

ABSTRACT

MOORBERG, COLBY JAMES. Phosphorus Release Mechanisms in Rhizospheres of Wetland Trees. (Under the direction of Michael Vepraskas).

Phosphorus release to ground or surface waters is commonly observed after restoration in wetlands that were drained and farmed, but the primary causal mechanism is unknown. This study examined whether the P release was originating from the rhizospheres, or soil matrix. Experiments were conducted in rhizotrons (glass-walled boxes) which were filled with Ap horizon material from a restored wetland. Phosphorus release was monitored from the rhizospheres of bald cypress roots (*Taxodium distichum* L.), and an unplanted control representing the soil matrix. Three water treatments (flooded, capillary fringe, and drained) were imposed on both planting treatments for 125 d, and soil water was collected twice monthly at three depths. Numbers of live and dead roots were determined monthly.

Rhizosphere processes did not increase P concentrations in the soil solution compared to the controls. In the planted treatment, TP concentrations were equal to or lower than the control (peak TP: 700-900 $\mu\text{g P L}^{-1}$) depending on sampling date and depth. The rhizospheres produced larger amounts of DOC and Fe(II) than the matrix. Dissolved P concentrations were equal to dissolved P concentrations in the matrix during the first 54 d, but lower than those of the matrix thereafter, most likely due to plant uptake. Tissue analysis and soil extractions showed that bald cypress are able to accumulate excess P equally under each water treatment, and immobilize the P to reduce potential export downstream. These findings indicate plant rhizospheres do not contribute to P release and may reduce it due to plant uptake.

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Phosphorus Release Mechanisms in Rhizospheres of Wetland Trees

by
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DEDICATION

I am proud to dedicate this thesis to my beautiful wife Stacy, who has been my compass wherever life leads me. Her never-ending encouragement and willingness to follow me to North Carolina so I could pursue my passion will always be remembered. Her love kept me grounded and her smile kept me motivated. I also want to dedicate this thesis to my parents, brothers, and grandparents. Your letters, phone calls, and visits have made me feel at home all through graduate school, and your support during my youth turned me into the man I am today, and for that I am thankful. Lastly I want to dedicate this thesis to all of my educators, grade school through graduate school. Teaching is a noble profession, and the academic building blocks you gave to me during the last two decades make research like this, and the career in my future possible.

BIOGRAPHY

Colby James Moorberg, son of Brian and Ruth Moorberg, was born in Spirit Lake, Iowa on February 24, 1986. He has an older brother, Matt, and a younger brother, Joe. He is married to his high school sweetheart Stacy Lee Moorberg, formerly Stacy Lee Schacherer. Colby grew up on a family farm near Estherville, Iowa. In high school Colby became involved in many activities including football, wrestling, tennis, the National Honors Society, and FFA. Through work on the farm, academics, and involvement in the FFA, Colby developed the building blocks of an education in agriculture and science, especially soil science. During high school, he discovered his academic and professional passion – wetlands. While duck hunting for the first time one cool October morning on Cheever Lake with his brother Matt, Colby (getting stuck waist deep in wetland muck without the use of waders) learned firsthand how dynamic, diverse, and integral wetlands are to the environment. It was that morning that he decided to follow a career that involved wetlands which eventually lead to a Bachelor of Science degree from Iowa State University in Ames, Iowa in 2008. While attending ISU, Colby became involved with Triangle Fraternity, Alpha Zeta agricultural honorary fraternity, Freemasonry, and the student chapter of the Soil and Water Conservation Society. His involvement with the SWCS student chapter eventually blossomed into a chapter presidency and two consecutive terms as the student director on the SWCS board of directors which he served while attending North Carolina State University. Inspired by soil scientists within the ISU faculty, and by scientists in the ISU Wetland Research Group, Colby continued his education in soil science as a Master of Science student in the NCSU department of soil science. Here, his interest in wetlands and wetland soils

continues to grow. Colby plans to continue his education through a PhD in soil science at NCSU where his research will focus on soil physics and phosphorus transport in wetland soils.

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TABLE OF CONTENTS

LIST OF TABLES..... vii

LIST OF FIGURES..... viii

CHAPTER ONE: Literature Review..... 1

 Introduction..... 1

 Wetland Restoration and Carolina Bays..... 1

 Phosphorus, Soil Reduction, and the Rhizosphere..... 4

 Study Species: Loblolly Pine and Bald Cypress..... 15

 References..... 19

CHAPTER TWO: Phosphorus Mobilization in the Rhizospheres of Bald
Cypress Grown in Wetland Soils..... 31

 Abstract..... 31

 Introduction..... 32

 Materials and Methods..... 36

 Results..... 47

 Discussion..... 57

 Conclusions..... 66

 References..... 68

LIST OF TABLES

Table 1.1	Common soil reduction half-reactions, redox potential calculations, standard state redox potential for half reactions, redox potentials at pH 6.....	30
Table 2.1	Summary of soil properties.....	96
Table 2.2	Test of effect slices for soil porewater analysis.....	97

LIST OF FIGURES

Figure 1.1	Eh-pH phase diagram for the reducing reactions shown in table 1.1.....	29
Figure 2.1	Map of Juniper Bay.....	77
Figure 2.2	Rhizotron design and construction.....	78
Figure 2.3	Soil porewater sampling and redox electrodes...	79
Figure 2.4	Root changes with time.....	80
Figure 2.5	Juniper Bay outflow dissolved reactive phosphorus.....	81
Figure 2.6	Changes in root concentration and root morbidity with time.....	82
Figure 2.7	Changes in redox potential (Eh) with time.....	83
Figure 2.8	Changes in the concentration of Fe(II) with time.....	84
Figure 2.9	Changes in the concentration of DOC with time.....	85
Figure 2.10	Changes in TP concentration with time.....	86
Figure 2.11	Regression of TP to Fe(II).	87
Figure 2.12	Regression of TP to DOC.....	88
Figure 2.13	Regression of DOC to Fe(II).	89
Figure 2.14	Change in tree height and tree trunk diameter.....	90
Figure 2.15	Biomass distribution in tree tops and roots.....	91

Figure 2.16	Mehlich III extractable P for all levels of the drained, flooded, and capillary fringe treatments.....	92
Figure 2.17	Mehlich III extractable Fe for all levels of the drained, flooded, and capillary fringe treatments.....	93
Figure 2.18	Microbial biomass C in the rhizosphere and matrix of all levels and water treatments.....	94
Figure 2.19	Microbial biomass P in the rhizosphere and matrix of all levels and water treatments.....	95

Chapter One

Literature Review

INTRODUCTION

Wetland restoration is an inherently complex process requiring consideration of hydrology, soil properties, and native plant species to be successful. Restoration activities on lands previously used for agriculture necessitate proper knowledge of wetland biogeochemical processes to minimize potential legacy pollution problems such as P dissolution. Numerous studies have examined the mechanisms of P dissolution in saturated soils, and the interactions of hydrophytic trees with their environments. In this chapter, P dissolution and plant-soil interactions will be discussed relative to the restoration of previously farmed wetlands.

WETLAND RESTORATION AND CAROLINA BAYS

Wetland Loss and Restoration

Wetlands provide a variety of functions that improve or protect the environment. Most wetlands provide habitat for a wide variety of plants and animals, while those on flood plains store water during floods, and many also improve water quality by removing sediment and nutrients from water passing through them (Galatowitsch and van der Valk, 1994). Despite these attributes, wetlands were drained since the 1760's and used for farmland or residential development. The U.S. government even provided incentives that encouraged wetland drainage for increasing agricultural production (Dahl and Allord, 1996).

Over two hundred years ago, at the time of early European settlement, there were approximately 89 million ha (221 million ac) of wetlands in the current conterminous US. By the 1980's only 42 million ha (103 million ac) remained (Dahl and Johnson, 1991). North Carolina has sustained an abnormally large alteration and loss of wetland area because it contained a high concentration of natural wetlands (Cashin et al., 1992b). It has been estimated that 3.2 million hectares (7.8 million ac) of wetlands existed in North Carolina in pre-colonial times (DEHNR, 1991). However, by the early 1990's approximately 50% of the wetlands in the NC Coastal Plain had been drained for agriculture or filled such that they ceased to perform their original functions (Cashin et al., 1992a).

Since the 1970's there has been an increased public awareness of the benefits of wetlands (Kean et al., 1988). Government policies have reversed stances on wetland drainage and now encourage "no net loss" of wetlands through policies such as the "Swampbuster" provisions of the 1985 Food Security Act that denied federal subsidies to any farm owner knowingly draining wetlands. The government has also increase awareness of wetlands through legislation like the Emergency Wetlands Resources Act of 1986 requiring the US Fish and Wildlife Service to publish any wetland trends (such as wetland loss or gain) (Dahl and Allord, 1996). The change in public opinion has resulted in an increase in wetland area on non-federal lands from 5,700 ha yr⁻¹ in 1992 to 20,600 ha yr⁻¹ in 2003 (USDA-NRCS, 2003).

Carolina Bays

One common wetland type in NC that has been drained for lumber and farmland is the Carolina bay. Ross (2000) described Carolina bays as distinctly elliptical, shallow, and

oriented depressions “sprinkled” across the Atlantic coastal plain from New Jersey to Georgia. All Carolina bays have an oval outline and are shallow, crater-like depressions bordered in whole or in part by rims of fairly white or buff-colored coarse sand (Johnson, 1942). The bays vary in size from hundreds of meters in diameter to 5 or 6 km long by 3 or 4 km wide. The depressions usually lie 5 to 15 m below the surrounding plain and are usually filled with dark clayey, organic-rich sand or silt, sandy or clay loam, and peat. The exact mode of their formation is unknown, but the leading hypothesis is that Carolina bays were created by wind blowing across shallow lakes on a beach plain (Kaczorowski, 1977). The long axis of the bays is oriented perpendicular to the direction of the prevailing winds.

Since many of these bays have been farmed, they have also accumulated P from agricultural fertilization. Fertilization often leads to over-saturation of inorganic nutrients in agricultural soils which occurs when more nutrients are applied than the crops can take up during the growing season (Bruland et al., 2003). In the examination of a previously drained and farmed Carolina bay wetland named Juniper Bay, Ewing (2003) observed extractable concentrations of P as high as 87 mg kg^{-1} in the mineral soils of the wetland compared to 0.3 mg kg^{-1} P in mineral soils of three nearby natural wetlands. Ewing (2003) also noted significantly more extractable P in the organic soils of Juniper Bay. Bruland et al. (2003) examined a Carolina bay where a portion of it had been restored, while the remainder was left in agricultural production. The section still in production had total P concentrations of 0.29 mg cm^{-3} compared to a reference pocosin wetland with 0.07 mg cm^{-3} of P.

PHOSPHORUS, SOIL REDUCTION, AND THE RHIZOSPHERE

Phosphorus as a Limiting Nutrient

Phosphorus is a limiting nutrient to primary production in many freshwater aquatic systems including lakes (Schindler, 1974), rivers, and streams (Reddy and DeLaune, 2008). Over-enrichment of nutrients, including P, to receiving waters causes eutrophication and excessive growth of aquatic plants and algae. When these plants and algae die, microbial decomposition decreases the dissolved oxygen in the water and this can result in fish kills and hypoxia (Correll, 1998). Concentrations of P as low as 0.100 mg L^{-1} in freshwater can cause eutrophication when other nutrients are not limiting (Correll, 1998).

Soil Reduction

When a soil is saturated, water fills most soil pores and limits gas exchange with the atmosphere. Because O_2 diffuses through water 10,000 times slower than it diffuses through air (Greenwood, 1961), the O_2 available for aerobic respiration of soil microbes and plant roots decreases precipitously in saturated conditions when biological activity occurs. Within hours of submergence, nearly all oxygen dissolved in the water is used through root and microorganism respiration and the soils become anaerobic (Ponnamperuma, 1972).

Oxidation-reduction reactions are chemical reactions where electrons are transferred from an electron donor (reducing agent) to an electron acceptor (oxidizing agent). This results in the reducing agent becoming oxidized through the loss of an electron, and the oxidizing agent becoming reduced by gaining an electron. These reactions are driven by the tendency of a system to decrease its free energy. If the redox reaction results in a net loss of free energy (ΔG) then the reaction is favorable and it will proceed (Ponnamperuma, 1972). In

reduced systems, the primary electron donor is organic matter through microbial respiration (Vepraskas and Faulkner, 2001).

When O₂ is present, it is the predominant electron acceptor for microbial oxidation of organic materials because its reduction provides the most free energy to the organism. When the supply of O₂ is depleted, microbial oxidation of organic materials will continue if alternate electron acceptors are available (Ponnamperuma, 1972). The most common alternate electron acceptors, in successive order of reduction, include: NO₃⁻, MnO₂, Fe(OH)₃, SO₄²⁻, and CO₂ (Kirk, 2004; Mitsch and Gosselink, 2007; Ponnamperuma, 1972; Vepraskas and Faulkner, 2001). Common reduction half reactions are shown in Table 1.1. The successive order of reduction is related to the redox potential. The redox potential (E_h) is a voltage that can be measured in the soil and used to predict the type of reduced species are present in the soil solution (Vepraskas and Faulkner, 2001). In strongly oxidizing systems the E_h is positive and high, while in strongly reducing systems the E_h is negative and low (Ponnamperuma, 1972). The thermodynamic basis for the redox potential is summarized by the Nernst equation:

$$E_h(V) = E_h^\circ - 0. \frac{059}{n} \log\left(\frac{(\text{reduced molecule})}{(\text{oxidized molecule})(H^+)^m}\right) \quad \text{[Equation 1]}$$

where E_h is the electrode potential (redox potential, volts, V) for the reaction, E_h° is the standard state potential for the half-reaction (volts, V) conditions, n is the moles of electrons involved in the reaction as written, m is the moles of protons involved in the reaction as written, and (reduced molecule) and (oxidized molecule) represent the activities of the reduced and oxidized species (McBride, 1994). The Nernst equation shows that each of the

main electron acceptors noted previously can produce a specific redox potential (Eh) which depends on the activity of the reduced species in solution, and on the activity of protons in solution. A modified version of the Nernst equation can be written using pH to substitute for the activity of protons (Vepraskas and Faulkner, 2001).

$$Eh(mV) = E^\circ - \frac{59}{n} \log + \frac{59m}{n} pH \quad \text{[Equation 2]}$$

For each of the reduction half-reactions in Table 1.1, there is a specific standard state redox potential (E°_h). The higher the E°_h the stronger the tendency for that half reaction to proceed as written (McBride, 1994). Under standard state conditions, thermodynamics predict that NO_3^- would be the first oxidant to become reduced, followed by MnO_2 and O_2 , respectively. However, standard state conditions are not realistic in soils. So, using the redox potential calculations in Table 1.1, An Eh-pH phase diagram is shown in Figure 1.1 illustrating the Eh values at which the reduction of each species would be favorable over a range of acidities (Vepraskas and Faulkner, 2001). In soils, where the pH is generally between 4 and 9 (McBride, 1994), the order would proceed as previously described with O_2 being reduced first, although the sequence can change for different pHs (Vepraskas and Faulkner, 2001). To further illustrate this, redox potentials at pH 6 are shown for each species in Table 1.1.

Reduction Microsites

In order for a soil to become reduced, it must first become anaerobic (Vepraskas and Faulkner, 2001). The extent that a soil becomes reduced – in terms of course, rate, and magnitude – depends on several factors including the kind of organic matter, the nature and content of electron acceptors, soil temperature, and duration of submergence

(Ponnamperuma, 1972). Considering this, and the heterogeneous character of soils, it is possible for reduction “microsites” or “hot-spots” to occur where the factors affecting reduction, such as availability of readily decomposable organic carbon, occur in small zones in the soil that may be less than 25 mm in diameter (Parkin, 1987). Such hotspots have been documented to form around dead roots and other actively decomposing organic matter with increased amounts of labile carbon (Christensen et al., 1990; Jacinthe et al., 1998; Parkin, 1987) where each study observed increased rates of anaerobic biogeochemical processes near a readily decomposable organic source – especially denitrification. Crozier et al (1995) observed increased production of CH₄ and reduced S gases in soils with increased labile C and noted that these gases may have been produced in microsites within each microcosm.

Microsites that form around particulate organic matter like dead roots can also lead to permanent soil morphological characteristics in hydric soils that persist during both wet and dry periods making them useful in the identification of hydric soils (Vepraskas, 1996). Hurt et al. (2001) stated that these hydric soil morphological characteristics form from the reduction, translocation, and/or accumulation of iron and other reducible elements during soil anaerobiosis when soil saturation is combined with microbial activity. The formation of these morphological characteristics is also discussed in detail by Vepraskas et al (2006). Example hydric soil field indicators, used in identifying hydric soils, that developed from these processes include F3, depleted matrix; F7, depleted dark surface; F12, iron-manganeses masses, and S6, stripped matrix (United States Department of Agriculture, Natural Resources Conservation Service, 2006; Vepraskas, 2010, personal communication).

Measuring Soil Reduction

The extent of soil reduction in a soil is characterized by determining which of the principal electron acceptors has been reduced in a soil at a particular time. Three methods are routinely used to evaluate the extent of reduction in the field: chemical analysis of the soil water, redox sensitive dyes, and redox potential measurements (Vepraskas and Faulkner, 2001). Chemical analysis of the soil solution identifies reduced species present. If Fe(II) or Mn(II) is observed in the soil solution then it can be assumed that the soil is reduced (Vepraskas and Faulkner, 2001).

Dyes, such as α,α' -dipyridyl are useful in field conditions (Childs, 1981). Heany and Davison (1977) demonstrated that α,α' -dipyridyl reliably identified the presence of reduced Fe in soils by turning from clear to a red color when it reacts with Fe(II). However, a test for Fe(II) using α,α' -dipyridyl dye will only tell us if the soil contains reduced Fe at the time the dye is applied. The soil may be reduced without the presence of Fe(II), and in such cases a positive reaction to the dye does not occur.

The third method to characterize the state of soil reduction is the measurement of redox potential through the use of Pt-tipped redox electrodes. The redox potential can be measured for a soil solution as a voltage that develops between the chemical species in a soil solution, and a set of reference conditions. Redox potentials in soils normally are measured using platinum microelectrodes. The Pt is a conductor of electrons from the reduced species in solution to a reference electrode. Redox (Pt-tipped) electrodes are constructed with a variety of methods, but the most common methods were described by Faulkner et al. (1989) and Patrick et al. (1996). A typical redox electrode consists of a Pt wire at the tip which is

welded or fused to a copper wire leading to a voltmeter. Non-Pt metal, such as Cu wire, in the electrode is coated with a non-conducting material such as heat-shrink tubing. Redox potential measurements are made in the field using calomel or silver/silver-chloride reference electrodes. If the soil is reduced, electrons will flow into the redox electrode through the Pt-tip. The potential produced against the reference electrode is measured using a voltmeter and is corrected accordingly depending on the type of reference electrode that is used.

Alternatively, if the soil is oxidized then electrons will be pulled from the redox electrode and a more positive redox potential will be measured. If the soil pH is known, the presence of oxidized or reduced chemical species in the soil can be predicted using an Eh-pH phase diagram (Figure 1.1) as described by Vepraskas and Faulkner (2001).

In reduced soils, pH usually changes following saturation and subsequent reduction because the reducing reactions consume electrons (Table 1.1). Ponnampereuma (1972) showed that acid soils tend to rise toward a pH of approximately 6.5, while in alkaline soils the pH tended to decrease toward a pH of 7. The pH can change as much as 3 units, but pH changes of less than 2 units is more probable (Vepraskas and Faulkner, 2001). The degree of change in pH depends on how much reduction is occurring and the amount of organic matter available for decomposition.

Phosphorus Dissolution Mechanisms in Flooded Soils

Understanding the mechanisms causing the release of P following wetland restoration on previously farmed soils is crucial to choosing management techniques that limit P released to drainage waters. When saturated soils become reduced, the release of P into solution has generally been attributed to five mechanisms including: 1) reduction of Fe that

binds phosphorus to the cation exchange sites; 2) ligand exchange competition for cations by organic anions produced under anaerobic conditions; 3) microbial mineralization of P bound by organic matter; 4) hydrolysis of iron and aluminum phosphates following an increase in pH in anaerobic, acidic soils; and 5) increased P diffusion in saturated, alkaline soils caused by decreased sinuosity.

Iron Reduction

The reduction of Fe is thought to be the dominant process controlling P solubility in anaerobic systems (Reddy and DeLaune, 2008). Phosphate is an anion and must bond to the negatively charged soil particles through the use of a cation bridge such as Fe, Al, or Ca. Under reducing conditions, Fe may be used by microbes as a terminal electron acceptor for their respiration. When this occurs, the Fe is reduced from ferric form (oxidized and non-soluble) to the ferrous Fe form (reduced and water soluble). When the Fe is reduced it dissolves into solution and releases the phosphate. The P may go into solution making it available to microbes or plants, or it may be transported through the soil to a drainage ditch and then be carried downstream. This P-release mechanism has been observed in many studies including Shenker et al (2005), Patrick and Khalid (1974), Vadas and Sims (1998), Sah and Mikkelsen (1986), and Holford and Patrick (1981).

Ligand Exchange

Phosphates (PO_4) can also be released into solution by a process called ligand exchange. In this mechanism, dissolved organic carbon (DOC) molecules compete with the negatively charged PO_4 for cations such as Fe and Al, because the DOC is a negatively charged organic compound like the phosphate. Thurman (1985) defines DOC as the organic

carbon in solution passing through a 0.45 µm filter. Under anaerobic conditions, DOC concentrations in the soil solution increases due to decreasing DOC adsorption, and inhibited decomposition of organic matter (Fiedler and Kalbitz, 2003). Dissolved organic carbon has been observed to form complexes with Fe and Al oxides (Dolfing et al., 1999) and to even solubilize Fe and Al (Gerke, 1992). Carboxylate anions from decaying organic matter and root exudates have been shown to compete with P for sorption sites on soils, and thereby displacing phosphate from the soil particle and into solution (Earl et al., 1979; Gerke, 1992; Lopez-Hernandez et al., 1986; Violante et al., 1991).

Phosphorus Mineralization

Phosphorus mineralization is the conversion of organic forms of P to inorganic forms of P, and the process is usually performed by microbes in the soil. Greaves and Webley (1965) found that the microbes with the ability to mineralize organic P were largely on the root surfaces and in the rhizosphere of pasture grasses, rather than the microbes in the soil matrix. Raghu and MacRae (1966) observed that in anaerobic environments, P mineralizing bacteria were stimulated by the rhizospheres around rice roots. The conversion of organic P to inorganic P is mediated by phosphatases which hydrolyze C-O-P ester bonds (Fox and Comerford, 1992). Fox and Comerford (1992) examined the activity of phosphatases in the rhizosphere of slash pine (*Pinus elliottii* L.) in a Leon fine sand (Aeric Haplaquod). Higher activity of phosphatases in the rhizosphere than in the matrix indicated that more P was mineralized in the rhizosphere. They also determined that the addition of inorganic P fertilizer inhibits phosphatases activity. Song et al. (2007) observed that repeated drying and

rewetting cycles of wetland soils increased phosphatase activity during the dry period. This high phosphatase activity increased amounts of inorganic P in solution after rewetting.

Changes in pH

The pH of a soil and soil solution can also contribute to P release. Phosphates associated with Fe(III) and Al(III) predominate in acidic soils while calcium phosphates are the dominant form of P in neutral or alkaline soils (Jackson, 1964; Ponnampetuma, 1972; Stumm and Morgan, 1981). In acidic systems the concentration of P in solution increases as the pH increases during reduction. In basic soils and basic solutions, P associated with calcium phosphates dissolves as the pH decreases (Stumm and Morgan, 1981), but the pH changes upon flooding of alkaline soils are smaller than the changes in pH of acidic soils (Turner and Gilliam, 1974a; 1974b). Thus, regardless of the initial pH, when a soil is saturated the reducing reactions cause the pH to move toward a pH of 7 and can cause release of P into solution.

Increased P-Diffusion

Turner and Gilliam (1974a and b) examined the release of P into the soil solution from several alkaline rice paddy soils. Using an anion exchange resin as a P sink, they found that more P was released in saturated soils than in moist soils. Turner and Gilliam (1974a) also observed that reducing environments did not increase the amount of P released into solution, and in fact P release peaked before any Fe reduction had occurred. They concluded that in alkaline systems where most P is bound to Ca not Fe, and where changes in pH following flooding were small due to low amounts of Fe being reduced, the increase in P concentration in the solution of flooded soils was caused by an increase in the rate of P

diffusion. Diffusion rates increased in saturated soils due to a lower sinuosity in the water filled pores, and this created shorter diffusion distances in the soils and a corresponding greater diffusion gradient. The results of these studies were in agreement of previous work done by Mahtab et al. (1971), and Olson et al. (1961).

The Rhizosphere

Curl and Truelove (1986) defined the rhizosphere as that narrow zone of soil subject to the influence of living roots as manifested by the leakage or exudation of substances that affect microbial activity. The 'bulk' soil is separate from the rhizosphere because it is generally not influenced by living roots except for the withdrawal of water and nutrients (Russell, 1977). Plant roots can have two very different effects on the rhizosphere under saturated conditions. In anaerobic soils, the roots may oxidize the rhizosphere through leakage of O_2 from the root. In addition, the root may act as a carbon source for microbes by releasing organic compounds (exudates) which can enhance further reduction of saturated soil.

Plant cells, including those in the roots, need oxygen to perform aerobic respiration (Cronk and Fennessy, 2001). Plants adapted to anaerobic soils have developed many physiological changes to increase O_2 in their roots and the rhizosphere. The most common aeration method is conduction of O_2 from the atmosphere to the roots through aerenchyma-plant tissue with large intercellular air spaces- that are contained within the leaves, stems, and roots. Molecular O_2 enters the plant through openings in the leaves or roots called lenticels, and moves by diffusion into the roots. In addition, other gases in the root can move in the reverse direction by diffusion and exit the plant through the lenticels (Colmer, 2003). The O_2 conducted to

the roots may then leak into the surrounding soil and oxygenate the rhizosphere. How far that O_2 penetrates into the soil depends on its rate of consumption and the rate of transport by diffusion out of the root and into the soil. Various aerobic processes take place in these aerated zones in the soil (Kirk, 2004). The aerenchyma also allow toxins such as ethanol and methane, products of anaerobic cellular respiration, to diffuse away from the roots minimizing their negative effect on root health (Vartepetian and Jackson, 1997). Other adaptations for growth in saturated soils, include adventitious roots, pneumatophores, prop roots and drop roots, and various stem adaptations (Cronk and Fennessy, (2001).

The rhizosphere can also be a place for increased rates of reduction in the soil, thereby acting as microsites. The types of exudates from plant roots that have been found in rhizospheres include carbohydrates, amino acids, organic acids, enzymatic proteins, and other various organic compounds (Russell, (1977). These exudates can be used by microbes in the rhizosphere as a C source resulting in further reduction of the rhizosphere in saturated soil to levels lower than the matrix where these exudates do not exist. Root death is also a common occurrence upon flooding when O_2 is deficient for too long a period. The O_2 demand is reduced when roots in deeper soil layers die (Schat, 1984) while the numbers of adventitious roots on the soil surface increase (Ernst, 1990; Schat, 1984). The roots that are sacrificed in deeper, less-oxidized portions of the soil may act as a carbon source for anaerobic microbes and drive continued reduction of the soil profile. Parkin (1987) described such sites of dead roots and plant residues as “hot-spots” where high rates of microbial activity due to the readily decomposable organic matter have been observed. These so-called “hot-spots” or “microsites” may contribute to additional P release from wetland soils by driving further reduction (enhancing mechanism 1) or by creating additional DOC (enhancing mechanism 2).

STUDY SPECIES: LOBLOLLY PINE AND BALD CYPRESS

Loblolly Pine

The trees of interest in this study are loblolly pine (*Pinus taeda* L.) and bald cypress (*Taxodium distichum* L.). Loblolly pine is abundant in the southeastern U.S. and is a common tree in the Coastal Plain, Piedmont, and the Mississippi River Valley land regions (Elias, 1980). Loblolly pine is the leading timber species in the southern U.S. (Harlow et al., 1991). This species grows in a range of soil types including low-lying, poorly drained soils to well-drained upland soils (Elias, 1980). This species does best in soils with deep surface layers having abundant moisture but poor surface drainage and fine-textured subsoils (Harlow et al., 1991). Loblolly pines often occur in pure stands but are commonly associated with a variety of hardwoods such as sweetgum (*Liquidambar styraciflua* L.), water oak (*Quercus nigra* L.), and sweetbay (*Magnolia virginiana* L.) on wet sites, and shortleaf pine (*Pinus echinata* Mill.) and southern red oak (*Quercus falcata* Michx.) on higher ground. Loblolly pines are important to wildlife due to the frequency of large seed crops (Elias, 1980).

The response of loblolly pine to saturated soil conditions has been well documented. In a review evaluating the flooding tolerance of southern U.S. trees, Hook (1983) described loblolly pine as moderately tolerant to waterlogging in comparison to all trees found in the south. Loblolly pine has been reported to exhibit temporary root injury and decreased growth following three months of saturation, but they can survive flooding durations that exceed one year but with some permanent root injury (Hunt, 1951). Injured roots are, however, still able to absorb water as long as they do not die (Hunt, 1951). Plant biomass, net photosynthesis,

and stomatal conductance of loblolly pine are reduced following soil saturation (Pezeshki, 1998). Survival rates of loblolly pine in anaerobic soils are not significantly reduced in severely anaerobic soils relative to moderately reduced soils (Pezeshki, 1998), but survival can be improved with P application in nutrient limited soils (Hook et al., 1983; McKee et al., 1984). This is attributed to improving the nutrient balance of P, K, Ca, and Mg due to improved function of roots (Hook et al., 1983).

Mechanisms used by loblolly pine to survive in anaerobic conditions include growth of adventitious roots, lenticel formation, and aerenchyma development with resulting decrease in root porosity (Topa and McLeod, 1986). These physiological changes lead to rhizosphere oxidation (Topa and McLeod, 1986). Topa et al. (1986) observed complete oxidation of the entire root system within 35 to 45 min. of the onset of saturation with the primary source of O₂ coming from the atmosphere and entering through the lenticels on the plant stem.

Bald Cypress

Bald Cypress is a conifer found in low, wet areas, primarily in the coastal regions of the southeastern U.S. It is generally found near streams, rivers, and particularly in swamps where it can occur in almost pure stands (Elias, 1980). The best growth of these trees is in deep, fine-sandy loams with adequate moisture in the surface layers and moderately good drainage. However, due to competition with hardwoods in well-drained soils, bald cypress is most commonly found in river bottomlands and swamps. This species is also known to extend its range into brackish tidewater areas but it grows poorly in these environments (Harlow et al., 1991).

The root system of the bald cypress can adapt quickly to changes in aeration status (1991). In poorly drained conditions, or where the water table fluctuates, the root system exhibits several descending roots providing anchorage and many shallow, wide spreading roots. Pneumatophores, commonly called “knees”, may protrude above the soil surface from these shallow roots which are commonly believed to anchor the tree or act as an aerating organ (Mitsch and Gosselink, 2007). However, Penfound (1938) argued that the knees do not occur when the tree is located in deep water – where knees would be needed most. Also, Kramer (1952) and Brown (1981) both observed that CO₂ is exchanged at the knees while O₂ is not, concluding that pneumatophores are not an aerating organ. Bald cypress is important to wildlife, especially waterfowl, as a food and cover source (Elias, 1980). In bottomlands and swamps, bald cypress is commonly associated with water tupelo (*Nyssa aquatic*), sweetgum (*Liquidambar styraciflua* L.), green ash (*Fraxinus pennsylvanica* L.), red maple (*Acer rubrum* L.), and American elm (*Ulmus Americana* L.) (Harlow et al., 1991).

Hook et al. (1983) described bald cypress as the most tolerant to flooding of any species found in the southern U.S. This tolerance is attributed to several physiological and metabolic changes that follow soil saturation. When soils become reduced below a redox potential of +200 mV, cells in the roots begin anaerobic metabolism and increase alcohol dehydrogenase activity (Pezeshki et al., 1996). This metabolic pathway limits the buildup of toxins and prevents root injury. Bald cypress also oxidize the rhizosphere (Anderson and Pezeshki, 2000; Colmer, 2003) to prevent buildup of phytotoxins such as sulfide (Koch et al., 1990). Aerenchyma also enhance rhizosphere oxidation (Pezeshki et al., 1996; Vartepetian and Jackson, 1997; Anderson and Pezeshki, 2000). Under sustained soil saturation, deep

roots may die while roots closer to the surface (adventitious roots) continue to live and grow (Schat, 1984; Conk, 2008).

Although root cells survive anaerobic conditions by undergoing anaerobic respiration (Pezeshki and DeLaune, 1990; Pezeshki, 1991), the energy generated is not adequate for root growth (Pezeshki, 1991). This results in limited root elongation in anaerobic environments (Pezeshki, 1991). Following soil saturation, net photosynthesis in bald cypress initially decreases for several weeks and then returns to normal rates (Pezeshki, 1998). The negative effects of waterlogged conditions on bald cypress is minimal (Pezeshki, 1998; Anderson and Pezeshki, 2000) in relation to the reduced survival rate, and reduced growth of other, less flood-tolerant species under saturated conditions (Conk, 2008).

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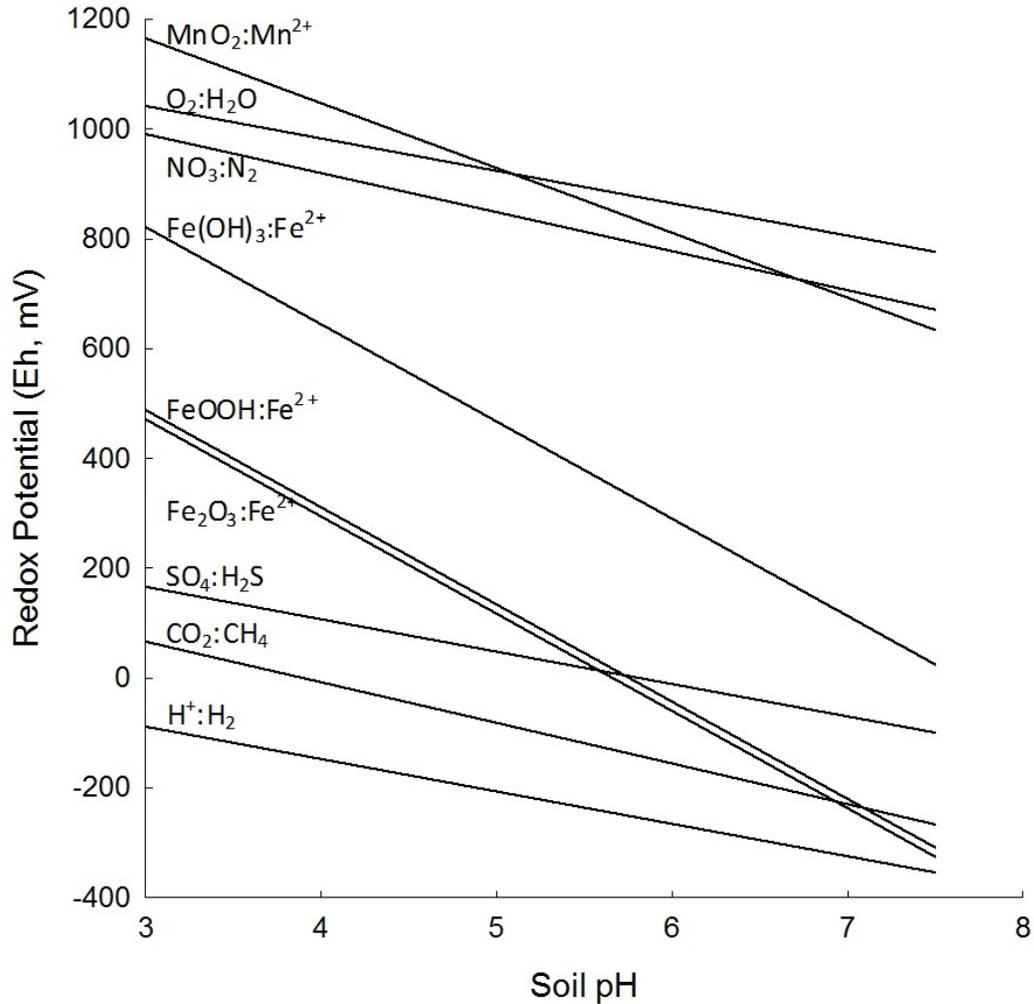


Figure 1.1. Eh-pH phase diagram for the reducing reactions shown in table 1.1. The lines were calculated for the following conditions: dissolved species were assumed to have an activity of 10^{-5} M, partial pressures for O₂ and CO₂ were 0.2 and 0.8 atmospheres, respectively, and partial pressures of the remaining gases were assumed to be 0.001 atm. This figure is from Vepraskas and Faulkner (2001) with permission.

Table 1.1 Common soil reduction half-reactions, redox potential calculations, standard state redox potential for half reactions, redox potentials at pH 6

Reaction ^a	Redox Potential (E _h) ^b =	E ^o _H ^a	E _h ^c , pH 6
	mV	mV	mV
$1/4\text{O}_2 + \text{H}^+ + \text{e}^- = 1/2\text{H}_2\text{O}$	$1229 + 59\log(P_{\text{O}_2})^{1/4} - 59\text{pH}$	1229	885
$1/5\text{NO}_3^- + 6/5\text{H}^+ + \text{e}^- = 1/10\text{N}_2 + 3/5\text{H}_2\text{O}$	$1245 - 59[\log(P_{\text{N}_2})^{1/10} - \log(\text{NO}_3)^{1/5}] - 71\text{pH}$	1245	778
$1/2\text{MnO}_2 + 2\text{H}^+ + \text{e}^- = 1/2\text{Mn}^{2+} + \text{H}_2\text{O}$	$1224 - 59\log(\text{Mn}^{2+})^{1/2} - 118\text{pH}$	1230	664
$\text{Fe}(\text{OH})_3 + 3\text{H}^+ + \text{e}^- = \text{Fe}^{2+} + 3\text{H}_2\text{O}$	$1057 - 59\log(\text{Fe}^{2+}) - 177\text{pH}$	935	290
$\text{FeOOH} + 3\text{H}^+ + \text{e}^- = \text{Fe}^{2+} + 2\text{H}_2\text{O}$	$724 - 59\log(\text{Fe}^{2+}) - 177\text{pH}$	667	-43
$1/2\text{Fe}_2\text{O}_3 + 3\text{H}^+ + \text{e}^- = \text{Fe}^{2+} + 3/2\text{H}_2\text{O}$	$707 - 59\log(\text{Fe}^{2+}) - 177\text{pH}$	793	-60
$1/8\text{SO}_4^{2-} + 5/4\text{H}^+ + \text{e}^- = 1/8\text{H}_2\text{S} + 1/2\text{H}_2\text{O}$	$303 - 59[\log(P_{\text{H}_2\text{S}})^{1/8} - \log(\text{SO}_4^{2-})^{1/8}] - 74\text{pH}$	308	-103
$1/8\text{CO}_2 + \text{H}^+ + \text{e}^- = 1/8\text{CH}_4 + 1/4\text{H}_2\text{O}$	$169 - 59[\log(P_{\text{CH}_4})^{1/8} - \log(P_{\text{CO}_2})^{1/8}] - 59\text{pH}$	172	-163
$\text{H}^+ + \text{e}^- = 1/2\text{H}_2$	$0.00 - 59\log(P_{\text{H}_2})^{1/2} - 59\text{pH}$	0	-266

Reactions and standard state redox potentials summarized from Essington (2004)

^bRedox potential calculations from Vepraskas and Faulkner (2001)

^cRedox potentials calculated using the redox potential equations listed and assumptions listed on Figure 1.1 at pH 6

Chapter Two

Phosphorus mobilization in the rhizospheres of bald cypress grown in wetland soils

ABSTRACT

Phosphorus release to ground or surface waters is commonly observed after restoration of wetlands that were drained and farmed, but the primary causal mechanism is unknown. This study examined whether the P release was originating from the rhizospheres, or soil matrix of restored wetland soils. Experiments were conducted in rhizotrons (glass-walled boxes) which were filled with Ap horizon material from a restored wetland. Phosphorus release was monitored from the rhizospheres of bald cypress roots (*Taxodium distichum* L.), and an unplanted control representing the soil matrix. Three water treatments (flooded, capillary fringe, and drained) were imposed on both planted and unplanted treatments for 125 d, and soil water was collected twice monthly at three depths. Numbers of live and dead roots were determined monthly. Rhizosphere processes did not increase P concentrations in the soil solution compared to the controls. In the planted treatment, TP concentrations were equal to or lower than the control (peak TP: 700-900 $\mu\text{g P L}^{-1}$) depending on sampling date and depth. The rhizospheres produced larger amounts of DOC and Fe(II) than the matrix. Dissolved P concentrations were equal to dissolved P concentrations in the matrix during the first 54 d, but lower than those of the matrix thereafter, most likely due to plant uptake. Tissue analysis and soil extractions showed that bald cypress was able to accumulate excess P equally under each water treatment, and to immobilize the P to reduce potential export downstream. These

findings indicate plant rhizospheres do not contribute to P release but actually reduce it, likely due to plant uptake.

INTRODUCTION

In recent years, wetlands have become recognized for their ecological services by the public (Kean et al., 1988), and by federal and state governments that have enacted legislation to protect wetlands. A major piece of legislation included the “Swampbuster” provisions of the 1985 Food Security Act that denied federal subsidies to any farm owner who knowingly drained wetlands (Dahl and Allord, 1996). Wetland protection has increased the amount of land being restored to wetland in the last two decades (USDA-NRCS, 2003) through wetland mitigation. In the southeastern U.S., wetland restoration efforts have focused on the reforestation of bottomlands that were cleared for agricultural production and later sold for restoration or were restored through federal wetland programs (Clewell and Lea, 1990). While such restoration efforts are believed to be beneficial, they may come at a cost to the environment. The annual addition of nutrients to farmland has caused high levels of nutrients such as P to remain in the soil after farming has ceased (Ewing, 2003). Once land is restored to wetland, residual nutrients may be leached from the soil and pollute surface waters downstream of the restored wetland.

Phosphorus is a major nutrient added to soils in fertilizer, and one that can be released to groundwater under anaerobic conditions (Ponnamperuma, 1972). Phosphorus is an essential nutrient for all living organisms and is often a limiting nutrient for growth of algae in freshwater systems such as lakes (Schindler, 1974), rivers, and streams (Reddy and

DeLaune, 2008). It is usually stable in the environment when it is held to soil particles by Fe and Al oxides in acidic soils, or incorporated into soil organic matter (SOM) (Dunne and Reddy, 2005). Phosphorus can accumulate in agricultural soils with regular fertilizer additions when P is applied at rates greater than what crops can utilize in one year (Bruland et al., 2003; Compton and Boone, 2000; Ewing, 2003). When soils that contain high levels of P are used in wetland restoration, the raised water table and resulting soil saturation creates anaerobic conditions that cause P to dissolve into the soil pore water (Ponnamperuma, 1972).

One wetland type that has been drained for agriculture extensively in the southeastern U.S. are Carolina bays. Carolina bays are elliptically shaped wetlands oriented in the northwest-southeast direction that are found in the southeastern Coastal Plain (Kaczorowski, 1977). Up to 50% of the Carolina bays in Bladen County, North Carolina were drained by 1982 primarily for row crop production, but are now being restored back to wetlands (Weakley and Scott, 1982). Fertilizer applications to farmland have led to the accumulation of P which must be taken into account in restoration efforts to prevent inadvertent pollution of drainage waters.

For the drained and farmed Carolina bay wetland named Juniper Bay in N.C., Ewing (2003) observed Mehlich III extractable P concentrations as high as 87 mg kg^{-1} in the mineral soils of the wetland after 30 years of agricultural production. These concentrations were significantly higher ($p < 0.1$) than the 0.3 mg kg^{-1} P found in mineral soils of three nearby natural wetlands. Ewing (2003) also noted significantly more ($p < 0.1$) Mehlich III extractable P in soils with histic epipedons of Juniper Bay. Bruland et al. (2003) examined a Carolina bay in Cumberland, County, N.C. where a portion of it had been restored, while the

remainder was left in agricultural production. The land still in production had total P concentrations of 0.29 mg cm^{-3} compared to a nearby, unfarmed, reference pocosin wetland with 0.07 mg cm^{-3} of P.

In anaerobic systems, the dissolution of P from acidic soils is commonly attributed to two main mechanisms – reduction of Fe that binds phosphorus to soil particles (mechanism no. 1); and ligand exchange of the anionic P molecule by organic anions produced under anaerobic conditions (mechanism no. 2). Mechanism no. 1 is considered to be the dominant mechanism controlling P solubility in anaerobic systems (Reddy and DeLaune, 2008), because P most commonly occurs as a phosphate anion (PO_4) that is bound to soil particles through cation bridges such as Fe(III), Al(III), and Ca(II). When the soil becomes anaerobic, Fe(III) becomes microbially-reduced to soluble Fe(II) and releases the P bonded to it to the soil solution. This mechanism has been well documented in many previous studies ((Holford and Patrick, 1981; Patrick and Khalid, 1974; Sah and Mikkelsen, 1986; Shenker et al., 2005; Vadas and Sims, 1998).

When O_2 becomes less available for root and microbial respiration in saturated, reduced systems, the decomposition of organic compounds is slowed and dissolved organic carbon (DOC) accumulates in the soil solution (Fiedler and Kalbitz, 2003). Thurman (1985) defined DOC as organic carbon in solution that passes through a $0.45 \text{ }\mu\text{m}$ filter. The DOC molecules are negatively charged, and in large concentrations can compete with anionic PO_4 compounds for bonding to cations such as Fe and Al. Dissolved organic carbon has been observed to form complexes with Fe and Al oxides (Dolfing et al., 1999) and to even solubilize Fe and Al (Gerke, 1992a). Carboxylate anions from decaying organic matter such

as dead roots, and from root exudates have been shown to displace P from the soil particle into solution (Earl et al., 1979; Gerke, 1992a; Lopez-Hernandez et al., 1986; Violante et al., 1991).

Other mechanisms of P dissolution in anaerobic systems that have been reported include P mineralization (Fox and Comerford, 1992; Greaves and Webley, 1965; Raghu and MacRae, 1966; Song et al., 2007), changes in pH (Ponnamperuma, 1972; Stumm and Morgan, 1981), and increased P diffusion (Mahtab et al., 1971; Olsen et al., 1961; Turner and Gilliam, 1974a; Turner and Gilliam, 1974b). These mechanisms are more applicable in alkaline soils and are limited in magnitude in acidic soils so they will not be discussed here. In acidic soils, Fe-reduction and DOC competition are considered the most likely mechanisms governing P release in soils and will be the focus of this study.

While it is well known that soil P dissolves into the solution when the soil becomes reduced, the location of release in the soil (e.g. matrix vs. rhizosphere) is not well documented. Reduction has been found to occur in “microsites” or “hot-spots” that develop around residues of readily decomposable organic carbon (Parkin, 1987). The microsites may be less than 25 mm in diameter (Parkin, 1987), and form around dead roots and other actively decomposing organic matter with increased amounts of labile carbon (Christensen et al., 1990; Jacinthe et al., 1998; Parkin, 1987). The microsites are where denitrification, CH₄ production, and reduced S gases have been produced in soils (Crozier et al., 1995; Parkin, 1987). It is likely that they are also the zones where P dissolution occurs as well.

We hypothesized that most soil P dissolution occurs in microsites following saturation and reduction, and that most active microsites will be those in the rhizosphere. To

examine these hypotheses, the objectives of this study were to: 1) determine the impact of rhizosphere processes on the production of dissolved P, Fe, and DOC in simulated wetland and drained wetland soils, and 2) evaluate the potential of plant uptake for decreasing P in the soil solution of restored wetlands. Because a restored wetland experiences periods when the soils are flooded and drained during the course of a year, the experiments included three water treatments-drained, capillary fringe, and flooded-that simulated the hydrologic conditions in a restored wetland.

MATERIALS AND METHODS

Site Description

The research consisted of two parts: 1) monitoring outflow water from a restored wetland to evaluate the magnitude of P released, and 2) evaluating the impacts of rhizosphere microsites in releasing P in the soil of the restored wetland. The field site was a Carolina bay wetland named Juniper Bay that was drained and used for agriculture from 1970 through 2000. It was fertilized and limed annually to meet soil test recommendations for crop production (Ewing, 2003). In 2000, it was acquired by the North Carolina Department of Transportation (NCDOT) which restored it back to wetland in two phases. Plants were established in 2001, and the ditches were filled in 2005 to restore the wetland hydrology.

Beginning in February 2002, water samples were taken from the single outflow ditch (Figure 2.1) of Juniper Bay using a 30 ml syringe. Water samples were then immediately passed through a 0.45 μm membrane filter into a 20 ml bottle containing one drop of 6 N HCl as a preservative. Dissolved reactive phosphorus (DRP) was measured colorimetrically

using a multi-channel Quick Chem 8000 (Lachat Instruments, Milwaukee, WI, USA) as described by Prokopy and Wendt (1994).

Rhizotron Construction

Greenhouse studies were conducted in rhizotrons (Plexiglas-walled boxes) to compare the effects of rhizosphere and matrix microsites on P dissolution. Twenty-four rhizotrons were constructed (Figure 2.2) using a design similar to that of Neufeld (1989). Each rhizotron consisted of a frame and two windows for root viewing, and was constructed to hold 8.7 L of soil (interior dimensions - 48.3 cm tall by 30.5 cm wide by 5.7 cm deep). The frame consisted of 2.5 cm thick expanded PVC (Aqua-Plas, Piedmont Plastics Inc., Raleigh, NC). The Aqua-Plas frame formed the two sides and the bottom, leaving the top open. Each piece was fastened together using PVC glue and sheetrock screws. During assembly, a bead of silicone sealant (Dow-Corning, Midland, MD, USA) was applied to the frame. A custom-made, black, neoprene gasket (Raleigh-Durham Rubber & Gasket Co., Inc, Raleigh, NC) was then placed on top of the silicone, and vacuum grease was applied to the outside of the gasket. The two polycarbonate (LEXAN, SABIC, Riyadh, Saudi Arabia) faces were attached to the front and back of the frame to allow for root observation and sample collection. Each face was pre-drilled and attached using sheetrock screws to provide a water-tight seal. Two holes, one on each side piece of the frame, were fitted with drains near the bottom which consisted of nylon elbows threaded on one end and barbed on the other. Landscape fabric was glued over each drain on the inside of the rhizotron to limit loss of soil during water drainage out of the rhizotron.

Soil and Tree Saplings

Following assembly, a layer of #2 well sand was added to each rhizotron to a depth of 2.5 cm from the bottom to facilitate uniform drainage. Each rhizotron was then packed with Ap horizon material collected from a Leon sand (Aeric Haplaquod) at Juniper Bay in February, 2009 (Figure 2.1). Soil was added in increments to the rhizotrons and tamped by hand to a bulk density of 1.45 g cm^{-3} (corrected for moisture content) that simulated field conditions at Juniper Bay. Soil was added to each rhizotron until approximately 2.5 to 5 cm of space was left between the soil surface and the top of the rhizotron.

Bald cypress (*Taxodium distichum*, L.) was chosen as the study species because it was used in the restoration of Juniper Bay and was well adapted to saturated conditions (Conk, 2008). One hundred bald cypress saplings were purchased from the North Carolina Division of Forest Resources Nursery (Goldsboro, NC, USA). Fifteen of these saplings with uniform root and shoot size were selected for this experiment. In planted rhizotrons, the soil was packed to the appropriate bulk density while taking care to avoid injuring the roots. During the initial growth phase the saplings were grown in the rhizotrons under field moisture conditions for four months and were watered using tap water.

Rhizotron Location and Growth Angle

Each rhizotron was supported at a 30° angle from the vertical during the growth phase to force the roots to grow downward against the front face for examination and sampling. To minimize light exposure on the faces of the rhizotrons, removable sheet-metal covers were placed over the front and back face of each rhizotron and were held in place using binder clips. Edges of the polycarbonate were also covered with aluminum tape to minimize light penetration through the sides of each rhizotron.

The rhizotrons were located on two adjacent greenhouse benches. Each rhizotron was numbered and randomly assigned to a spot on the bench. Locations of each rhizotron were randomly reassigned monthly to avoid any confounding effect associated with locations in the greenhouse.

Preliminary Redox and Water Tension Measurements

Redox potential and soil matric potential were monitored during the initial growth phase to ensure that the trees had adequate moisture but not enough to cause anaerobic conditions. Redox potential was measured using platinum-tipped electrodes constructed by a method similar to that of Wafer (2004). Prior to installation, each electrode was tested in Light solution (Light, 1972) and those registering within 10 mV of 420 mV were selected for use. Each electrode was installed to a depth of 24 cm from the soil surface in each rhizotron. A calomel reference electrode and voltmeter were used during measurement of redox potential. Redox measurements were made weekly to ensure over-watering and soil reduction was not occurring during the growth stage. All voltage readings were converted to redox potential (Eh) by adding a 250 mV correction factor.

One tensiometer was installed in each rhizotron to monitor soil water potential. Tensiometers were constructed following the method of Cassel and Klute (1986). The soil water potential was measured four times or more weekly using a vacuum gage (Tensimeter, Soil Measurement Systems, Tucson, AZ). Rhizotrons were kept at field capacity, defined as the soil matric potential between -100 and -300 cm for this experiment.

Statistical Design

The experiment was a completely randomized design. Prior to packing and planting, each of the 24 rhizotrons was randomly assigned to a plant treatment (planted or unplanted). To simulate the conditions in the rhizosphere, 15 rhizotrons were planted with cypress saplings, one sapling per rhizotron. The remaining nine rhizotrons were packed without a tree to simulate the matrix. Both planted and unplanted rhizotrons were watered similarly and kept under the same greenhouse conditions during the growth phase.

One of three water treatments were imposed on each rhizotron to establish different levels of reducing conditions and, we hypothesized, different concentrations of P released: (i) drained, (ii) capillary fringe, and (iii) flooded. Water treatments were randomly assigned to each of the rhizotrons yielding five planted and three unplanted replicates for each water treatment. The water treatments were established in May of 2009, marking the end of the growth period, and each treatment was maintained through completion of the experiment on September 2009.

In the drained water treatment the conditions that were imposed during the growth period were continued to the end of the experiment. In the capillary fringe treatment, each rhizotron was connected to a Marriot bottle via one drain outlet and Teflon tubing. A vertical

viewing tube was installed on the other drain outlet using clear Teflon tubing. This viewing tube allowed measurement of the simulated groundwater table in each rhizotron. For the capillary fringe treatment, no water was added from the surface during the duration of the experiment to avoid inadvertently raising the water table. The water table in all five capillary fringe rhizotrons was controlled by raising or lowering the glass tube in the Marriot bottle. For the duration of the treatment, the water table remained at a depth of approximately 41 cm. In the flooded treatment, water was added to the rhizotron through the drains so that air within the soil was expelled out the top of the rhizotron. Water was added to the rhizotron from the bottom up until several cm of water were ponded on the surface. Additional water was added daily to maintain 1 cm of water on the surface.

Rhizon Sampler and Redox Electrode Installation

Samples were collected from three layers in each rhizotron during the course of the experiments: top (0-22 cm), middle (22 to 41 cm), and bottom (41 to 59 cm). One Rhizon Flex Pore water sampler (Item # 19.21.26, Rhizosphere Research Products, Dolderstraat 62, NL 6706JG Wageningen, The Netherlands) was installed within each layer of each rhizotron to collect water samples. In planted treatments, the Rhizon sampler was placed adjacent to a root to collect samples from the rhizosphere. In unplanted treatments, a Rhizon sampler was placed in each of the three layers to collect samples from the soil matrix.

Rhizon samplers were used because they do not absorb P from solution as do porous ceramic samplers (Bottcher et al., 1984; Nagpal, 1982; Zimmermann et al., 1978). The sampling tips of the Rhizon samplers are made of hydrophilic plastic that does not affect solution P concentration (Shotbolt, 2009). The sampling tips have a pore size of 0.1 μm

making additional sample filtration unnecessary (2009). The Rhizon samplers also do not change sample redox conditions under normal sampling environments, and provide many other improvements over porous cups for pore water sampling as described by Shotbolt (2009).

Samplers were shortened to 3 cm to ensure samples were only extracted from the rhizosphere of the root that was visible through the rhizotron face. Each sampler tube was cut to the appropriate length and then one end dipped in epoxy to seal it (Epoxy Kit, Item #0980V, Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Prior to sampler installation, rhizon samplers were stored for 24 hours in deionized water with the hydrophilic tips submerged to saturate them for use. One day prior to the start of the water treatments, samplers were installed by inserting them through a hole that was drilled through the face of each rhizotron (as shown in Figure 2.3. Silicon was applied around the portion of the sampler tube that protruded from the rhizotron face to ensure water-tight and air-tight seals.

Redox electrodes were also installed within 1 cm of each rhizon sampler to monitor Eh in both the matrix and rhizospheres. These redox electrodes were constructed as described previously and were installed through a hole that was drilled through the face of each rhizotron. The heat-shrink tubing that covered each redox probe created a waterproof seal around each sampler.

Sampling

Soil pore-water sampling was performed twice monthly beginning one day after water treatments were established, and continuing through the end of the experiment. Two amber-colored serum bottles (100 ml and 30 ml) were used to collect soil pore water samples

from each sampler. Prior to sampling, each bottle was acid-washed and dried, acidified with 0.250 ml of 12 M H₂SO₄, capped with a rubber septa and aluminum cap, and evacuated to -700 cm H₂O or greater pressure using an electric vacuum pump. The 100ml bottle was used for collecting samples for the analysis of dissolved Fe(II), reactive P (DRP), and total P (TP). The second bottle was left un-acidified and used to collect samples to measure DOC and pH. To ensure samples were collected from the drained treatment, each drained rhizotron was saturated with water for 4 hours prior to sampling to allow the soil solution to come to equilibrium with the soil. After sample collection, the excess water was removed from the rhizotrons in the drained treatment drained before reducing conditions developed.

Water samples were collected through a 25-gauge, 3.8 cm long, Luer-lock needle that was attached to a Rhizon sampler (Figure 2.3). The sampling tube was first purged by inserting the needle into an evacuated serum bottle to collect one Rhizon sampler volume (0.187ml) (Soilmoisture Equipment Corp., 2008) or more of water. After purging, the sampler needle was inserted into the septa of the 30 ml serum bottle to collect 15 ml of solution. Following this, the needle was inserted into the 100 ml bottle to collect approximately 30 ml of solution. In cases where 30 ml of solution were not collected within an 8 hour period, the sample collection was stopped and the collected solution analyzed. All serum bottles used in sampling were suspended from the top of the rhizotron using string and binder clips to reduce stress on the sampler (Figure 2.3).

Samples contained in the 30 ml bottle were frozen within 30 min. of collection, and were not thawed until analyzed. Samples in the 100 ml bottles were not frozen but were stored in the dark at room temperature. Redox potential was measured on all Pt electrodes

using a voltmeter and calomel reference electrode that was placed in contact with the soil surface at the top of the rhizotron. All voltage readings were corrected to Eh by adding 250 mV to account for the calomel reference electrode.

Sample Analyses

Total phosphorus (TP) was analyzed colorimetrically using a multi-channel Quick Chem 8000 (Lachat Instruments, Milwaukee, WI, USA) as described by Liao (2001).

Dissolved reactive phosphorus (DRP) was measured colorimetrically, simultaneously with TP using a multi-channel Quick Chem 8000 (Lachat Instruments, Milwaukee, WI, USA) as described by Prokopy and Wendt (1994).

The concentrations of Fe(II) in solution were measured colorimetrically using the phenanthroline method (Joint Task Group: 20th Edition, 2005). Phenanthroline reagent was added to all samples within 24 hr of collection, and the samples were analyzed using a Shimadzu UV-2101PC spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) with absorbance measured at 510 nm within 48 hr of sampling. Reactions took place in low light in a fume hood with care being taken to limit exposure of samples to oxygen before phenanthroline reaction. A blank sample not containing phenanthroline was used to correct the absorbance reading for the impact of dissolved organic matter. A standard curve was produced prior to each sampling. Standards were made using ammonium iron(II) sulfate hexahydrate (99.997%) and stored at room temperature in the dark until use.

Solution pH and DOC were determined using samples in the unacidified 30 ml serum bottles. The frozen samples were thawed and allowed to return to room temperature.

Solution pH was measured using an Accumet Portable AP62 pH/mV Meter (Fisher

Scientific, Pittsburgh, PA, USA) calibrated to pH range 4-7. The sample solution was then immediately analyzed for DOC using a Shimadzu TOC-5050 total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Dissolved organic carbon is organic C that passes through a 0.45 μm filter, while rhizon samplers used in this study have a smaller pore size (0.1 μm). The DOC in this study will be operationally defined to the smaller pore size.

Tree and Soil Measurements

Tree height was measured monthly by recording the distance (cm) from the top of the rhizotron to the uppermost piece of foliage using a meter stick. The diameter of the base of the tree was recorded 1 cm above the soil surface using a caliper that was accurate to the nearest mm.

Root counts began prior to the period when water treatments were established and were continued monthly thereafter. A grid consisting of 5 cm by 5 cm squares was drawn on one window of each planted rhizotron (Figure 2.4). The number of roots that had lengths greater than or equal to 2 cm within each grid square were counted manually. The counted roots were also classified as dead or alive based on appearance. Roots that were entirely black in color were considered dead, while those that were white or brown and appeared healthy were considered alive. The concentration of live and dead roots in each rhizotron level was calculated by summing the total number of roots counted in each grid square in each level and dividing by the total area of each level. At the conclusion of the experiments the rhizotrons were opened and the roots examined to ensure that these color criteria for root morbidity were valid.

Plant tissue samples from the leaves, tops, and roots of each tree were dried, ground, and submitted to the North Carolina State University Environmental and Agricultural Testing Service Laboratory, Raleigh, NC for analysis. Extractable soil P and Fe were measured using the Mehlich-III extractant (Mehlich, 1984). Filtered extraction solutions were submitted to the North Carolina State University Environmental and Agricultural Testing Service Laboratory, Raleigh, NC where they were analyzed for TP and total Fe (TFe) using an inductively coupled plasma-atomic emission spectrometer (Model Optima 2000, Perkin-Elmer, Waltham, MA, USA).

A chloroform fumigation method described in detail by Witt et al. (2000) was used to estimate the microbial biomass C and P in soil samples retrieved from each sampling site. This method uses direct addition of chloroform to the soil sample and is appropriate for saturated soils. The amounts of microbial biomass C (MBC) and P (MBP) measured for each sample were reported on a dry-soil basis. Correction factors of 0.35 and 0.4 were used for MBC and MBP, respectively, as recommended by Witt et al. (2000).

Statistical Analysis

This study used a completely randomized design. Rhizotron levels were nested within each individual rhizotron in the PROC MIXED analysis performed in SAS version 9.2 software (The SAS Institute, Cary, NC 2010) for the analysis of the repeated soil porewater samples measurements. The difference of least square means with a Tukey-Kramer adjustment for multiple comparisons was used to analyze for differences among the plant growth and tissue, soil extraction, microbial biomass analyses. Log-transformations were used in the analysis of DOC, Fe(II), TP, and DRP, MBC, and MBP though original means

are reported. A square root transformation was used on the root concentration data. All graphing and regression analysis was performed using Sigma Plot version 11.0 (Systat Software Inc., San Jose, CA, USA).

RESULTS

Juniper Bay Outflow DRP

The concentration of DRP in the outflow water from Juniper Bay is shown in Figure 2.5. Prior to restoration the concentrations of DRP in the drainage water rarely exceeded $100 \mu\text{g P L}^{-1}$, the threshold concentration of P at which eutrophication of freshwaters is likely (Correll, 1998). Juniper Bay was restored to wetland hydrology in September of 2005 by plugging each drainage ditch leaving only the perimeter ditch open. After restoration the concentrations of DRP regularly exceeded $100 \mu\text{g P L}^{-1}$ indicating that the elevated concentration of P in this agricultural soil, combined with the newly-formed saturated conditions from restoration, were causing Juniper Bay to act as a P source for downstream pollution. Our hypothesis was that the elevated P levels originated in microsites around rhizospheres of the wetland vegetation.

Rhizotron Studies

While three water treatments were evaluated, the discussion of results will focus on the flooded treatment because it was the treatment that produced the most TP in solution. The drained and capillary fringe treatments will be discussed as comparisons to the flooded treatment for the various measurements of the saplings, microbial community, and soil extractions.

Root Distribution

All treatments had the same mean concentration of roots (approximately 0.2 roots cm⁻²) in each of the three sampling levels prior to imposition of the water treatments.

Distributions of bald cypress roots in the rhizotrons are shown in Figure 2.6 for the flooded treatment, both before and after the flooding was imposed. Changes in root distribution following imposition of water treatments were most apparent in the flooded treatment as illustrated in Figure 2.4. Root concentrations changed over time after flooding, with different responses in the top, middle, and bottom layers of the rhizotrons. Following flooding (on day 0), all roots in the top layer remained alive throughout the study, and new roots continued to grow raising the root concentration to a maximum of 1 root cm⁻² by the end of the study. New roots, as visually noted with white root tips, grew horizontally from the tap root to the edges of the rhizotron, and then proceeded downward to the levels below. In the middle layer there was little root death, and healthy living roots elongated until completion of the experiment.

Roots continued to die in the bottom layer through 28 d as the anaerobic soil environment caused root injury and killed approximately one third of the original living roots (Figure 2.6). After 50 d, new white-tipped roots, presumably adapted to the anaerobic conditions, grew into the lower level raising the concentration of live roots. These results showed that the distribution of bald cypress roots shifted from the bottom level to the top following flooding. However, over time roots growing in the top section elongated sufficiently to reach the bottom level. The rhizospheres of the new roots entering the bottom level were not sampled in this study.

No roots died in the drained treatment in any of the three sampling levels. Root concentrations increased to 0.09, 0.34, 0.64 roots cm⁻² in the top, middle, and bottom levels, respectively, after 122 d showing that the most growth had occurred in the bottom level while little growth had occurred near the surface. Root distributions for both living and dead roots in the capillary fringe treatment were similar to those observed in the flooded water regime for the middle level (capillary fringe) and bottom level (saturated conditions). The top level of the capillary fringe showed less growth than the flooded treatment and more growth than the drained treatment at that level.

Chemical Analyses

The analysis of fixed effects of the soil porewater chemistry measurements are shown in Table 2.2 where significance and variability are shown using p-values. The controlled effect is the labeled effect in that row while the effect being analyzed is the variable left out of each row.

Redox Potential

Prior to imposition of the water treatments, all Eh values in the matrix and rhizosphere of all treatments ranged between 100 to 300 mV. In the flooded treatment, the Eh in the matrix decreased into the Fe reduced range in all sampling layers after flooding was imposed (Figure 2.7), and the matrix Ehs were not significantly different among layers ($p=0.7474$) during any single day.

On the other hand, rhizosphere Eh values in the flooded treatment did differ significantly with depth ($p<0.0001$). The rhizospheres in the top layer remained the most oxidized throughout the experiment (Figure. 2.7), where the mean Eh never fell below -43

mV which is the Eh at which goethite (FeOOH) is reduced to Fe(II) at pH 6 (Vepraskas and Faulkner, 2001). Rhizosphere Ehs were also significantly ($p=0.0029$) higher than those of the matrix in the top layer. This suggested that the rhizosphere in the top layer was being oxygenated by the roots. In the bottom layer, the rhizosphere Eh declined to a minimum of -200 mV by 30 d and then increased to -100 mV at 111 d. The rhizosphere Eh was significantly higher ($p<0.0001$) than that of the matrix for selected time periods in the bottom layer, possibly caused by roots from the top layer growing into the bottom layer and oxidizing the rhizospheres of the new roots. This could not be confirmed because Ehs in the rhizospheres of the new roots were not monitored in the bottom layer. The Ehs in the rhizospheres and matrix of the middle layer of the flooded treatment were not significantly different on any sampling day ($p=0.1156$ and $p=0.8104$ respectively).

The drained treatment maintained oxygenated conditions in most layers with Eh values ranging between 450 and 715 mV for the duration of the study in both the rhizosphere and matrix. The matrix in the bottom layer was an exception as the Eh fluctuated between 25 and 490 mV due to overwatering and the absence of transpiring plants in this treatment. In the capillary fringe treatment, the Eh values in the bottom layer were similar to those in the flooded treatment while Eh values in the upper layer in were similar to those in the top layer of the drained treatment. In the capillary fringe itself (middle layer at depths of 22 to 41 cm), the rhizosphere was less reduced than those in the flooded treatment and reached a minimum of -25 mV, while in the matrix the Eh values were similar to those in the flooded treatment.

Ferrous Iron

As shown in Figure 2.8, concentrations of Fe(II) in solution reflected changes in Eh. In the flooded treatment, the Fe(II) concentration in the matrix increased steadily through 84 d and reached a maximum concentration of approximately 3.0 mg Fe(II) L⁻¹ in all layers before declining thereafter. While matrix Fe(II) concentrations differed significantly (p=0.0008) with depth for certain times, the differences were small. On the other hand, rhizosphere Fe(II) concentrations did differ with depth (p<0.0001). In the top level, where conditions were the least reduced, the concentration of Fe(II) in the rhizosphere was low and mean concentration never exceeded 0.5 mg Fe(II) L⁻¹. In the bottom layer, the mean concentration of Fe(II) in the rhizosphere reached a maxima of approximately 3 mg Fe(II) L⁻¹ and this was significantly higher (p=0.0006) than the matrix concentrations when variance was controlled for time. The rhizosphere in the middle layer had intermediate concentrations, approximately halfway between the top and bottom concentrations, while the concentration of Fe(II) in the rhizosphere was significantly lower (p=0.0145) than the matrix when accounting for the effect of time.

The drained treatment had very low concentrations of Fe (II) (less than 0.3 mg L⁻¹) throughout the experiment for both the rhizosphere and the matrix in all layers. In the capillary fringe treatment, the highest concentrations of Fe(II) occurred in the matrices of the middle and bottom levels which reached peaks of 2.50 and 1.60 mg Fe(II) L⁻¹ at 84 d. The rhizosphere concentrations at all levels remained below 1.00 mg Fe(II) L⁻¹ through the duration of the experiment. The top level matrix Fe(II) concentrations were below the detection limit throughout the experiment.

Dissolved Organic Carbon

Changes in DOC over time are shown for the matrix and rhizospheres in the flooded treatment in Figure 2.9. Matrix DOC concentrations were not significantly ($p=0.3291$) different among layers, and increased to a maximum of approximately $125 \text{ mg DOC L}^{-1}$ by day 56 then declined thereafter. In the rhizospheres of the flooded treatment, DOC concentrations were significantly higher in the bottom layer. The DOC concentrations in the top layer declined through 40 d then remained at low concentrations.

The rhizospheres of the flooded treatment had significantly less DOC than the matrix in the top and middle layers ($p<0.0001$ and $p=0.0377$ respectively). In the bottom layer, the rhizosphere DOC concentration was significantly higher ($p=0.0032$) than the matrix, probably due, in part, to the number of roots that died after flooding was imposed. Microorganisms utilizing the labile C in the dead roots may have generated the DOC concentrations observed in the bottom layer. The interaction of level, plant treatment, and time was highly significant ($p<0.0001$) for all levels (Table 2.1) indicating the differences in DOC concentrations between the rhizosphere and matrix were affected by how long the rhizotron had been saturated.

In the drained treatment, aerated conditions kept the DOC concentrations relatively constant through the experiment ($<50 \text{ mg DOC L}^{-1}$) in both the rhizosphere and matrix for all layers. In the capillary fringe treatment, matrix DOC concentrations mirrored the flooded treatment in the middle and bottom layers, while in the top layer the accumulation of DOC was similar to that found in the drained treatment. The rhizosphere DOC concentrations in the capillary fringe treatment had an initial peak of approximately $100 \text{ mg DOC L}^{-1}$ at 15 to

28 d in all three layers followed by a decline to less than 10 mg DOC L⁻¹ by 111 d.

Rhizospheres in the top layer maintained DOC concentrations between 100 to 150 mg DOC L⁻¹ after the initial increase.

Total Phosphorus

Changes in TP over time are shown for the flooded treatment in Figure 2.10. In the matrix, TP concentrations increased in the bottom layer for the first 28 d (56 d in the top level) and reached a maximum level of 700 to 900 µg P L⁻¹ across the three sampling depths. There were no significant differences in the matrix TP concentration across depths (p=0.8538). On the other hand, TP concentrations in the rhizosphere did differ significantly (p<0.0001) in the three layers. Rhizospheres in the top layer had the lowest TP concentration with a maximum of 201 µg P L⁻¹ at 15 days that declined thereafter. In the bottom layer, TP concentration closely paralleled that found in the matrix through day 56 reaching a peak in TP at 818 µg P L⁻¹ that declined until 111 d. The differences in TP between the matrix and rhizosphere were significant for the top and middle levels only (p<0.0001, p=0.0018 respectively). However, differences in TP concentration were highly significant (p<0.0001) for all levels when variability was controlled for the effect of time. This indicated that the decline in TP noted in the bottom level after 60 d may be due to immobilization of P by plant uptake.

In the drained treatment TP concentrations were low relative to the flooded and capillary fringe treatments. Mean matrix TP concentrations ranged from 70 to 230 µg P L⁻¹, and rhizosphere concentrations remained below 100 µg P L⁻¹ except for the bottom rhizosphere which started at 337 µg P L⁻¹ and declined below 100 µg P L⁻¹ thereafter. The

capillary fringe matrix middle and bottom level TP concentrations mirrored those in the flooded treatment. The TP concentration in the matrix of the top level remained less than $175 \mu\text{g P L}^{-1}$ for the entire experiment. Rhizosphere TP concentrations peaked on 28 d. at 324, 326, and $930 \mu\text{g P L}^{-1}$ for the top middle and bottom, respectively. The TP concentrations in all three layers then declined to approximately $200 \mu\text{g P L}^{-1}$ or less by 111 d.

The TP concentration consisted of approximately 75% DRP (data not shown) regardless of treatment and level. Patterns in DRP concentrations acted similarly to the TP concentrations for all treatments and levels. Further discussion of P will focus on TP, and DRP measurements will not be presented or discussed further.

The correlations of TP to Fe(II) and DOC, and the correlation of DOC to Fe are shown in Figures 2.11, 2.12, and 2.13 respectively. The correlation of TP to DOC was the highest ($R^2=0.73$) which was closely followed by the correlation of TP to Fe(II) ($R^2=0.67$). The concentrations of Fe(II) and DOC were also correlated ($R^2=0.67$). These results show that it is not possible to identify the dominant mechanism of P-dissolution.

Tree Growth and Tissue Analysis

Changes in tree height and diameter are compared among water treatments in Figure 2.14 after 122 d. The change in height in the capillary fringe treatment was significantly larger than the drained treatment ($p=0.0200$), however differences among the capillary fringe and flooded, and the drained and flooded treatments were not significant ($p=0.3664$, and $p=0.2689$ respectively).

The flooded treatment exhibited the most buttressing as indicated by the greatest change in diameter at the trunk base (Figure 2.14). Multiple comparisons of the changes in

tree diameter showed that the mean diameter of trees in the flooded treatment was greater than for the other treatments ($p < 0.0001$), with trees in the drained treatment having the smallest diameters ($p = 0.0003$).

Dry weight measurements (Figure 2.15) showed that the capillary fringe and flooded treatments both had significantly more above ground biomass than the drained treatment ($p = 0.0016$ and $p = 0.0007$, respectively) while no significant difference occurred between the flooded and capillary fringe treatments ($p = 0.9908$). No significant differences were observed in the belowground plant matter among the three water treatments. The flooded treatment above-ground biomass was greater than the below-ground biomass ($p = 0.0046$). No differences in above and below-ground biomass were observed for the capillary fringe and drained treatments.

Organic C content for the leaves, stems and roots were not significantly different (0.05 level) and all were approximately 0.5 g C g^{-1} . There was no significant difference in P accumulation in tree tissues from each water treatment. No significant differences in N accumulation were observed for the stems and leaves of each water treatment. The capillary fringe treatment did accumulate 16 mg more N in the roots than the flooded treatment ($p = 0.0496$) while in the drained treatment the root N accumulation was not different in the capillary fringe and flooded treatments. No differences in Fe accumulation were observed in the leaves and stems of each water treatment. There was more Fe in the roots than in the stems and leaves of each treatment but root sample contamination from soil Fe was suspected due to very high variability.

Soil Extractions

Mehlich III-P extractions are shown in Figure 2.16. The flooded treatment had significantly more ($p=0.0145$, $0.256 \text{ mg P g}^{-1} \text{ soil}$) Mehlich III-extractable P than the drained treatment. The capillary fringe treatment was not significantly different from either the flooded or the drained treatment. Across all water treatments and levels, the rhizosphere had significantly less ($p=0.007$, $0.202 \text{ mg P g}^{-1} \text{ soil}$) Mehlich III-extractable P than the matrix. PROC MIXED tests of fixed effects analysis showed that this significance due to plant treatment was mostly attributed to differences in extractable P in top, middle, and bottom levels of the capillary fringe treatment ($p=0.022$, $p=0.031$, $p=0.005$, respectively), and the top level of the drained treatment ($p=0.0120$).

Mehlich III-Fe extractions are shown in Figure 2.17. Across all levels and each plant treatment, both the flooded and capillary fringe treatments had more Mehlich III extractable Fe than the drained treatment ($p<0.0001$ and $p=0.0003$, respectively). The bottom and middle levels both had more Mehlich III extractable Fe than the top level ($p<0.0001$ and $p=0.0002$, respectively). PROC MIXED tests of fixed effects analysis showed that this significance due to layer was mostly attributed to differences in extractable Fe in the rhizosphere and matrix of the capillary fringe treatment, and the rhizosphere in the flooded treatment ($p=0.0009$, $p=.0003$, $p=0.0002$, respectively).

Microbial Biomass

Microbial biomass C measurements are shown in Figure 2.18. A multiple comparison analysis showed that both the drained and capillary fringe treatments had more MBC than the flooded treatment ($p=0.0018$ and $p=0.0423$, respectively) while the difference between the

drained and capillary fringe treatments was not significant ($p=0.3246$). PROC MIXED tests of fixed effects analysis showed that this significance due to the water treatment is attributed to differences in MBC within the rhizospheres of the three treatments in the top and middle levels ($p=0.0005$ and 0.0397 respectively) and less significantly the bottom level ($p=0.0524$). The MBC in the rhizosphere was significantly greater than the matrix in the drained treatment top and bottom levels ($p=0.039$ and $p=0.006$, respectively). In the drained treatment middle level, and all other treatments and levels there was no significant difference between the rhizosphere and matrix MBC contents.

Microbial biomass P measurements are shown in Figure 2.19. Multiple comparison analysis showed differences in MBP among all three water treatments and between the rhizosphere and matrix. The drained treatment had more MBP than the capillary fringe and flooded treatments ($p=0.0029$ and $p<0.0001$, respectively) while the capillary fringe treatment had more MBP than the flooded ($p=0.0095$). The matrix had more MBP than the rhizosphere ($p<0.0001$) averaged across all levels and water treatments, and this difference was most significant in the drained fringe top, middle and bottom levels ($p=0.0296$, $p<0.0001$, and $p=0.0785$, respectively), and the capillary fringe top ($p=0.0092$).

DISCUSSION

Juniper Bay P Export

The changes in concentrations of DRP in the drainage water from Juniper Bay before and after restoration showed that following restoration of wetland hydrology the DRP levels increased to reach twice the $100 \mu\text{g P L}^{-1}$ threshold at which eutrophication of freshwaters is likely (Correll, 1998). The elevated amounts of P in the Juniper Bay soil observed by Ewing

(Ewing, 2003) are likely leaving the wetland due to the change in hydrology which increased durations of saturation and reducing conditions. Because Juniper Bay is exporting P, proper management of this wetland requires further knowledge of the causes of P-dissolution in this and other acidic, organic-rich wetland soils.

Matrix vs. Rhizosphere as Sources of P

The rhizotron experiments showed that TP is being solubilized in both the rhizospheres and the matrix when the soils are saturated and reduced. The redox potential in the matrix in all layers of the flooded treatment fell low enough for Fe reduction to occur indicating that carbon levels in the matrix were not limiting the amount of reduction that developed in these soils. The organic C concentration in the matrix was 4%. We hypothesized originally that most reduction would occur in “hotspots” that formed around tissues of roots that died during the experiments, and predicted that these hotspots would occur preferentially in the rhizospheres around dead roots. Under sustained soil saturation, portions of bald cypress roots have been reported to die while roots closer to the soil surface continue to live and grow (Schat, 1984; Conk, 2008). These dead roots are sources of carbon for the reduction “hot spots” in the soil as discussed by Parkin (1987) where increased rates of reduction occur within a small area surrounding a piece of decaying plant tissue. The result is a high rate of biogeochemical processes such as denitrification and iron reduction. Such hot spots have been reported to form around dead roots and other actively decomposing organic matter with increased amounts of labile carbon (Christensen et al., 1990; Jacinthe et al., 1998; Parkin, 1987). However, while hot spots can form in the rhizosphere when dead

root tissue is present, they apparently also can form in the matrix around organic tissues that were not identified in this study.

We had hoped that microbial C would be useful for identifying the active hotspots in these soils, because reduction processes in soils are controlled by microorganisms, but such was not the case. Microbial biomass C was greater in the drained treatment and capillary fringe treatments than in the flooded treatment because the aerobic conditions were more favorable for growth of soil microbes. More MBC was expected in the rhizospheres than matrices of the flooded and capillary fringe treatments because of the larger amounts of dead roots and readily decomposable organic matter in the rhizospheres; however there were no significant differences. It is likely that during the time after root death and before soil sampling, the microbe community may have already consumed the readily decomposable organic matter as evidenced by the decline in DOC in the flooded treatment rhizosphere (Figure 2.9). As the food source declined so did the microbial community resulting in no difference in MBC among rhizosphere and matrix. In the bottom layer of the flooded treatment, the MBP was higher in the matrix than in the rhizosphere. Under saturated conditions the microbial community would consist primarily of bacteria rather than fungi. Plant roots can more effectively compete with a bacteria-dominated microbial community for available P in these conditions resulting in less MBP, though the effect of microbe competition for P was likely irrelevant because of the equal rates of P-accumulation in all three water treatments. A correction for the amount of P immediately adsorbed to the soil following cell lyses was not performed in this experiment. However, relative differences in MBP are noteworthy and useful for interpretation despite uncorrected MBP.

Processes Affecting P Dissolution

The amounts of TP dissolved in these soils were related to redox potential (Figure 2.7). The lowest amounts of TP were present in the oxidized rhizosphere in the top layers, while similar amounts were found in the matrix (all levels) and in the rhizospheres in the bottom section which had the most dead roots. The TP was released wherever the redox potential was low enough to reduce Fe. This occurred in the matrix as well in the rhizospheres along dead roots. Furthermore, as shown in Figure 2.10, the amounts of TP decreased in the rhizosphere after day 60 in the bottom level, probably due in part to plant uptake of the TP. Therefore, by the end of the experiment the rhizospheres were the locus of P uptake rather than P release from soil particles.

Mehlich III extractions of the soil surrounding each Rhizon sampler, that was collected at the end of the experiment and allowed to oxidize, showed more extractable P occurred in the flooded treatment than in the drained treatment – especially in the middle and bottom layers which exhibited the most reduced conditions. The Fe(II) that was produced following soil saturation and reduction (Figures 2.7 and 2.8) likely converted to amorphous forms of Fe (e.g. ferrihydrite) when the flooded soils were sampled and oxidized at the end of the study. The amorphous forms of Fe are more readily soluble in the Mehlich III extractant than more crystalline forms of Fe (e.g. goethite). It is probable that the soil P was attached to the amorphous forms of Fe and was therefore more readily extracted as was the Fe. Also, less Mehlich III-extractable P was found in the rhizosphere across all water treatments showing that the trees had noticeably reduced the concentration of P in the soil through plant accumulation.

We could not clearly show which of the two mechanisms governing release of TP was active in the rhizotrons because TP was strongly correlated with both Fe(II) and DOC (Figures 2.17 and 2.18), and Fe(II) and DOC were also highly correlated with each other. Both mechanisms were probably active, and possibly interactive, in these soils. As noted earlier, TP was released when Fe reduction was occurring. Iron reduction occurs when microorganisms are oxidizing plant tissues and use Fe(III) as their electron acceptors. It is likely that some DOC is produced as the organic tissues are being oxidized in the Fe reduction process. While TP may be released in some portions of the soil when Fe is reduced, the soil particles may be surrounded by DOC which can also be releasing TP that is bonded to oxidized Fe minerals that are not reduced, as well as TP bonded to Al minerals.

Response of Bald Cypress to Flooding

During the course of a year, the hydrology of a restored wetland such as Juniper Bay will cycle through periods when the soils are drained, transition to a capillary fringe condition as the water table rises, and then become flooded. The rhizotron studies showed that the distribution of bald cypress roots will change to adapt to the different hydrologic conditions. In the drained condition, most of the bald cypress roots grew in the middle and bottom layers (Figs 2.4 and 2.6). Following imposition of the flooding treatment, approximately one third of the roots present prior to flooding died in the bottom and middle layers.

In the top layer of the flooded treatment, no root death was observed and root numbers increased as new roots developed. As time progressed these new roots were able to extend into lower depths along with other new roots extending from the tap root. We believe

that the new roots in the top layer contained aerenchyma that enabled them to oxidize the rhizosphere to prevent these roots from dying. This was shown by the redox potential measurements in the rhizosphere of the top layer (Fig. 2.7) which remained nearly constant over time after the flooding was imposed. In addition, the redox potential in the rhizospheres of the top layer were consistently greater than those in the rhizosphere of the lower layers, and greater than those in the matrix of all layers, indicating that the roots had developed a mechanism for oxidizing the rhizosphere. Roots in the middle and lower layers that were not able to survive the anaerobic conditions that developed probably did not possess mechanisms for aerating their rhizospheres. Only roots that developed after the flooding was imposed seemed to have developed the ability to oxidize the rhizosphere.

In studies that preceded the experiment presented here using the same tree stock and rhizotron design, Conk (2008) reported that bald cypress saplings grown in flooded rhizotrons produced aerenchyma-containing-roots following the initial flooding of the soil. It was presumed that the roots produced following flooding in this experiment were similar to those studied by Conk (2008) and did contain aerenchyma. Bald cypress are known to oxidize the rhizosphere (Anderson and Pezeshki, 2000; Colmer, 2003) to prevent buildup of phytotoxins such as sulfide (Koch et al., 1990). Aerenchyma enhance rhizosphere oxidation (Pezeshki et al., 1996); (Vartepetian and Jackson, 1997; Anderson and Pezeshki, 2000) and will result in changes in soil redox potential.

After flooding began, the redox potentials decreased the quickest in the rhizospheres in the middle and the bottom levels where dead roots provided a readily-digestible food source for microbial activity. The redox potential stayed relatively stable in the top level

rhizosphere where aerenchyma-containing roots kept the rhizosphere oxidized relative to the other levels. As these roots grew into the lower levels with time there was an apparent increase in Eh which indicates that redox potentials rise following growth of new roots that are adapted to anaerobic conditions. Although new roots were not instrumented with redox electrodes, we believe that the redox potential near these new roots would be less reduced than the middle and bottom rhizospheres, with Eh values potentially as high as the top level rhizosphere measurements. Because the redox potentials in the matrix were not significantly different among layers, it is likely that diffusion of atmospheric O₂ from the soil surface was minimal in these rhizotrons, and that the differences in Eh between the levels in the rhizosphere was due to the effect of roots alone.

Root growth in the top layers of the drained and capillary fringe treatments was less vigorous than in the flooded treatment. In the flooded treatment, root growth was greater in the less-reduced soil zones, while the largest increase in root concentration in the drained treatment was in the lower level as roots extended into lower levels for increased water access. The capillary fringe treatment did incur root death of up to 0.1 dead roots cm⁻¹ but not enough to cause a decrease in living root concentration over time.

If the root system must change in response to changing hydrology as shown here, then root death will occur in restored sites as hydrology changes from a drained to flooded condition. A significant portion of the deep roots of bald cypress will probably die during the transition and create reducing conditions that release P to the soil solution. As shown here, the deeper roots that did not die will probably not be active in extracting significant amounts of TP in solution. After 60 days, however, the new roots that developed near the

surface and which contain aerenchyma will grow into the deeper layers and be able to extract P. The process might be sped up if the trees planted in a restored site are “conditioned” early in their growth by short saturation events that allow them to adapt to reduced soil conditions.

Impact of Trees to Remove P

The concentration of TP was decreased below concentrations in the matrix in the presence of live, active roots. This decline in TP concentration may be attributed to plant uptake of solution P, microbial uptake of P, or by an unknown mechanism of P-precipitation out of solution. The uptake of P by plants would control the concentration of P in solution at Juniper Bay for long periods of time as it is retained in tree biomass. Mitsch et al. (1979) reported net P-uptake by bald cypress in an Illinois floodplain at rates of $0.10 \text{ g P m}^{-2} \text{ yr}^{-1}$ ($1.00 \text{ kg P ha}^{-1} \text{ yr}^{-1}$), similar to a reported $0.23 \text{ g P m}^{-2} \text{ yr}^{-1}$ ($2.28 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) reported by Schlesinger (1978) in the bald cypress dominated Okefenokee Swamp, Georgia. As the bald cypress stand at Juniper Bay matures, the P-uptake rates are expected to be similar to those reported.

The oxidation of the rhizosphere is another way P can be kept out of solution. If enough O_2 can be introduced into the reduced soil by hydrophytic plants such as bald cypress, then the concentrations of P in solution can be expected to reach levels as low as is shown in the top level rhizosphere where the soil was the least reduced and where the most living, adapted roots were present. Through these observations, it appears that a promising tool in the management of wetlands constructed on P-rich farmland would be to plant hydrophytic plants like bald cypress that can both oxidize the soil, and stabilize P for long

periods of time. Further research would be warranted to limit the amount of root death at depth following soil saturation.

Because the capillary fringe and flooded treatments had higher TP concentrations in the soil solution and exhibited greater tree growth than the drained treatment, more available P was expected to be the cause of the increased growth. However, the tissue analysis showed that the amount of P and N accumulated by the trees in each treatment was not significantly different. This indicated that soil fertility was not limiting plant growth and was not the cause in the differences in growth among water treatments. One potential cause of decreased growth in the drained treatment is drought stress. Although bald cypress are extremely flood-tolerant, the species has very low drought tolerance (Megonigal and Day, 1992) and plant shoots can even be irreparably damaged in as little as 3 to 4 hr of drought stress (Dickson and Broyer, 1972). Even though the drained treatment was watered four or more times per week in an effort to keep the soil at field capacity, the potential exists that short term drought stress may have caused damage to the stems resulting in stunted growth in comparison to the flooded and capillary fringe treatments that had ample water. Despite the potential of drought stress in the drained treatment, all three treatments did accumulate the same amount of P. In terms of future management of Juniper Bay, immediate and long term storage of P in biomass to prevent P loss from the wetland should be a major consideration. Removal of excess soil P by bald cypress and other flood-tolerant tree species is a potentially economical solution. The P accumulation under three different water regimes in this study showed that under P-rich conditions, sapling-age cypress trees will accumulate P at the same rate, regardless of water conditions. Given this information, there is a need for further research

examining planting and hydrology conditions that maximize P-uptake in similar wetland restorations.

CONCLUSIONS

The export of P from Juniper Bay was increased following the restoration of wetland hydrology and reducing soil conditions. Proper management of Juniper Bay, and similar wetland restorations of previously farmed soils, require knowledge of the causes of P dissolution and the location of P dissolution at the soil profile scale. The goals of this study were to: 1) determine the impact of rhizosphere processes on the production of P, Fe(II), and DOC; and 2) evaluate the potential of plant P-uptake as wetland management technique in Juniper Bay and other previously farmed wetland restorations.

We hypothesized that the rhizosphere and microsites containing dead root material would act as hot-spots of P dissolution. Our results indicate that this is not the case. Total P concentrations in the rhizosphere did not exceed TP concentrations in the matrix. The rhizosphere actually decreased TP concentrations in locations where high concentrations of live roots were present which both accumulated P and aerated the soil. The aeration of the soil lowered the reduction of Fe to Fe(II) and reduced the accumulation of DOC, thus suppressing the effects of the P dissolution mechanisms no. 1 and 2 respectively.

Lower amounts of Mehlich III-extractable P were found in the rhizosphere indicating that trees were able to accumulate P and reduce the potential of P dissolution into groundwater. However, the total amount of P accumulated by a plant was not affected by the hydrology. This indicates that plant accumulation of excess P into the tree biomass may be a useful remediation tool in wetland restoration of previously farmed land, and that hydrology

should be managed to foster the highest tree survival rates while meeting wetland hydrology criteria.

There is a need for further research on methods to maximize the amounts of P accumulated by flood-tolerant trees. These methods may include avoiding drought stress to limit tree death and stem damage, and planting saplings at close intervals to maximize the volume of soil explored by tree roots. In addition, the affects of the rhizosphere on P dissolution in the low Fe and Al, organic soils of Juniper Bay is still unknown. Future research should include a focus on these organic soils as a potential additional source of P export. Lastly, the future duration of P export from Juniper Bay is unknown. A P balance of this study site that forecasts the magnitude and duration of P export would be useful in prescribing the intensity of wetland management needed to minimize P dissolution and export.

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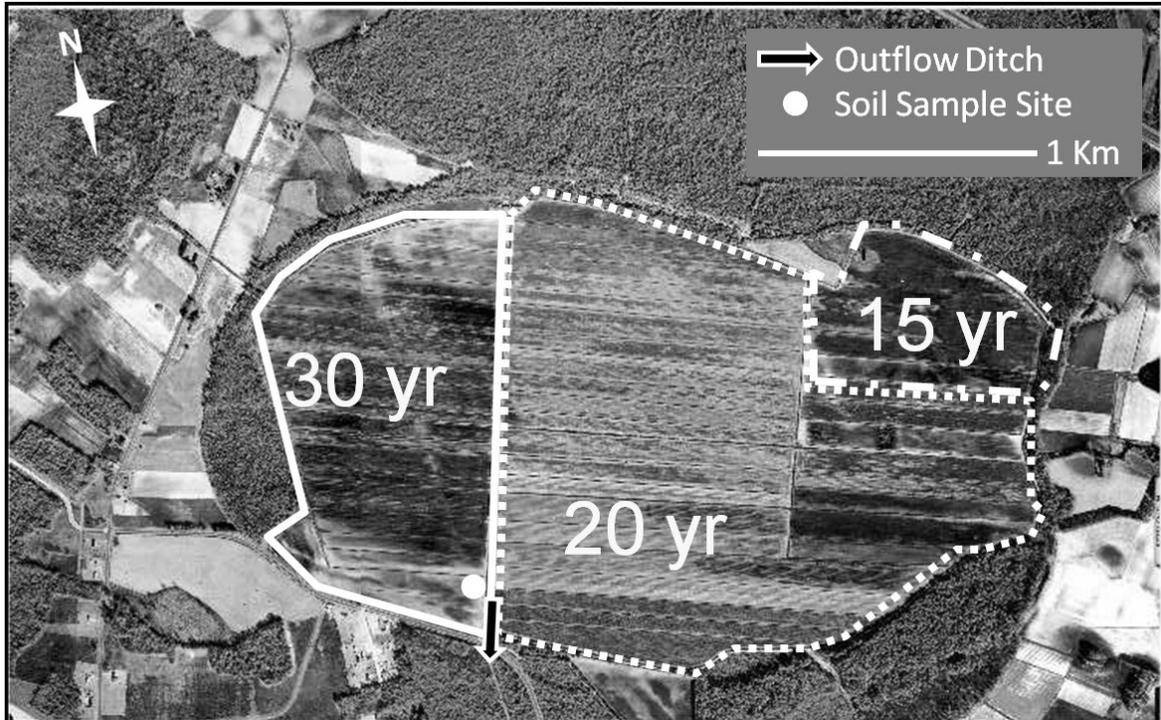


Figure 2.1 Map of Juniper Bay. Juniper Bay is located in Robeson County, NC. It was cleared and farmed in three stages. The northwest side of the bay was under production for 30 years. The middle section was under production for 20 years. The east corner was under production for 15 years. Juniper Bay has organic soils in the middle and mineral soils along the edge. The aerial photo shown here was taken prior to restoration. The white dot indicates the location where soil was taken for this experiment which is in the mineral portion of the 30 year section. A perimeter ditch circumscribes the wetland and drains out at a single outflow depicted by the arrow. Water samples from the J.B. drainage water were taken at the single outflow.

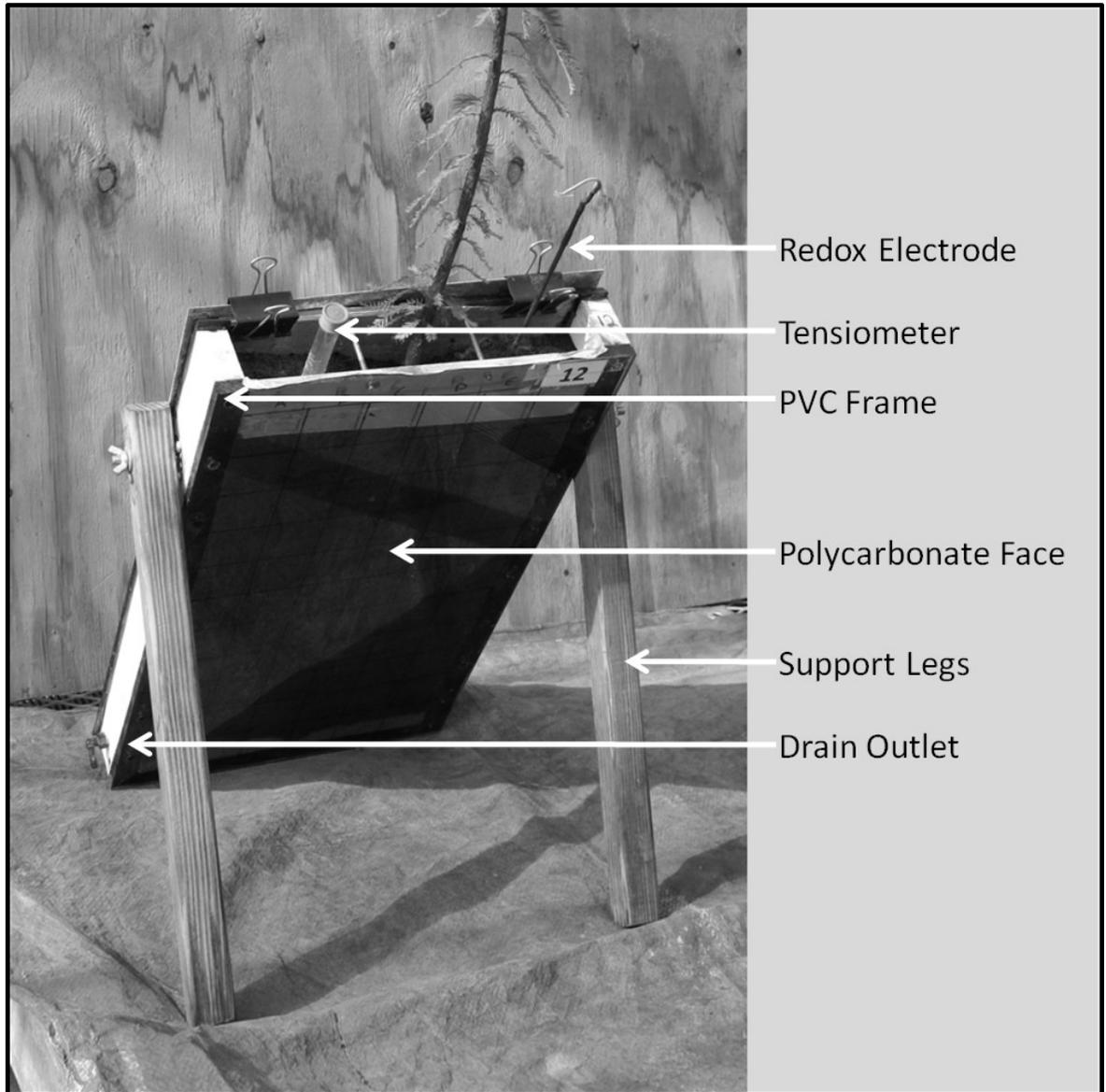


Figure 2.2 Rhizotron design and construction. Rhizotrons were constructed as shown above similarly to Neufeld (1989). Each rhizotron was kept at 30° from the vertical during the growth period causing roots to grow against the face for visual inspection. A redox electrode and tensiometer were used to ensure proper soil moisture.

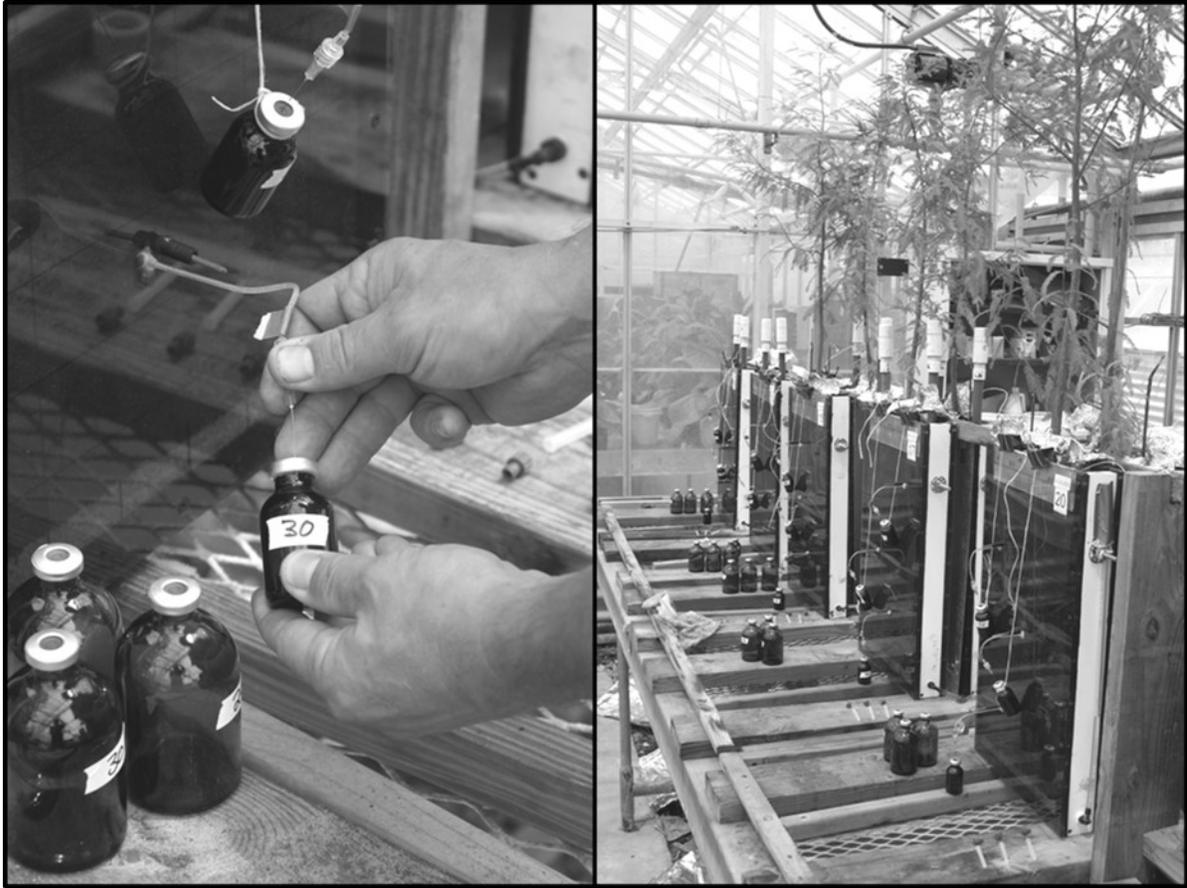


Figure 2.3 Soil porewater sampling and redox electrodes. On the left, rhizon samplers and redox electrodes were inserted through the rhizotron face adjacent to roots and in the matrix. Samples were extracted using evacuated serum bottles and Luer-lock needles. Redox measurements were taken with the Pt-tipped redox electrodes, a calomel reference electrode, and a voltmeter. On the right, rhizotrons are shown in their upright position. Serum bottles were suspended with string during sampling.

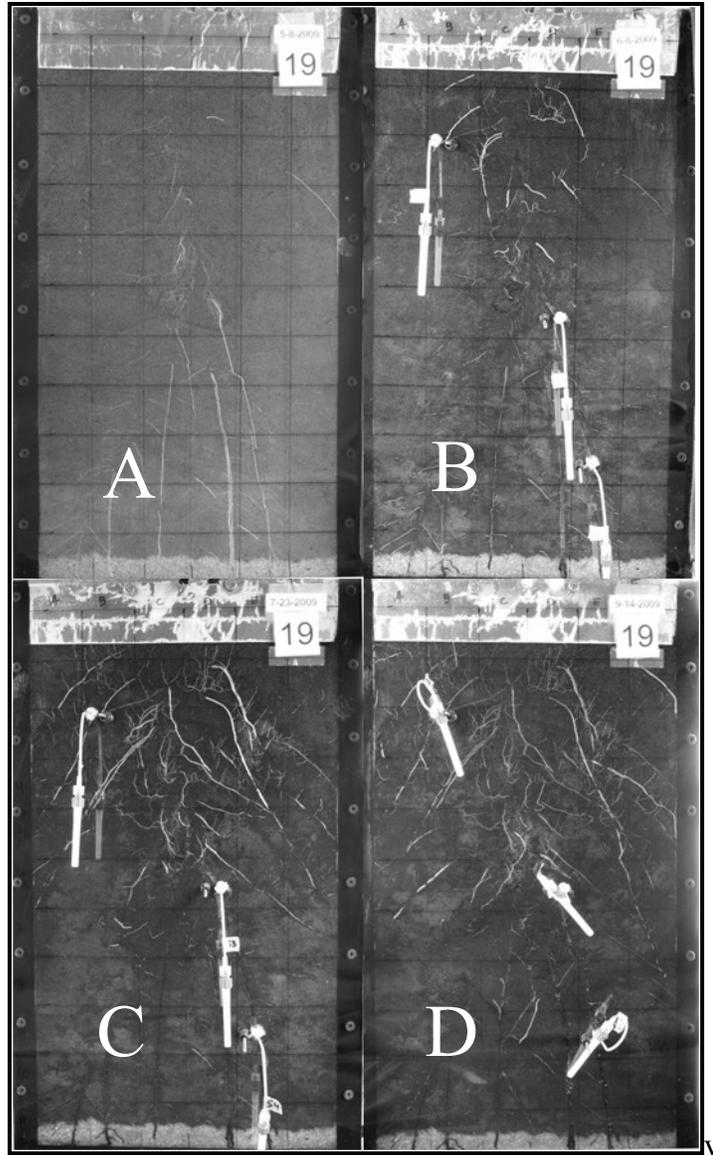


Figure 2.4 Root changes with time. The flooding of the rhizotron caused both root death and root growth in different depths of the rhizotron. The root system prior to flooding (A) indicated most roots were in the lower half of the rhizotron. Immediately following flooding (B) roots in the lower section began to die. Root numbers in the upper half of the rhizotron (C) increased as root numbers in the lower section decreased. By the end of the study, roots from the upper layer had grown into the lower layer (D).

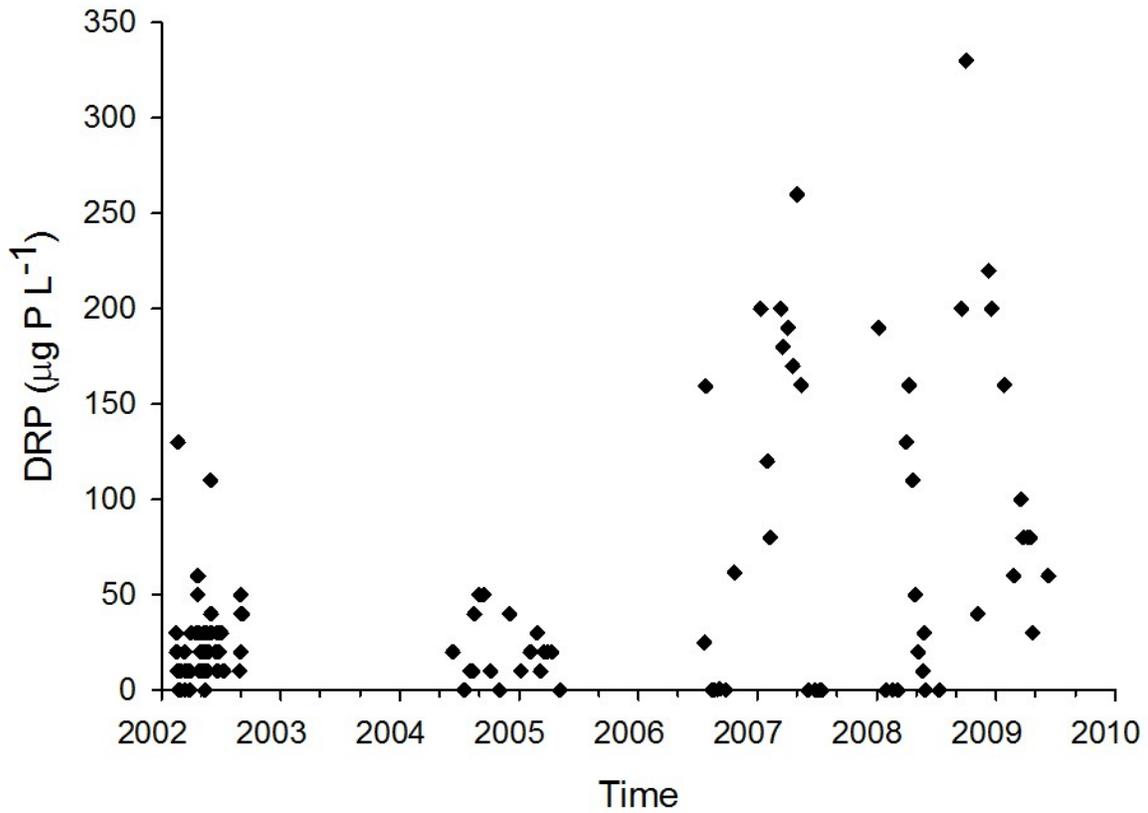


Figure 2.5 Juniper Bay outflow dissolved reactive phosphorus. The concentration of DRP in the drainage water of Juniper Bay is shown starting in 2002 prior to restoration through 2010. Wetland hydrology was restored in Juniper Bay in the beginning of 2006. Prior to restoration the concentration of DRP rarely exceeded 100 $\mu\text{g P L}^{-1}$. Following restoration, DRP concentrations regularly exceeded that mark, showing that the saturation of the P-rich soils of Juniper Bay has caused it to become an excessive source of P for downstream aquatic systems.

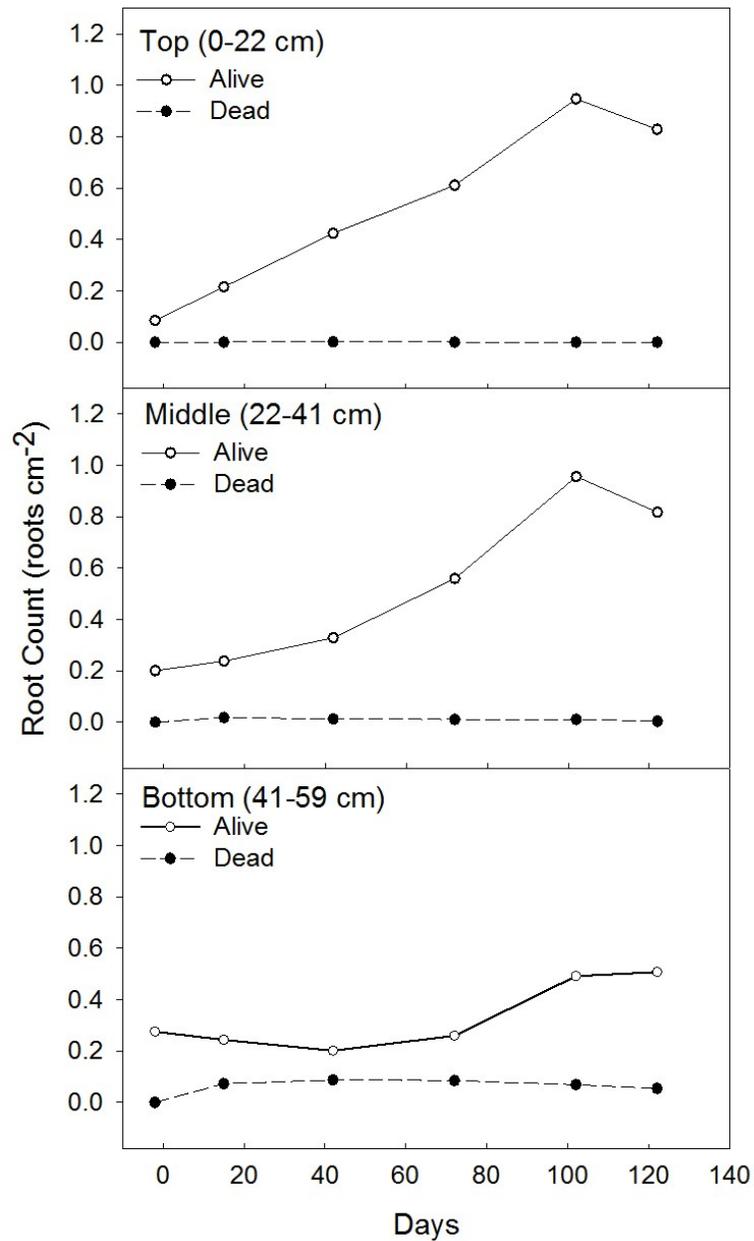


Figure 2.6 Changes in root concentration and root mortality with time. The concentration of roots classified as alive or dead are shown with changes over time for all three depths in the flooded treatment. Most root death occurred in the bottom layer. The highest concentration of living roots was in the top layer.

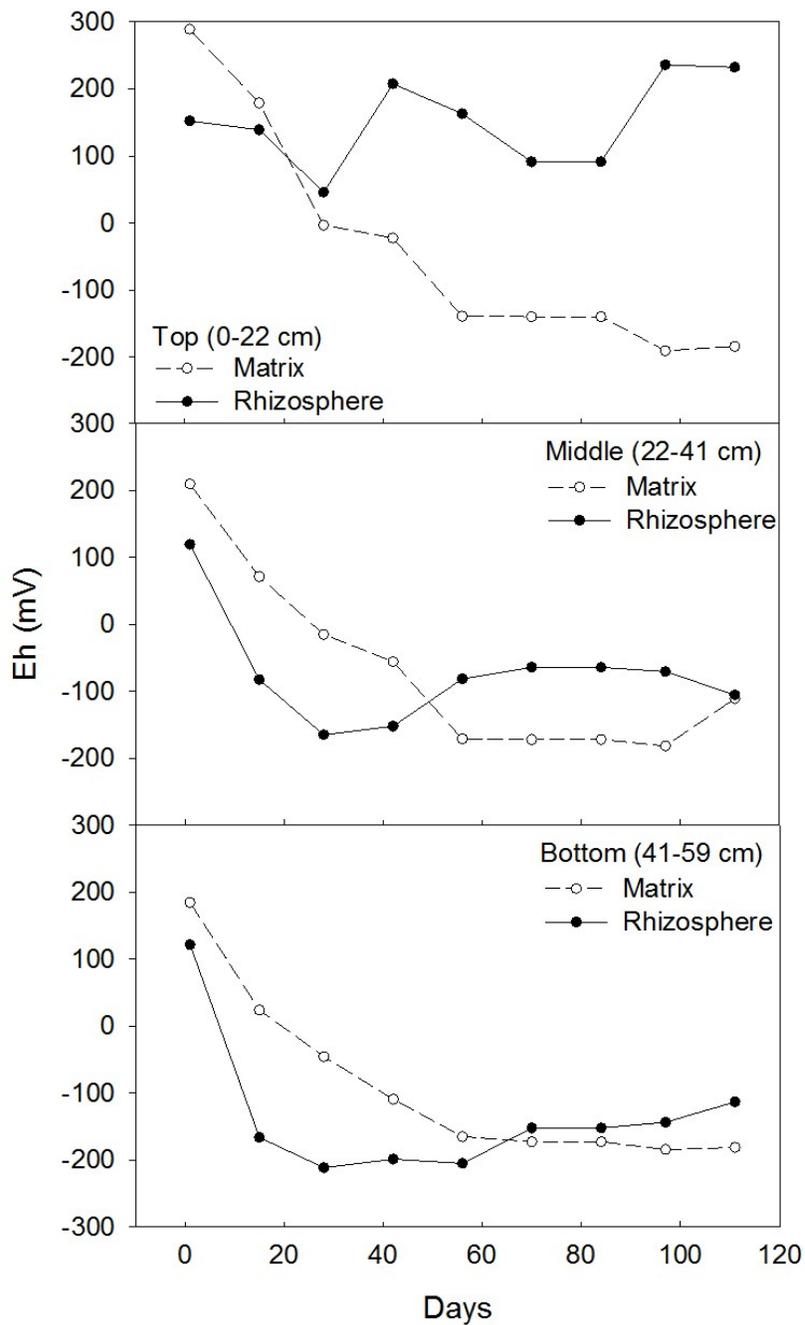


Figure 2.7 Changes in redox potential (Eh) with time. The Eh in the rhizosphere and matrix of the three levels are shown over time. The fastest decrease in Eh occurred in the bottom and middle rhizosphere while the top level rhizosphere exhibited the highest Eh.

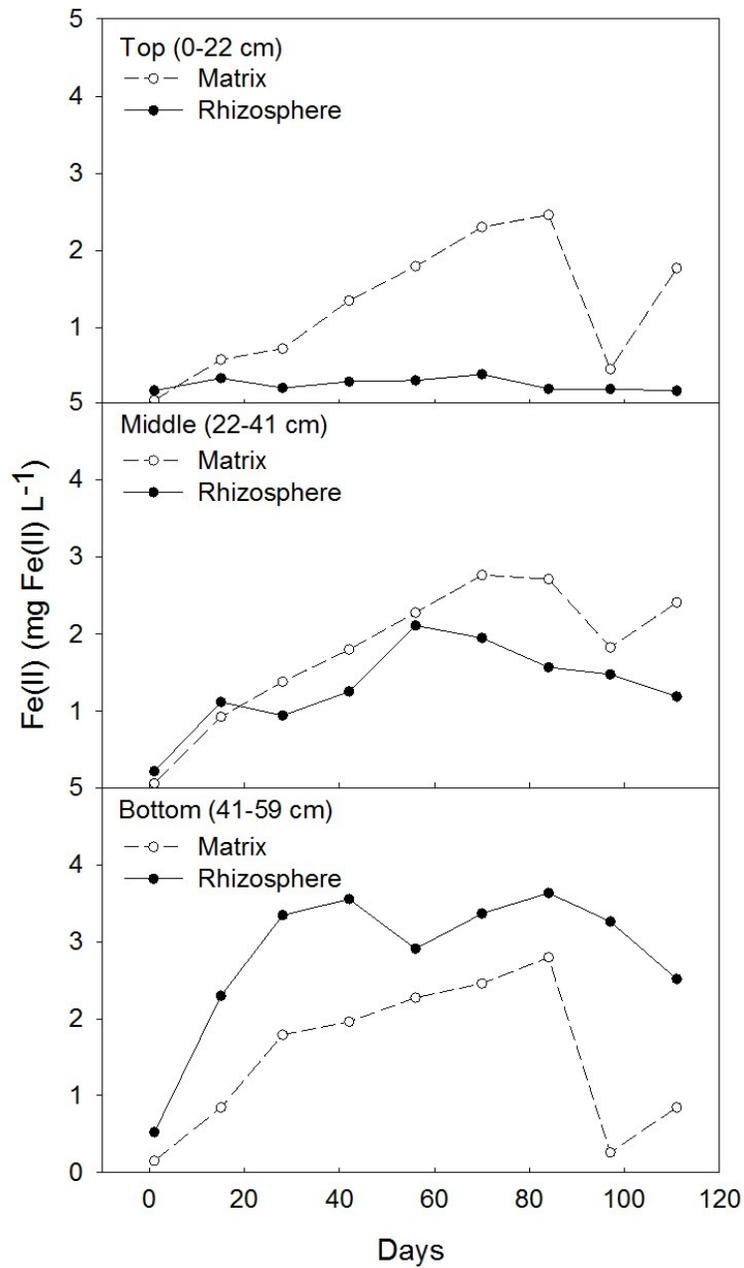


Figure 2.8 Changes in the concentration of Fe(II) with time. Changes in the concentration of Fe(II) in solution are shown over time for each level. The most Fe(II) occurred in the bottom level rhizosphere where the system was most reduced, while the least Fe(II) occurred in the top level rhizosphere where the system was least reduced.

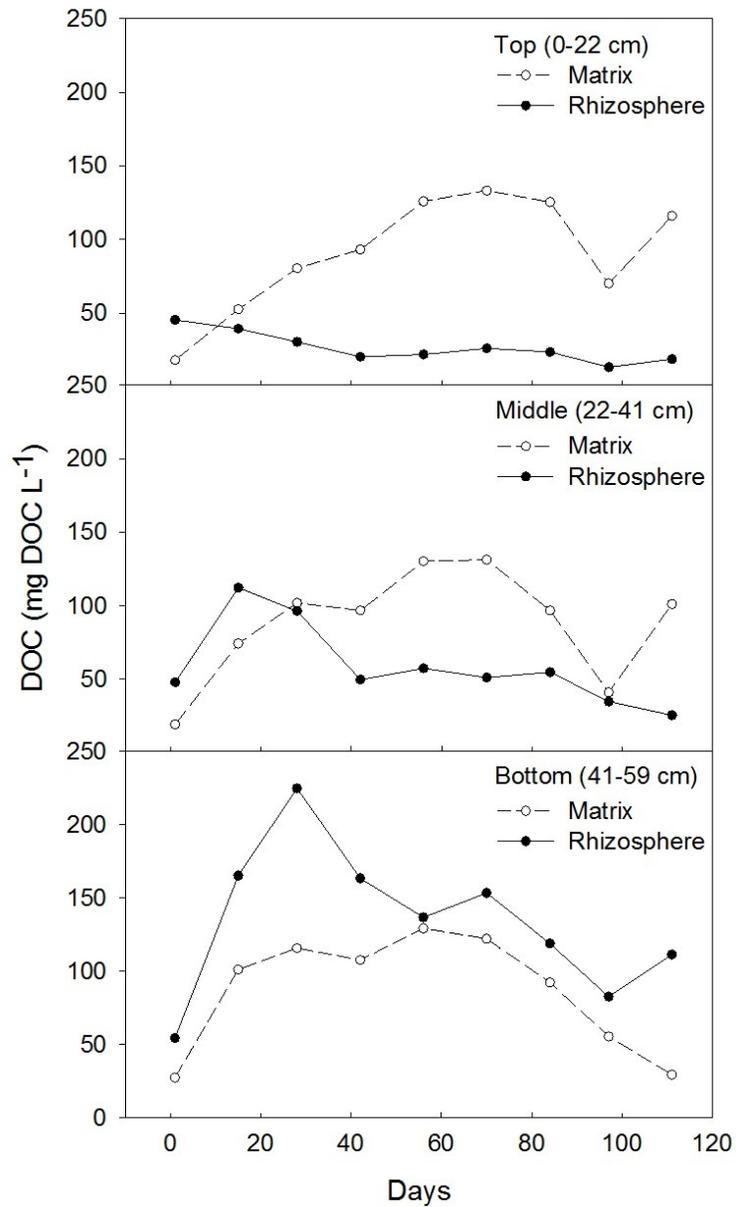


Figure 2.9 Changes in the concentration of DOC with time. The concentration of DOC was higher in the rhizosphere than in the matrix when roots were dying (middle and bottom levels) and lower in the rhizosphere than in the matrix when living, active roots adapted to anaerobic conditions were present (top and middle levels). Dead roots can act as a DOC source, while live roots can act as an aerating agent to reduce DOC accumulation.

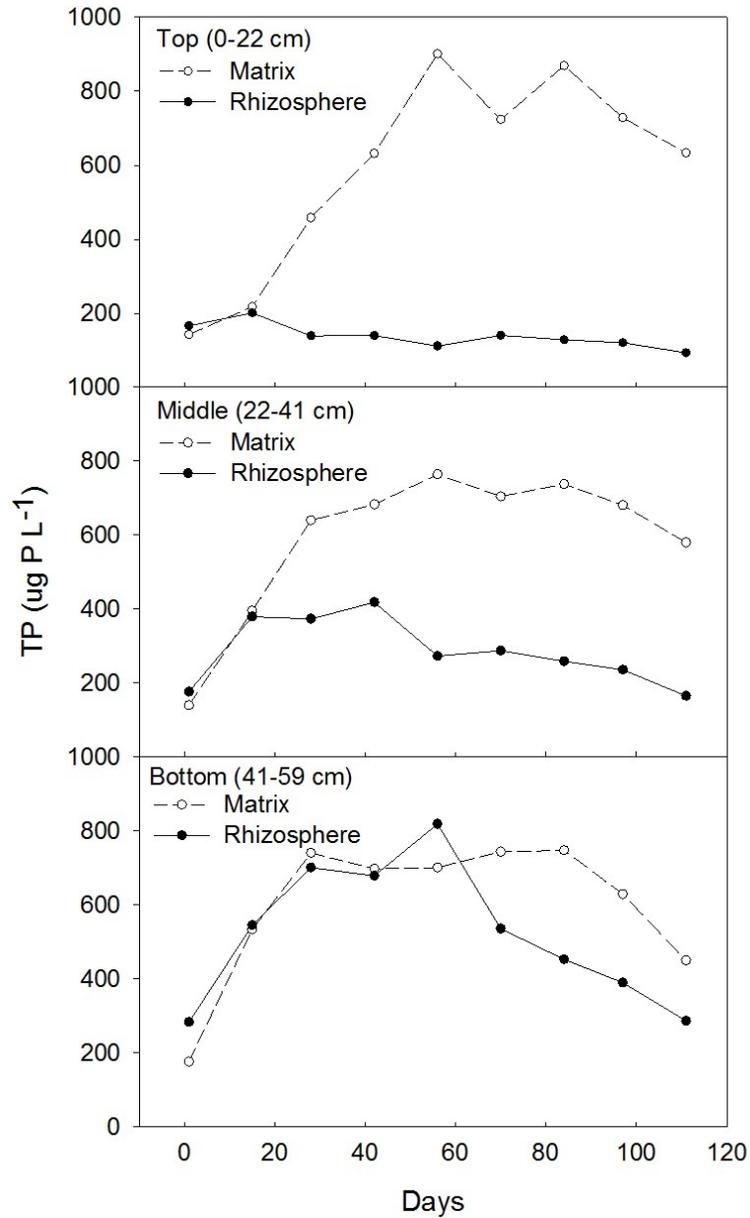


Figure 2.10 Changes in TP concentration with time. The concentration of TP was not increased over concentrations in the matrix by the presence of roots. The concentration of TP in the rhizosphere was actually lowered below the concentration of TP in the matrix due to plant uptake, microbial uptake, soil aeration from root- O_2 loss, or other unknown P-precipitation mechanisms.

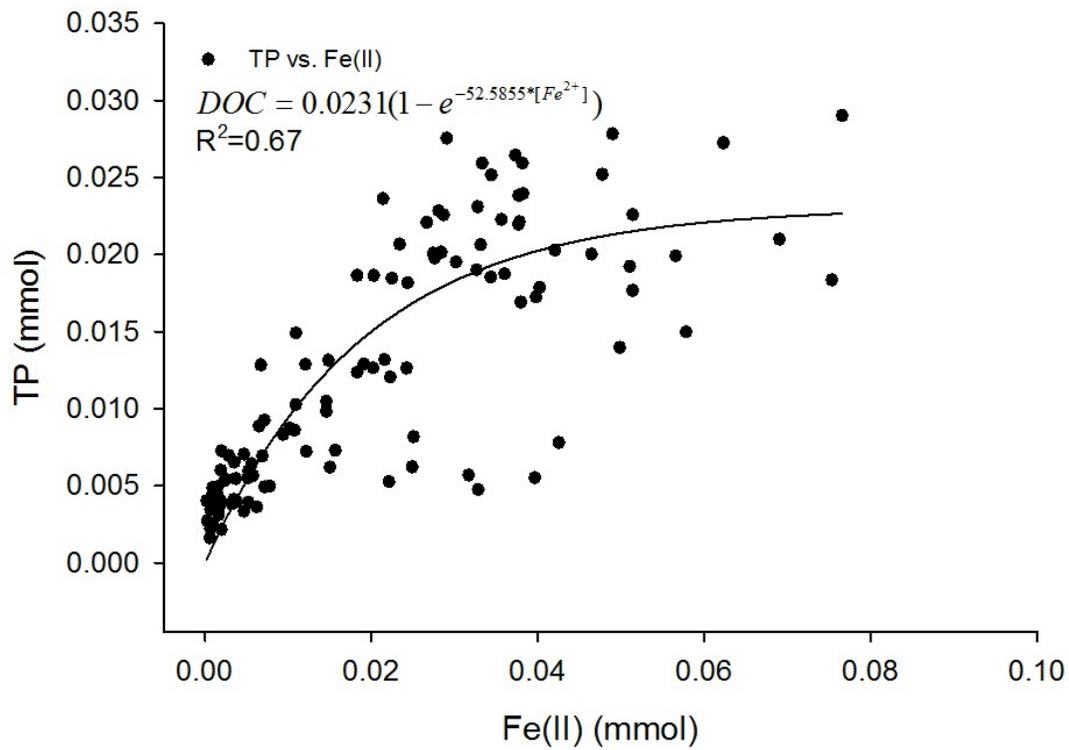


Figure 2.11. Regression of TP to Fe(II). This graph depicts the relationship of P dissolution and the reduction of Fe to Fe(II) in the first 58 d. in flooded rhizotron porewater samples.

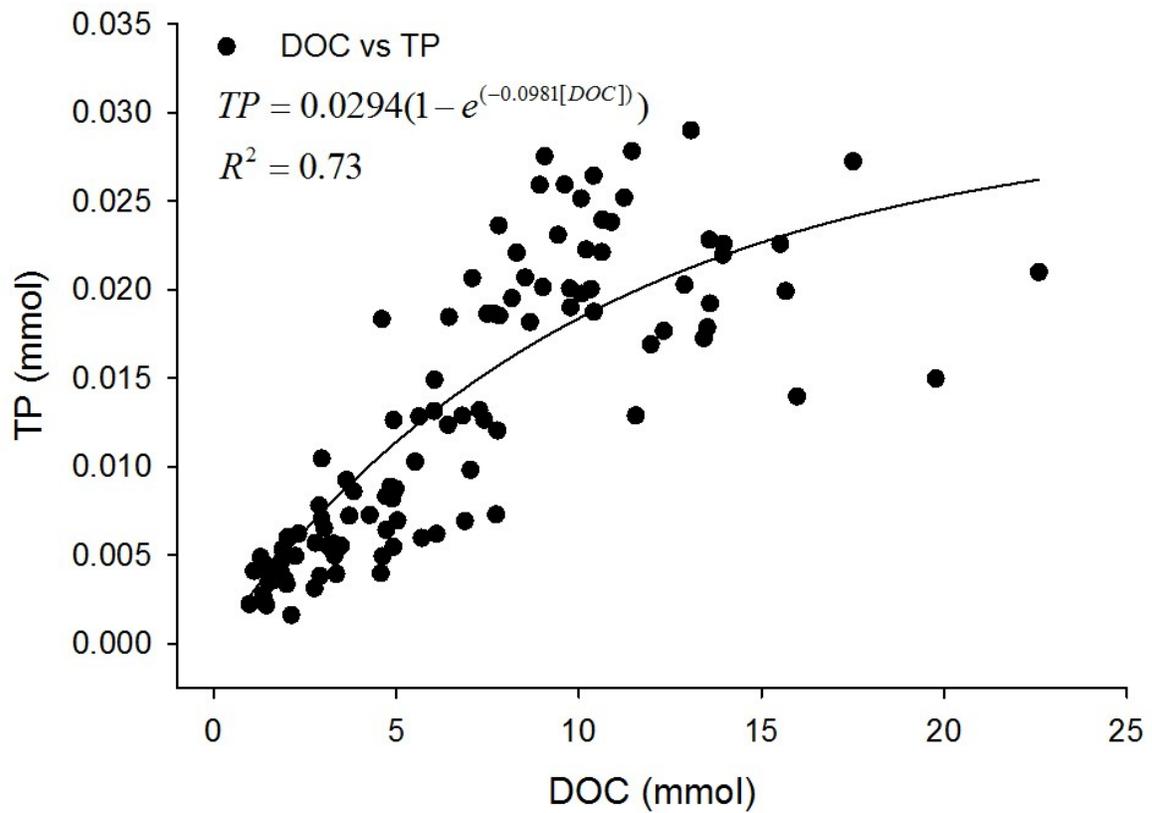


Figure 2.12. Regression of TP to DOC. This graph shows the relationship of P dissolution and the accumulation of DOC in the first 58 d. in flooded rhizotron porewater samples.

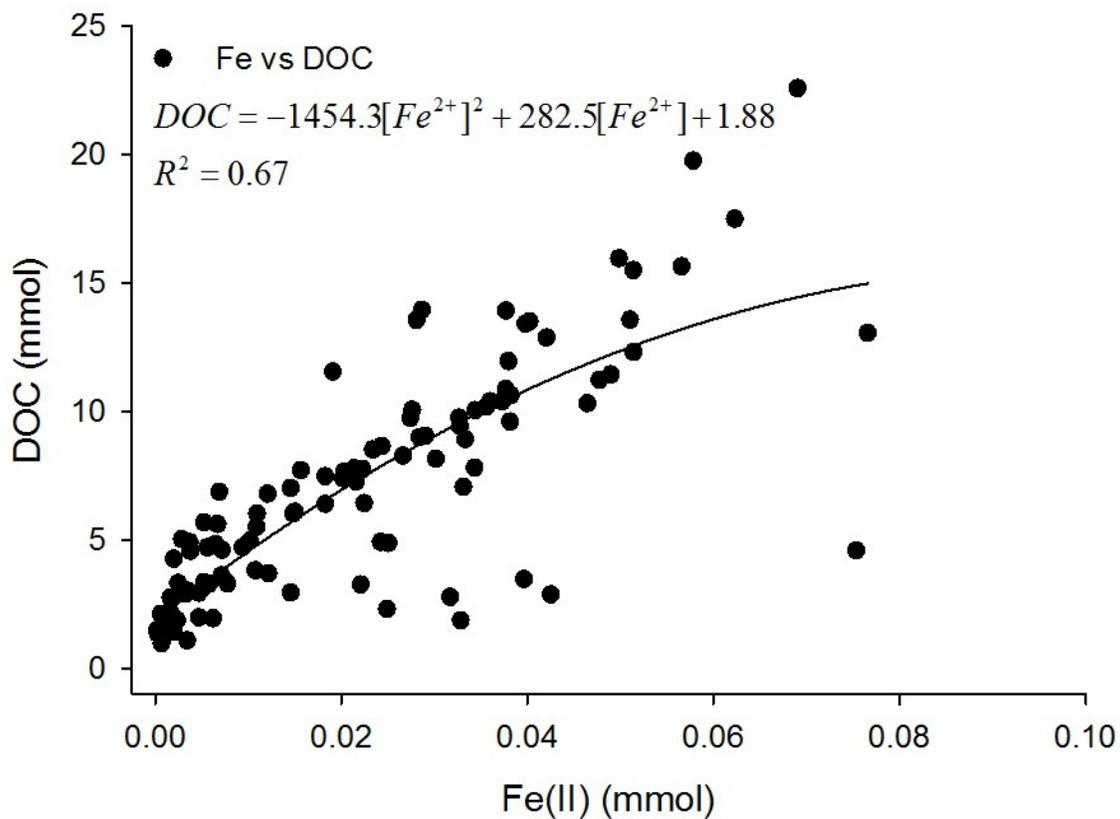


Figure 2.13. Regression of DOC to Fe(II). This graph depicts the relationship of DOC accumulation and the reduction of Fe to Fe(II) in the first 58 d. in flooded rhizotron porewater samples.

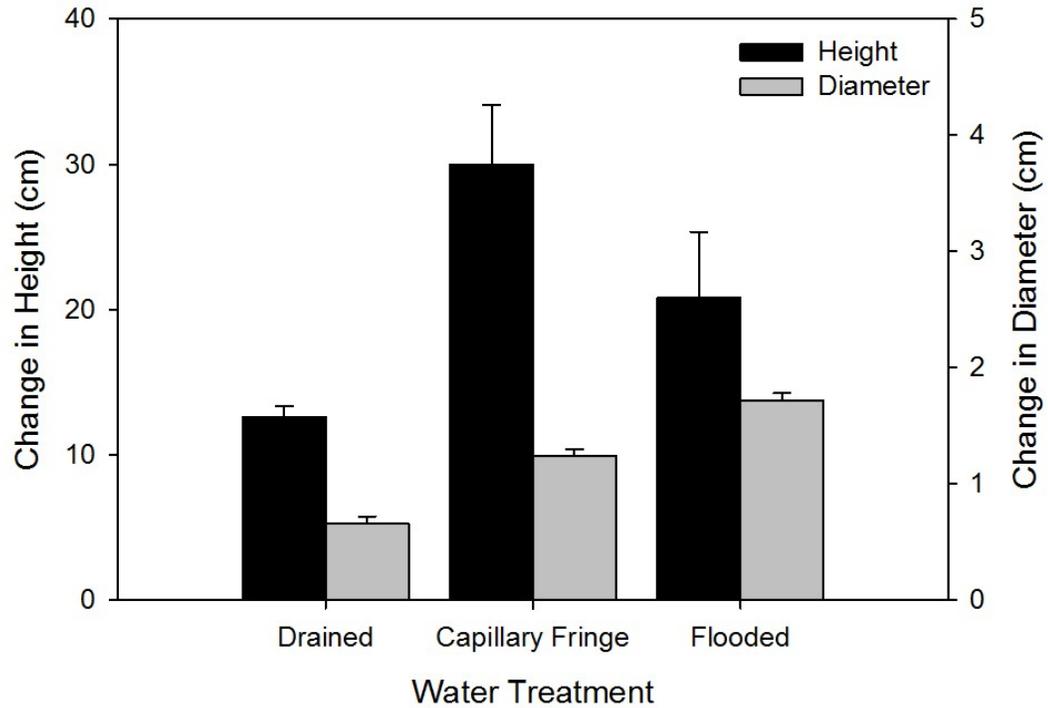


Figure 2.14 Change in tree height and tree trunk diameter. Changes were measured as the difference between the beginning measurement and the end of the experiment after 122 days of each prescribed water treatment during the growing season. The growth of the capillary fringe and the flooded treatments were each significantly greater than the drained treatment growth, though the capillary fringe and drained treatment were not different. The flooded treatment had significantly greater diameters than both the capillary fringe and drained treatment. The capillary fringe treatment had significantly wider diameters than the drained treatment.

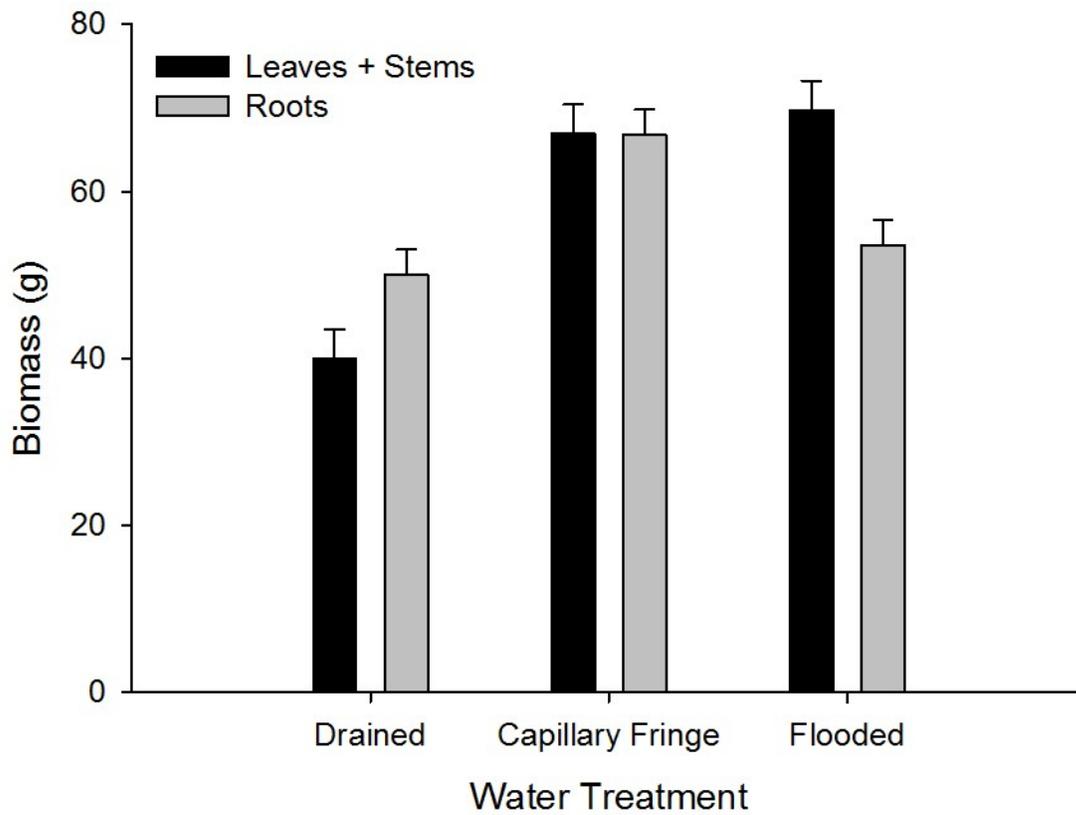


Figure 2.15. Biomass distribution in tree tops and roots. The flooded treatment was the only water treatment to have more biomass in the leaves and stems than in the roots. The capillary fringe and flooded treatment both had significantly more total biomass than the drained treatment.

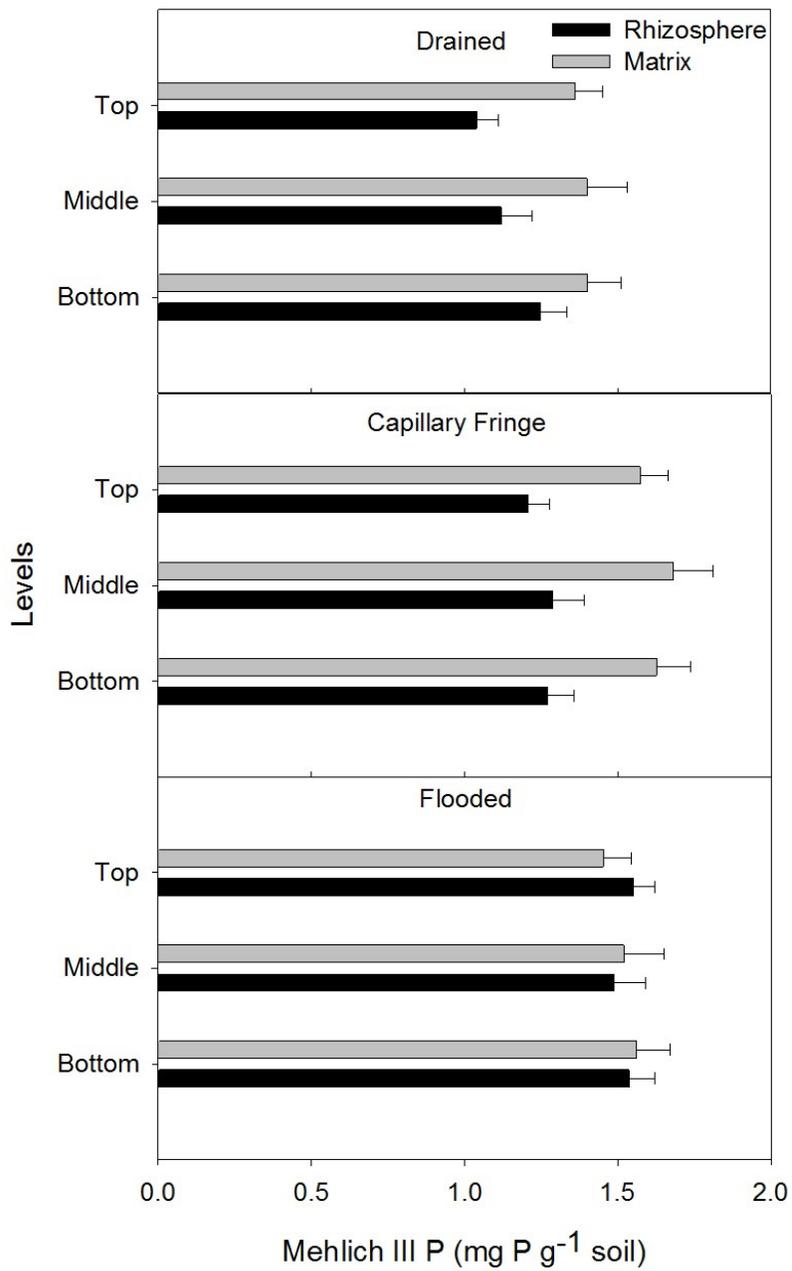


Figure 2.16. Mehlich III extractable P for all levels of the drained, flooded, and capillary fringe treatments. The flooded treatment had more Mehlich III extractable P than the drained treatment. Rhizosphere soil samples had less extractable P than the matrix due to plant uptake.

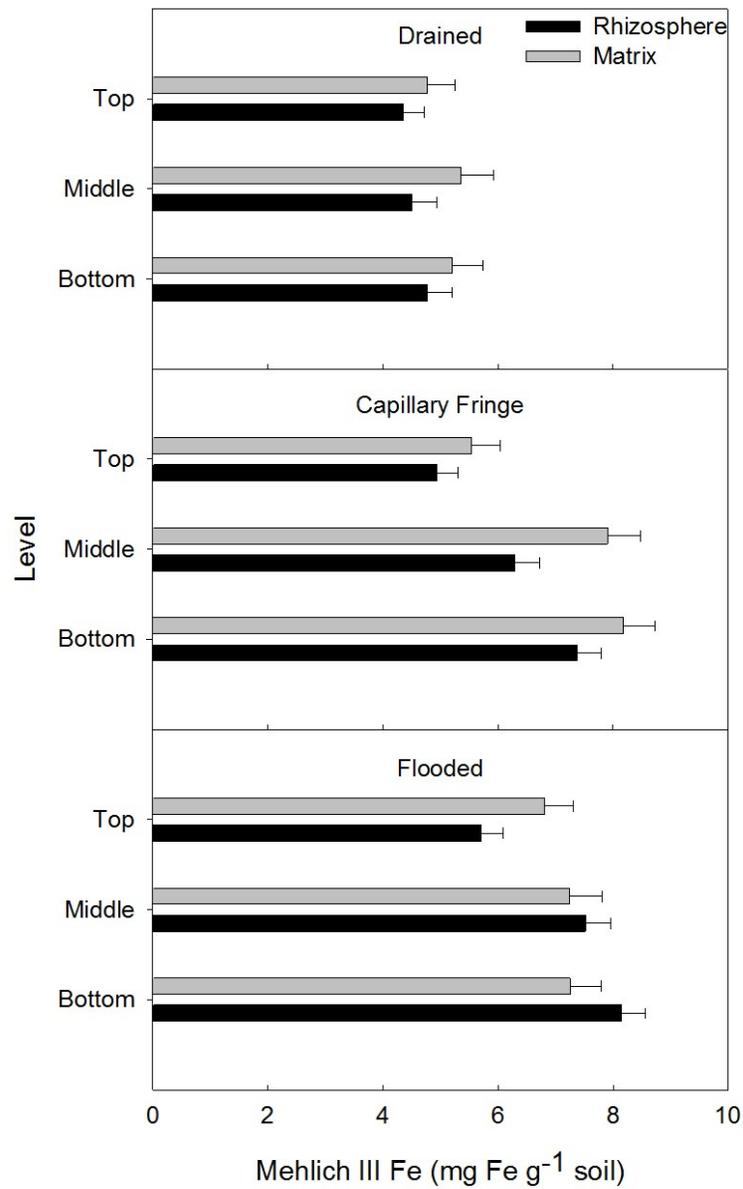


Figure 2.17. Mehlich III extractable Fe for all levels of the drained, flooded, and capillary fringe treatments. The highest amount of Mehlich III extractable Fe was found in the flooded treatment while the least was found in the drained which is likely due to more amorphous forms of Fe found in reducing conditions.

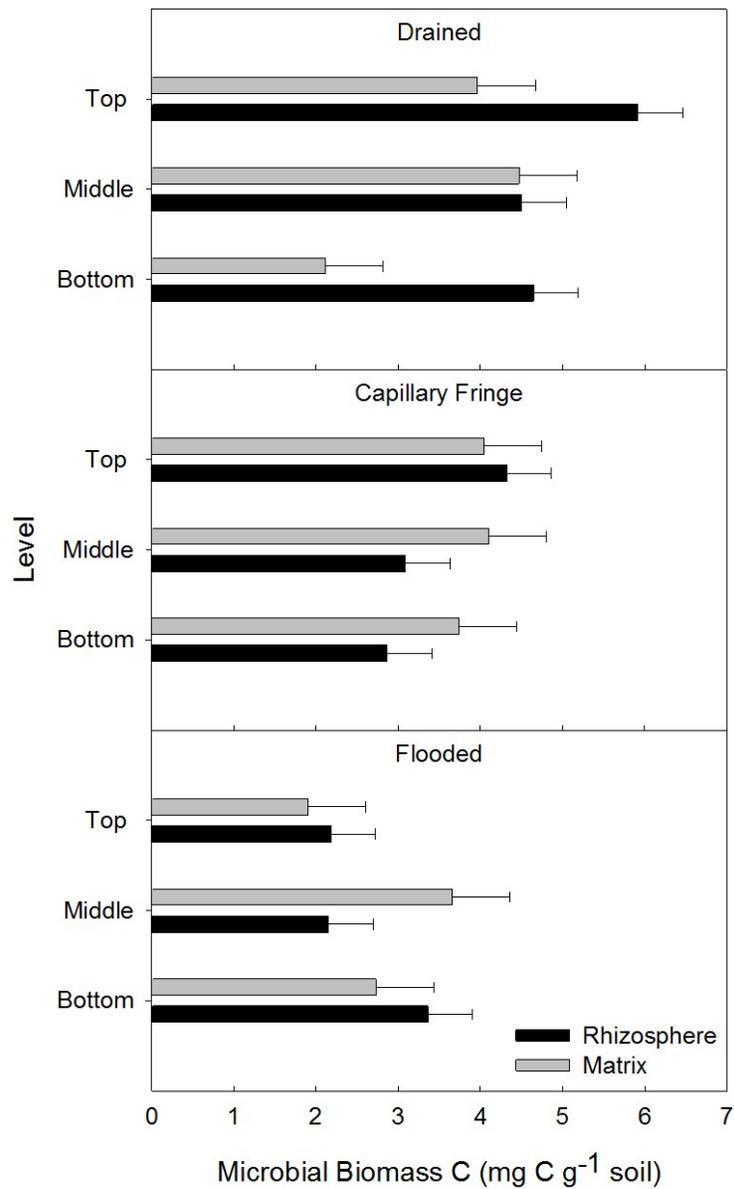


Figure 2.18. Microbial biomass C in the rhizosphere and matrix of all levels and water treatments. No difference was found in the matrix where dead roots were observed, potentially indicating that most easily decomposable organic matter had already been consumed, or that enough organic C diffused into the matrix to support an equally large microbe community.

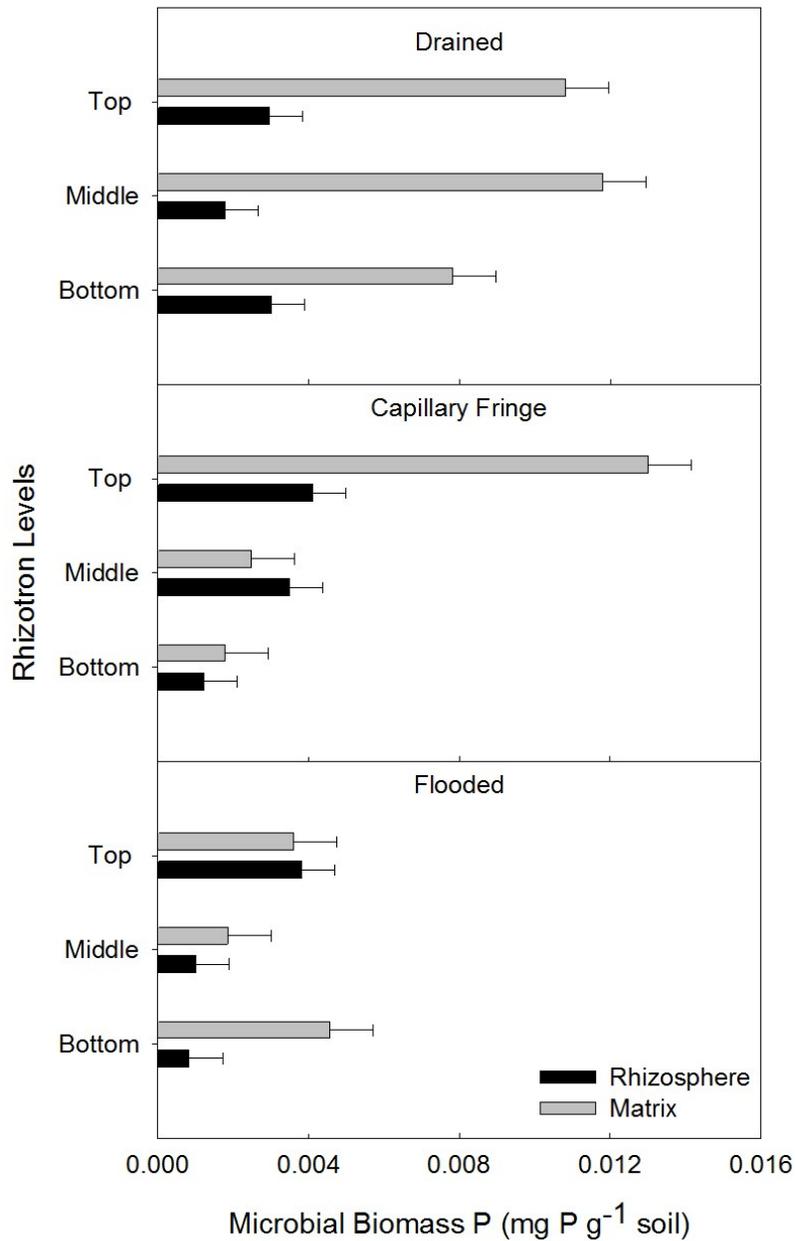


Figure 2.19. Microbial biomass P in the rhizosphere and matrix of all levels and water treatments. More MBP was found in the matrix in the bottom level (most reduced) of the flooded treatment, indicating that the likely bacteria-dominated community was less able to compete with the tree roots for P.

Table 2.1.

Summary of soil properties. This table describes some of the important chemical and physical properties of the soil used in this study. Abit (2009) used the same soil source and sampling location.

Soil Properties		
Property		
Sub Group		Aeric Haplaquod
Texture		LS
Total Organic C	(g C kg ⁻¹)	26.1
Total N	(g N kg ⁻¹)	1.1
Mehlich III P	(mg Fe cm ⁻³)	2.86
Mehlich III Fe	(mg P cm ⁻³)	11.10
pH*		5.30
Bulk Density	(g cm ⁻³)	1.45
Porosity*	(cm ³ cm ⁻³)	0.48
Ksat*	(m d ⁻¹)	3.00

*from Abit, 2009.

Table 2.2 Test of effect slices for soil porewater analysis. This table describes the significance of the analysis of variance using the PROC MIXED procedure in SAS. Where the plant treatment or level column includes a label, it indicates that for that row, the variance was controlled for that plant treatment or level. The column that is left blank is the effect that is being analyzed for significance.

Test of Effect Slices

Effects	Plant Treatment	Level	Pr > F					
			Log ₁₀ TP	Log ₁₀ DRP	Log ₁₀ Fe(II)	Log ₁₀ DOC	Redox	pH
Plant_Treatment*Level		Top	<0.0001	<0.0001	0.0018	<0.0001	0.0029	0.0115
Plant_Treatment*Level		Middle	0.0018	0.0005	0.6797	0.0377	0.8104	0.0022
Plant_Treatment*Level		Bottom	0.5291	0.2591	0.0822	0.0032	0.0029	0.4146
Plant_Treatment*Level	Matrix		0.8538	0.9056	0.6559	0.3291	0.7474	0.1592
Plant_Treatment*Level	Rhizosphere		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
Plant_Treatment*Days	Matrix		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Plant_Treatment*Days	Rhizosphere		<0.0001	<0.0001	0.0197	<0.0001	0.0012	<0.0001
Level*Days		Top	0.0002	0.0003	0.2859	0.2986	0.0285	0.0765
Level*Days		Middle	<0.0001	<0.0001	0.0010	<0.0001	0.0146	<0.0001
Level*Days		Bottom	<0.0001	<0.0001	0.0080	<0.0001	<0.0001	<0.0001
Plant_Treatment*Level*Days	Matrix		<0.0001	<0.0001	0.0008	<0.0001	0.0003	<0.0001
Plant_Treatment*Level*Days	Rhizosphere		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Plant_Treatment*Level*Days		Top	<0.0001	<0.0001	0.0029	<0.0001	0.0016	0.0012
Plant_Treatment*Level*Days		Middle	<0.0001	<0.0001	0.0145	<0.0001	0.1156	<0.0001
Plant_Treatment*Level*Days		Bottom	<0.0001	<0.0001	0.0006	<0.0001	<0.0001	<0.0001