission spectroscopy. Some samples yielded  $P_T$  concentrations lower than DRP concentrations; these were digested by adding 0.01 g mL<sup>-1</sup> potassium persulfate, autoclaving, and measuring again (Greenberg et al., 1992) (Appendix E). Nitrate and  $SO_4^{2-}$  concentrations were measured in selected (oxidized) samples by ion chromatography (Appendix F).

Base traps filled with freshly standardized NaOH were titrated for un-reacted hydroxide at  $t = 0$  and  $t = 25$  d, and sometimes at 4 to 6 d intervals when time permitted. Using a volumetric pipette, triplicate  $20.00 \pm 0.05$  mL subsamples of NaOH trapping solution were transferred to a 200 mL Erlenmeyer flask, and 3 to 5 drops of phenolphthalein pH indicator solution were added. The solutions were titrated with standardized 0.1 M HCl until color changed from pink to colorless.

### **Solids Characterization**

Less than 53  $\mu$ m solids from reactor stock suspensions (stored in the dark at 3 °C during experimentation) were freeze dried and used to determine Total P ( $P_{tot}$ ) and inorganic P ( $P_i$ ) in the < 53  $\mu$ m solids by the ignition method of Kuo (1996), modified to include filtration of the acid extract through 0.2  $\mu$ m Isopore filters and analysis of dissolved components using ICP emission spectrometry. Organic P ( $P_o$ ) was calculated by the difference between  $P_{tot}$  and  $P_i$ . The freeze-dried solids were also used to determine the amount of Fe and Al extractable with citrate-bicarbonate-dithionite, acid ammonium oxalate, and Na-pyrophosphate solutions according to the methods of Jackson et al. (1986). For each

extractant, 0.15 g of < 53  $\mu\text{m}$  solids were used, and separate extractions were performed on separate samples, not in sequence on a single sample. The extracts were filtered through 0.2  $\mu\text{m}$  Millipore filters and stored in capped 15 mL borosilicate glass test tubes at 3°C until analysis by atomic absorption spectrometry. Total organic carbon (TOC) concentration of the < 53  $\mu\text{m}$  freeze-dried solids was determined with a Perkin-Elmer Model 2400 Elemental CN Analyzer (Perkin Elmer Corporation, Norwalk, CT). Total P,  $P_{\text{in}}$ , and TOC were determined in the < 2 mm fraction using the same methods used for the < 53  $\mu\text{m}$  fraction.

### **Statistical Analysis**

Statistical analyses were performed with SAS version 9.0 (SAS Institute, Cary, NC, 2005). PROC REG was used for regression analyses. PROC GLM was used to test for differences among solid-phase Fe and Al, using the model:

$$\text{Extractable Fe/Al} = \text{Soil} \quad \text{Extractant} \quad \text{Soil} \cdot \text{Extractant}.$$

PROC GLM was also used to test for differences in  $P_{\text{tot}}$ ,  $P_{\text{o}}$ , particle size distribution, TOC,  $\rho_{\text{B}}$ , and pH for < 2 mm and < 53  $\mu\text{m}$  solids. PROC TTEST was used to determine differences in mean values for 2 and 62 d aqueous solution measurements in the static incubation experiment.

## RESULTS

### Soil Characteristics

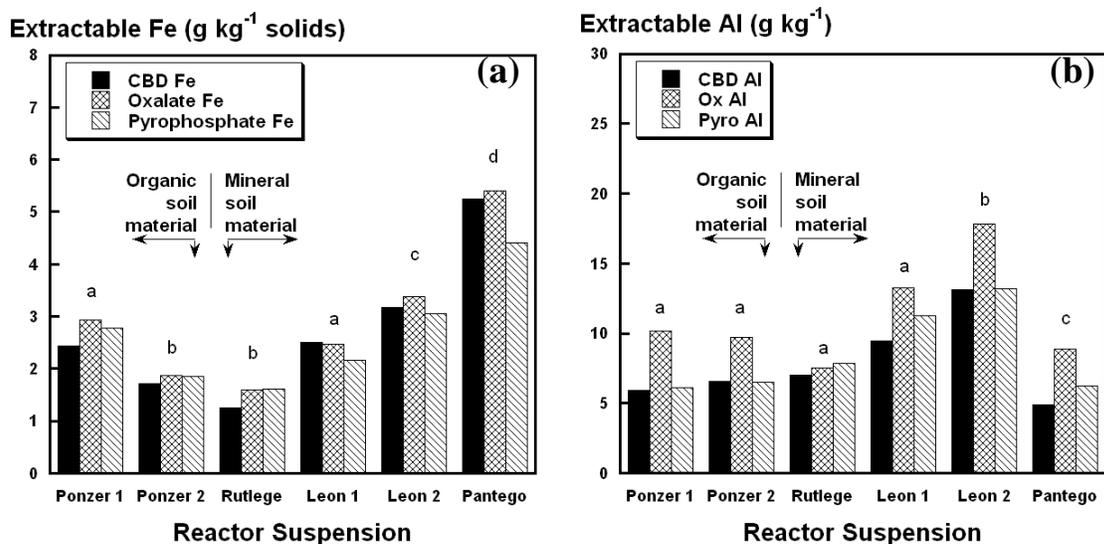
Table 2.1 shows that P and C were more concentrated in the  $< 53 \mu\text{m}$  fraction than in the  $< 2 \text{ mm}$  fraction. Phosphorus concentrations in the  $< 53 \mu\text{m}$ , freeze-dried sub-samples used for laboratory experiments showed highly significant ( $p < 0.001$ ) differences both between the amounts of P among soil series and the types of P within each soil series. The highest concentrations of  $P_{\text{tot}}$  were in the Leon 1 and Leon 2 solids, which contained  $2400 \pm 500 \text{ mg } P_{\text{tot}} \text{ kg}^{-1}$  and  $2600 \pm 180 \text{ mg } P_{\text{tot}} \text{ kg}^{-1}$ , respectively. The Ponzer 2 sample contained the lowest  $P_{\text{tot}}$  concentration,  $1000 \pm 180 \text{ mg } P_{\text{tot}} \text{ kg}^{-1}$ , while the Ponzer 1, Rutlege, and Pantego samples had intermediate  $P_{\text{tot}}$  concentrations. On average, the mineral surface soil fines had twice as much total P ( $2000 \pm 650 \text{ mg P kg}^{-1}$ ) as the organic surface soil fines ( $1100 \pm 150 \text{ mg P kg}^{-1}$ ). Organic P constituted between one half and about two thirds of  $P_{\text{tot}}$  in the  $< 53 \mu\text{m}$  fractions from both organic and inorganic soils. The bulk densities of the Ponzer 1, Ponzer 2 are typical of organic soil material. The bulk densities of the Rutlege, Leon 1, Leon 2, and Pantego samples were lower than typical mineral soil material, probably because they were also rich in organic matter.

Extractable forms of Fe in the  $< 53 \mu\text{m}$  surface soil fines used for the continuously stirred reactor experiment are shown in Figure 2.2.

**Table 2.1. Characterization of whole soil and < 53  $\mu\text{m}$  separates used in 62 d static incubation and 25 d continuously stirred reactor experiments, respectively.  $P_{\text{tot}}$  = Total P,  $P_0$  = Organic P, TOC = Total Organic Carbon,  $\rho_B$  = bulk density (oven-dry basis), NA = not applicable. Results within < 2mm and < 53  $\mu\text{m}$  size fractions that are followed by the same letter are not statistically different at the  $p < 0.05$  level. Standard deviations of triplicate measurements are shown in parentheses. Ponzer 1 replicates were combined into one settling column for particle size analysis due to the large sample volume required.  $P_{\text{tot}}$  results for the < 2 mm size class were derived from duplicate samples.**

| <b>Sample</b>                           | <b><math>P_{\text{tot}}</math><br/>(<math>\text{mg kg}^{-1}</math>)</b> | <b><math>P_0/P_{\text{tot}}</math></b> | <b>TOC<br/>(<math>\text{g kg}^{-1}</math>)</b> | <b>pH</b>     | <b><math>\rho_B</math><br/>(<math>\text{g kg}^{-1}</math>)</b> | <b>Sand<br/>(%)</b> | <b>Clay<br/>(%)</b> |
|---|---|--|--|---------------|--|---------------------|---------------------|
| <u>&lt; 2 mm</u>                        |   |  |  |               |  |                     |                     |
| <u>mineral</u>                          |   |  |  |               |  |                     |                     |
| Leon 1                                  | 400 (100) a   | 0.68 a                                 | 80 (1) a                                       | 6.2 (0.06) a  | 0.99 (0.08) a  | 94(1) a             | 1(0.1) a            |
| Leon 2                                  | 870 (70) b  | 0.55 b                                 | 70 (1) b                                       | 4.9 (0.04) b  | 1.12 (0.07) b  | 82(1) b             | 2(0.3) b            |
| Pantego <sup>†</sup>                    | 390 (24) a  | 0.60 bc                                | 60 (1) c                                       | 6.4 (0.01) c  | 0.96 (0.01) a  | 92(1) a             | 1(0.1) a            |
| Rutlege                                 | 350 (30) a  | 0.80 d                                 | 110 (1) d                                      | 5.4 (0.03) d  | 0.81 (0.06) c  | 81(1) b             | 3(0.1) b            |
| <u>organic</u>                          |   |  |  |               |  |                     |                     |
| Ponzer 1                                | 700 (170) c   | 0.86 d                                 | 470 (1) e                                      | 5.2 (0.02) e  | 0.45 (0.05) d  | 61 c                | 7 c                 |
| Ponzer 2 <sup>†</sup>                   | 770 (30) bc   | 0.62 ac                                | 350 (1) f                                      | 4.5 (0.06) f  | 0.51 (0.03) d  | 48(4) d             | 4(0.8) d            |
| <u>&lt; 53 <math>\mu\text{m}</math></u> |   |  |  |               |  |                     |                     |
| <u>mineral</u>                          |   |  |  |               |  |                     |                     |
| Leon 1                                  | 2400 (500) a  | 0.51 ab                                | 380 (10) a                                     | 5.9 (0.46) a  | NA   | NA                  | NA                  |
| Leon 2                                  | 2600 (180) a  | 0.49 b                                 | 230 (10) b                                     | 5.2 (0.03) ab | NA   | NA                  | NA                  |
| Pantego                                 | 1700 (80) b   | 0.64 ab                                | 200 (10) b                                     | 6.2 (0.12) c  | NA   | NA                  | NA                  |
| Rutlege                                 | 1200 (80) bc  | 0.63 ab                                | 200 (10) c                                     | 4.6 (0.07) d  | NA   | NA                  | NA                  |
| <u>organic</u>                          |   |  |  |               |  |                     |                     |
| Ponzer 1                                | 1200 (70) bc  | 0.70 a                                 | 460 (10) a                                     | 5.5 (0.07) a  | NA   | NA                  | NA                  |
| Ponzer 2                                | 1000 (180) c  | 0.50 ab                                | 210 (20) c                                     | 4.7 (0.17) bd | NA   | NA                  | NA                  |

<sup>†</sup> Samples were not included in static incubation study due to insufficient sample volumes.

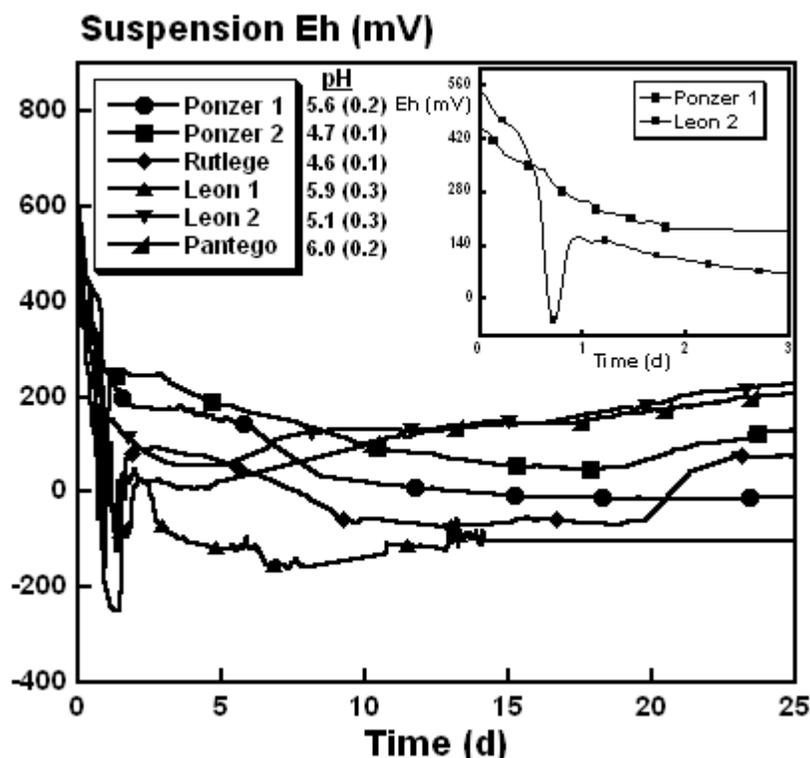


**Figure 2.2. Extractable forms of Fe (a) and Al (b) ( $\text{mg kg}^{-1}$ ) in the  $< 53 \mu\text{m}$  solids used in stirred reactor incubation studies. Extractions performed on separate samples. CBD = citrate bicarbonate dithionite.**

Concentrations of CBD, oxalate, and pyrophosphate extractable Fe and Al were indistinguishable ( $p > 0.05$ ) within each sample. Hence, most free Fe within each sample was in pyrophosphate-extractable form, suggesting that it is organically bound. Concentrations of extractable Fe varied significantly ( $p < 0.05$ ) between samples labeled with different letters in Figure 2.2, and ranged from  $1710 \pm 530 \text{ mg Fe}_{\text{cbd}} \text{ kg}^{-1}$  in the Ponzer 2 solids to  $5240 \pm 300 \text{ mg Fe}_{\text{cbd}} \text{ kg}^{-1}$  in the Pantego solids. Comparably, concentrations of  $\text{Al}_{\text{cbd}}$ ,  $\text{Al}_{\text{ox}}$ , and  $\text{Al}_{\text{pyr}}$  were approximately three times higher than extractable Fe(III) concentrations. Like forms of extractable Fe(III), forms of extractable Al(III) were not significantly distinguishable ( $p < 0.05$ ) in individual samples. Concentrations of extractable Al(III) were similar in the Rutlege, Ponzer 1, Ponzer 2, and Pantego samples, while the Leon 1 and Leon 2 solids contained significantly ( $p < 0.05$ ) more extractable Al than other samples.

## CSR Experiment

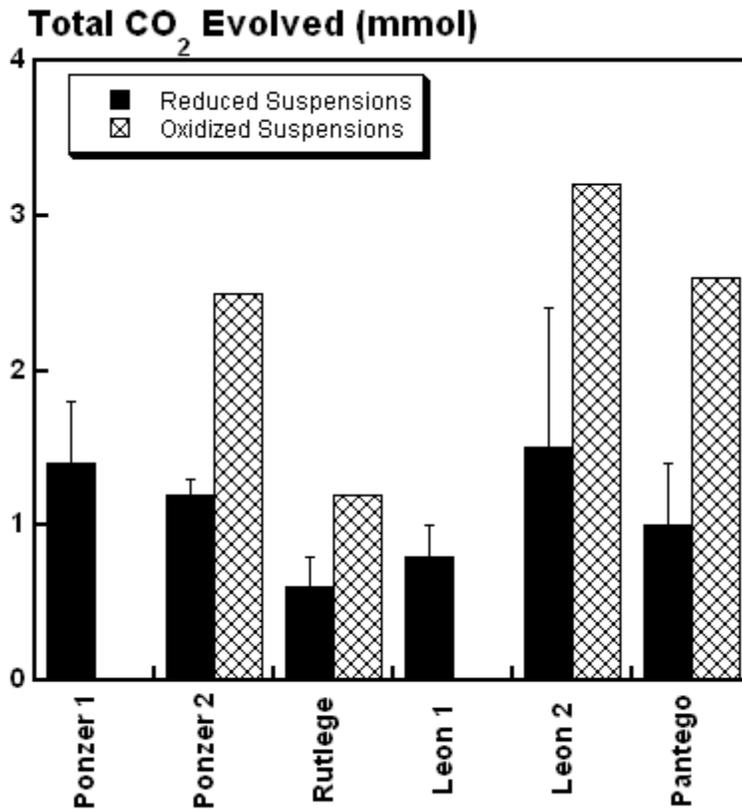
### *Suspension Reduction*



**Figure 2.3. Temporal trends in Eh (mV) plotted continuously for selected replicates of all suspensions. Inset plot shows a typical trough feature produced during initial reduction of Leon 2 suspension and lack thereof in a Ponzer 1 suspension. Standard deviation of duplicate pH measurements are shown in parentheses.**

Figure 2.3 shows that the Eh of all suspensions was  $\sim 500$  mV (mean =  $480 \pm 60$  mV) at  $t = 0$  d. After adding a  $2 \text{ g dextrose kg}^{-1}$  solids spike at  $t = 0$  to initiate reduction, an Eh drop of at least 200 mV occurred rapidly in all samples. This rapid Eh drop mimicked observed reduction rates following initial saturation of soils at Juniper Bay (Smith, 2004). An Eh of  $< 200$  mV was achieved within 4-5 days and maintained during the remainder of the experiment. In some samples initial rapid reduction was followed by a rapid, un-

sustained increase in Eh, which may have been caused by the reduction of glucose in solution (Appendix C).



**Figure 2.4. Total CO<sub>2</sub> evolved (mmol) from reduced and oxidized suspensions over 25 d. Error bars show standard deviations from duplicate reactors. No error bars are shown for oxidized samples, which were not replicated. Data from Ponzer 1 and Leon 1 oxidized suspensions are not available.**

Figure 2.4 shows that CO<sub>2</sub> evolution occurred in the reduced treatments, suggesting that reduction was microbially mediated. Furthermore, average CO<sub>2</sub> evolution rates from ranged from 20 to 60  $\mu\text{mol d}^{-1}$  in reduced suspensions, and 50 to 130  $\mu\text{mol d}^{-1}$  in oxidized suspensions, suggesting that reduction suppressed microbial respiration. Such suppression is consistent with slower mineralization rates caused by the exclusion of O<sub>2</sub> from soil pores (Oades, 1995).

## Suspension pH

Figure 2.5 shows representative pH trends in selected suspension replicates during 25 d of reduction.

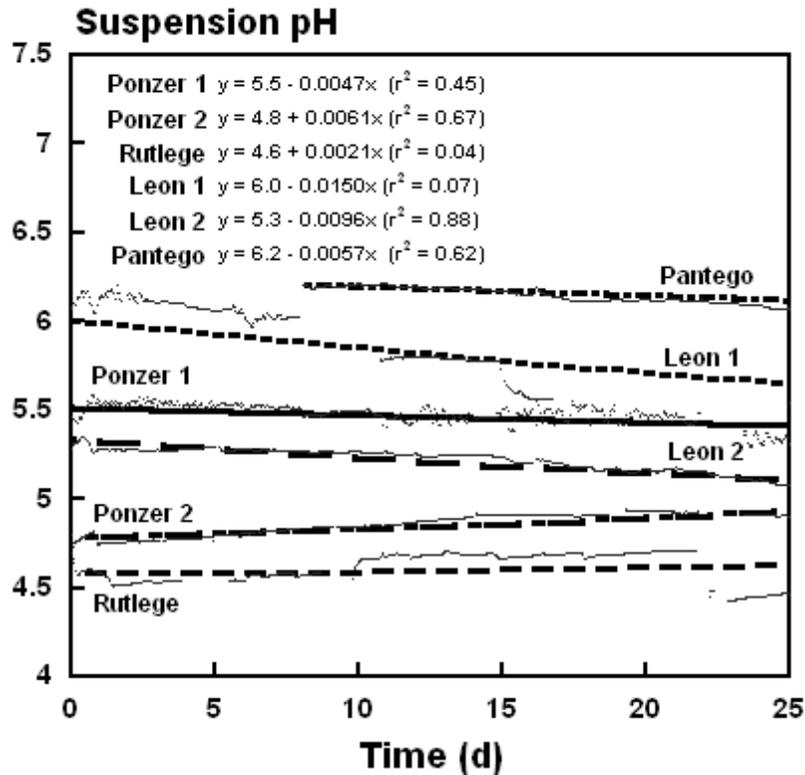
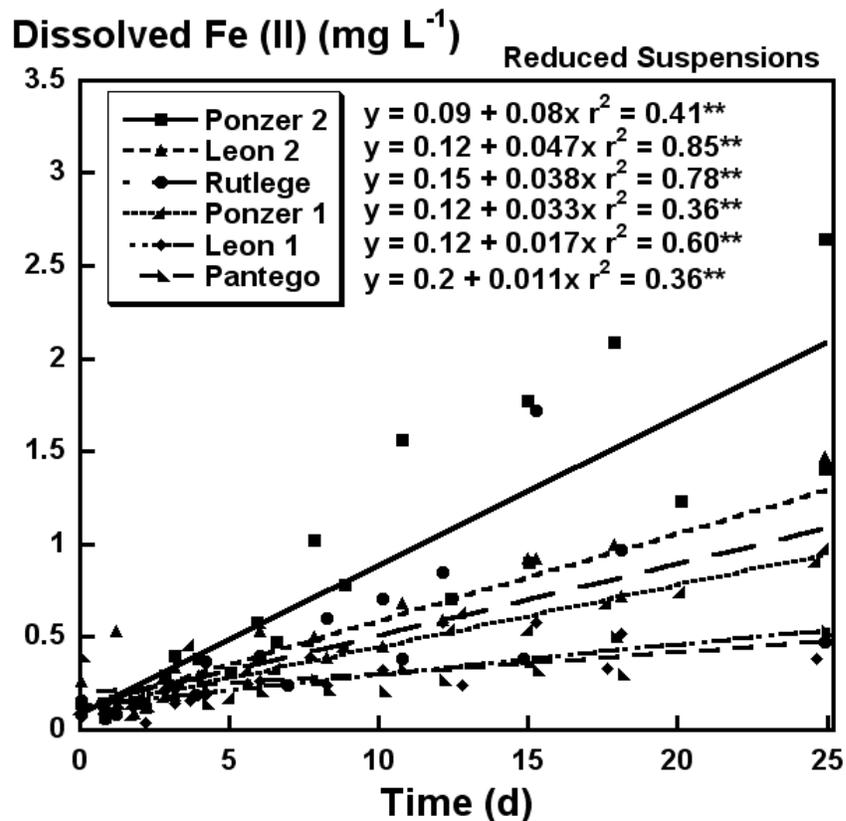


Figure 2.5. Temporal trends in pH for selected replicates of all suspensions. Data for Pantego suspension before  $t = 7$  d is not available due to equipment malfunction. Actual data in gray.

The pH of all suspensions changed less than 0.2 units during the experiment with the exception of the “Leon 2” suspension, which decreased by 0.5 pH units. Since all suspensions were undergoing reduction, which typically consumes protons, one or more buffering processes must have been occurring concurrently. Soils that are low in reducible iron may not exhibit a pH greater than 6.5 even after weeks of submergence (Ponnamperuma, 1972), presumably because of the pH buffering capacity of organic matter (Ponnamperuma, 1972; Ruttner, 1963).

### *Fe(II) Dissolution*

Figure 2.6 shows trends in dissolved Fe(II) concentrations in suspensions of mineral and organic separates and proves that Fe(III) reducing conditions were maintained during the experiment. The fastest rate of Fe(II) dissolution occurred in the Leon 2 suspension, in which Fe(II) increased twelve fold from approximately  $0.15 \pm 0.03 \text{ mg L}^{-1}$  to about  $0.96 \pm 0.32 \text{ mg L}^{-1}$  during the experiment. The highest concentration of Fe(II) occurred in the Ponzer 2 suspension, which, despite much scatter in the data, significantly ( $p < 0.05$ ) increased from about  $0.5 \text{ mg L}^{-1}$  to about  $2 \text{ mg L}^{-1}$ . A nine fold increase in Fe(II) occurred in the Ponzer 1 and the Rutlege soil suspension, with final suspension concentrations close to  $1 \text{ mg L}^{-1}$ .

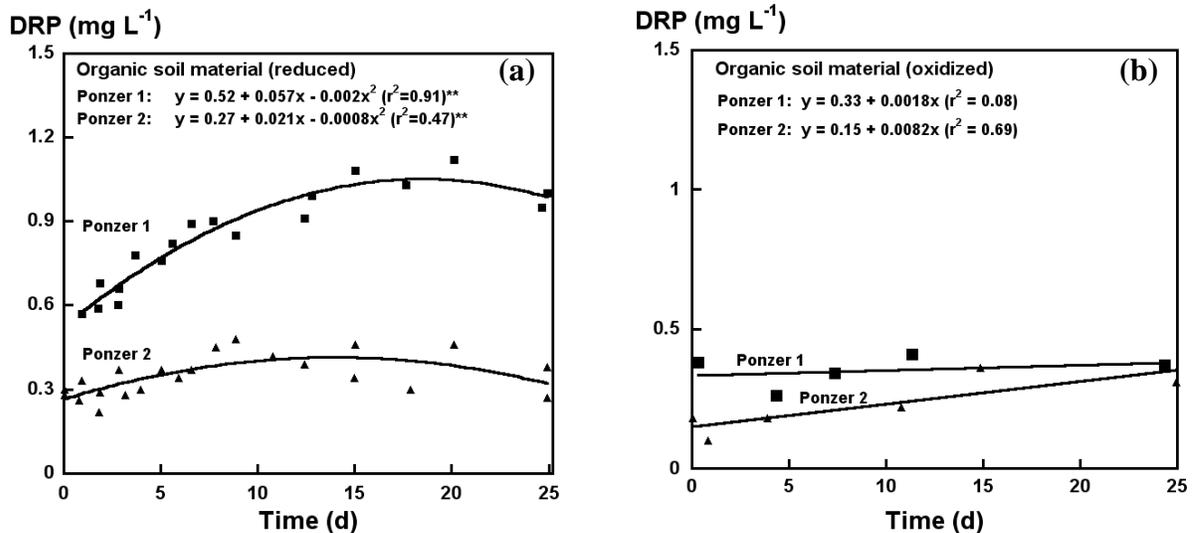


**Figure 2.6. Fe(II) dissolution in reduced suspensions. Significant linear fits at the  $p < 0.01$  level are denoted by “\*\*\*”.**

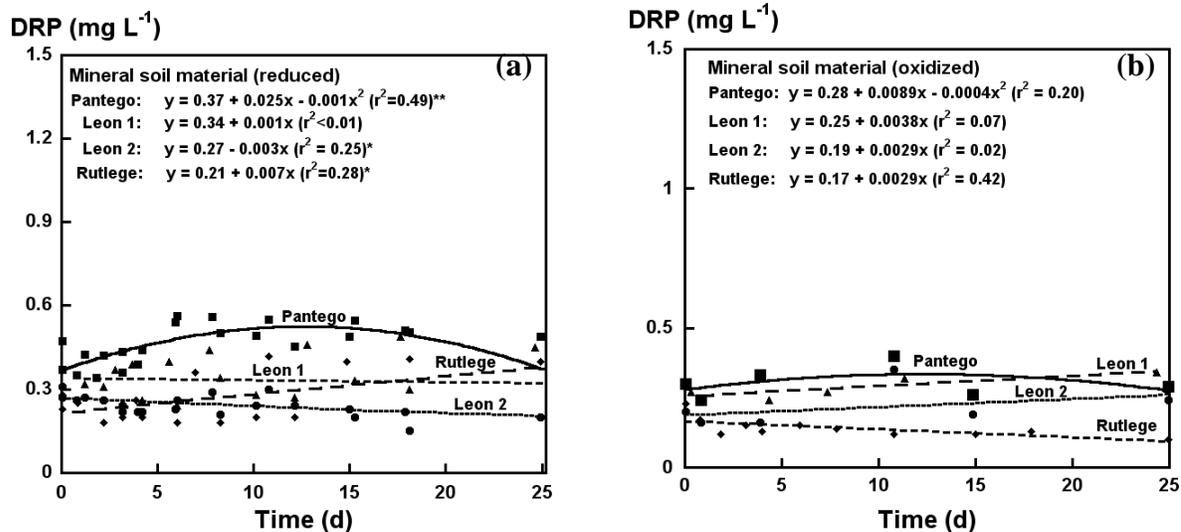
## Phosphorus Dissolution

Figures 2.7 and 2.8 show the temporal trends in concentrations of DRP in organic and mineral soil suspensions, respectively, during 25 d of reduction. Either a linear or a quadratic model was first to fit the data, whichever gave the greater coefficient of determination. A quadratic model significantly ( $p < 0.01$ ) fit the Pantego, Ponzer 1, and Ponzer 2 data, while a linear model fit ( $p < 0.05$ ) the Leon 2 and Rutlege soils. Neither model fit the Leon 1 soil.

After 25 d of reduction, DRP concentrations increased 1.9 fold in the Ponzer 1 suspension, from  $0.59 \text{ mg L}^{-1}$  to  $1.1 \text{ mg L}^{-1}$ . No net change in phosphate dissolution was observed in either of the other two organic suspensions or any of the mineral suspensions. For the solids concentration used in this experiment ( $15 \text{ g kg}^{-1}$ ), up to 6 % (for Ponzer 1) of the total solid-phase P dissolved during reduction. Trends in  $P_T$  closely followed DRP trends, indicating that DRP constituted a major fraction of the total dissolved P (Appendix E).



**Figures 2.7a and 2.7b. Dissolved reactive phosphorus (DRP) concentrations in reduced (b) and oxidized (a) suspensions of < 53  $\mu\text{m}$  organic soil separates. Data from reduced treatments were statistically fit by the better of linear and quadratic models.**



Figures 2.8a and 2.8b. Dissolved reactive phosphorus (DRP) concentrations in reduced (a) and oxidized (b) suspensions of < 53  $\mu\text{m}$  mineral soil separates. Data from reduced treatments were statistically fit by the better of linear and quadratic models.

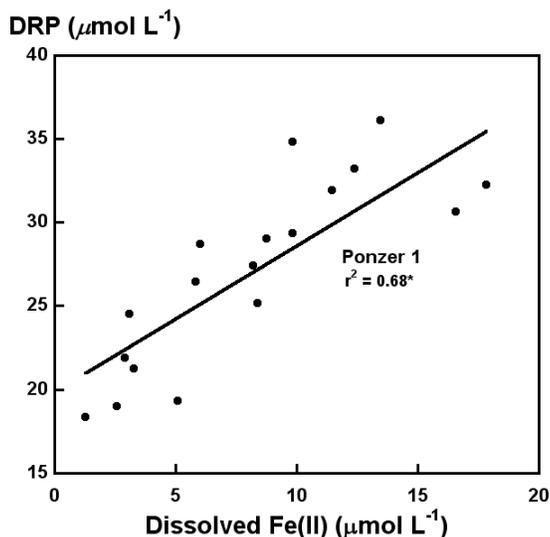


Figure 2.9. Scatter plots of DRP and Fe(II) in the Ponzer 1 sample, which was the only sample in which a significant ( $p < 0.05$ ) correlation was found.

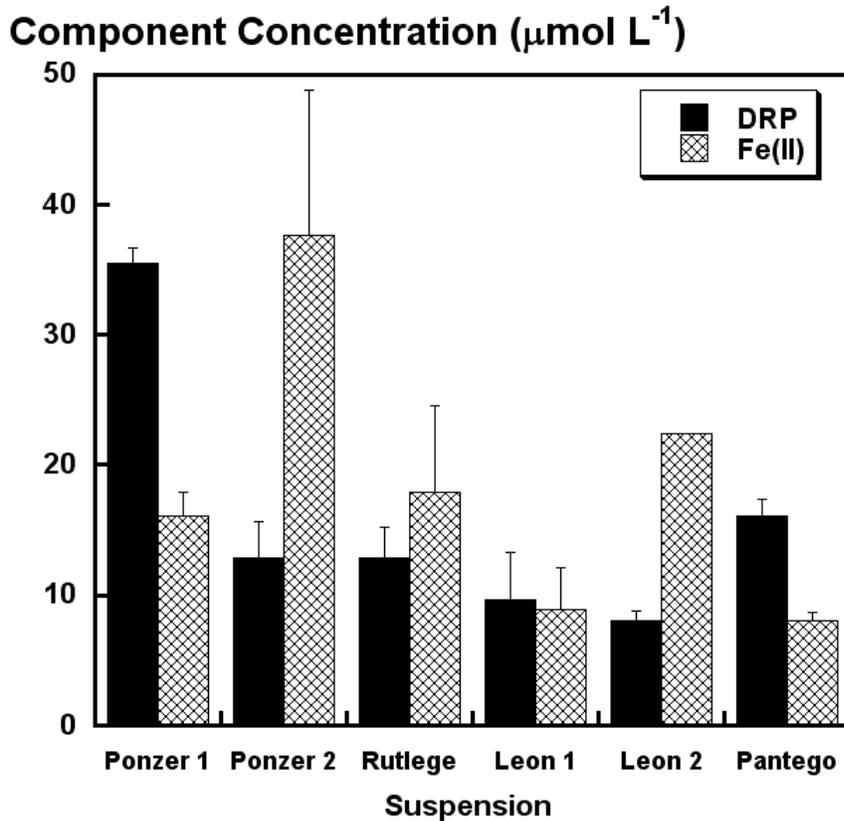
Models of DRP vs. Time for Ponzer 1, Ponzer 2, and Pantego suspensions showed that DRP concentrations decreased approximately  $0.1 \text{ mg L}^{-1}$  between 15 and 25d. Precipitation of vivianite ( $\text{Fe}_3(\text{PO}_4)_2 \cdot 8 \text{ H}_2\text{O}$ )<sup>2</sup> could not be ruled out as a source for decreases in DRP, nor could the immobilization of dissolved P by microbial activity since  $\text{CO}_2$  evolution occurred during reduction.

Figure 2.9 shows correlations of DRP with Fe(II) in suspensions of organic and mineral soil material. Significant correlations ( $p < 0.05$ ) showed a relationship between DRP and Fe(II) in the Ponzer 1 suspension only, which was also the suspension with the most notable P dissolution. Iron(II) dissolution in the Rutlege, Leon 2, and Ponzer 2 suspensions was 1.25, 1.75, and 2.5-fold higher, respectively, than in the Ponzer 1 suspension; yet no net P dissolution occurred in any of the former suspensions. The lack of correlation between DRP and Fe(II) in the suspensions that exhibited the most Fe(II) dissolution suggests that Fe(III) reduction was a necessary-but-not-sufficient condition for P dissolution in the reactor suspensions.

The insufficiency of Fe(III) reduction as the single source of P dissolution is shown more definitively in Figure 2.10, which compares the concentrations of DRP and Fe(II) in the reduced suspensions at the peak [DRP]. Hutchison and Hesterberg (2004) reasoned that dissolved, molar P:Fe(II) ratios  $> 1$  could not result solely from the congruent dissolution of Fe(III)-bound P in the absence of reactions that substantially remove Fe(II) from solution. For example, the molar ratios of P to Fe in strengite ( $\text{Fe}(\text{PO}_4) \cdot 2\text{H}_2\text{O}$ ) and vivianite ( $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ) are 1 and 0.67, respectively, so congruent dissolution of strengite or vivianite would result in P:Fe(II) ratios less than or equal to one. Adsorbed phases have even lower P:Fe since P bonding occurs only at the Fe-oxide mineral surface. Figure 2.11 shows

---

that the Ponzer 2, Rutlege, Leon 1, and Leon 2 suspensions, molar P:Fe was less than 1. However, molar P:Fe(II) in the Ponzer 1 and Pantego suspensions was 2.3 and 2.0, respectively, suggesting that Fe(III) reduction alone could not have accounted for all P dissolution in these samples.



**Figure 2.10. Concentrations of P and Fe(II) ( $\mu\text{mol L}^{-1}$ ) in the reactor solutions at  $t = 20\text{d}$ .**

Figures 2.11 and 2.12 show [DOC] in reduced and oxidized (control) suspensions of organic and mineral samples. Concentrations of DOC in the Ponzer 1 suspension were 1.8 fold higher (up to  $55 \text{ mg L}^{-1}$ ) than any other suspension, although DOC did increase significantly as a result of reduction in all soils.

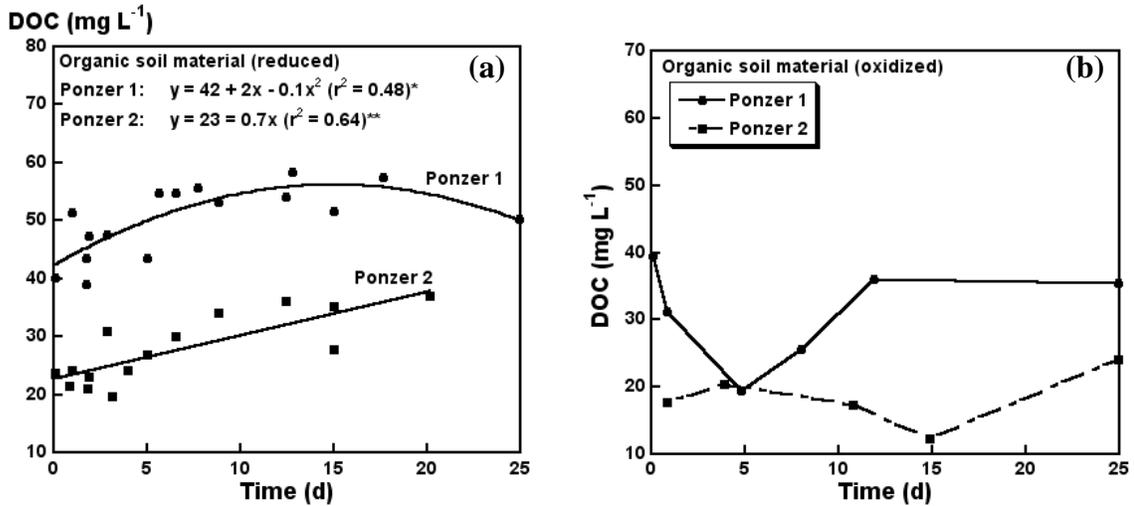


Figure 2.11a and 2.11b. Dissolved organic carbon (DOC) during 25 d in reduced (a) and oxidized (b) suspensions. Reduction data are plotted using the better of linear and quadratic models for duplicate data sets. Oxidation data were not replicated. DOC increased 1.6 fold in the Ponzer 2 suspension and 1.3 fold in the Ponzer 1 suspension during 25 d of reduction. DOC increased slightly in the Rutlege suspension during reduction.

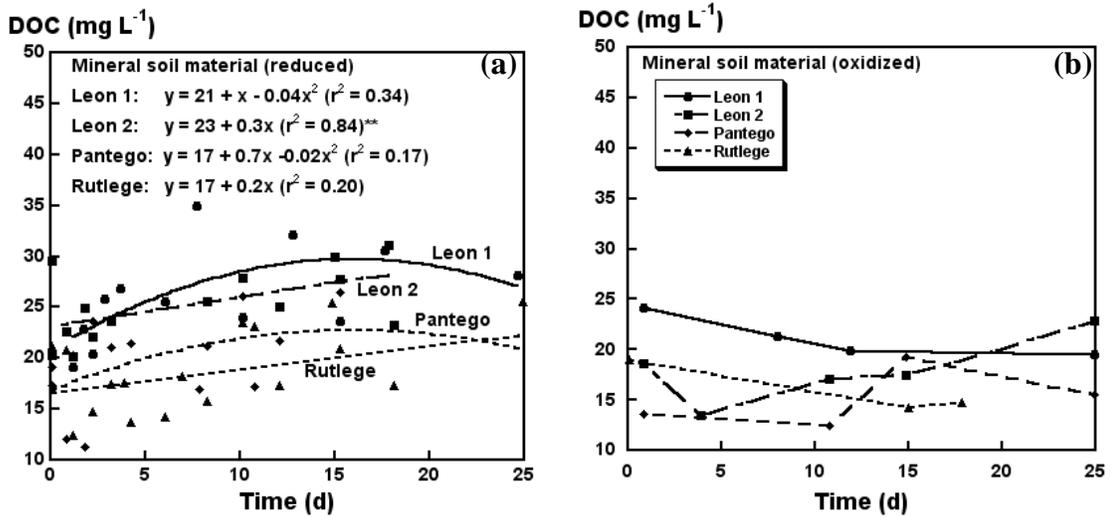
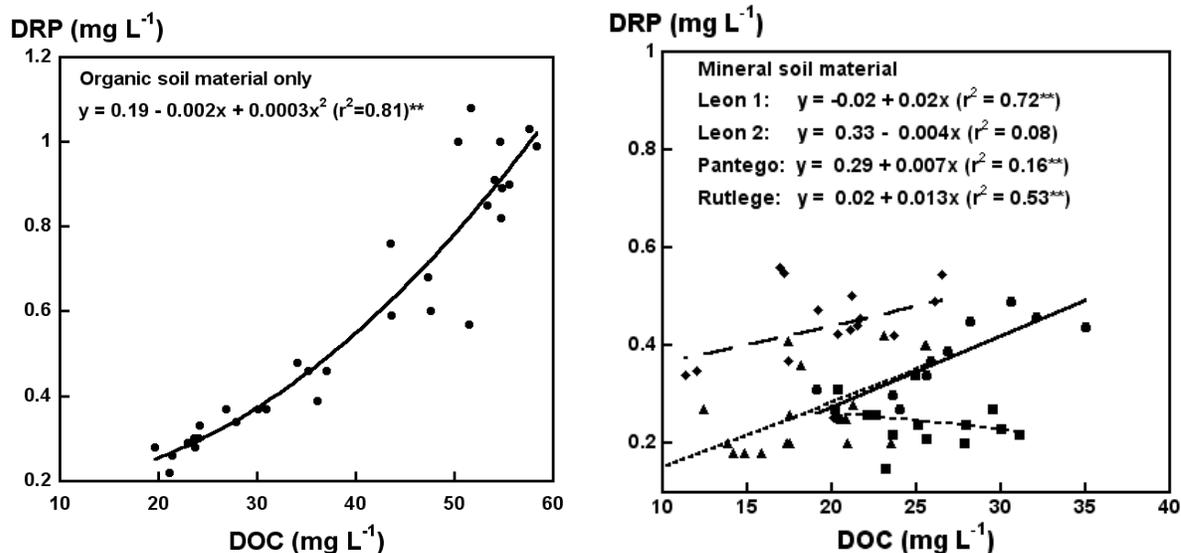
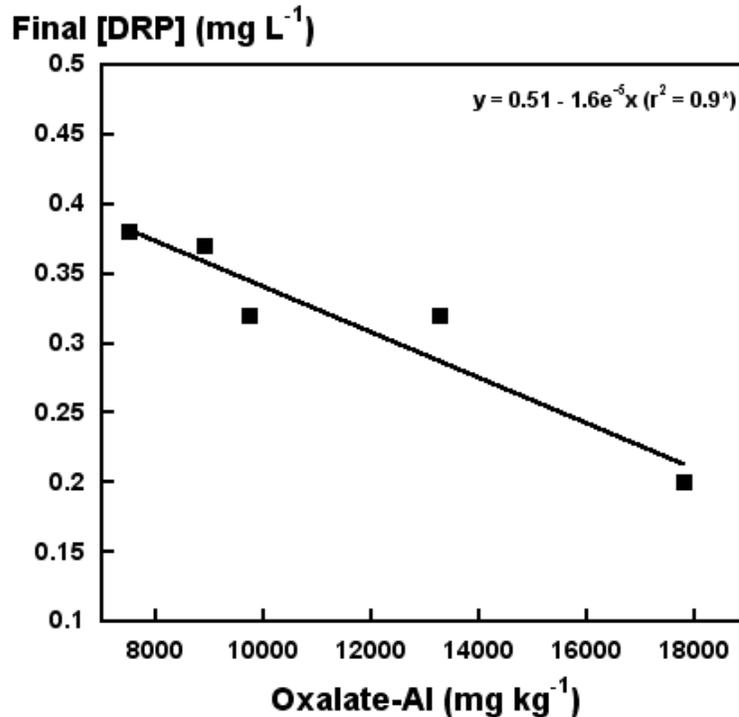


Figure 2.12a and 2.12b. Dissolved organic carbon (DOC) during 25 d in reduced (a) and oxidized (b) suspensions. Reduction data are plotted using the better of linear and quadratic models for duplicate data sets. Oxidation data were not replicated. DOC increased 1.5 fold and 1.3 fold during reduction of the Leon 1 and Pantego suspensions, respectively. DOC increased 1.2 fold during reduction of the Leon 2 suspension.



**Figures 2.13a and 2.13b. Correlations of DRP and DOC. Dissolved reactive phosphate was highly correlated with DOC in the organic suspensions (a). In the mineral suspensions (b), DRP was highly correlated with DOC in the Leon 1 and the Pantego suspensions.**

When plotted together, data from all organic samples show a highly significant ( $p < 0.01$ ) positive relationship between DRP and DOC (Fig. 2.13). A similar relationship was reported by Hutchison and Hesterberg (2004). Data from the Rutlege, Leon 1 and Pantego samples show significant linear relationships between DRP and DOC, while there was no significant relationship identified between DRP and DOC in the Leon 2 sample (Fig 2.12). If data from the Rutlege mineral samples are pooled with data from the organic samples (data not shown), the coefficient of determination increases to 0.90 and remains highly significant ( $p < 0.01$ ). Based on the strength of the regression analysis shown in Fig. 2.13, DOC was a more reliable predictor than Fe(II) for P dissolution.



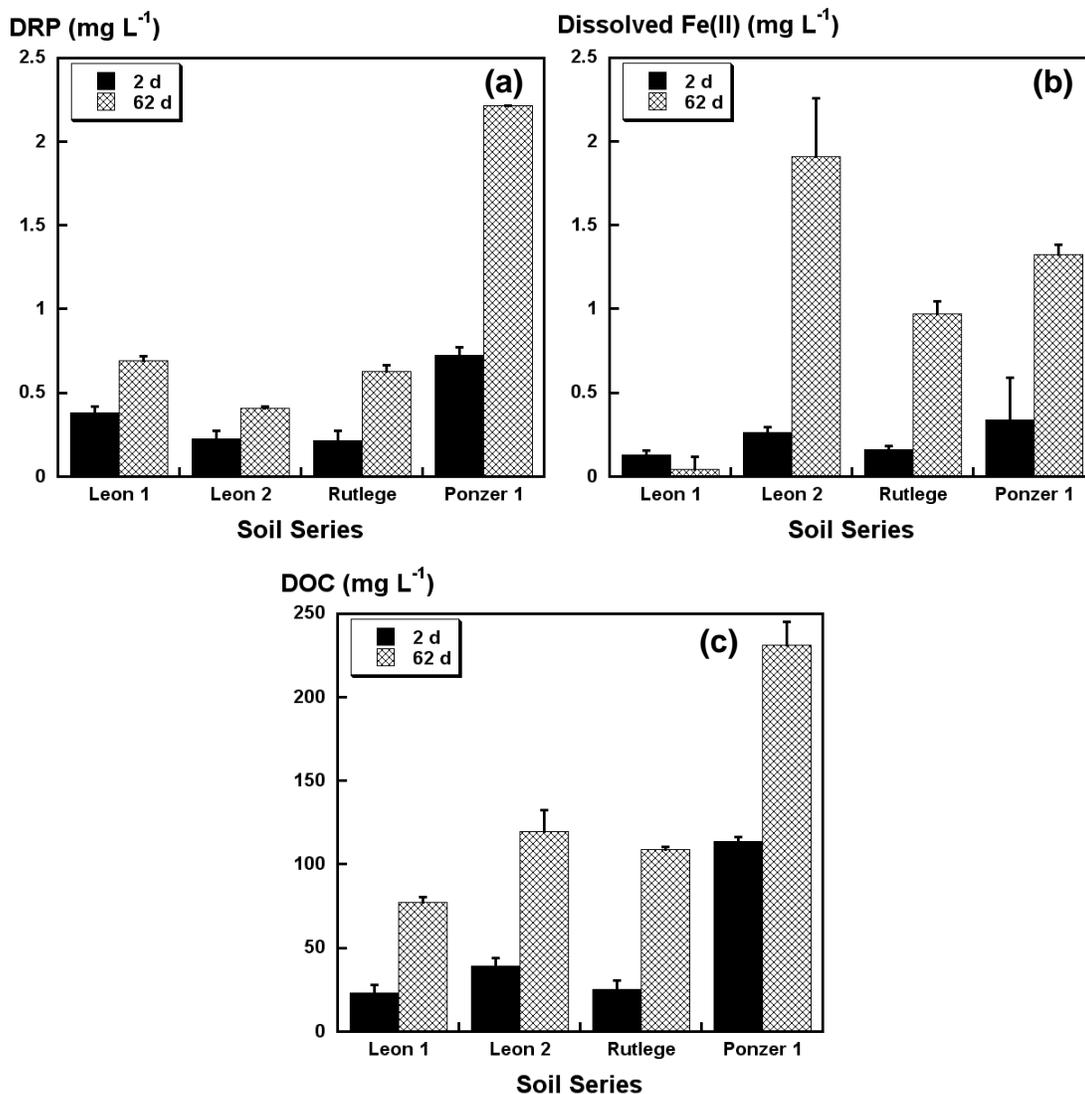
**Figure 2.14.** Final [DRP] as predicted by  $Al_{ox}$  concentrations in the reactor solids. DRP concentrations shown are from best fit models (Figures 2.11 and 2.12) of duplicate reduced suspensions. Ponzer 1 data ( $x = 10,200 \text{ mg kg}^{-1}$ ,  $y = 0.98 \text{ mg L}^{-1}$ ) not displayed.

Oxalate extractable Al was a poor predictor of final DRP concentrations when considering all samples ( $p > 0.05$ , data not shown). When considering only samples in which P dissolution did not occur, however,  $Al_{ox}$  significantly ( $p = 0.013$ ) explained 90 % of the variability in final DRP concentrations (Fig. 2.14). A similar result was reported by Richardson (1984), while Hogan et al. (2004) correlated P dissolution in natural wetlands with  $Al_{pyr}$ . The high coefficient of determination in Fig. 2.14 suggests that poorly crystalline and short-range ordered Al-hydroxides may have served to inhibit P dissolution among the Ponzer 2, Rutlege, Leon 1, Leon 2, and Pantego samples, samples in which DRP was always  $\leq 0.6 \text{ mg L}^{-1}$ .

## Static Incubation Experiment

The static incubation experiment differed from the stirred reactor experiment in three key ways. Firstly, soil samples were incubated in static chambers, not continuously stirred reactors, enabling the use of entire soil samples (0 to 2 mm) instead of just the  $< 53 \mu\text{m}$  separates. Secondly, samples were allowed to react under oxygen-free conditions for 62 d rather than 25 d, extending the time period over which results were relevant. Thirdly, a 19-fold higher solid:suspension was used in the static incubation experiment ( $285 \text{ g kg}^{-1}$ ) than in the stirred reactor experiment ( $15 \text{ g kg}^{-1}$ ), more-closely mimicking a saturated soil at Juniper Bay. Moreover, the static incubation experiment was designed to be one step closer to reasonable field conditions than was the stirred reactor experiment. Hence, results from the static incubation experiment are more useful for predicting P dissolution from reduced soils at Juniper Bay than results from the stirred reactor experiment, even if their usefulness in understanding why P dissolution occurs is comparably limited.

Initial and final concentrations of DRP, Fe(II), and DOC are displayed in Figure 2.15, while trends in all experimental variables are summarized in Table 2.2. At the beginning of the static incubation experiment, the mean Eh of all four samples was  $380 \pm 20 \text{ mV}$  (data not shown). After 62 d of anaerobic incubation, the Eh of all samples significantly ( $p < 0.01$ ) decreased to  $180 \pm 30 \text{ mV}$ ; their pH did not change significantly ( $p > 0.05$ ). As in the continuously stirred reactor experiment, these same Eh and pH trends indicate that reduction-associated proton consumption was offset by a pH buffering process.



Figures 2.15a, 2.15b, and 2.15c. Concentrations of DRP (a), dissolved Fe(II) (b), and DOC (c) after 2 and 62 d of incubation at 25 °C.

Table 2.2. Changes in pH, Eh, DRP, Fe(II) and DOC concentrations of incubated soil samples after 62 d of anaerobic incubation at 25°C. “\*\*\*” and “\*” denote differences at the  $p < 0.01$  and  $p < 0.05$  levels of significance..

| Soil     | pH  | $\Delta$ pH | $\Delta$ Eh | $\Delta$ DRP                                 | $\Delta$ Fe(II) | $\Delta$ DOC       |
|----------|-----|-------------|-------------|--|-----------------|--------------------|
|          |     |             | mV          | $\mu\text{mol L}^{-1}$ (mg L <sup>-1</sup> ) |                 | mg L <sup>-1</sup> |
| Leon 1   | 6.0 | 0.05 (0.03) | -184 (29)** | 10.0 (0.31)**                                | -1.43 (-0.08)   | 54 (4)**           |
| Leon 2   | 5.6 | 0.08 (0.04) | -200 (20)** | 6.13 (0.19)*                                 | 29.5 (1.65)**   | 80 (8)**           |
| Rutlege  | 5.3 | 0.17 (0.14) | -179 (40)** | 13.2 (0.41)**                                | 14.5 (0.81)**   | 84 (4)**           |
| Ponzer 1 | 5.5 | 0.07 (0.04) | -210 (15)** | 48.4 (1.50)**                                | 17.7 (0.99)**   | 118 (12)**         |

Reducing redox conditions resulted in significant increases in [DRP] ( $p < 0.05$ ) and [DOC] ( $p < 0.01$ ) in all four samples. The greatest increase and highest [DRP] occurred in the Ponzer 1 sample, in which DRP increased about three fold to  $2.2 \text{ mg L}^{-1}$  after 62 d. Concentrations of DRP in the Leon 1, Leon 2, and Rutlege samples were 3 to 5-fold lower than in the Ponzer 1 sample after 62 d. Concentrations of DOC after 62 d ranged from  $80 \pm 3 \text{ mg L}^{-1}$  in the Leon 1 sample to  $230 \pm 15 \text{ mg L}^{-1}$  in the Ponzer 1 sample. Significant ( $p < 0.01$ ) net dissolution of Fe(II) occurred in the Leon 2, Rutlege, and Ponzer 1 samples, but no net Fe(II) dissolution occurred in the Leon 1 sample. Molar  $\Delta\text{P}:\Delta\text{Fe}$  in the Ponzer 1 sample was 2.7 (Table 2.2). As discussed by Hutchison and Hesterberg (2004), molar  $\text{P}:\text{Fe(II)} > 1$  imply that the congruent dissolution of Fe(III)-bound P cannot account for all  $\text{PO}_4$  dissolution.

## DISCUSSION

### The Meaning of Static Incubation and CSR Results

The static incubation experiment and the CSR experiment differed in several key ways. The objective of the static incubation experiment was to determine long term trends in P dissolution in a way that could be used to infer the potential for P to dissolve in reduced soils of a restored wetland. The objective of the CSR experiment was to investigate the causes of P dissolution, and specifically to evaluate the relative importance of Fe(II) and organic carbon dissolution to the dissolution of P. Thus, samples were allowed to react under oxygen-free conditions for 25 d in the CSR experiment and for 62 d in the static incubation experiment.

Diffusive limitations to P dissolution reactions were removed by stirring in the CSR experiment, but were not in the static incubation experiment. Stirring required the separation of < 53  $\mu\text{m}$  particles for the CSR experiment, while the entire soil samples (0 to 2 mm) were used in the static incubation experiment. Furthermore, a 19-fold higher solid:suspension was used in the static incubation experiment ( $285 \text{ g kg}^{-1}$ ) than in the CSR experiment ( $15 \text{ g kg}^{-1}$ ) to mimic more realistic field conditions in the former and to ensure proper mixing in the latter. In sum, results from the static incubation experiment are more useful for predicting P dissolution from reduced soils in the restored wetland, while results from the stirred reactor experiment provide insight into chemical processes contributing to the dissolution of P.

Differences in P dissolution that occurred in samples evaluated in the static incubation experiment might be explained by a 2 to 4-fold higher concentrations of DOC, a 2.5-fold longer incubation time, or a 19-fold higher solids:suspension compared with stirred reactor experiment. However, concentrations of DRP (and DOC) were highest in the Ponzer

1 sample in both experiments. In the CSR experiment, relationships between DRP and dissolved Fe(II), and DRP and DOC, indicate that DOC accounts for trends in P dissolution better than Fe(II).

The dependence of DRP on DOC in reduced suspensions was also reported by Hutchison and Hesterberg (2004), who saw up to sevenfold increases in DRP (from 1.5 up to 10 mg/L) after 40 days of microbial reduction of a high-P Umbraquilt in a continuously-stirred redox reactor. Their results indicated that DOM produced during soil reduction contributed to the increase in DRP and suggested that this might have been due to competitive adsorption between phosphate and DOM for Fe and Al oxide minerals or to the formation of ternary DOM-Fe-PO<sub>4</sub> or DOM-Al-PO<sub>4</sub> complexes.

In light of the P dissolution that also accompanied reduction, the best explanation for increases in DOC might be reductive dissolution of Fe(III)-cemented organic colloids (Ryan and Gschwend, 1992; Tadanier et al., 2005). Considering both the stirred incubation and CSR experiments, samples in which P dissolution occurred tended to have higher DOC concentrations than samples in which no P dissolution occurred. In fact, no net P dissolution occurred in samples with a final [DOC] of less than 40 mg L<sup>-1</sup>. Once dissolved, colloidal organic ions can preferentially bind to mineral Al(III) or Fe(III) surfaces rather than remaining chelated to Fe(II) (Jansen et al., 2002). Preferential binding of DOM to Al(III) and Fe(III) surfaces can result in ligand exchange reactions where P occupies adsorption sites on Al(III) and Fe(III) mineral surfaces (Kreller, 2003). Alternatively, liberated DOM can remove Al(III) and Fe(III) from mineral surfaces, exposing otherwise occluded P (Gerke, 1992; Nierop et al., 2002). Because both Fe(II) and DOC increased in all soils upon

reduction, either of these pathways could have contributed to P dissolution in my experiments.

### **Implications for Wetland Restoration at Juniper Bay**

Concentrations of DRP measured in reduced samples from the Ponzer 1 samples were 12 and 22 times more concentrated in P than concentrations known to cause eutrophication in P limited freshwaters. Because the highest concentration of DRP was measured in the Ponzer 1 sample in both the stirred reactor and static incubation experiment, P dissolution is expected to be most pronounced in the center of the Ponzer soil unit at Juniper Bay. However the results of the static incubation experiment suggest that  $\text{PO}_4$  dissolution is likely to occur upon reduction of soils at Juniper Bay near the Leon 1, Leon 2, and Rutlege samples, as well. Even so, the impact of P immobilization by plants and long-term (> 62 d) P sorption processes were not evaluated, so a prediction of [DRP] in waters that drain Juniper Bay remains difficult to assess. Rather, results from this study indicate that the organic and organic-rich soils at Juniper Bay present a significant risk for eutrophication after reducing conditions are established. A prudent management strategy would therefore include monitoring of P concentrations at all points where surface water exits the property boundary, and prevention of short-circuits in the drainage network. Short circuits, such as areas where surface water currently ponds near still-open drainages, could potentially conduct dissolved P directly to drainage waters without exposure to plant uptake or long-term sorption processes.

## CONCLUSIONS

In the static incubation study, reducing redox conditions resulted in 2 to 3-fold increases in DRP after 62 d in all samples. Concentrations of DRP were greatest in the Ponzer 1 sample (max. DRP = 2.2 mg L<sup>-1</sup>) and decreased in the order Ponzer 1 >> Leon 1 ≈ Rutlege < Leon 2. In the CSR experiment, which was designed to investigate chemical processes that occurred in the static incubation experiment, an increase in DRP (2-fold, up to 1.1 mg L<sup>-1</sup>) was observed for only the Ponzer 1 sample. Dissolved Fe(II) was significantly correlated with DRP in the Ponzer 1 CSR sample, although molar P:Fe implied that congruent dissolution of Fe(III)-bound P could not wholly account for the P that dissolved. A strong ( $r^2 = 0.81$ ) linear relationship between DRP and DOC was found in the organic CSR samples. For CSR samples in which no P dissolution occurred (all except Ponzer 1), final [DRP] after reduction was significantly ( $p < 0.05$ ) negatively correlated with oxalate-extractable Al(III), and the maximum [DOC] was 2 to 8- fold lower than for samples in which P dissolved. These results suggest that non redox-active Al(III) inhibited the dissolution of PO<sub>4</sub> at low [DOC] (below 40 mg L<sup>-1</sup>). Furthermore, these results imply that the P dissolution that occurred was not likely the result of only Fe(III)-bound P dissolution, and that interactions of DOM with PO<sub>4</sub> or minerals that bind PO<sub>4</sub> plays an important role in P dissolution under reducing conditions. Hence, the dissolution of P from reduced, organic-rich soils in restored wetlands presents an environmental concern.

## REFERENCES

- Bartlett, R.J., and B.R. James. 1993. Redox Chemistry of Soils. *Adv. Agron.* 50:151-208.
- Bertsch, P.M. and P.R. Bloom. 1996. Aluminum. p. 517-550. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part 3. Chemical and microbiological methods.* SSSA, Inc. Madison, WI.
- Blake, G.R. and K.H. Hartge. 1986. Bulk Density. *In* A. Klute (Ed.) *Methods of Soil Analysis Part 1: Physical and mineralogical methods*, 2<sup>nd</sup> ed. Soil Science Society of America, Inc. Madison, Wisconsin.
- Chacon, N., S. Flores, and A. Gonzalez. 2006. Implications of iron solubilization on soil phosphorus release in seasonally flooded forests of the lower Orinoco River, Venezuela. *Soil Bio. Biochem.* 38:1494-1499.
- Correll, D. 1998. The role of phosphorus in the eutrophication of receiving waters: a review. *J. Environ. Qual.* 27:261-266.
- Darke, A., and M.R. Walbridge. 2000. Al and Fe biogeochemistry in a floodplain forest: implications for P retention. *Biogeochemistry.* 51:1-32.
- Ewing, J.M. 2003. Characteristics of soils in a drained Carolina bay wetland prior to restoration. Ph.D. diss. North Carolina State Univ., Raleigh.
- Ewing, J.M., M.J. Vepraskas, and C.W. Zanner. 2004. Using Historical Records of Land Use to Improve Wetland Mitigation. *Southeast. Geog.* 45:25-43.
- Fox, R.L., and E.J. Kamprath. 1970. Phosphorus sorption isotherms for evaluating the phosphate requirement of soils. *Soil Sci. Soc. Am. Proc.* 34:902-907.
- Gee, G.W. and J.W. Bauder. 1986. Particle size analysis. *In* A. Klute (Ed.) *Methods of Soil Analysis Part 1: Physical and mineralogical methods*, 2<sup>nd</sup> ed. Soil Science Society of America, Inc. Madison, Wisconsin.
- Gerke, J. 1993. Phosphate adsorption by humic/Fe-oxide mixtures aged at pH 4 and 7 and by poorly ordered Fe-oxide. *Geoderma.* 59:279-288.
- Gerke, J., and R. Hermann. 1992. Adsorption of orthophosphate to humic-Fe-complexes and to amorphous Fe-oxide. *Z Pflanzenernaehr. Bodenkd.* 160:427-432.
- Greenberg, A., L. Clesceri, A. Eaton, and M. Franson. 1992. Persulfate Digestion Method. *In* *Standard methods for the examination of water and wastewater*, 18<sup>th</sup> ed. American Public Health Association, Washington, D.C. p.4-112.

- Gustafson, J.P. 2007. Visual Minteq [Online]. Available at [www.lwr.kth.se/English/OurSoftware/vminteq/](http://www.lwr.kth.se/English/OurSoftware/vminteq/) (verified 19 Apr 2007). Dep. Land and Water Resour. Eng., Stockholm.
- Hermann, R., and J. Gerke. 1992. Complexation of iron (III) to humic substances of a humic podzol at pH 2.5-6.4 – quantification of the organically complexed iron by pyrophosphate extraction. *Z. Pflanzernahr. Bodenk.* 155:229-232.
- Hogan, D.M., T.E. Jordan, and M.R. Walbridge. 2004. Phosphorus retention and soil organic carbon in restored and natural freshwater wetlands. *Wetlands.* 24(3):573-585.
- Hutchison, K.N. 2003. Dissolution of phosphate in a phosphorus-enriched ultisol as affected by microbial reduction. M.S. Thesis. North Carolina State Univ., Raleigh.
- Hutchison, K.N., and D. Hesterberg. 2004. Dissolution of phosphate in a phosphorus-enriched ultisol as affected by microbial reduction. *J. Environ. Qual.* 33:1793-1802.
- Jansen, B., K.G.J. Nierop, and J.M. Verstraten. 2002. Influence of pH and metal/carbon ratios on soluble organic complexation of Fe(II), Fe(III) and Al(III) in soil solutions determined by diffusive gradients in thin films. *Anal. Chim. Acta.* 454(2):259-270.
- Kreller, D.I., G. Gibson, W. Novak, G.W. van Loon, J.H. Horton. 2003. Competitive adsorption of phosphate and carboxylate with natural organic matter on hydrous iron oxides as investigated by chemical force microscopy. *Colloids and Surfaces A: Physicochem. Eng. Aspects.* 212:249-264.
- Kuo, S. 1996. Phosphorus. P.869-919. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part 3: Chemical Methods.* SSSA, Inc. and ASA, Inc., Madison, WI.
- Kuo, S., and D.S. Mikkelsen. 1978. Distribution of iron and phosphorus in flooded and unflooded soil profiles and their relation to phosphorus adsorption. *Soil Sci.* 127/1:18-25.
- Levesque, M. and M. Schnitzer. 1967. Organo-metallic interactions in soils: 6. Preparation and properties of fulvic acid-metal phosphates. *Soil Sci.* 103:183-190.
- Lindsay, W.L. 1979. *Chemical equilibria in soils.* John Wiley & Sons, New York.
- Loeppert, R.H., and W.P. Inskeep. 1996. P. 639-664. Iron. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part 3 Chemical Methods.* SSSA, Inc. and ASA, Inc., Madison, WI.
- McBride, M.B. 1994. *Environmental Chemistry of Soils.* Oxford University Press, New York.
- Mortimer, C.H. 1941. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.* 29:280-329.

- Murray, G.C., and D. Hesterberg. 2006. Iron and phosphate dissolution during abiotic reduction of ferrihydrite-boehmite mixtures. *70*:1318-1327.
- Nierop, K., B. Jansen, and J. Verstraten. 2002. Dissolved organic matter, aluminium and iron interactions: precipitation induced by metal/carbon ratio, pH and competition. *Sci. Tot. Environ.* 300:201-211.
- Oades, J.M. 1995. An overview of processes affecting the cycling of organic carbon in soils. *In* R.G. Zepp and Ch. Sonntag (Eds.) *Role of nonliving organic matter in the Earth's carbon cycle*. John Wiley and Sons Ltd., New York.
- Osmond, D. 2005. Personal Communication. Raleigh, North Carolina.
- Patrick, W.H., and I.C. Mahapatra. 1968. Transformations and availability to rice of nitrogen and phosphorus in waterlogged soils. *Adv. Agron.* 20:323-360.
- Patrick, W.H., and R.A. Khalid. 1974. Phosphate release and sorption by soils and sediments: effect of aerobic and anaerobic conditions. *Science.* 186:53-55.
- Patrick, W.H., B.G. Williams, and J.T. Moraghan. 1973. A simple system for controlling redox potential and pH in soil suspensions. *Soil Sci. Soc. Am. Proc.* 37:331-332.
- Patrick, W.H., R.P. Gambrell, and S.P. Faulkner. 1996. Redox measurements of soils. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part 3.* SSSA Book Ser. 5. SSSA, Madison, WI.
- Ponnamperuma, F.N. 1972. The chemistry of submerged soils. *Adv. Agron.* 24:29-96.
- Reddy, K.R., R.H. Kadlec, E. Flaig, and P.M. Gale. 1999. Phosphorus retention in streams and wetlands: a review. *Crit. Rev. in Environ. Sci. Tech.* 29(1): 83-146.
- Richardson, C.J. 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science.* 228:1424-1427.
- Richardson, C.J. 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science.* 228:1424-1427.
- Ruttner, F. 1963. *Fundamentals of Limnology*, 3<sup>rd</sup> Ed. University of Toronto Press, Toronto.
- Ryan, J.N., and P.M. Gschwend. 1992. Effect of iron diagenesis on the transport of colloidal clay in an unconfined sand aquifer. *Geochim. Cosmochim. Acta.* 56:1507-1521.
- Sallade, Y.E., and J.T. Sims. 1997. Phosphorus transformations in the sediments of Delaware's agricultural drainageways: I. Effect of reducing conditions on phosphorus release. *J. Environ. Qual.* 26:1579-1588.
- SAS Institute. 2005. SAS Version 9.1. SAS Inst., Cary, NC.

- Sawyer, C.N. 1947. Fertilization of lakes by agricultural and urban drainage. *J. New England Water Works Assoc.* 69:109-127.
- Shenker, M., S. Seitelbach, S. Brand, A. Haim, and M.I. Litaor. 2004. Redox reactions and phosphorus release in re-flooded soils of an altered wetland. *Eur. J. Soil Sci.* 56(4):515-525.
- Smith, H. 2004. Time required for anaerobic conditions to develop in saturated soils. M.S. Thesis. North Carolina State Univ., Raleigh.
- Stumm, W., and J.J. Morgan. 1996. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*. 3<sup>rd</sup> Edition. John Wiley and Sons, New York.
- Tadanier C.J., M.E. Schreiber, and J.W. Roller. 2005. Arsenic mobilization through microbially mediated deflocculation of ferrihydrite. *Environ. Sci. Tech.* 39 (9): 3061-3068.
- Thompson, A., O.A. Chadwick, S. Boman, and J. Chorover. 2006. Colloid mobilization during soil iron redox oscillations. *Environ. Sci. Tech.* 40:5743-5749.
- Tjedor-Tjedor, M.I., and M.A. Anderson. 1990. Protonation of phosphate on the surface of goethite as studied by CIR-FTIR and electrophoretic mobility. *Langmuir* 6:602-611.
- Vollenweider, R.A. 1976. Advances in defining critical loading levels of phosphorus in lake eutrophication. *Mem. 1<sup>st</sup>. Ital. Idrobiol.* 33:53-83.
- White, R.E. and G.W. Thomas. 1981. Hydrolysis of aluminum on weakly acidic organic exchanges: implications for phosphate adsorption. *Fert. Res.* 2:159-167.
- Willet, I.R. 1989. Causes and reduction of changes in extractable phosphorus during flooding. *Aust. J. Soil Res.* 27:45-54.
- Young, E.O., and D.S. Ross. 2001. Phosphate Release from Seasonally Flooded Soils: A Laboratory Microcosm Study. *J. Environ. Qual.* 30:91-101.

## APPENDICES

## APPENDIX A: THE ORIGIN OF CAROLINA BAYS AND THE PROCESS OF RECLAIMING THEM

Carolina bays are one type of wetland in North Carolina that have been extensively reclaimed to support timber production, agriculture, or rural housing (Bennet and Nelson, 1991; Ewing, 2003; Shartz and Gibbons, 1982). Carolina bays are oval-shaped depressions that support a variety of wetland plant communities and occur on the Atlantic Coastal Plain from Florida to Delaware, especially in North and South Carolina (Ewing, 2004; Lees, 2003; Schafale and Weakley 1990). Because they occur on inter-stream divides, bay boundaries are often contiguous with pocosins, which, like bays, are characterized by slow drainage conditions (years) that result in the accumulation of soil organic matter, but unlike bays are not depressed in the landscape (Ewing, 2004). Dozens of theories have been proposed to explain their origin (Ross, 2000). According to the so-called “theory of complex origin” (Johnson, 1942), confining clay layers created artesian conditions in groundwater and perforations in the clay layers allowed groundwater to upwell, forming shallow ponds or lakes on the land surface. Over thousands of years, prevailing winds elongated the shallow lakes, uniformly oriented them along a southeast to northwest axis, and contributed to the concentration of sand around its edges (Johnson, 1942; Kaczorowski, 1977). As the artesian conditions were relieved, the water table leveled with the ground surface, and thickly-vegetated swamplands were formed (Johnson, 1942).

Preparing Carolina bay soils for agricultural management was a monumental task, and many bay soils were permanently altered during the process (Ewing, 2003; Lilly, 2003). According to Ewing (2003), the first steps included establishment of a skeletal drainage network and initial timber harvesting, followed by timber-raking of the plow layer to remove

large roots and trees. Debris from the raking process was windrowed and then incinerated. Next, extensive ditch networks were established to provide adequate drainage, and ditch spoils were used to crown field centers in a process called “turtle-backing”. Although the soils were ready to be plowed at that point, soil fertility often had to be improved before crops yields became profitable. For example, Ewing (2003) reported selected NCDA soil test information for three natural (unaltered) bays in North Carolina. The pH of their surface soils ranged from 3.5 – 4.0, which is low enough to cause H toxicity to many crops in organic soils (Kamprath and Smyth, 2005). Hence, many bays currently used for agriculture required extensive liming (Lilly, 2003). In some cases, special fertilizers were also required because soils high in organic matter often contribute to crop micronutrient deficiencies (Lilly, 2003).

References:

Bennet, S.H. and J.B. Nelson. 1991. Distribution and status of Carolina bays in South Carolina. Nongame and Heritage Trust Pub. No. 1. South Carolina Wildlife and Marine Resources Department, Columbia.

Ewing, J.M. 2003. Characteristics of soils in a drained Carolina bay wetland prior to restoration. Ph.D. diss. North Carolina State Univ., Raleigh.

Ewing, J.M., M.J. Vepraskas, and C.W. Zanner. 2004. Using Historical Records of Land Use to Improve Wetland Mitigation. *Southeast. Geog.* 45:25-43.

Johnson, D.W. 1942. The origin of Carolina bays. Columbia Univ. Press, New York.

Kaczorowski, R.T. 1977. The Carolina bays: a comparison with modern oriented lakes. Ph.D. dissertation. Univ. of South Carolina, Columbia.

Kamprath, E.J. and J.T. Smyth. Liming. *In* D. Hillel (Ed.) *Encyclopedia of Soils in the Environment*. Elsevier Ltd., Oxford, U.K.

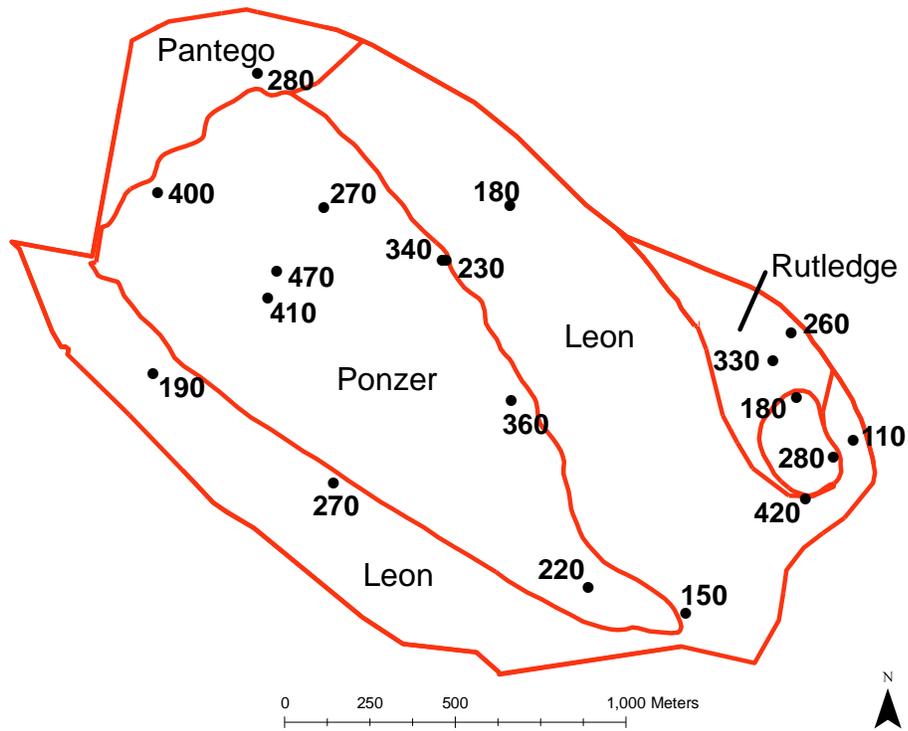
Lees, B. 2003. Vegetation of three reference Carolina bays in relation to soils and hydrology. M.S. thesis. North Carolina State Univ., Raleigh.

Ross, T.E. 2000. Carolina bays: an annotated and comprehensive bibliography 1844-2000. Carolina Press, Southern Pines, NC.

Schafale, M.P. and A.S. Weakly. 1990. Classification of the natural communities of North Carolina. 3rd approximation. North Carolina Natural Heritage Program, Raleigh, NC.

Shartz, R.R. and J.W. Gibbons. 1982. The ecology of southeastern shrub bogs (pocosins) and Carolina bays: A community profile. U.S. Fish and Wildlife Service WS/OBS-82/04.93.

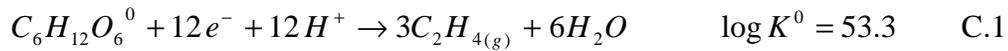
## APPENDIX B: $P_T$ SURVEY OF SOILS AT JUNIPER BAY



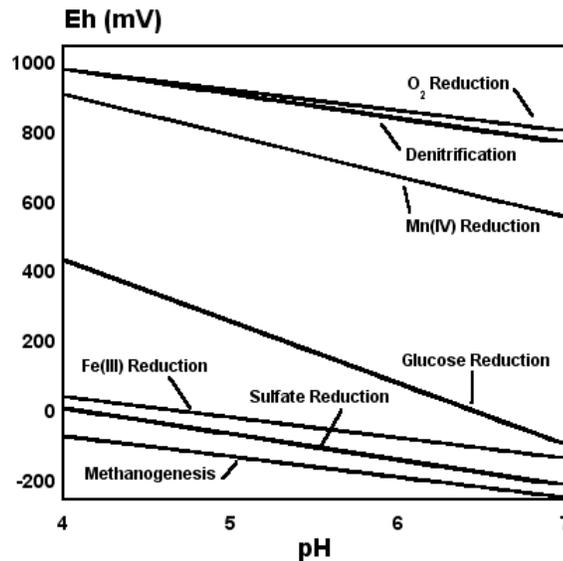
**Figure B.1.**  $P_{tot}$  concentrations (mg kg<sup>-1</sup>) in air-dried surface soil samples (0-10 cm) from Juniper Bay taken at the start of my study to identify P-enriched soils. Lines approximately delineate soil map units.  $P_T$  concentrations were between 110 and 470 mg P kg<sup>-1</sup> soil.

## APPENDIX C: GLUCOSE REDUCTION IN RELATION TO OTHER REDOX COUPLES

One explanation for the trough feature is that it is a reduction of aqueous glucose to ethane, as written in Equation C.1.



This transformation is predicted by thermodynamics to occur after Fe(III) reduction but before sulfate reduction (Dassonville and Renault, 2002 ;Lindsay, 1979), as is shown in Figure C1.



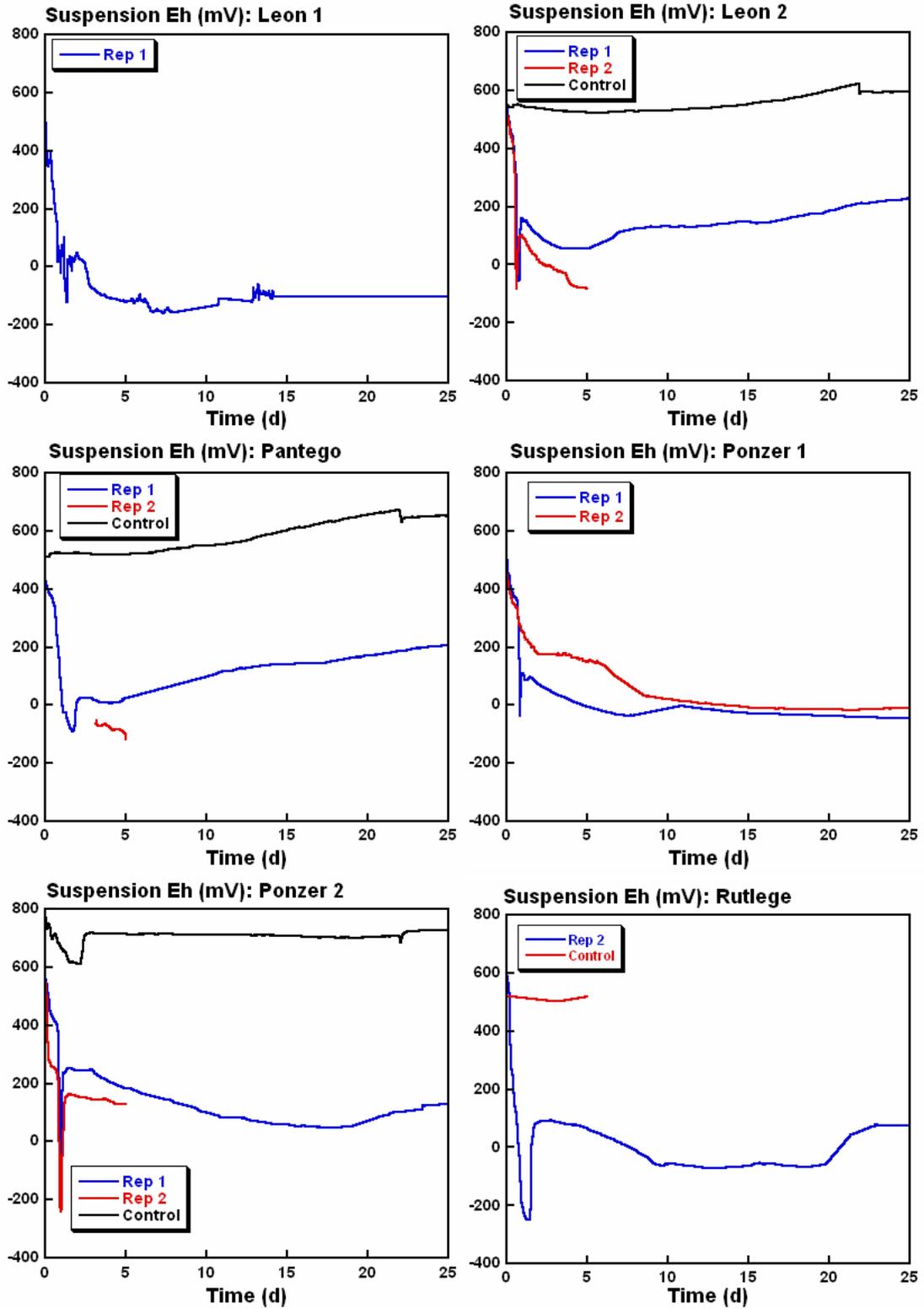
**Figure C.1. Theoretical reduction sequence of important aqueous species in soil suspensions as predicted by thermodynamics. Assumptions:  $[NO_3^-] = 10^{-4}$ ,  $[Mn^{2+}] = 10^{-5}$ ,  $\rho O_2 = 0.2$  for  $O_2$  reduction,  $\rho C_2H_6 = 0.2$ ,  $[SO_4^{2-}] = [H_2S]$ ,  $[CO_2] = [CH_4]$ . (Lindsay, 1979)**

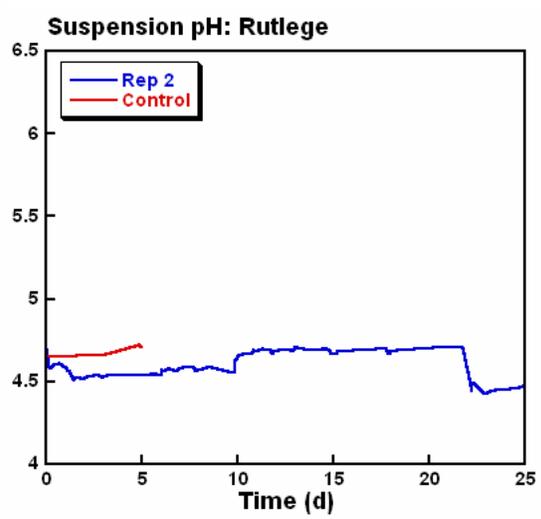
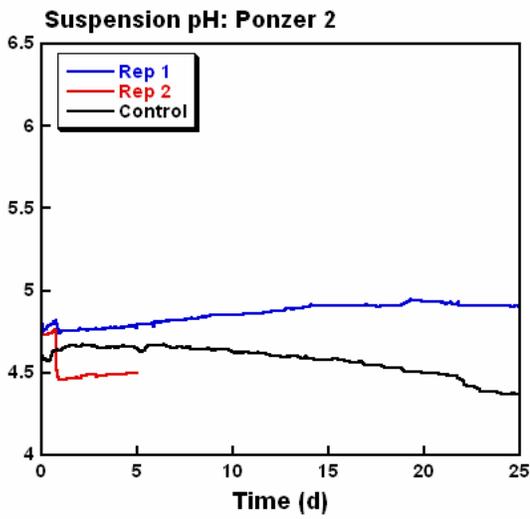
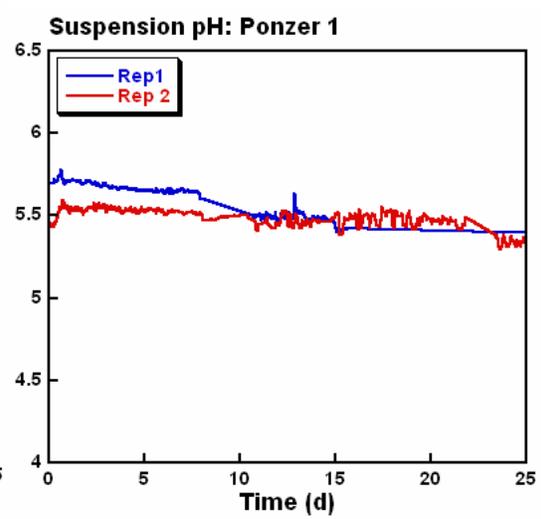
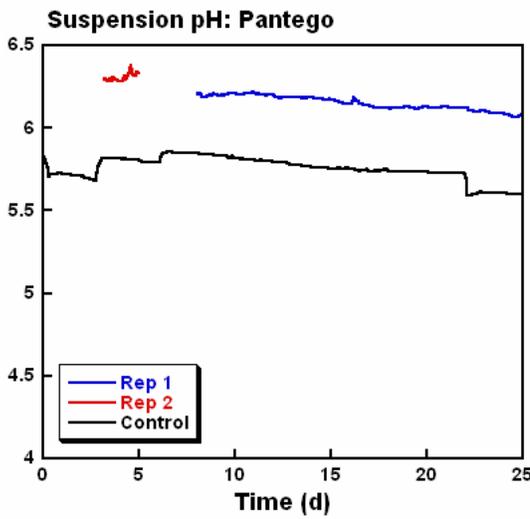
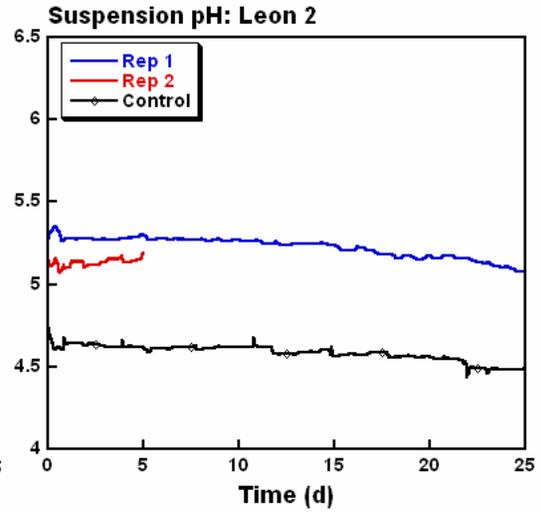
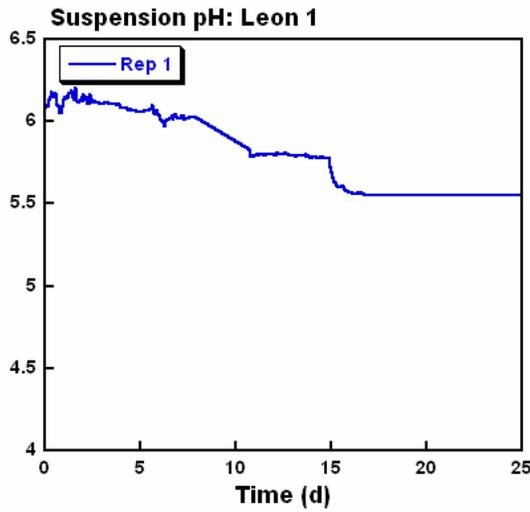
### References:

Dassonville, F. and P. Renault. 2002. Interactions between microbial processes and geochemical transformations under anaerobic conditions: a review. *Agronomie*. 22:51-68.

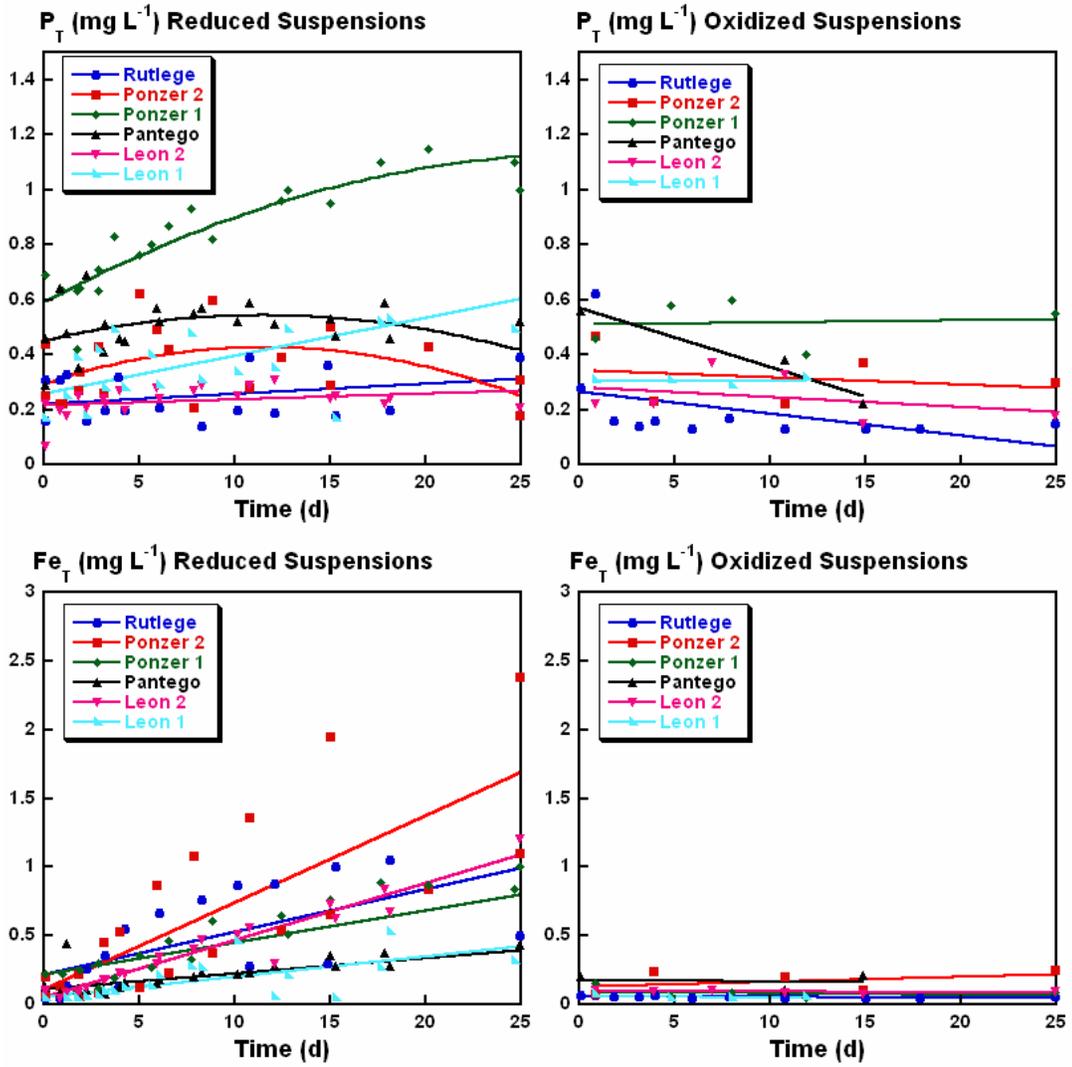
Lindsay, W.L. 1979. *Chemical equilibria in soils*. John Wiley & Sons, New York.

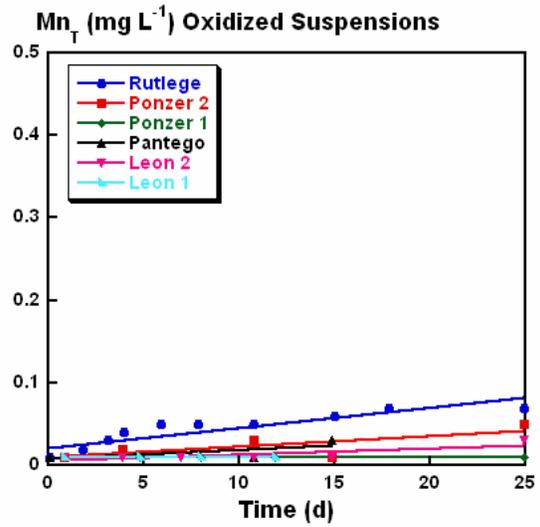
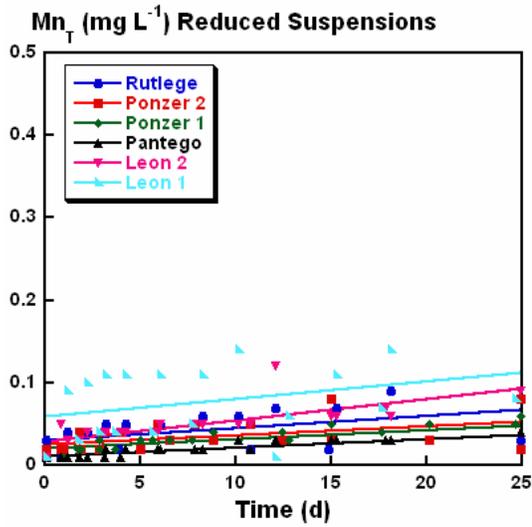
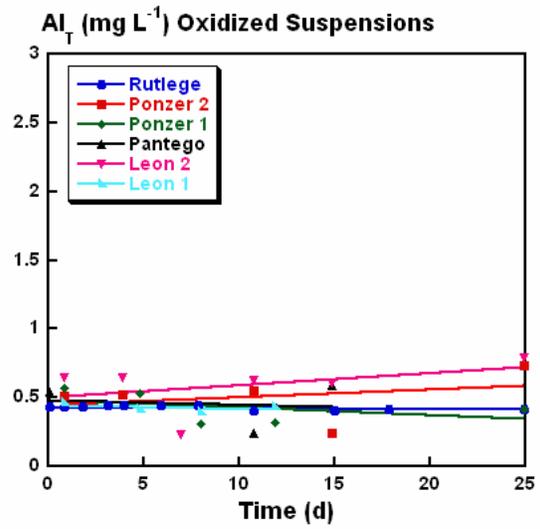
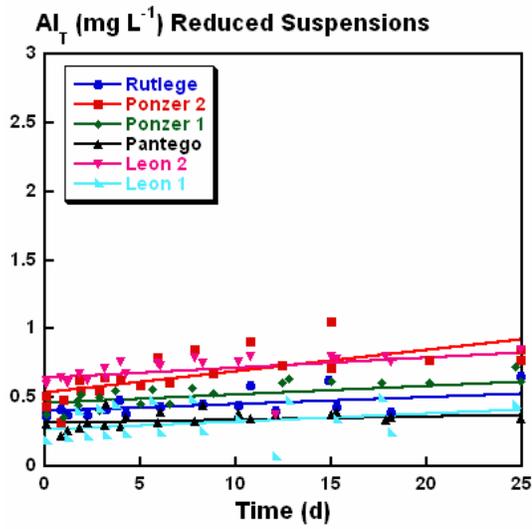
## APPENDIX D: pH AND Eh DATA





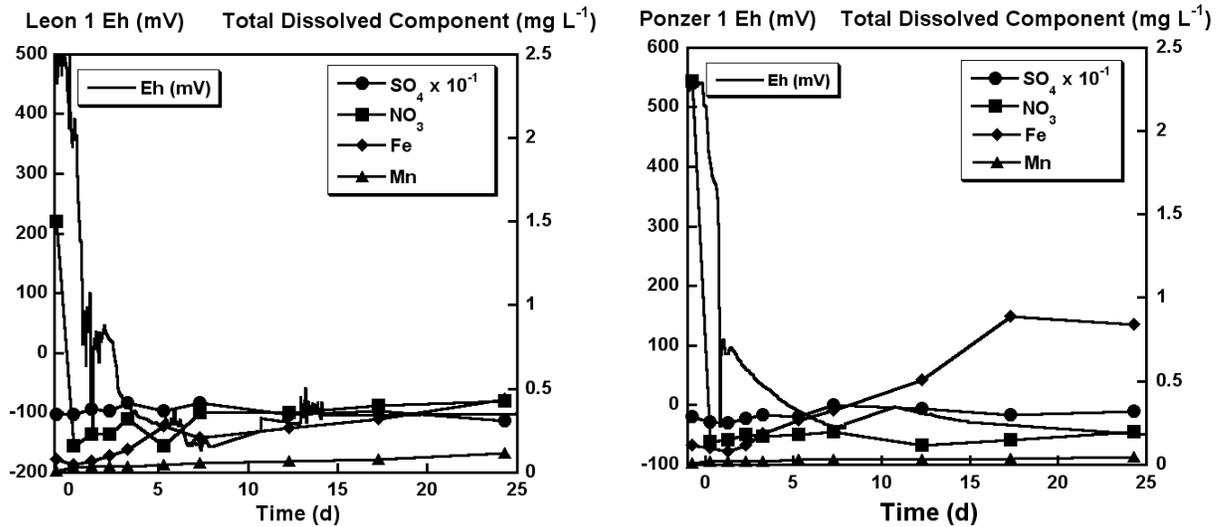
# APPENDIX E: TOTAL P, Fe, Al, Mn DATA





## APPENDIX F: REDOX-AFFECTED AQUEOUS COMPONENT CONCENTRATIONS

Figure F.1 shows the effect of reduction on the dissolved concentration of common, redox-active species in one replicate of Leon 1 and Ponzer 1 suspensions.



**Figure F.1. Eh (mV) and concentrations ( $\text{mg L}^{-1}$ ) of redox-active species for a suspension of mineral (left) and organic (right) soil material.**

Nitrate concentrations decreased from between 1.5 and 2.3  $\text{mg L}^{-1}$  before reduction to near detection limits immediately after adding dextrose. Nitrate reduction to  $\text{N}_{2(\text{g})}$  is thermodynamically favorable below 530 mV (pH 6.5), so the disappearance of  $\text{NO}_3^-_{(\text{aq})}$  can be explained by a combination of denitrification and immobilization by microbes. Dissolved  $\text{Mn}_i$  concentrations increased from below detectable amounts to 0.05 and 0.12  $\text{mg L}^{-1}$  in the organic and mineral suspension, respectively. Dissolved  $\text{Fe}_i$  increased 7-fold following reduction, from 0.12  $\text{mg L}^{-1}$  to 0.84  $\text{mg L}^{-1}$  in the organic suspension to 0.07  $\text{mg L}^{-1}$  to 0.5  $\text{mg L}^{-1}$  in the mineral suspension. Sulfate concentrations (note scale) were not affected by reduction in either suspension, supporting the idea that the trough features in Figure 2.3 were created by a redox couple that occurs between the  $\text{Fe(III)/Fe(II)}$  and  $\text{SO}_4^{2-}/\text{H}_2\text{S}$  couples (Appendix C).