ABSTRACT

MOORBERG, COLBY JAMES. Dynamics of P Release from Wetlands Restored on Agricultural Land. (Under the direction of Michael J. Vepraskas).

Wetland restoration is done, in part, to improve water quality. However, P release to surface waters has been observed in many wetlands restored from agricultural land. Phosphorus is a limiting nutrient in many freshwater systems, so it is important to understand how wetland restoration on agricultural land may affect surface water quality. Numerous studies have examined such water quality impacts of wetland restorations, but the role that plants play in governing the rate of P loss in isolated wetlands is not well understood. The general goal of this research was to examine the role of wetland vegetation in P cycling within restored wetlands. Special attention was paid to the effects of the rhizosphere of bald cypress (*Taxodium distichum* L.) roots on P dissolution in greenhouse and field settings in soils from a 291 ha Carolina bay wetland restored from agricultural land named Juniper Bay. The role of plants in P cycling in restored wetlands was assessed by developing a P budget for that same wetland.

The first study used root-box rhizotrons to examine rhizosphere effects on P dissolution. Rhizotrons were planted with bald cypress saplings or left unplanted to simulate rhizosphere and matrix conditions, respectively. Two soil treatments were imposed to simulate the two dominant soil types – mineral (Aeric Alaquods) and organic (Terric Haplosaprists). The rhizosphere treatment did not cause higher P concentrations in solution than matrix values for either soil type. This was because labile C was not limiting to reduction processes in the matrix of these two soils. Redistribution of roots was observed with root death in deep, reduced soil layers and root growth in oxidizing surface soil layers.

A second study was conducted at Juniper Bay to “field-truth” the root-box rhizotron results. Bald cypress trees were instrumented with minirhizotron tubes, porewater samplers, and groundwater monitoring wells at sites located on mineral or organic soils. The trees exhibited vigorous root growth
during drought conditions in 2011, then root death during the wet seasons of 2012 and 2013. However, redistribution of roots from the deeper subsoil to the surface, as was seen in the root-box rhizotron study, was not observed in this minirhizotron study. This is attributed to a difference in plant age and prior exposure to reducing conditions between the two studies. Soil solution chemistry measurements corresponded closely with results from the root-box rhizotron study and suggested that P dissolution was dependent on Fe reduction under saturated conditions.

A third study examined if Juniper Bay is contributing P to surface waters using a P balance. The change in soil P was evaluated between archived samples taken at restoration (2005), and eight years after restoration (2013). The P pool at the time of restoration was 800 kg P ha\(^{-1}\). After eight years of restoration that P pool declined to 740 kg P ha\(^{-1}\), but that difference was not significant at the \(\alpha=0.05\) level. Atmospheric deposition contributed 7 kg P ha\(^{-1}\), plants extracted 27 kg P ha\(^{-1}\) and incorporated it into woody biomass, and 0.5 Mg P was lost to surface waters draining the site. Because P loss to surface waters was small, and that P concentrations were not high enough to cause eutrophication (< 0.1 mg/L), we concluded that Juniper Bay is not contributing to the degradation of surface water quality of nearby streams following restoration. This is due to little groundwater flowing either into or out of the site as a result of the small hydrologic gradient that exists in this flat wetland system. Further, “isolated” wetlands such as this Carolina bay are ideal sites for future wetland mitigation projects due to limited impacts on surface water quality.
Dynamics of P Release from Wetlands Restored on Agricultural Land

by
Colby James Moorberg

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APPROVED BY:

_______________________________  ______________________________
Michael Vepraskas                Aziz Amoozegar
Committee Chair

_______________________________  ______________________________
Ryan Emanuel                    Daniel Richter
DEDICATION

To Stacy
BIOGRAPHY

Colby James Moorberg, son of Brian Moorberg and Ruth Mroch, 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 (Schacherer) Moorberg of Wallingford, Iowa. 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. After getting stuck waist-deep in muck during his first ever duck hunt as a teenager, Colby discovered his academic and professional passion – wetlands. This eventually led to a Bachelor of Science degree in environmental science from Iowa State University of Science and Technology in Ames, Iowa in 2008. Inspired by soil scientists within the ISU faculty, and by scientists in the ISU Wetland Research Group, Colby continued his education at North Carolina State University, in Raleigh, North Carolina. There he earned a Master of Science degree in soil science in 2010 with a thesis entitled: “Phosphorus Release Mechanisms in Rhizospheres of Wetland Trees”. He continued his graduate work through a PhD in soil science, also at NCSU, studying P dissolution in wetlands at the rhizosphere and field scales. In his free time Colby enjoys cycling, camping, hunting, and fishing. He is also an avid sports fan, cheering on the football teams of his two Alma Maters, and following the Minnesota Twins and Vikings. He enjoys participating with his Masonic Lodges and their respective philanthropies, volunteering for various professional societies, and fostering and training rescued dogs with his wife in their home. Following completion of his PhD, Colby plans to begin his career as a postdoctoral researcher, and aspires to eventually become a professor of soil science.
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CHAPTER 1. LITERATURE REVIEW

1. INTRODUCTION

A common objective of wetland restoration is improvement of water quality. However, wetlands restored from previously farmed and fertilized land can sometimes contribute P to surface and drainage water, thus further impairing water quality. Numerous studies have examined such water quality impacts of wetland restorations, and the underlying mechanisms of P dissolution such as Fe reduction, and dissolved organic C (DOC) competition. In this chapter, plant-soil interactions with P dissolution will be discussed with regard to wetlands restored on previously farmed lands, and to the overall impact on water quality.

2. WETLAND RESTORATION AND CAROLINA BAYS

2.1 Wetland Drainage and Restoration

Wetlands contribute a variety of ecosystem services, such as providing habitat for plants and animals, improving water quality, supplying baseflow to surface waters, and storing water during times of high rainfall (Galatowitsch and van der Valk, 1994). However, these ecosystem services have only recently been recognized in the U.S. Between 1760 and 1970, US wetlands were drained to increase agricultural production and urban development, as well as for mosquito control (Dahl and Allord, 1996). During that time, total wetland area in the contiguous U.S. decreased from 93.5 million ha to 41.7 million ha (221 million ac to 103 million ac, respectively) (Dahl and Allord, 1996). North Carolina, in particular, sustained large wetland alterations and areal losses due to drainage of its abundant wetlands (Cashin et al., 1992). Of the 3.2 million ha (7.8 million ac) estimated pre-colonial wetlands...
in North Carolina (N.C. Department of Environment, Health, and Natural Resources (DEHNR), 1991), 50% have been drained or filled to the extent that they no longer perform their original ecosystem services (Cashin et al., 1992).

Since the 1970s, public awareness of the importance of wetlands has increased (Kean et al., 1988). As a result, state and federal programs have been enacted to encourage “no net loss” of wetlands through legislation that limits filling and draining of wetlands, and encourages restoring artificially drained areas to their original wetland condition (Dahl and Allord, 1996). Examples of such policies include Section 404 of the Clean Water Act (US EPA, 2013) which regulates wetland loss on non-agricultural land, the “Swampbuster” provisions of the 1985 Food Security Act (U.S. Fish and Wildlife Service, 2003) that denies federal subsidies to farmers who knowingly drain wetlands, and the Emergency Wetlands Resources Act of 1986 (U.S. Fish and Wildlife Service, 2013) which requires the Fish and Wildlife Service to publish any trends in wetland gains or losses, thus increasing public awareness of wetland loss. As a result of these and similar policies, wetland area in the U.S. increased by 60,200 ha (148,800 ac) between 1997 and 2002, and by 41,000 ha (101,400 ac) between 2002 and 2007, though the latter increase was not significant (USDA-NRCS, 2013b, 2013). Of the gains to wetland area between 1997 and 2007, 166,000 ha (410,200 ac) came from converted agricultural land (USDA-NRCS, 2013b). There have been 12,000 ha (30,000 ac) total wetlands conserved, restored, or enhanced in North Carolina (N.C. Department of Environment, Health, and Natural Resources (DEHNR), 2012). Based on estimates from the North Carolina Ecosystem Enhancement Program, approximately two-thirds of restored wetlands in North Carolina originated from drained and fertilized
agricultural lands. Since 1999 wetlands restored from agricultural land in North Carolina totals approximately 1500 ha since 1999 (Smith, 2011, personal communication).

2.2 Carolina Bays

One type of wetland in North Carolina that has been commonly drained for forestry and agricultural production is the Carolina bay. These wetlands are elliptical, flat depressions that are oriented along their long axis in a northwest-southeast direction (Ross, 1987). The depressions are bordered in whole or in part by rims of white, or buff-colored coarse sand (Johnson, 1942) and can vary in size from hundreds of meters in diameter to 6 km long by 4 km wide (Kaczorowski, 1977). The bays commonly lie 5 to 15 m below the surrounding plain, and are usually filled with dark, organic-rich mineral soils and organic soils. The leading hypothesis on Carolina bay formation is that they were created by wind blowing across shallow lakes on a beach plain, with the long axis of the bay oriented perpendicular to the direction of the prevailing winds (Kaczorowski, 1977). Carolina bays are not naturally connected to streams or other surface water bodies, thus hydrology is driven largely by rainfall and evapotranspiration (Sharitz, 2003).

Many of these bays have been drained or filled for agriculture. Bennett and Nelson (1991) reported that of the 2,651 Carolina bays larger than 0.8 ha in area in South Carolina, 97% had been disturbed by agriculture (71%), logging (34%), or both. Fertilization practices can create a surplus of inorganic nutrients in agricultural soils when more nutrients are applied than crops can take up during the growing season (Bruland et al., 2003). Bruland et al. (2003) examined a Carolina bay that had one portion restored to wetland conditions, and another portion that remained under agricultural production. The portion still in production
had total P concentrations of 0.29 kg m$^{-3}$ in the soil, compared to 0.07 kg m$^{-3}$ total P in a reference wetland that was never used for agriculture. Ewing et al. (2012a) observed Mehlich III extractable P concentrations as high as 0.095 and 0.077 kg m$^{-3}$ in mineral and organic soils of a restored Carolina bay, respectively; compared to 0.008 and 0.005 kg m$^{-3}$ for analogous soils in three reference Carolina bay wetlands. Phosphorus is a limiting nutrient in many freshwater aquatic systems, such as lakes (Schindler, 1974), rivers, and streams (Reddy and DeLaune, 2008), and additions of P to these systems can cause eutrophication as seen by excessive growth of aquatic plants and algae. Because Carolina bays restored from agricultural land have been shown to contain substantial amounts of P, it is important to understand how chemical changes that occur following restoration of wetland hydrology and reduced soil conditions can cause P release to nearby surface waters.

3. **STUDY SPECIES: BALD CYPRESS**

3.1 **Bald Cypress**

Bald cypress ( Taxodium distichum, L.) is a deciduous conifer found in the coastal regions of the southeastern U.S., and is commonly found in swamps such as Carolina bays, as well as low-lying areas near rivers and streams (Elias, 1980). Bald cypress trees exhibit the highest growth in fine sandy loams with adequate moisture and moderately good drainage, however competition with other tree species generally limit the range of bald cypress to wetland areas (Elias, 1980). Bald cypress are commonly associated with water tupelo ( Nyssa aquatica L.), sweetgum ( Liquidumbar styraciflua L.), green ash ( Fraxinus pennsylvanica L.), red maple ( Acer rubrum L.), and American elm ( Ulmus Americana L.)
(Harlow et al., 1991). The species is also important to wildlife, particularly waterfowl, as a source of food and cover (Elias, 1980).

3.2 Flooding Tolerance and Adaptations

Hook (1984) described bald cypress as the most flood-tolerant tree species of the southeastern U.S., and attributed that tolerance to several physiological and metabolic adaptations exhibited following soil saturation. Pezeschki (1996) observed that below an Eh of +200 mV, bald cypress root cells began anaerobic respiration, and increased alcohol dehydrogenase activity, thus limiting the buildup of toxins known to cause root injury. They have also been observed to accumulate malate and shikimate (a cyclohexanecarboxylic acid) in the root system under saturated and reduced conditions (Li et al., 2010). These organic acids are believed to act as a carbon sink in the roots (Pradet, 1978), thus acting as a CO$_2$ store and as a means of reducing the CO$_2$ partial pressure in the roots under saturated conditions (Crecelius et al., 2003).

Bald cypress can oxidize the rhizosphere (Anderson and Pezeshki, 2000; Colmer, 2003), preventing the buildup of toxins like sulfides (Koch et al., 1990). In addition, bald cypress trees have been observed to redistribute their root system under waterlogged conditions from deep, reduced soil layers to shallow, more oxidized soil layers through the development of adventitious roots near the surface, and root death at depth (Schat, 1984; Slusher et al., 2013; Moorberg et al., 2013). The duration of flooding can also affect root production and plant biomass allocation. Megonigal and Day (1992) observed that periodic flooding caused increased root production, likely allowing the tree adequate moisture during
times when the soil was not waterlogged. A continuously flooded treatment had greater shoot production, while net production was equal between the two hydrologic treatments.

Pneumatophores, commonly called “knees”, are another root adaptation exhibited by bald cypress in ponded environments. These root features protrude above the soil surface, and are believed to anchor the tree, or mediate gas exchange (Mitsch and Gosselink, 2007). Kramer (1952) and Brown (1981) noted that CO$_2$ was exchanged at the knees, while O$_2$ was not, thus concluding that knees do not function as an aerating organ. In addition to mediating CO$_2$ exchange, pneumatophores facilitate the diffusion of methane out of the rhizosphere and into the atmosphere (Sebacher et al., 1985; Vann and Megonigal, 2003; Purvaja et al., 2004).

While these root adaptations allow bald cypress to be more competitive under flooded conditions relative to other trees, growth and photosynthetic gas exchange are affected by flooded conditions. Energy produced via anaerobic respiration is adequate for cell survival in anaerobic conditions (Pezeshki and DeLaune, 1990; Pezeshki, 1991), but is not sufficient for further root growth (Pezeshki, 1991). Net photosynthesis in bald cypress initially decreases for several weeks following soil saturation, but then returns to normal (Pezeshki, 1998). Bald cypress growth rates in saturated and reduced soils has been shown to be greater than that of other less flood-tolerant species (Hook, 1984; Slusher et al., 2013).

3.3 Drought Tolerance

Bald cypress grows well in wetland habitats that experience periodic flooding as well as mild drought (Elcan and Pezeshki, 2002). Photosynthesis in bald cypress decreases under drought stress (Nash and Graves, 1993; Elcan and Pezeshki, 2002; Stiller, 2009; Li et
al., 2010). This has primarily been attributed to stomatal closure (Elcan and Pezeshki, 2002; Stiller, 2009), and not to changes in photosynthesis biochemistry (Stiller, 2009). Reduced net photosynthesis was also attributed to increased specific leaf mass caused by an increase in leaf thickness. This resulted in decreased transpirational water loss per unit mass of leaves (Nash and Graves, 1993). Under drought stress, bald cypress stem growth decreased (Mitsch and Ewel, 1979), and stem hydraulic conductivity was reduced, but wood density increased (Stiller, 2009). A higher wood density makes trees more drought tolerant, but will also decrease stem hydraulic conductivity which can slow growth during periods when water is not limiting (Stiller, 2009).

4. Soil Reduction and the Rhizosphere

4.1 Soil Reduction
In saturated soils, soil pore space is filled with water which, in turn, limits gas exchange with the atmosphere. Oxygen diffuses 10,000 times slower through water than through air (Greenwood, 1961); therefore O₂ available for aerobic respiration decreases precipitously in the presence of biological activity when soils become saturated. Ponnamperuma (1972) reported that within hours of submergence, nearly all oxygen dissolved in water is used by root and microbial respiration, causing the soil to become anoxic.

Oxidation-reduction reactions are chemical reactions in which electrons are transferred from an electron donor (reducing agent) to an electron acceptor (oxidizing agent). Oxidation-reduction reactions are driven by the tendency of a system to decrease in
free energy ($\Delta G$). Reactions that result in the most net loss of free energy are favored. In soil systems, the electron donor is organic matter (Vepraskas and Faulkner, 2001).

Under aerobic conditions, $O_2$ is the predominant electron acceptor for microbial oxidation of organic matter, because the reaction provides the most free energy to the organisms. When $O_2$ is depleted, microbial oxidation of organic matter will continue if alternate electron acceptors are available, though at a slower rate (Ponnamperuma, 1972). Common alternate electron acceptors in soils, in successive order of reduction at a soil pH of 6.5, include $NO_3^-$, $MnO_2$, $Fe(OH)_3$, $SO_4^{2-}$, and $CO_2$ (Ponnamperuma, 1972; Vepraskas and Faulkner, 2001; Kirk, 2004; Mitsch and Gosselink, 2007). The successive order in which these electron acceptors are reduced is related to redox potential ($Eh$), which is a voltage that can be measured in the soil and, in practical application, can be used to predict which reduced species are present in solution (Vepraskas and Faulkner, 2001). High $Eh$ is indicative of oxidized conditions, while low $Eh$ is indicative of reduced conditions. The Nernst equation (Equation 1) summarizes the thermodynamic basis for redox potential:

$$Eh = E^o - \frac{RT}{nF} \ln \left( \frac{\text{(red)}}{\text{(ox)}} \right) - \frac{mRT}{nF} \ln(H^+)$$  \hspace{1cm} [Equation 1]

where $Eh$ is the electrode potential (redox potential, volts, V) for the reaction; $E^o$ is the standard state potential for the half reaction (volts, V) conditions; $R$ is the gas constant; $T$ is the absolute temperature; $n$ is the moles of electrons involved in the reaction; $F$ is the Faraday constant; $(\text{red})$, $(\text{ox})$, and $(H^+)$ are the activities of the reduced species, oxidized species, and $H^+$, respectively; and $m$ is the number of moles of protons involved in the reaction (McBride, 1994). This equation shows that each species mentioned previously
produces a specific Eh, depending on the activity of the reduced species in solution, and the activity of protons in solution (Vepraskas and Faulkner, 2001). This equation can be modified by substituting pH for the activity of protons, and inserting the constants to produce the following equation:

\[ Eh \ (mV) = E^\circ - \frac{59}{n} \log \frac{(red)}{(ox)} + \frac{59m}{n} pH \]  

[Equation 2]

Equation 2 can be used to produce a phase diagram to depict the dominant electron acceptors under a typical soil pH values which, under the assumptions listed by Vepraskas and Faulkner (2001), show a successive order of reduction of electron acceptors mentioned earlier is generally the case, but is pH dependent.

**4.2 Reduction Microsites**

The chemical species that are reduced in saturated soils depend on the type of organic matter, the nature and concentration of electron acceptors, soil temperature, and duration of submergence (Ponnamperuma, 1972). Because of the natural heterogeneity of soils, it is possible to create soil “reduction microsites” when factors affecting reduction are ideal in zones of soil as small as 25 mm in diameter (Parkin, 1987). Such microsites have been observed in areas high in labile carbon, such as around decaying roots, and have been attributed with a majority of denitrification (Parkin, 1987; Christensen et al., 1990; Jacinthe et al., 1998), and increased production of methane and H$_2$S gasses (Crozier et al., 1995). The reduction of Fe and Mn in reduction microsites is attributed to the development of permanent, year-round soil morphological characteristics making them useful for identifying hydric soils (Vepraskas, 1996). These processes result in the reduction, translocation, and/or
accumulation of Fe, Mn, and other reducible elements (Hurt and Carlisle, 2001) providing the basis for several hydric soil field indicators. Examples of hydric soil field indicators include the stripped matrix; depleted matrix; and depleted dark surface (USDA NRCS), 2010).

4.3 Reduction Microsites and the Rhizosphere

Because roots are the primary source of labile carbon to fuel reduction in microsites, it is important to consider the effects of the rhizosphere on reduced soils. The rhizosphere is a narrow zone of soil around the root that is rich in organic compounds excreted from the roots which are a food source for soil microbes (Curl and Truelove, 1986). It is separate from the bulk soil which is not influenced by roots other than the withdrawal of water and nutrients (Russell, 1977). Under anaerobic conditions roots can either facilitate the diffusion of O₂ from the atmosphere into the rhizosphere, or act as a carbon source for microbes in the form of root exudates and/or decaying roots. These conditions have even been observed at different depths with the same root system (Moorberg et al., 2013).

Root exudates include carbohydrates, amino acids, organic acids, enzymatic proteins, and other organic compounds (Russell, 1977). These compounds can be used as a C source by microbes resulting in a rhizosphere that is more reduced than the matrix where these exudates do not exist. Reduced soil conditions sustained over a long period of time (e.g., 14 d) can result in root death at depth, while increasing the number of adventitious roots near the surface (Schat, 1984; Ernst, 1990). The organic C released from roots that die within the deepest, most reduced portions of the soil can, in turn, drive further soil reduction as the root tissue forms the loci for reduction microsites, or “hot spots” (Parkin, 1987).
Plant cells require O\(_2\) to perform aerobic respiration, and to grow at optimal rates (Cronk and Fennessy, 2001). In order to maintain high rates of respiration under anaerobic conditions, plant roots have adapted to anaerobic conditions through the development of many physiological changes. Those physiological changes help plants survive in anaerobic soils by increasing O\(_2\) diffusion to the roots, decreasing respiration rates, or by removing byproducts of anaerobic respiration. Aerenchyma, one of the physiological adaptations, are large intercellular air spaces within the stem and roots that conduct O\(_2\) from leaves and lenticels down to the roots (Colmer, 2003). In addition, aerenchyma facilitate the diffusion of methane, sulfides, and other toxic gases out of the plant through lenticels located on the stem (Vartepetian and Jackson, 1997). The O\(_2\) that diffuses to the roots can then leak into the surrounding soil and oxygenate the rhizosphere. The distance that O\(_2\) penetrates the soil depends on the rate of transport out of the root and into the soil, and by the rate of consumption within the rhizosphere (Cronk and Fennessy, 2001). Common plant adaptations for growth in reduced soils are summarized in Table 1.1.

### 4.4 Root Monitoring Methods

Because roots can both facilitate O\(_2\) diffusion into the rhizosphere, and act as a source of labile carbon to drive soil reduction, it is important to monitor root systems in real time in wetland soil studies. Böhm (1979) summarized many of the common methods of observing roots, including root excavations, monolith methods, auger methods, and profile wall methods. However, many of these are undesirable for wetland soil studies because they require a well-drained site, directly impact the plant in question, or cannot be performed on a repeated basis for ongoing studies. Glass wall methods do allow for the examination of
roots through time with minimal disturbance to the plant (Böhm, 1979), however root cellar and root laboratory methods still require relatively dry sites and can be expensive to construct.

The use of glass-faced containers, or now more commonly referred to as root-box rhizotrons, is a container-study adaption of the glass wall method. Root-box rhizotrons were first introduced by Sachs (1873), and come in a variety of shapes and sizes, depending on the needs of the researcher and plant species, but generally consist of two transparent faces attached to a frame made of metal, wood, or other materials (Böhm, 1979). The faces are covered with a removable sheet made of opaque material such as aluminum foil to limit root exposure to light. The root-box rhizotrons are commonly tilted at an angle to force roots to grow against the transparent face (Böhm, 1979). Limitations of this method include the size of the plants being studied, and that soil materials in the boxes usually must be disturbed (Böhm, 1979). Root-box rhizotrons allow researchers more control over conditions to which the plants and roots are exposed, such as soil saturation and ponding for wetland soil studies. In addition, soil solution properties and redox potential can be monitored over time using instrumentation inserted through the root-box rhizotron face (Moorberg et al., 2013).

A further adaption of the glass wall method is to make observations of roots under field conditions using “glass tubes” (Böhm, 1979), today commonly referred to as “minirhizotrons”. First proposed by Bates (1937), minirhizotrons allow for repeated, in situ root observations and measurements with minimal disturbance to the plant (Böhm, 1979) using cameras and scanners inserted into the tube (Hendrick and Pregitzer, 1996). Minirhizotron tubes are commonly installed at a 45° angle to limit artifacts of vertical
installation, such as missing vertically growing roots or creating artificial conditions caused by preferential water flow along the tube (Johnson et al., 2001). Iverson et al. (2012) presented a thorough review of problems associated with the installation and use of minirhizotrons in wetlands, and the common methods used to overcome them. Recommendations include: i) installation of the minirhizotron so that the tube opening is above the potential flooding or ponding depth of the site, ii) the use of plugs at the buried end of the minirhizotron that will remain water-tight for the duration of the study, iii) consideration of microtopography during minirhizotron installation, and iv) the need to anchor minirhizotron tubes in peat soils with low soil strength. A common limiting factor in minirhizotron studies is the number of tubes that can be analyzed. However, advances in imaging and root analysis technology have given researchers the ability to install and utilize more minirhizotrons for their studies, thus improving the overall quality of the data (Iversen et al., 2012). Minirhizotrons are suitable for fine-root (< 2 mm diam.) studies, but course-root studies in wetlands will require other methods. Overall, minirhizotrons are useful for monitoring dynamics of ephemeral roots (Iversen et al., 2012), a primary source of labile carbon for soil reduction microsites (Parkin, 1987).

5. PHOSPHORUS AND WETLAND RESTORATION

5.1 Phosphorus Export Following Wetland Restoration

While wetlands are commonly associated with improvements to water quality, wetland restoration on agricultural land containing large amounts of legacy P from fertilization can result in the release of P from the recently flooded wetland (Van Dijk et al., 2004; Aldous et al., 2005; Ardon et al., 2010b; Vepraskas et al., 2010). That solubilized P
can contribute to eutrophication and water quality impairments in surface water bodies downstream of the restored wetland (Aldous et al., 2005; Duff et al., 2009; Ardon et al., 2010b).

Restored wetlands exhibiting releases of P have come from a wide variety of agricultural lands, including wetlands restored from agricultural grasslands in the Netherlands (Van Dijk et al., 2004), grazing and crop lands in Upper Klamath Lake, Oregon, USA (Aldous et al., 2005, 2007; Duff et al., 2009), croplands in a riverine wetland complex in the North Carolina Coastal Plain subject to tidal fluxes (Ardon et al., 2010a; b) Carolina bay wetlands in North Carolina, USA (Bruland et al., 2003; Vepraskas et al., 2010), subtropical peat soils restored from dairy land (Dunne et al., 2011), and lakeside wetlands restored from rice paddy soils in the Yangtze-Huaihe region, China (Zhou et al., 2010). To limit P export from wetlands restored from cropland in future restorations, it is important to understand the mechanisms through which P is released into solution.

5.2 Mechanisms of Phosphorus Dissolution

Dissolution of P in restored wetlands has been attributed to several mechanisms, including Fe reduction (Reddy and DeLaune, 2008), competition with organic acids (Earl et al., 1979; Lopez-Hernandez et al., 1986; Violante et al., 1991; Gerke, 1992a; Borggaard et al., 2005), P mineralization (Greaves and Webley, 1965; Raghu and MacRae, 1966), changes in acidity (Jackson, 1964; Ponnamperuma, 1972; Stumm and Morgan, 1981), and increased P diffusion (Turner and Gilliam, 1974a; b). Iron reduction is considered to be the dominant mechanism controlling P solubility in anaerobic systems (Reddy and DeLaune, 2008). When the soil becomes anaerobic, Fe$^{3+}$ is microbially-reduced, and the solubilized
Fe$^{2+}$ releases phosphate bonded to it into the soil solution (Patrick and Khalid, 1974; Holford and Patrick, 1981; Sah and Mikkelsen, 1986; Vadas and Sims, 1998; Shenker et al., 2005).

Organic acids may also affect phosphate adsorption. Borggaard et al. (2005) summarized the mechanisms by which organic acids, or DOC, can affect phosphate adsorption. These include: (i) competition for adsorption sites, (ii) dissolution of adsorbents (e.g. Al oxide, ferrihydrite, goethite), (iii) change of the surface charge of adsorbents, (iv) creation of new adsorption sites through adsorption of metal ions, and (v) retardation of crystal growth of poorly ordered aluminum and iron oxides. There is disagreement in the literature in regard to the competitive effects of DOC and phosphate under field conditions. Weng et al. (2008) found these mechanisms relevant in explaining changes in phosphate adsorption in relation to changes in DOC while Borggaard et al. (2005) and Guppy et al. (2005) did not.

Mineralization of organic P within the rhizosphere may also contribute to increased concentrations of P in solution (Greaves and Webley, 1965; Raghu and MacRae, 1966). Phosphorus mineralizing bacteria have been found primarily within the rhizosphere and on root surfaces (Greaves and Webley, 1965). Raghu and MacRae (1966) found that root exudates stimulate these microbes. In addition, the activity of phosphatases, the microbial enzymes that mineralize organic P, has been observed to be highest in the rhizosphere of slash pine (*Pinus elliottii* L.) (Fox and Comerford, 1992), and phosphatase activity can be stimulated by drying and rewetting cycles of wetland soils (Song et al., 2007).
A change in acidity towards a more neutral pH can affect P concentrations in both acidic and alkaline soils. In acidic soils, phosphates associated with Fe$^{3+}$ and Al$^{3+}$ predominate; while in alkaline soils calcium phosphates predominate (Jackson, 1964; Ponnamperuma, 1972; Stumm and Morgan, 1981). In acidic soils, pH increases as conditions become more reduced and P concentration also increases. As pH decreases in alkaline soils during reduction, calcium phosphates dissolve causing P to increase. Turner and Gilliam (1974a; b) noted that smaller changes in pH occurs in alkaline soils. Turner and Gilliam (1974a; b) offered a physical explanation for why P concentration in solution increases following saturation. In examining several rice paddy soils, they used an anion exchange resin as a P sink, and compared moist and saturated soils under both reduced and oxidized conditions. Regardless of the oxidation status, more P was absorbed on the resin under saturated conditions than in moist soil conditions. However, the highest amounts of absorbed P were under saturated, reduced conditions. They concluded that under saturated conditions P can diffuse faster through the soil making more P being available for plants over a given time period, but acknowledged that changes in pH or iron reductions may have also influenced P release under reducing conditions.

5.3 Evaluating P Dissolution in Wetlands
Researchers have approached the problem of P dissolution using several different techniques. In restorations where drainage water exits the restoration site, the quality of the water draining from the site has been compared to drainage water from nearby sites still under agricultural production, and/or unaltered reference wetlands (Bruland et al., 2003; Ardon et al., 2010a). Phosphorus balances can also be determined to study P fluxes into, out
of, and within restored wetlands (Ardon et al., 2010a). Such P balances can also be employed by researchers to estimate the time and magnitude in which restored wetlands will contribute P to surface or drainage waters. Ardon et al. (2010a) estimated that soils from a riverine wetland subject to tidal flux in the North Carolina Coastal Plain soil will continue to release P for 3 to 16 years. Reddy et al. (2011) projected that wetlands restored from dairy pastures and cropland will contribute P between 20 and 120 years after restoration. Aldous et al. (2007) used a mesocosm study to predict that between 1 and 16% of the total P contained within restored lake-fringe wetland soils would be released to Upper Klamath Lake as a one-time P loading event following restoration of wetland hydrology.

The time frame in which P discharges return to pre-agriculture rates can be expected to vary largely due to differences in fertilization history, soil properties, and hydrology. At this time no long-term studies have observed the complete release or removal of all legacy P from a restored wetland. More long-term studies examining P discharges from wetland restorations are needed to help guide future wetland restorations and to limit possible negative effects on surface water quality.
6. SOURCES CITED


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Smith, H. 2011. Personal communication.


7. **Table**

**Table 1.1. Summary of root adaptations to saturated, reduced soils (from Cronk and Fennessy, 2001).**

<table>
<thead>
<tr>
<th>Root Adaption</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerenchyma</td>
<td>Aerenchymous tissue forms cavities in stems and roots called lacunae which facilitate gas transport within the plant</td>
</tr>
<tr>
<td>Adventitious Roots</td>
<td>Roots that grow laterally from the base of the stem and grow into the soil or along the soil surface</td>
</tr>
<tr>
<td>Shallow Rooting</td>
<td>Roots grow on the soil surface, or near the surface where soil is most consistently oxygenated</td>
</tr>
<tr>
<td>Pneumatophores</td>
<td>Modified, erect roots that grow upwards and assist in exchange of CO₂ and other gases with the atmosphere</td>
</tr>
<tr>
<td>Prop &amp; Drop Roots</td>
<td>Prop roots and drop roots extend from the lower portion of the stem and branches, respectively, towards the substrate, and contain a large number of lenticels that facilitate gas exchange with the atmosphere</td>
</tr>
</tbody>
</table>
CHAPTER 2. PHOSPHORUS DISSOLUTION IN THE RHIZOSPHERE OF BALD CYPRESS TREES IN RESTORED WETLAND SOILS

1. ABSTRACT

Phosphorus release to ground or surface waters has been observed in wetlands restored from farmland, but the causal mechanism is not fully understood. This study examined whether rhizospheres caused by the roots of bald cypress (*Taxodium distichum* L.) are a source of increased P dissolution as compared to the soil matrix. The study was conducted in root-box rhizotrons filled with mineral and organic soil materials (Aeric Alaquods and Terric Haplosaprists, respectively) from a Carolina bay wetland recently restored from row crop agriculture. Rhizotrons were either planted with bald cypress saplings or left unplanted to simulate rhizosphere and matrix conditions, respectively. Ponding was imposed for 128 d. Soil porewater was sampled at three layers (0-22 cm, 22-41 cm, and 41-59 cm) in each rhizotron twice-monthly for dissolved total and reactive P (DTP and DRP, respectively), dissolved organic C (DOC), and ferrous Fe (Fe$^{2+}$). Redox potential (Eh) was also monitored. Monthly root counts were performed manually to monitor growth and death. Root death was most prevalent at 41-59 cm, while vigorous root growth was observed near the surface. Reduced conditions were observed across all layers in the rhizosphere over the first 30 d following saturation. Elevated concentrations of DOC over matrix concentrations were observed in the rhizosphere treatment of both soils at the 22-41 and 41-59 cm layers. The mineral soil also had an initial peak in Fe$^{2+}$ above matrix concentrations in the first 30 d. Despite conditions favoring a possible increase in P
dissolution, no corresponding P increase occurred above matrix concentrations. Near the surface, rhizosphere P concentrations declined below matrix concentrations after 60 days of ponding. Our results show that the rhizosphere of bald cypress did not cause higher P concentrations than matrix values for mineral and organic soils with 3.5 and 19.5 % C, respectively. In addition, vigorous root growth near the surface resulted in more oxidizing conditions and/or plant uptake of P which decreased P concentration below matrix values.
2. INTRODUCTION

Over 50% of the original wetlands in the lower 48 states of the U.S. were drained for food production between 1780 and 1980 (Dahl, 2006). Since 1977, state and federal programs have been enacted to reverse wetland loss by restoring artificially drained areas to their original condition, frequently by plugging or filling drainage ditches. A common goal in wetland restoration is the improvement of water quality. However, in cases where wetlands are restored from agricultural land that contain large amounts of residual P leftover from years of fertilization, the saturated and reduced soil conditions created in wetlands can cause the release of P from the newly flooded wetland (Van Dijk et al., 2004; Aldous et al., 2007; Ardon et al., 2010a; Vepraskas et al., 2010). This exported P can contribute to eutrophication in surface water bodies that lie downstream of the restored wetland (Aldous et al., 2007; Duff et al., 2009; Ardon et al., 2010a).

There are several mechanisms by which P can be dissolved in restored wetlands, including Fe reduction (Reddy and DeLaune, 2008), and ligand exchange (Earl et al., 1979; Lopez-Hernandez et al., 1986; Violante et al., 1991; Gerke, 1992a). Iron reduction is considered to be the dominant mechanism controlling P solubility in anaerobic systems (Reddy and DeLaune, 2008). Under anaerobic conditions, Fe$^{3+}$ is microbially reduced to soluble Fe$^{2+}$, which releases phosphate bound to ferric iron into solution (Patrick and Khalid, 1974; Holford and Patrick, 1981; Sah and Mikkelsen, 1986; Vadas and Sims, 1998; Shenker et al., 2005). Organic acids may also affect phosphate adsorption. Borggaard et al. (2005) summarized the mechanisms by which organic acids can affect phosphate adsorption, including: (i) competition for adsorption sites, (ii) dissolution of adsorbents, (iii)
change of the surface charge of adsorbents, (iv) creation of new adsorption sites through adsorption of metal ions, and (v) retardation of crystal growth of poorly ordered aluminum and iron oxides. However, there is disagreement in the literature in regard to the competitive effects of DOC and phosphate under field conditions. Guppy et al. (2005) and Borggaard et al. (2005) did not find these mechanisms relevant in explaining changes in phosphate adsorption, while Weng et al. (2008) did.

As O$_2$ becomes less available for root and microbial respiration in saturated soils, the soils become more reduced, and the decomposition of organic compounds is slowed. As a result, DOC accumulates in the soil solution (Fiedler and Kalbitz, 2003). [Thurman (1985) defined DOC as organic C in solution that passes through a 0.45 μm filter.] The DOC molecules are negatively charged, and in large concentrations can compete with PO$_4^{3-}$ compounds for cation bridges. Dissolved organic C has also been observed to form complexes with Fe and Al oxides (Dolfing et al., 1999) and/or solubilize Fe and Al (Gerke, 1992a). Dissolved organic C from root exudates and decaying roots contain carboxylate functional groups which have been shown to displace P from the soil particle, releasing the P into solution (Earl et al., 1979; Lopez-Hernandez et al., 1986; Violante et al., 1991; Gerke, 1992a).

Although it is well known that soil P dissolves into the soil solution under reducing conditions, the precise location of P release in the soil (matrix vs. rhizosphere) is not well documented. Reduction can occur in “microsites” or “hot-spots” that develop around residues of readily decomposable organic C in zones as small as 25 mm in diameter (Parkin, 1987). Such residues include dead roots, buried leaves, and other actively decomposing
organic matter containing labile C (Parkin, 1987; Christensen et al., 1990; Jacinthe et al., 1998). Denitrification, CH$_4$ production, and S reduction have been found to occur at higher rates in microsites than in the matrix (Parkin, 1987; Crozier et al., 1995). In a rhizotron root-box study similar to the one presented here, Moorberg et al. (2013) examined P dissolution during ponding in the matrix and rhizosphere of bald cypress (*Taxodium distichum*, L) in a sand with 3.5% organic C. They found that despite increased Fe$^{2+}$ and DOC concentrations and lower Eh values in the rhizosphere, there was no increase in P concentration over matrix concentrations. In addition, near the surface (0-22 cm), Eh was moderated at higher potentials by the rhizosphere causing a decrease in Fe$^{2+}$ and DOC concentration, and a significant decrease in dissolved P. It was hypothesized that the organic C concentrations of the matrix were high enough to overcome to support Fe reduction at levels found in the rhizosphere.

Some tree species such as bald cypress that are adapted to anaerobic soil conditions and have moderate drought tolerance for survival during dry periods are used to restore wetlands (Li et al., 2010). Under drought stress, bald cypress exhibits significant decreases in net photosynthesis (Nash and Graves, 1993; Elcan and Pezeshki, 2002; Stiller, 2009; Li et al., 2010). This has primarily been attributed to stomatal closure (Elcan and Pezeshki, 2002; Stiller, 2009), but not changes in photosynthesis biochemistry (Stiller, 2009). Under drought stress bald cypress has been shown to have decreased stem growth (Mitsch and Ewel, 1979) and root growth (Palta et al., 2012), lower stem hydraulic conductivity, and higher wood density (Stiller, 2009).
Bald cypress metabolic adaptations to flooded conditions include anaerobic respiration and the increase of alcohol dehydrogenase activity (Pezeshki et al., 1996), which limits the buildup of toxins known to cause root injury. Bald cypress can also accumulate shikimate and malate (metabolites associated with anaerobic respiration) under reducing conditions (Li et al., 2010). Physiological adaptations to flooded conditions include the development of pneumatophores, or “knees”, that are believed to help anchor the tree (Mitsch and Gosselink, 2007), or to facilitate CO$_2$ (Kramer et al., 1952; Brown, 1981) and CH$_4$ (Sebacher et al., 1985; Vann and Megonigal, 2003; Purvaja et al., 2004) exchange with the atmosphere. Bald cypress also express trunk buttressing, adventitious root growth, hypertrophied lenticels, and aerenchyma (Slusher et al., 2013). Aerenchyma facilitate the diffusion of atmospheric O$_2$ down to the roots, permitting aerobic respiration (Pezeshki et al., 1996; Vartepetian and Jackson, 1997; Anderson and Pezeshki, 2000). As a result, some O$_2$ is diffused into the rhizosphere allowing for a zone of elevated redox potential in the rhizosphere (Anderson and Pezeshki, 2000; Colmer, 2003).

In a root-box rhizotron study, Slusher (2013) observed the formation of aerenchyma in existing and newly formed roots of bald cypress saplings during sustained ponding. During flooded conditions, deep roots die while roots closer to the surface continue to live and grow because they are able to develop aerenchyma (Schat, 1984; Slusher et al., 2013). The presence of dead roots serving as a C source deeper in the soil, with living roots containing aerenchyma near the surface, creates both reduced and oxidized microsites in the soil which may be zones of P release and P immobilization. This process has been observed in mineral wetland soils from restored Carolina bay (Moorberg et al., 2013), but it is not
known if this also occurs in Histosols (organic soils) – a common soil in Carolina bays and pocosin wetlands in the Southeastern US, and wetlands around the world.

We hypothesized that within the root zone of trees adapted to anaerobic conditions, soil reduction microsites will form near dead roots, and that these dead roots will be the foci of further dissolution of P by Fe reduction and/or DOC competition for binding sites. We also hypothesized that P concentrations will be lower in areas of increased root growth near the surface due to plant uptake or precipitation of P through Fe oxidation. Our study objectives were to: 1) compare concentrations of P, Fe\(^{2+}\), and DOC in the matrix and rhizosphere of bald cypress saplings, 2) evaluate the effect of soil type (mineral vs. organic) on P dissolution over time, and 3) evaluate the potential of plant uptake and/or rhizosphere oxidation on reducing P concentrations in reduced soils.

3. METHODS

The methods used for this study largely followed those described by Moorberg et al. (2013) with the primary changes relating to the experimental design.

3.1. Site Description

The two soil materials used in this study were collected from a Carolina Bay wetland (Juniper Bay) located approximately 10 km southeast of Lumberton, NC in Robeson County (34°30’30”N, 79°01’30”E). Ewing et al. (2012) reported that the wetland was drained and used for agriculture from 1970 through 2000. During that period it was fertilized and limed annually to meet soil test recommendations for crop production (Ewing et al., 2012b). In 2005, the site was restored to wetland by filling ditches and planting bald cypress and other tree species. Vepraskas et al. (2010) reported that prior to restoration, drainage water
concentrations of dissolved reactive P (DRP) rarely exceeded 100 μg P L\(^{-1}\)—the threshold concentration of P at which eutrophication of freshwaters is likely to occur (Correll, 1998). However, once wetland hydrology was restored in 2005 the concentrations of DRP regularly exceeded 100 μg 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.

Representative soil materials from Juniper Bay were procured from two locations in the wetland that have been studied previously by Taggart et al. (2011, 2012), Ewing et al. (2012), Moorberg et al. (2013) and Slusher et al. (2013). The respective soil properties are summarized in Table 2.1. Approximately 0.3 m\(^3\) of soil was collected from both the Ap horizon (0-20 cm layer) of a Leon sand (sandy, siliceous, thermic Aeric Alaquods), and the Oa horizon of a Ponzer muck (loamy, mixed, dysic, thermic Terric Haplosaprists) by shovel and transported to the greenhouse in plastic buckets. The moist soil was passed through a 1-cm-mesh screen to remove large plant material. After sieving, the material was thoroughly mixed by hand and stored in its moist state.

Particle-size distribution was determined on the soil material by the hydrometer method described by Gee and Or (2002). Total organic C and total N were determined by dry combustion with a Perkin-Elmer PE2400 CHN Elemental Analyzer (Culmo, 1988). Soil pH was determined by Abit (2009) using a 1:1 soil to water mass ratio. Bulk density was measured by the core method (Grossman and Reinsch, 2002).
3.2. Planting and Rhizotron Construction

This study was conducted in a greenhouse using rhizotrons similar to that of Neufeld (1989) to monitor root development. The rhizotrons’ construction was described in detail by Moorberg et al. (2013) and consisted of a frame made of expanded PVC and two polycarbonate windows for viewing roots, as shown in Figure 2.1. The interior volume was 8.4 L of soil (interior dimensions were 48.3 cm tall by 30.5 cm wide by 5.7 cm deep). Stopcocks were installed near the bottom of the frame for drainage. A layer of no. 2 well sand was added to each rhizotron to a depth of 2.5 cm from the bottom to facilitate uniform drainage. Then, each rhizotron was packed with soil material. Soil was added in increments to the rhizotrons and manually tamped to a bulk density of 1.45 g cm\(^{-1}\) in the mineral soil, and 0.62 g cm\(^{-1}\) in the organic soil simulating field conditions at Juniper Bay (Ewing et al., 2012b). Approximately 2.5 to 5 cm of space was left between the soil surface and the top of the rhizotrons to allow for at least 1 cm of ponding depth.

3.3. Experimental Design

The study included two repetitions – one conducted in 2010, and the second in 2011. In each repetition, a split-split-plot design was used with two soil treatments (mineral and organic soil), and two planting treatments (a planted treatment to simulate rhizosphere conditions, and an unplanted treatment to simulate matrix conditions). Sixteen rhizotrons were used in both repetitions. Of those, eight rhizotrons were packed with mineral soil material, and eight were packed with organic soil material. Within each group of eight rhizotrons, five were planted with one bald cypress sapling each and three were left as unplanted controls. Rhizotrons in the planted treatment were used for sampling the rhizospheres around roots, while those in the unplanted treatment were used for sampling
the soil matrix. One planted rhizotron from the 2010 repetition was removed due to the tree being injured while the rhizotron was being moved.

Bald cypress was used in this study because the species was planted in the restoration of Juniper Bay and has been previously studied in rhizotron and container studies using Juniper Bay soils (Slusher et al., 2013; Moorberg et al., 2013). In 2010, saplings were acquired from the North Carolina Division of Forest Resources Nursery (Goldsboro, NC, USA). In 2011 that nursery did not have bald cypress seedlings available so seedlings were attained from South Carolina SuperTree Nursery® (Blenheim, SC, USA). Five saplings for each repetition were selected based on a distance of approximately 60 cm from root collar to shoot tip, and uniform root ball size and stem diameter. During planting in the rhizotrons, each sapling was suspended during the addition and packing of the soil material so that the root collar remained at what would be the final soil surface. The soil was tamped by hand to the bulk density noted above, very carefully to avoid root injury. The rhizotrons were packed and planted in March at the start of the North Carolina growing season.

The saplings were grown in the rhizotrons for five months to allow roots to reach the bottom of the rhizotrons, and to provide a sufficient number of roots for sampling. During this phase, each rhizotron was supported at a 30° angle from the vertical (Figure 2.2) to allow downward root growth against the front face for examination and sampling. The polycarbonate faces were covered with steel panels (Figure 2.2) prior to sampler installation and with Al foil after sampler installation to minimize light exposure below the soil surface. Both planted and unplanted rhizotrons were watered similarly with tap water (City of Raleigh, 2013) and kept under the same greenhouse conditions during the growth phase.
Rhizotrons were moved to new locations on the bench monthly by randomly assigning a bench-top location to avoid potential confounding effects associated with locations in the greenhouse.

Tensiometers were installed in each rhizotron to monitor soil water potential (Figure 2.1). Tensiometers were constructed according to Young and Sisson (2002) and installed vertically with the porous cups located 7.5 cm below the surface. The soil water matric potential was measured four times or more weekly using a vacuum gage (Tensimeter, Soil Measurement Systems, Tucson, AZ). After trees were planted, all rhizotrons were kept at field capacity moisture, defined as the soil matric potential between -100 and -300 cm for this experiment during the growth phase.

Redox potential was monitored in each rhizotron during the growth phase to ensure watering rates would not cause anaerobic conditions. Redox potential was measured using platinum-tipped electrodes constructed by a method similar to that of Wafer et al. (2004) (Figure 2.1). 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. Using a calomel reference electrode and voltmeter, redox measurements were made weekly to ensure over-watering and soil reduction were not occurring during the growth stage. Voltage readings were corrected relative to a standard hydrogen electrode (Vepraskas and Faulkner, 2001).

The roots reached the bottom of the rhizotrons by early August and late July for the 2010 and 2011 repetitions, respectively. At that time, one 2.5 mm Standard Rhizon Sampler (Rhizosphere Research Products, Dolderstraat 62, NL 6706JG Wageningen, The
Netherlands) was installed within the top (0-22 cm), middle (22-41 cm), and bottom (41-59 cm) layers of each rhizotron to collect soil porewater samples. The Rhizon samplers were placed adjacent to a root in planted rhizotrons, and in the matrix in unplanted rhizotrons (Figure 2.3).

Rhizon samplers were shortened to 3 cm prior to installation to ensure that the water extracted included the solution near the rhizosphere of the root that was visible through the rhizotron face. Each sampler tube was cut to length and then dipped in epoxy to seal the cut tip (Soil Moisture Epoxy Kit, Soilmoisture Equipment Corp. Santa Barbara, California, USA). Before installation, Rhizon samplers were stored for 24 h in deionized water with the hydrophilic tips submerged to saturate them for use. One day before ponding, samplers were installed by inserting them through holes drilled through the face of each rhizotron. Silicone caulk was applied around the portion of the sampler tube that protruded from the rhizotron face to ensure a tight seal.

Redox electrodes were installed within 1 cm of each Rhizon sampler to monitor Eh in both the matrix and rhizospheres (Figure 2.3). These electrodes were approximately 10 cm in length, and were installed through a hole drilled through the face of each rhizotron so that the Pt tip of each electrode was in contact with the soil inside the rhizotron. In addition to redox measurements, rhizotrons were checked for the presence of Fe$^{2+}$ and H$_2$S at each depth using α, α, dipyridyle dye, and by smelling soil samples from each horizon, respectively. This was performed during destructive sampling at the completion of each repetition.
3.4. Saturation and Sampling

One day after installation of redox electrodes and Rhizon samplers, each rhizotron was placed in a vertical position and remained vertical for the remainder of the study. Each rhizotron was saturated with tap water from the bottom to the top until 2.5 cm of water was ponded on the surface. The ponded condition was maintained for the remainder of the experiment. Rhizotrons remained saturated for a total of 127 d. These ponding durations are representative of the two plant communities established at Juniper Bay--bay forest (104 d yr\(^{-1}\)) and non-riverine swamp forest (123 d yr\(^{-1}\)) (Caldwell et al., 2011).

Starting one day after the rhizotrons were flooded, soil pore-water sampling was performed twice monthly and continued through the end of the experiment. Two amber-colored serum bottles (100 ml and 30 ml) were used to collect the samples. Prior to sampling, each 100 ml bottle was acid-washed and dried, acidified with 0.2 ml of 12 M \(\text{H}_2\text{SO}_4\), capped with a rubber septa and aluminum cap, and evacuated to approximately -800 cm H\(_2\)O using an electric vacuum pump. The sample in the 100 ml bottle was analyzed for dissolved Fe\(^{2+}\), reactive P (DRP), and dissolved total P (DTP). Each 30 ml bottle was similarly washed, dried, capped, and evacuated, but left unacidified. These samples were analyzed for DOC and pH. All porewater samples were collected through a 25-gauge, 3.8 cm long, Luer-lock needle that was attached to each Rhizon sampler (Figure 2.3). After purging one sampler volume (0.187 ml), approximate sample volumes of 15 ml and 30 ml were collected in the 30 ml and 100 ml bottles, respectively. Sample collection was generally finished within 8 hours of the start of sampling, but some large bottles were allowed to remain collecting overnight if the flow rate was low. Each 30 ml bottle sample
was frozen within 30 min of collection and was not thawed until the sample was ready for analysis. Flocculation of DOC due to freezing has been reported by Giesy and Briese (1978), but was not observed in this study. Samples in the 100 ml bottles were stored in the dark at room temperature. Redox potential was measured at the beginning of each sampling event using the Pt electrodes, a calomel reference electrode, and a voltmeter.

3.5. Sample Analyses

Dissolved total phosphorus (DTP) was analyzed colorimetrically using a multi-channel Quick Chem 8000 (Lachat Instruments, Milwaukee, WI, USA) using the method described by Liao (2001). Dissolved reactive phosphorus (DRP) was measured colorimetrically, simultaneously with DTP, on the same instrument using the method of Prokopy and Wendt (1994). Dissolved organic P was found by difference between DTP and DRP.

The concentration of Fe$^{2+}$ in solution was determined colorimetrically using the phenanthroline method (Joint Task Group: 20th Edition, 2005). The samples were reacted with the phenanthroline reagent within 24 h of collection, and analyzed using a Shimadzu UV-2101PC spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) with absorbance measured at 510 nm within 48 h of sampling. Reactions were conducted under low light in a fume hood. A sample aliquot without phenanthroline reagent was used as a blank to correct the absorbance reading for the impact of dissolved organic matter. A standard curve was produced prior to each sampling using ammonium iron (II) sulfate hexahydrate (99.997%) and stored at room temperature in the dark until use.
The frozen samples were thawed and allowed to return to room temperature. Solution pH was measured with a pH electrode. The sample solution was then immediately analyzed for DOC using a Shimadzu TOC-5050 total organic C analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Because the Rhizon samplers used in this study have a smaller average pore size (0.15 µm), DOC will be defined as dissolved organic C that passes through a 0.15 µm filter which is smaller than the that passing through a 0.45 µm filter as noted earlier.

3.6. Tree Measurements

Tree height was determined monthly by measuring the distance (cm) from the top of the rhizotron face to the uppermost piece of foliage. The diameter of the base of each sapling was recorded 1 cm above the soil surface using a caliper. Root counts commenced after ponding and continued approximately monthly thereafter. A grid of 5 cm by 5 cm squares were marked on one window of each planted rhizotron (Figure 2.4). Roots greater than 2 cm in length within each grid square were counted manually, and then classified as dead or alive based on appearance. Roots entirely black in color were considered dead, whereas those that were white or brown and appeared healthy were considered alive. Root density within each layer was calculated by summing the total numbers of each root type (alive and dead) counted in each grid square and layer and dividing by the total area. At the conclusion of each repetition, the rhizotrons were opened and the roots examined to ensure that these color criteria for root morbidity were valid (Figure 2.5). Tree roots, stems, and leaves were harvested for biomass determination and nutrient analysis. Roots were washed thoroughly to remove attached soil. Roots, stems, and leaves were dried in an oven at 70°C
for 24 h and weighed for biomass determination before being ground in a Wiley mill. Each ground sample was submitted to the NC State University Environmental and Agricultural Testing Service for further nutrient analysis (C, N, P). Carbon and N were analyzed using a Perkin Elmer model 2400 CHN Elemental Analyzer (Waltham, MA, USA). Phosphorus was determined with a dry-ash method.

3.7. Statistical Analysis

Tree nutrient and biomass statistical analyses were performed using a two-way ANOVA in SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA) and normality was confirmed using the SigmaPlot 12.5 Shapiro-Wilk normality statistic. Tree height, diameter, and all soil solution chemistry data were analyzed in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) using the PROC MIXED procedure. The SAS programs used for each variable are shown in Appendix I. Means depicted in each figure are LSMeans from the outputs of each SAS program or SigmaPlot result. The error bars are standard errors of the means. Natural log transformations were used for Fe and P data, and a square root transformation was used for the root count data to conform to the normality and equal variance assumptions of the PROC MIXED procedure.

4. RESULTS

4.1 Tree Measurements and Root Counts
The seedlings from the 2010 and 2011 repetitions had similar tree height, stem biomass, and leaf biomass (Table 2.2). The 2011 (SuperTree Nursery®) saplings exhibited greater root biomass (p<0.001), resulting in greater total biomass (p=0.038). Stem diameters were also larger in 2011, though not significant (p=0.062). Plant tissue analysis revealed no
large differences in nutrient content between the mineral and organic soil, or between years, that would suggest nutrient deficiencies between treatments or repetitions (Appendix A.). However, saplings grown in the organic soil had significantly higher total, leaf, stem, and root biomass than in the mineral soil.

Root counts following ponding for 2010 are shown in Figure 2.6. Initially, the surface layer had the lowest concentration of roots, while the bottom layer had the most. Following ponding, the surface (0-22 cm) and middle (22-41 cm) depths exhibited sustained increases in root concentration (middle depth not shown), while the bottom depth (41-59 cm) exhibited almost no new root growth. Across all depths, trees planted in the organic soil had higher concentrations of living roots than in the mineral soil. The trees planted in the mineral soil had the highest concentration of dead roots for both the 22-41 cm and 41-59 cm depths. Most root death occurred within 14 days for the mineral soil, and 36 days for the organic soil. This pattern of root death at depth in the first few weeks after ponding, and root growth near the surface was also observed in 2011.

4.2 Chemical Measurements

4.2.1 Acidity

The organic soil had a significantly lower (p<0.0001) soil solution pH than the mineral soil (4.43 and 5.72, respectively). This difference is representative of soil pH observed by Ewing (2012b) across the Juniper Bay mineral and organic soils. The rhizosphere did increase solution pH slightly from 4.96 to 5.18 (p=0.0001). The 2011 pH of 5.02 was a decrease from the 2010 pH of 5.12 (p=0.0445). Because soils from each year were collected approximately one year apart, the change may be due from a progression of
the restored wetland back to acidic conditions following agricultural production and liming. Effects of depth and time after ponding on solution pH were limited.

4.2.2 Redox Potential

There was no effect of soil type on redox potential, and minimal effect of depth. However, the planting treatment had a large effect on redox potential over time (Figure 2.7). The presence of roots caused the soil to become more reduced at a much faster rate than the matrix treatment at all depths. Redox potentials for the rhizosphere and matrix are shown for the 2010 repetition in Figure 2.7. Redox potential decreased sharply between days 1 and 15 for both the matrix and the rhizosphere, and continued to decrease thereafter. The rhizosphere was significantly more reduced (lower redox potential) than the matrix until 86 d. The rhizosphere reached a redox potential of -50 mV and remained there through the duration of the experiment. The matrix steadily decreased in redox potential throughout the entire study, reaching a minimum of -30 mV on the final sampling day (118 d). Redox potential became lower with increasing depth. The soil treatment did not have a significant effect on redox potential. The 2011 repetition exhibited similar rhizosphere effects on redox potential over time. The interaction of soil treatment, plant treatment, depth, and time was not significant (p=0.24), but is depicted in Appendix B.

4.2.3 Ferrous Iron

Ferrous iron concentrations increased rapidly following ponding. The 2010 matrix and rhizosphere log-transformed concentrations for the mineral and organic soils are shown in Figure 2.8. The mineral rhizosphere concentration peaked at around 3 mg L\(^{-1}\) (0.99 ln(mg Fe\(^{2+}\) L\(^{-1}\))) at 30 d then decreased to matrix concentrations at 0.5-1.0 mg L\(^{-1}\) (-0.69-0.00
ln(mg Fe\textsuperscript{2+} L\textsuperscript{-1})) at 58 d. This peak in rhizosphere ferrous Fe was not observed in the organic soil. In that soil, matrix and rhizosphere Fe\textsuperscript{2+} reached a maximum concentration of 1.3-1.5 mg Fe\textsuperscript{2+} L\textsuperscript{-1} (0.40 ln(mg Fe\textsuperscript{2+} L\textsuperscript{-1})). Both the rhizosphere and matrix Fe\textsuperscript{2+} concentrations in both the mineral and organic soils decreased towards the end of the study to approximately 0.5 mg Fe\textsuperscript{2+} L\textsuperscript{-1} (-0.69 ln(mg Fe\textsuperscript{2+} L\textsuperscript{-1})). There was no significant interaction of depth with the plant or soil treatments over time. The 2011 repetition (Appendix C.) exhibited similar Fe\textsuperscript{2+} concentrations and a significant peak in rhizosphere Fe\textsuperscript{2+} above that found in the matrix in the first 30 d. However, in 2011 the Fe\textsuperscript{2+} concentrations remained at their maximum concentration through the conclusion of the study.

The presence of Fe\textsuperscript{2+} in all layers was further confirmed in all depths of all soil and plant treatments with positive reactions to α, α, dipyridyle dye. A smell of rotten eggs, a sign of H\textsubscript{2}S, was also noted at all depths of planted treatments, and in the 41-59 cm depth of matrix rhizotrons.

Matrix DOC steadily increased throughout the study starting at approximately 75 mg DOC L\textsuperscript{-1}, and reaching a maximum concentration of approximately 200 mg L\textsuperscript{-1} at 118 d (Figure 2.9). There was no significant effect of depth in the matrix on DOC. In the rhizosphere, DOC concentration quickly increased to around 200 mg L\textsuperscript{-1} at all depths within the first 15 d. After 15 d, DOC concentrations decreased in the surface layer (0-22 cm) (Figure 2.9), which corresponded with an increase in root growth at that depth (Figure 2.6). In the 22-41 cm and 41-59 cm depths DOC was significantly higher than matrix concentrations, and those concentrations remained high, or increased slightly through the duration of the experiment. This rhizosphere effect on DOC was present in both soil
treatments, although the organic soil exhibited approximately 50% higher concentrations of 
DOC than the mineral soil in both the rhizosphere and the matrix. The interaction of soil 
treatment, plant treatment, depth, and time was not significant (p=0.47), but is shown in 
Appendix D.

4.2.5 Phosphorus

The log-transformed DTP concentrations over time for the matrix and rhizosphere 
are shown in Figure 2.10 for the 0-22 cm and 41-59 cm depths. In both plant treatments, P 
concentrations in the 41-59 cm layer started at 170 μg L⁻¹ (5.1 ln(μg L⁻¹)) at 1 d and 
increased to over 600 μg L⁻¹ (6.3 ln(μg L⁻¹)) by the completion of the experiment. In the 0- 
22 cm depth, DTP concentration increased from 150 μg L⁻¹ (5.0 ln(μg L⁻¹)) to 325 μg L⁻¹ 
(5.7 ln(μg L⁻¹)) at 43 d. Thereafter, the rhizosphere DTP concentrations declined to 120 μg 
L⁻¹ (4.7 ln(μg L⁻¹)), while the matrix concentrations continued to increase to 410 μg L⁻¹ (6.0 
ln(μg L⁻¹)). The 22-41 cm depth also exhibited a decrease in DTP after 43 d, but not to the 
extent that was observed in the surface (data not shown). The significant decrease in 
rhizosphere DTP below matrix DTP concentrations in the surface depth was observed in 
both the mineral and organic soils.

Across all depths, plant treatments, and repetitions, the average DTP concentration 
in organic soil (417 μg L⁻¹, 5.9 ln(μg L⁻¹)) was significantly higher (p<0.0001) than the 
mineral soil (287 μg L⁻¹, 5.8 ln(μg L⁻¹)). This higher DTP concentration in the organic soil 
was consistent over time. Similar results for the soil and plant treatments, and depth were 
also observed for 2011 (Appendix E). The effect of soil treatment, plant treatment, depth, 
and time was not significant, but is shown in Appendix F. Dissolved reactive P
concentrations, and the respective plant and soil treatment effects mirrored DTP results, though at lower concentrations (Appendices G and H).

5. DISCUSSION

Although the saplings in mineral and organic soils were significantly different in biomass for both years of the study, a nutrient limitation was unlikely because plant tissue nutrient content did not differ. After the study was concluded, plant available water in the rhizotrons was estimated to be 0.5 L in the mineral soil, and 2.0 L in the organic soil based on soil water retention curves determined by Ewing (2003) for similar soil materials. It is likely that the growth of the saplings in the mineral soil was water limited. Plant growth conditions in this study are likely still representative of field conditions for restored wetlands such as Juniper Bay. Juniper Bay experienced severe drought in 2007, as well as five years of below average rainfall since restoration in 2005, based on monthly and annual rainfall ranges from the USDA-NRCS (2013). As a result, trees monitored in a related study at Juniper Bay were significantly shorter on mineral soils than on organic soils as of January 2012. The organic soils are lower in elevation, and exhibit higher water tables throughout the years making water more available during times of drought.

As a physiological adaption to reduced soil conditions, the bald cypress saplings in this study reallocated root biomass from deep, reduced soil horizons to surface soil horizons. This has been observed in other bald cypress container studies (Megenigal and Day, 1992; Slusher et al., 2013; Moorberg et al., 2013). Roots closer to the surface are likely more able to oxidize the rhizosphere via diffusion of O₂ through aerenchyma (Pezeshki et al., 1996; Vartepetian and Jackson, 1997; Anderson and Pezeshki, 2000). Roots deeper in the profile
are exposed to more reducing conditions (Moorberg et al., 2013), forcing the roots to undergo anaerobic respiration (Pezeshki and DeLaune, 1990; Pezeshki, 1991). This, in turn, reduces energy available for root growth causing a cessation in root elongation (Pezeshki, 1991). Reducing conditions also resulted in root death below 22 cm, which is also common for bald cypress during sustained periods of flooding (Schat, 1984; Ernst, 1990; Moorberg et al., 2013).

The faster rate of reduction in the rhizosphere treatment is attributed to the creation of reduction microsites. The source of labile C for enhanced soil reduction was likely root exudates in the 0-22 cm depth, and both root exudates and dead, decomposing roots in the 22-41 and 41-59 cm depths. The difference in reduction between the rhizosphere and the matrix for the 22-41 and 41-59 cm depths is representative of what was reported by Moorberg et al. (2013). However, there was a difference in the surface. Moorberg et al. (2013) observed moderate redox potentials in the 0-22 cm depth, with Eh values between 0 and 250 mV – significantly higher than what was observed in the matrix at that depth. That was not observed in this study. Moorberg et al. (2013) did observe much lower redox potentials at all depths of the matrix and lower depths of the rhizosphere treatments though. It may be that in this study redox potential did not decline to a point where aerenchyma were prevalent enough to cause a measureable difference in Eh in the surface depth.

With the redox potentials and pH values observed here, Fe would be expected to be in a reduced form within 15 d under the assumptions and phase diagram presented by Vepraskas and Faulkner (2001). The presence of Fe$^{2+}$ was confirmed with positive reactions with α, α, dipyridyle dye, and through 1, 10 phenanthroline determinations of Fe$^{3+}$ over time.
in the rhizosphere and in the matrix at all depths. There was a rhizosphere effect on Fe concentration in the mineral soil where Fe\(^{2+}\) concentrations peaked through the first 30 d at 3 mg Fe\(^{2+}\) L\(^{-1}\) – significantly higher than the matrix during those same sampling events. This rhizosphere effect was not observed in the organic soil. Because the organic soil had significantly higher dissolved organic C in both the rhizosphere and matrix treatments, it is likely that labile C available for the microbial reduction of Fe was sufficient in the organic soil, but not in the mineral soil. In the mineral soil, releases of C through exudates or decomposing roots caused further soil reduction, and increased concentrations of Fe\(^{2+}\). The decline in Fe\(^{2+}\) concentration toward the conclusion of the study is likely due to Fe precipitation. Because the presence of H\(_2\)S was confirmed qualitatively in all depths in the planted treatment, and the lower depths in the matrix treatment, it is likely that some Fe\(^{2+}\) was precipitating as FeS. The diagnostic black color of FeS was not seen in the rhizotrons of this study because the soil materials had a black color. We have observed it in earlier studies using lighter colored materials. In addition, if some roots were able to form aerenchyma to sufficiently oxidize localized areas, the Fe\(^{2+}\) concentration would decrease without a measured change in redox potential, as the sampler measures a “bulk” soil solution sample, and the redox electrode measures redox at a specific point. Because dissolved P was also present in solution, along with Fe\(^{2+}\), the precipitation of vivianite is also a possibility (Heiberg et al., 2012; Walpersdorf et al., 2013). The presence of FeS or vivianite was not tested for in this study.

The increase in DOC in the rhizosphere can be attributed to three potential processes – root exudation from living roots, decomposition of dead roots, and accumulation of C that
would normally be respired under more oxidizing conditions. There was a depth effect on rhizosphere DOC concentrations, but not on matrix DOC concentrations. In the 22-41 and 41-59 cm depths, DOC concentrations were significantly higher than matrix concentrations throughout the study. The 41-59 cm depth had minimal root growth, and the highest concentrations of dead roots. The 22-41 cm depth had both living and dead roots present. At both of these depths root exudates, root decomposition, and accumulation of DOC all likely played a part in increasing DOC levels above matrix concentrations. In the surface layer, DOC was prevented from reaching concentrations found in the rhizosphere in lower depths, or even to concentrations found in the matrix near the surface. This suggests that localized sites with oxidizing conditions are allowing for more efficient decomposition of DOC – likely in rhizospheres of tree roots that developed aerenchyma following ponding.

A steady increase in dissolved P (both DRP and DTP) was observed in the matrix at all depths in both the mineral and organic soils. The rhizosphere did impact DTP and DRP concentrations, but did not increase concentrations above matrix values. Increases through 58 d followed concentrations observed in the matrix at all depths. Thereafter, rhizosphere DTP decreased down to concentrations observed at the time of initial ponding, significantly lower than the matrix concentration. This decrease in P concentration coincides with decreases in Fe$^{2+}$ and DOC. Although Eh did not decrease at the points measured in this study, the decline in P, Fe$^{2+}$, and DOC in the bulk soil solution suggests that roots near the surface are facilitating the oxidation of the rhizosphere leading to the oxidation of Fe$^{2+}$, increased DOC decomposition, and thus P precipitation. This was not observed in the lower depths where P concentrations followed those found in the matrix.
6. CONCLUSIONS

Plant roots, both living and dead, can contribute labile C for the creation of soil reduction microsites in saturated soils. It was unknown whether these soil reduction microsites are the cause of the dissolution of P following wetland restorations on agricultural soils through the promotion of several P dissolution mechanisms. The objectives of this study were to: 1) compare concentrations of P, Fe$^{2+}$, DOC in the matrix and rhizosphere of bald cypress saplings, 2) evaluate the effect of soil type (mineral vs. organic) on P dissolution over time, and 3) evaluate the potential of plant uptake and/or rhizosphere oxidation.

We hypothesized that within the root zone of trees adapted to anaerobic conditions, soil reduction microsites will form near dead roots, and that these dead roots will be the foci of further dissolution of P by Fe reduction and/or DOC competition for binding sites. Despite there being more DOC in the rhizosphere of both soil types, and more Fe reduction in the rhizosphere of the mineral soil, DTP and DRP in the rhizosphere were not observed in significantly higher concentrations in the rhizosphere than was found in the matrix. We also hypothesized that P concentrations would be lower in areas of increased root growth near the surface due to plant uptake or precipitation of P through Fe oxidation. A significant reduction in DTP and DRP below matrix concentrations was observed in the 0-22 cm depth following 60 days of continuous ponding. Those declines eventually led to rhizosphere concentrations near pre-flooded conditions by 120 days.

These results show that soil reduction microsites caused by bald cypress growing in agricultural soils did not lead to increased rates of P dissolution, likely because the high
labile C contents of the soils studied (3.5% and 19.5% for the mineral and organic soil, respectively). Further, root growth and the oxidation of rhizospheres near the soil surface during long periods of saturation caused a significant reduction in the amount of P in solution. Thus, the use of bald cypress as a means of remediating high soil P contents in restored wetlands through plant uptake and rhizosphere oxidation seems promising for future restorations. It is not known whether the results found here are representative of wetlands restored on soils with low C soils, so further research on the effect of the rhizosphere on P dissolution is needed.
7. SOURCES CITED


City of Raleigh. Public Utilities Reports - The Official City of Raleigh Portal. Available at http://www.raleighnc.gov/home/content/PubUtilAdmin/Articles/WaterQualityReports.html (verified 15 September 2013).


Smith, H. 2011. Personal communication.


8. **TABLES**

**Table 2.1. Summary of soil properties.**

<table>
<thead>
<tr>
<th>Soil Properties</th>
<th>Unit</th>
<th>Mineral Soil</th>
<th>Organic Soil</th>
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<tr>
<td>†Total P</td>
<td>mg cm(^{-3})</td>
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<tr>
<td>Mehlich III P</td>
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<td>pH</td>
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<td>Bulk Density</td>
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<td>‡Saturated Conductivity</td>
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†Average TP value for each respective soil type from 0-15 cm from Chapter 4
‡Values from Abit (2009)
Table 2.2. Tree measurements.

<table>
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<th>Source of Variation</th>
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<tr>
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<tr>
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<tr>
<td><strong>Stem Biomass (g)</strong></td>
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<tr>
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<tr>
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</tr>
<tr>
<td>Year</td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>3.87</td>
<td>0.973</td>
</tr>
<tr>
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<tr>
<td><strong>Leaf Biomass (g)</strong></td>
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<tr>
<td>Soil</td>
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<tr>
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</tr>
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</tr>
<tr>
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<td>1.46</td>
<td>0.129</td>
</tr>
<tr>
<td>2011</td>
<td>15.70</td>
<td>1.38</td>
<td></td>
</tr>
</tbody>
</table>

†Standard error of the mean.
Figure 2.1. Rhizotron design.

Rhizotron root-boxes used in this study are shown in the growth stage position, 30° from the vertical.
Figure 2.2. Benchtop setup.

Rhizotrons were set at a 30° from the vertical (right row) during the growth stage, and were set vertically during the ponding stage of the experiment (left). The polycarbonate faces of each rhizotron were covered with steel plates prior to sampler installation (shown), and with Al foil thereafter. Rhizotron positions on the greenhouse bench were randomized once a month.
Figure 2.3. Rhizon samplers and redox electrodes.

A soil porewater sampler and a redox electrode are shown installed in the planted (rhizosphere) treatment, adjacent to a root. A syringe needle is attached to one sampler tube for sample extraction using an evacuated serum bottle. Bottles were suspended by string during sampling.
Figure 2.4. Sapling roots from mineral and organic soils.

Root counts were performed throughout the study to quantify root growth and depth over time. Root system of a sapling grown in mineral and organic soil are shown in the top and bottom rows, respectively. The left column shows roots prior to sampler installation and flooding. The middle column shows roots prior to destructive sampling at the end of the experiment. The right column shows rinsed root systems during destructive analysis.
Figure 2.5. Examination of dead roots.

During root counts, roots that were dark black in color were classified as dead. This was confirmed through inspections during destructive sampling at the end of the experiment, shown here. Dead roots were very fragile and dark black in color.
Figure 2.6. Root counts by soil type and depth over time.

The concentration of living and dead roots for 2010 are shown for the 0-22 cm (top) and 41-59 cm (bottom) depths with a square root transformation. There were no dead roots observed in the 0-22 cm depth for both the mineral and organic soil treatments; the lines for which overlap, but are both shown. The error bars depict standard error of the means.
Figure 2.7. Rhizosphere Effect on Redox Potential.

Redox potential declined for both soils at all depths over time. This decline was more pronounced in the rhizosphere. The error bars depict the standard error of the means.
Figure 2.8. Rhizosphere Effect on Ferrous Fe Concentration.

Concentrations of Fe$^{2+}$ for the matrix and rhizosphere are shown for the mineral soil (top) and organic soil (bottom). The error bars depict the standard error of the means.
Figure 2.9. Dissolved Organic Carbon by Depth and Plant Treatment.
Concentrations of DOC for the matrix and rhizosphere are shown for the 0-22 cm depth (top) and 41-59 cm depth (bottom). The error bars depict the standard error of the means.
Figure 2.10. Dissolved Total P by Depth and Plant Treatment.

Concentrations of DTP for the 2010 matrix and rhizosphere are shown for the 0-22 cm depth (top) and 41-59 cm (bottom) with a natural log transformation. The error bars depict the standard error of the means.
10. APPENDICES
Appendix A. Plant Tissue Nutrient Content

<table>
<thead>
<tr>
<th>Tree Part</th>
<th>Soil</th>
<th>C</th>
<th>Standard Error</th>
<th>N</th>
<th>Standard Error</th>
<th>P</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt. %</td>
<td>wt. %</td>
<td>%</td>
<td>wt. %</td>
<td>wt. %</td>
<td>wt. %</td>
<td>wt. %</td>
</tr>
<tr>
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<td>A</td>
<td>0.069</td>
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<td>B</td>
<td>0.67</td>
<td>0.04</td>
<td>B</td>
<td>0.066</td>
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<tr>
<td>Stem</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral</td>
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<td>0.10</td>
<td>A</td>
<td>0.64</td>
<td>0.03</td>
<td>A</td>
<td>0.044</td>
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<tr>
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<td>A</td>
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<td>0.03</td>
<td>B</td>
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<tr>
<td>Mineral</td>
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<td>0.07</td>
<td>A</td>
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<tr>
<td>Organic</td>
<td>51.94</td>
<td>0.38</td>
<td>B</td>
<td>1.17</td>
<td>0.07</td>
<td>A</td>
<td>0.109</td>
</tr>
</tbody>
</table>

Significance at the $\alpha=0.05$ level, values with different letters within the plant part are significantly different.
Appendix B. 2010 Effect of Soil treatment, Plant Treatment, and Depth on Eh

Redox potentials (Eh) for the matrix and rhizosphere of the mineral and organic soil treatments are shown for each of the three depths. The error bars depict the standard error of the means.
Appendix C. 2011 Rhizosphere effect on Fe$^{2+}$ concentration

Concentrations of Fe$^{2+}$ for the matrix and rhizosphere are shown for the mineral soil (top) and organic soil (bottom). The error bars depict the standard error of the means.
Concentrations of DOC for the matrix and rhizosphere of the mineral and organic soil treatments are shown for each of the three depths. The error bars depict the standard error of the means.
Appendix E. 2011 Rhizosphere Effect on DTP Concentration

Concentrations of DTP for the 2011 matrix and rhizosphere are shown for the 0-22 cm depth (top) and 41-59 cm (bottom) with a natural log transformation. The error bars depict the standard error of the means.
Appendix F. 2010 Rhizosphere Effect on DTP Concentration by Soil Type

![Graph showing DTP concentration over days for 0-22 cm and 41-59 cm depths, with different lines representing mineral matrix, mineral rhizosphere, organic matrix, and organic rhizosphere.](image-url)
Appendix G. 2010 Rhizosphere Effect on DRP Concentration

Concentrations of DRP for the 2010 matrix and rhizosphere are shown for the 0-22 cm depth (top) and 41-59 cm (bottom) with a natural log transformation. The error bars depict the standard error of the means.
Appendix H. 2011 Rhizosphere Effect on DRP Concentration

Concentrations of DRP for the 2011 matrix and rhizosphere are shown for the 0-22 cm depth (top) and 41-59 cm (bottom) with a natural log transformation. The error bars depict the standard error of the means.
Appendix I. SAS Code

Tree Diameter and Height

PROC SORT DATA=treediam;
by year date days soil rhizotron;
r
run;
ods listing close;
ODS tagsets.RTF STYLE=seaside file= "Output_TreeDiameter_sep232013.rtf";
ODS GRAPHICS ON;
TITLE "Split-Split plot analysis for days ";
PROC MIXED data=treediam plots=all; *by year;
class year date days soil rhizotron;
model Diam= year| soil days(year) / ddfm=kr outp=outmx1 outpm=outmnx1
residual;
random int/subject= rhizotron(soil*year);
repeated / subject = rhizotron*days(soil*year) group=year;
lsmeans year soil / cl diff adjust=Tukey ;
lsmeans year * soil/slice=(year soil) cl ; * diff adjust=Tukey ;
lsmeans days(year)/slice=(year ) cl ; *diff adjust=Tukey ;
lsmeans soil * days(year) /slice=(soil year) cl ; * diff adjust=Tukey ;
ods output lsmeans=lsmnds1 diffs=diffds1;
run;
title "potential conditional outliers";
proc print data =outmx1;
where abs(StudentResid) gt 3.5;
run;
title "potential marginal outliers";
proc print data =outmnx1;
where abs(StudentResid) gt 3.5;
run;

data lsmnds1sy;
set lsmnds1;
where effect in ('soil' 'year');
run;

data diffds1sy;
set diffds1;
where effect in ('soil' 'year');
run;
pdmi xt800(diffds1sy, lsmnds1sy)
ods graphics off;
ODS tagsets.rtf CLOSE ;
ODS listing;
run;
ods listing close;
ODS tagsets.RTF STYLE=seaside file= "Output_TreeHeight_sep232013.rtf.rtf";
ODS GRAPHICS ON;
PROC MIXED data=treediam plots=all; *by year;
class year date days soil rhizotron;
model height= year| soil days(year) / ddfm=kr outp=outmx2 outpm=outmnx2
residual;
random int/subject= rhizotron(soil*year);
repeated / subject = rhizotron*days(soil*year) group=year;
lsmeans year soil / cl diff adjust=Tukey ;
lsmeans year * soil/slice=(year soil) cl ; * diff adjust=Tukey ;
lsmeans days(year)/slice=(year) cl; *diff adjust=Tukey;
lsmeans soil*days(year)/slice=(soil year) cl; * diff adjust=Tukey;
ods output lsmeans=lsmnds2 diffs=diffds2;
run;
title "potential conditional outliers";
proc print data =outmx2;
where abs(StudentResid) gt 3.5;
run;
title "potential marginal outliers";
proc print data =outmx2;
where abs(StudentResid) gt 3.5;
run;
data lsmnds2s;
set lsmnds2;
where effect = 'soil';
run;
data diffds2s;
set diffds2;
where effect = 'soil';
run;
pdmi800(diffds2s, lsmnds2s)
run;
quilt;
ods graphics off;
ODS tagsets.rtf CLOSE;
ODS LISTING ;
run;
**Root Counts**

```sas
proc sort data=all2;
by year soil_treatment rhizotron level daf status;
run;

title "3. sqrt combined data Alive and Dead roots combining years";
proc mixed data= all2 plots(only) = StudentPanel;
class year rhizotron soil_treatment level DAF status;
model sqrt_density=year soil_treatment level
                    year * soil_treatment
                    year * level
                    soil_treatment * level
                    year * soil_treatment * level
                    DAF (year)
                    DAF*soil_treatment(year)
                    DAF*level(year)
                    DAF*soil_treatment*level(year)
                    status
                    status*year
                    status*soil_treatment
                    status *level
                    status*soil_treatment*level
                    status*year*soil_treatment
                    status*year*level
                    status*year*soil_treatment*level
                    DAF *status(year)
                    DAF*soil_treatment*status(year)
                    DAF*level *status(year)
                    DAF*soil_treatment*level *status(year)
/subject=rhizotron soiltreatment*year;
random int /subject=rhizotron soiltreatment*year;
random level /subject=rhizotron soiltreatment*year;
repeated status / subject= Year*soil_treatment*rhizotron*level*DAF
                      type= un rcorr;
lsmeans DAF*soil_treatment DAF*level DAF*soil_treatment*level
       /slice=( level soil_treatment DAF ) diff cl;
lsmeans status*soil_treatment status*level status*soil_treatment*level
       /slice=( level soil_treatment status ) diff cl;
ods output lsmeans=lsmnds3 diffs= diffds3;
ods exclude diffs;
run;
proc print data=outmx5;
where abs(StudentResid) gt 3.5;
run;
```
pH

proc sort data=a1011;
by Year Soil_treatment Plant_Treatment rhizotron Level Sampler Days;
run;

%macro analysCombYTreeCS(dataset, depvar);
title "Combined analysis both years days type=cs";
title " &depvar";
proc mixed data= &dataset plots(only)=studentpanel;
class Year Soil_treatment Plant_Treatment rhizotron Level Sampler Days wk10;
model &depvar= Year| Soil_treatment | Plant_Treatment | Level | Days(Year) / ddfm=kr
outp=outmx residual;
random int / subject=rhizotron (Year*Soil_treatment * Plant_Treatment );
repeated wk10/subject=rhizotron *Level(YEAR*Soil_treatment * Plant_Treatment ) type=cs rcorr
group=year;
lsmeans Year Soil_treatment Plant_Treatment level Days(Year);
lsmeans Soil_treatment * Plant_Treatment /slice=(Soil_treatment Plant_Treatment );
lsmeans Soil_treatment * Level /slice=(Soil_treatment Level );
lsmeans Soil_treatment * Days(Year) /slice=(Soil_treatment Days(year) );
lsmeans Plant_Treatment*Level /slice=( Plant_Treatment Level );
lsmeans Plant_Treatment*Days(Year) /slice=( Plant_Treatment Days );
lsmeans Soil_treatment*Plant_Treatment*Days(Year)/slice=(Soil_treatment Plant_Treatment );
run;

proc print data= outmx;
where abs(StudentResid) gt 3.5;
run;
quit;
%mend;

ods listing close;
ods tagsets.rtf style=seaside file = "output_pHFinal_WK10_Sep272013.rtf";
ods graphics on;
%analysCombYTree(a1011, pH);
ods graphics off;
ods tagsets.rtf close;
ods listing;
Redox

proc sort data=a1011;
  by Year Soil_treatment Plant_Treatment rhizotron Level Sampler Days;
run;

%macro analysCombYTree(dataset, depvar);
title "Combined analysis both years as Split-Split factor";
title " &depvar";
proc mixed data= &dataset plots(only)=studentpanel;
  class Year Soil_treatment Plant_Treatment rhizotron Level Sampler Days wk10;
  model &depvar= Year Soil_treatment Plant_Treatment Level Days(Year) / ddfm=kr
    outp=outmx residual;
  random int level/ subject=rhizotron (Year Soil_treatment Plant_Treatment);
  lsmeans Year Soil_treatment Plant_Treatment level Days(Year) / diff cl adjust=tukey;
  lsmeans Soil_treatment * Plant_Treatment /slice=(Soil_treatment Plant_Treatment
    Soil_treatment*Plant_Treatment) diff cl adjust=tukey;
  lsmeans Plant_Treatment*Days(Year) /slice=( Plant_Treatment Days Plant_Treatment*Days )diff
    cl adjust=tukey;
  lsmeans Soil_treatment * Plant_Treatment *Level /slice=(Soil_treatment Plant_Treatment
    Level ) diff cl adjust=tukey;
  lsmeans Soil_treatment * Plant_Treatment *Days(Year) /slice=(Soil_treatment Plant_Treatment
    Days(Year) Soil_treatment*Plant_Treatment*Days(Year)) diff cl adjust=tukey;
  lsmeans Soil_treatment * Level * Days(Year) /slice=(Soil_treatment Level Days(Year)
    Soil_treatment*Level*Days(Year) ) diff cl adjust=tukey;
run;

proc print data= outmx;
  where abs(StudentResid) gt 3.5;
run;
quit;
%mend;
ods listing close ;
ods tagsets.rtf style=seaside file = "output_RedoxFinal_WK10_5oct2013.rtf" ;
ods graphics on;
%analysCombYTree(a1011, Redox );
ods graphics off;
ods tagsets.rtf close;
ods listing;
proc sort data=a1011;
by Year Soil_treatment Plant_Treatment rhizotron Level Sampler Days;
run;

%macro analysCombYTree(dataset, depvar);
title "Combined analysis both years days as Split-Split factor";
title "; &depvar";
proc mixed data= &dataset plots(only)=studentpanel;
class Year Soil_treatment Plant_Treatment rhizotron Level Sampler Days wk10;
model &depvar= Year| Soil_treatment | Plant_Treatment | Level |Days(Year) / ddfm=kr
outp=outmx residual;
random int level/ subject=rhizotron (Year*Soil_treatment * Plant_Treatment );
lsmeans Year Soil_treatment Plant_Treatment level Days(Year);
lsmeans Soil_treatment * Plant_Treatment /slice=(Soil_treatment Plant_Treatment );
lsmeans Soil_treatment * Level /slice=(Soil_treatment Level );
lsmeans Plant_Treatment*Level /slice=( Plant_Treatment Level );
lsmeans Plant_Treatment*Days(Year) /slice=( Plant_Treatment Days );
lsmeans Soil_Treatment*Plant_Treatment*Level/slice=(Soil_treatment Plant_Treatment level );
lsmeans Soil_treatment * Plant_Treatment *Days(Year)/slice=(Soil_treatment Plant_Treatment Days(Year) );
lsmeans Level *Days(Year) /slice=(Level Days(Year));
lsmeans Plant_Treatment*Level*Days(Year)/slice=( Plant_Treatment Level Days(Year) );
run;

proc print data= outmx;
where abs(StudentResid) gt 3.5;
run;
quitos;
%mend;
ods listing close;
ods tags=seaside file = "output_DOCFinal_WK10_Sep272013.rtf";
ods graphics on;
%analysCombYTree(a1011, DOC );
ods graphics off;
ods tags=seaside close;
ods listing;
Log-Transformed Fe\textsuperscript{2+}, DRP, DTP, DOP

```sas
%macro analysCombYTreeCS5(dataset, depvar);
title "Combined analysis both years days type=cs";
title " &depvar;";
proc mixed data= &dataset plots(only)=studentpanel; where days ne 89;
class Year Soil_treatment Plant_Treatment rhizotron Level Sampler Days wk10;
model &depvar= Year| Soil_treatment | Plant_Treatment | Level | Days(Year)/ddfm=kr
outp=outmx5 residual;
random int / subject=rhizotron (Year*Soil_treatment * Plant_Treatment);
repeated wk10/subject=rhizotron *Level(YEAR*Soil_treatment * Plant_Treatment)type=cs rcorr
group=year;
lsmeans Year Soil_treatment Plant_Treatment level Days(Year );
lsmeans Year* Soil_treatment /slice=( Year Soil_treatment);
lsmeans Year* Plant_Treatment /slice=( Year Plant_Treatment );
lsmeans Soil_treatment * Plant_Treatment /slice=(Soil_treatment Plant_Treatment);
lsmeans Soil_treatment * Level /slice=(Soil_treatment Level);
lsmeans Soil_treatment * Days(Year) /slice=(Soil_treatment Days(year));
lsmeans Plant_Treatment*Level /slice=( Plant_Treatment Level );
lsmeans Plant_Treatment*Days(Year) /slice=( Plant_Treatment Days );
lsmeans level*Days(Year) /slice=( level Days );
lsmeans Plant_Treatment * Level * Days(Year) /slice=( Plant_Treatment Level Days(Year));
lsmeans Soil_treatment*Plant_Treatment*Level/slice=(Soil_treatment Plant_Treatment level);
lsmeans Year*Soil_treatment*Plant_Treatment*Level/slice=(Year Soil_treatment Plant_Treatment level);
lsmeans Soil_treatment * Plant_Treatment *Days(Year)/slice=(Soil_treatment Plant_Treatment );
lsmeans Soil_treatment * Level *Days(Year) /slice=(Soil_treatment Level Plant_Treatment );
lsmeans Soil_treatment * Plant_Treatment *Level /slice=(Soil_treatment Plant_Treatment Level);
run;

proc print data= outmx5;
where abs(StudentResid) gt 3.5;
run;
quit;
%mend;
ods listing close;
ods tagsets.rtf style=seaside file = "output_mdl5_LogDRPFe2Final_WK10_OCT192013b.rtf";
ods graphics on;
%analysCombYTreeCS5(a1011, LogDRP );
%analysCombYTreeCS5(a1011, LogDTP );
%analysCombYTreeCS5(a1011, LogORGp );
%analysCombYTreeCS5(a1011, LogFe2 );
ods graphics off;
ods tagsets.rtf close;
ods listing;
```
CHAPTER 3. AN IN-SITU MINIRHIZOTRON STUDY EXAMINING PHOSPHORUS DYNAMICS NEAR BALD CYPRUS ROOTS IN A RESTORED WETLAND

1. ABSTRACT

Phosphorus dissolution has been observed in soils of wetlands restored from agricultural land, but the causal mechanism is not fully understood. This minirhizotron field study was conducted in order to field-truth a concurrent root-box rhizotron study examining the effect of the rhizosphere of bald cypress (*Taxodium distichum* L.) roots on P dissolution. This minirhizotron study was conducted in a Carolina bay wetland that had recently been restored after 30 years of agricultural production. Minirhizotrons were installed to observe the root systems of 16, six-year-old bald cypress on organic and mineral soils (Terric Haplosaprist and Aeric Alaquods, respectively). Root growth and death was monitored on a monthly basis for two years using a minirhizotron camera for depth intervals of 0-20, 20-40, and 40-60 cm. Soil solution chemistry and redox potential were also monitored in the root system on a monthly basis using rhizon samplers and redox electrodes, respectively, at depths of 15 and 30 cm. Solution samples were analyzed for pH, dissolved organic C (DOC), dissolved reactive P (DRP), and dissolved total P (DTP). Water table depths were monitored manually using observation wells. Soil solution chemistry results corresponded with observations made with root-box rhizotrons. Phosphorus dissolution was controlled by Fe-reduction processes. Dissolved total P concentrations reached up to 750 μg L⁻¹ during extended periods of saturated conditions, and 100 μg L⁻¹ during the dry seasons. A high rate
of root growth occurred at all depths during dry conditions, while root death occurred at all depths during sustained periods of saturation. The concurrent root-box rhizotron study showed a redistribution of roots with death in deep, reduced soil layers, and growth near the surface. Root redistribution was not observed in this field study because the six year old trees had already been accustomed to saturated conditions. This minirhizotron study confirmed that root-box rhizotrons do successfully simulate saturated field conditions, and that P dissolution was controlled by Fe-reduction in high-organic C soils restored from agricultural land.
2. INTRODUCTION

Wetlands provide crucial ecosystem services such as wildlife habitat, groundwater recharge, and surface water quality improvement (Galatowitsch and van der Valk, 1994). To protect those services federal and state regulations encourage wetland restoration to mitigate for the loss of existing wetlands (Dahl and Allord, 1996). However, wetlands restored from agricultural land may contribute P to surface and drainage water, thus further impairing water quality. Previous studies have examined the mechanisms of P dissolution, as well as the relationship of root dynamics on the creation of soil reduction microsites and P dissolution. In this chapter, a minirhizotron study is presented to examine root dynamics, soil reduction, and P dissolution of wetland soils restored from agricultural land in-situ.

Phosphorus release from restored wetland soils has been observed in a variety of systems, including wetlands restored from agricultural grasslands in the Netherlands (Van Dijk et al., 2004), grazing and crop lands in Upper Klamath Lake, Oregon, USA (Aldous et al., 2005; Duff et al., 2009), croplands in a riverine wetland complex in the North Carolina Coastal Plain subject to tidal fluxes (Ardon et al., 2010a; b), Carolina bay wetlands in North Carolina, USA (Bruland et al., 2003; Vepraskas et al., 2010), subtropical peat soils restored from dairy land (Dunne et al., 2011), and lakeside wetlands restored from rice paddy soils in the Yangtze-Huaihe region, China (Zhou et al., 2010). In most of these restored wetlands, P dissolution was attributed to Fe reduction (Reddy and DeLaune, 2008). When the soil becomes anaerobic, Fe$^{3+}$ can be microbially reduced to soluble Fe$^{2+}$. As a result, phosphate that was bound to Fe$^{3+}$ can be released into solution, making it available for plant uptake, reprecipitation, or transport to nearby surface waters potentially contributing to
eutrophication (Patrick and Khalid, 1974; Holford and Patrick, 1981; Sah and Mikkelsen, 1986; Vadas and Sims, 1998; Shenker et al., 2005). While Fe reduction is the most common mechanism of P dissolution, other mechanisms have also been proposed, including effects of organic acids (dissolved organic C, DOC) on phosphate adsorption (Borggaard et al., 2005), mineralization of organic P (Greaves and Webley, 1965; Raghu and MacRae, 1966), changes in pH (Jackson, 1964; Ponnamperuma, 1972; Stumm and Morgan, 1981), and increases P gradients caused by a shortened path length (Turner and Gilliam, 1974a; b).

Each of these mechanisms is directly related to the soil redox potential (Eh), a measure of how oxidized or reduced the soil is at a given point in time. Because of the natural heterogeneity of soils, soil reduction microsites (approximately 25 mm in diameter) may form near areas of high concentrations of labile C, such as around dead roots or in the rhizosphere where root exudates are high in concentration (Parkin, 1987). Soil reduction microsites have been attributed with a majority of soil reduction biogeochemical processes, such as denitrification (Parkin, 1987; Christensen et al., 1990; Jacinthe et al., 1998), production of methane and S gases (Crozier et al., 1995), and the reduction of Fe and Mn leading to the development of soil morphological characteristics in hydric soils (Vepraskas, 1996). Because P dissolution in reduced soils is associated closely to Fe reduction, it is likely that P dissolution can occur at higher rates in soil reduction microsites formed in the presence of dead and decaying roots, or in the rhizosphere of living roots where high concentrations of root exudates occur.

Bald cypress (*Taxodium distichum* L.) is the species of interest in this chapter, and was used heavily in the restoration of the study site, Juniper Bay. Bald cypress is a
deciduous conifer commonly found in the coastal southeastern U.S. in Carolina bays and other low-lying areas (Elias, 1980). The species is known for extreme tolerance to flooding conditions due to its multiple metabolic and physiological adaptations (Hook, 1984).

Metabolic adaptations include anaerobic respiration and increased alcohol dehydrogenase activity (Pezeshki et al., 1996) and the ability to accumulate malate and shikimate in its roots (Li et al., 2010). Physiological adaptations include the development of aerenchyma and pneumatophores, among others. Aerenchyma is porous tissues in the stem and roots that allow diffusion of oxygen into the roots and rhizosphere, and diffusion of sulfides, methane, and other toxic gases out to the atmosphere (Anderson and Pezeshki, 2000; Colmer, 2003). Pneumatophores (knees) also allow CO₂, methane, and sulfide exchange with the atmosphere (Brown, 1981; Purvaja et al., 2004; Mitsch and Gosselink, 2007).

Most studies examining root dynamics of bald cypress have focused on container or root-box rhizotron methods to study the roots (e.g. Megonigal and Day, 1992; Pezeshki et al., 1996; Slusher et al., 2013; Moorberg et al., 2013). These studies allow the researcher to control environmental conditions during the experiment, such as water table depth or salinity. However they are limited to seedlings and saplings due to constraints on the size of the trees that can be studied (Böhm, 1979). In-situ monitoring of roots can be performed using minirhizotron tubes, which are clear tubes installed in the soil at an angle into the root system (Iversen et al., 2012). Through the use of modified cameras, allow for imaging of roots at a specified depth over time (Böhm, 1979; Iversen et al., 2012). Use of minirhizotron tubes has proven useful in wetland systems which experience large fluctuations of root growth and death due to shallow water tables (Iversen et al., 2012). Root dynamics of bald
cypress have not previously been examined using minirhizotron tubes in-situ. Further, the effects of tree root dynamics on the creation of soil reduction microsites and the resulting dissolution of P has not been examined in the field in conjunction with root-box rhizotrons.

In Chapter 2, a root-box rhizotron study was used to examine the effects of the rhizosphere of bald cypress on P dissolution, in both a mineral and an organic soil, simulating flooded conditions of restored Carolina bay wetlands. This study was executed concurrently with that presented in Chapter 2 with the primary goal of examining the validity of the observations made using root-box rhizotrons. The hypotheses tested were: i) saturated and reduced conditions would result in root death in deep soil layers and concurrent root growth near the soil surface; ii) saturated and reduced conditions would cause Fe reduction, DOC accumulation, and increased P dissolution; and iii) root dynamics and related soil solution chemistry measurements conducted using root-box rhizotrons are representative of field measurements using minirhizotron tubes and similar soil solution sampling procedures. Research objectives of this study were to: 1) determine if field measurements of soil solution chemistry reflects observations from a concurrent root-box rhizotron experiment, 2) determine if bald cypress root dynamics observed in-situ using minirhizotron tubes under saturated conditions reflect observations made using root-box rhizotrons, 3) provide recommendations for further root studies in wetland soils, and 4) monitor soil reduction processes in-situ on a monthly basis over two years to elucidate the effects of high and low water tables on P dissolution.
3. METHODS

3.1. Site Description

Juniper Bay (Figure 3.1) is a Carolina bay in Robeson County, NC, located approximately 10 km south of Lumberton, NC (34°30′30″N 79°01′30″E). In 1999, the North Carolina Department of Transportation (NCDOT) purchased this drained Carolina Bay wetland to mitigate the destruction of nearby wetlands caused by highway construction (Ewing, 2003). Juniper Bay is oval-shaped, oriented lengthwise along a northwest-southeast transect, and is virtually flat with an area of 256 ha. The NCDOT, with the aid of USDA-NRCS personnel, mapped Juniper Bay soils. Organic soils occupy approximately 60% of Juniper Bay, largely in its center; mineral soils occupy the remainder. It was drained for agriculture beginning in 1971 by excavating a perimeter ditch around the edge of the bay, and installing primary and secondary ditches within the bay to facilitate drainage into a single surface water outlet on the southern edge of the wetland (Ewing, 2003). Juniper Bay was fertilized annually to meet soil-test recommendations. It remained in crop production until 2001 (Ewing, 2003).

In 2000, the NCDOT commissioned wetland soil scientists, foresters, botanists and engineers at North Carolina State University (NCSU) to study the feasibility and success of their restoration efforts. The project provided extensive background characterization of soils and monitoring of hydrology in the bay for 5 years prior to restoration. Preliminary restoration efforts started in June 2003 and ditch filling began in late 2005.

During and after restoration the water quality has been monitored at the single, surface-water outflow structure on the southern edge of the bay, and in groundwater samples.
throughout the site. Concentrations of P in the surface water outflow have shown that P is being lost from the bay since restoration of wetland hydrology.

### 3.2. Experimental Design

The plot locations were determined by placing an equilateral triangle grid over the soils map of the bay, and then selecting eight locations that were distributed across the organic and mineral soil units (Figure 3.1). Four plots were in organic soils and four in mineral soil.

Bald cypress was chosen as the study species because it is one of the more common trees planted at Juniper Bay during the restoration (N.C. Department of Environment, Health, and Natural Resources (DEHNR), 2010), and has been previously studied in rhizotron and pot studies using Juniper Bay soils (Slusher et al., 2013; Moorberg et al., 2013). At each plot, an initial tree survey was performed within 30 m of the existing groundwater monitoring to identify bald cypress trees that were in good health, and were 3 m in height or taller. Of the eligible trees, two per plot were randomly selected for instrumentation.

### 3.3. Minirhizotron Construction and Installation

A minirhizotron system was installed for monitoring root growth and death throughout the study (Figure 3.2). The minirhizotron tubes were 1.5 m long, acrylic, 2 in. ID x 3.25 in. OD (5.08 cm, 5.72 cm, respectively, Piedmont Plastics, Morrisville, NC, USA). A hole was drilled at the top of each tube to engage the locking mechanism of the minirhizotron camera indexing handle system. A 2 in. diam. (5.08 cm) mechanical test plug (Oatey Supply Chain Services, Cleveland, Ohio, USA) was used to seal the bottom of the
rhizotron tube. The test plug was flush with the outside of the minirhizotron tube and installed with vacuum grease to ensure a water-tight seal. The tubes were installed at each instrumented tree by boring a auger hole 60 cm from the base of the tree trunk with a 2 in.-diam. (5.08 cm) soil auger held at a 45° angle. A jig was used to hold the auger as close to 45° as possible. Each rhizotron tube was then inserted into the auger hole, and driven to the required depth with a mallet and wooden block when necessary. The exposed portion of each minirhizotron tube was then covered with adhesive aluminum flashing foil to limit light entering the tube, and to offer some insulation. Each tube was secured with zip ties to wooden stakes.

3.4. Root Analysis and Tree Measurements

Roots were photographed monthly for a 2-yr period on the days soil porewater was collected. Images were obtained with a BTC-2 Camera System (Figure 3.2), BTC I-CAP software, and the Indexing Handle System (Bartz Technology Corporation, Santa Barbara, CA, USA). This system captures images of roots within “windows” 13.5 mm vertical by 18 mm horizontal in size. Within the BTC I-CAP software, each root image is tagged with a minirhizotron tube number, a session number, and a window number for future analysis.

Each image was analyzed using RootFly 2.0.2, a free, open-source software application designed for minirhizotron image analysis. RootFly tracks root length, diameter, color, growth, and death for each individual root for a given tube, session, and window. RootFly does offer an automated image analysis algorithm to aid in identifying and tracing new roots. That feature was determined to have limited utility for this application, so all roots were identified and traced manually. The dataset from RootFly was then summarized
for depth intervals of 0-20 cm, 20-40 cm, and 40-60 cm for all tubes over all sessions. Root depth was determined using the following equation:

\[ D_r = W_n \times 1.35 \times \tan(\alpha) \]  

Eq. 1

where \( D_r \) is the vertical root depth in cm, \( W_n \) is the window number, 1.35 is the window height in cm, and \( \alpha \) is the angle at which the minirhizotron tube is installed. An \( \alpha \) of \( \pi/4 \) was used in this study to account for the 45° angle from the soil surface. Summary data included total root number, total root length, and average width for each depth.

The diameter at breast height (DBH) and tree height were determined for each instrumented tree on January 23, 2012, and again on June 17, 2013 using a clinometer, measuring tape, and calipers.

3.5. Soil Solution Sampling and Redox Measurements

Soil solution was collected using Rhizon soil porewater samplers (standard 2.5 mm rhizon sampler, 5 cm length, Rhizosphere Research Products, Wageningen, The Netherlands). Before installation, Rhizon samplers were stored for 24 hours in deionized water with the hydrophilic tips submerged to saturate them for use. The tubing of each sampler was extended using approximately 40 cm of PTFE tubing (0.8mm I.D.) to keep the Luer lock of each sampler above the soil surface and/or ponded water. Rhizon sampler porous tips are made of hydrophilic plastic (Shotbolt, 2009) which do not absorb P from solution as do porous ceramic samplers (Zimmermann et al., 1978; Nagpal, 1982; Bottcher et al., 1984). The sampling tips have a mean pore size of 0.15 μm making additional sample filtration unnecessary (Shotbolt, 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).

Platinum-tipped redox electrodes similar to that of Wafer et al. (2004) were used to monitor redox potential. 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. Three electrodes were installed at both the 15 and 30 cm depths adjacent to the rhizon sampler as depicted in Figure 3.2. Measurements were made using a calomel reference electrode and voltmeter. Voltage readings were corrected relative to a standard hydrogen electrode (Vepraskas and Faulkner, 2001).

Soil solution samplers and redox electrodes were installed at depths of 15 and 30 cm at a distance of 30 cm from a tree trunk. A polycarbonate plate (LEXAN, SABIC, Riyadh, Saudi Arabia) was first installed vertically in the soil to hold the Rhizon samplers and redox electrodes at the desired depths. Each plate was driven to the proper depth with a rubber mallet. Care was taken to limit root injury during the installation of the plates, samplers, or electrodes. After the plate was inserted, an access hole was excavated on one side of the plate to allow insertion of samplers and electrodes through holes that were pre-drilled into the plate at the proper depths. The plates also allowed soil on one side to remain undisturbed, while the access hole was refilled.

One sampler and three electrodes were installed at both depths for each instrumented tree. After installation, the access hole was backfilled with soil. The tubing and wires from the samplers and redox electrodes were secured to a support constructed of polyvinyl chloride (PVC) pipe. This ensured that wire and tube tips would remain out of ponded
water. Rhizon samplers were replaced every six months as recommended by the manufacturer.

3.6. Sampling and Analyses

Soil pore-water sampling was performed monthly beginning in May 2011 and continued for 2 yrs. 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 100 mL 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 -800 cm H₂O or greater pressure using an electric vacuum pump. The 100ml bottle was used for collecting samples for the analysis of dissolved Fe²⁺, dissolved reactive P (DRP), and dissolved total P (DTP). The second bottle was left un-acidified and used to collect samples to measure dissolved organic C (DOC) and pH.

Water samples were collected through a 25-gauge, 3.8 cm long, Luer-lock needle that was attached to each Rhizon sampler. The sampling tube was first purged by inserting the needle into an evacuated serum “purge” 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 approximately 15 ml of solution over the course of 4 to 6 h. Following this, the needle was inserted into the 100 ml bottle to collect approximately 30 ml of solution overnight and collected first thing the next morning. The unacidified samples were frozen upon the return from the field, and remained frozen until ready for analysis. Flocculation of DOC due to freezing has been reported by Giesy and Briese (1978), but was not observed in this study.
The acidified serum bottles were stored at room temperature in the dark until they were analyzed for Fe$^{2+}$.

The concentration of Fe$^{2+}$ in solution was determined colorimetrically using the phenanthroline method (Joint Task Group: 20th Edition, 2005). The samples were reacted with the phenanthroline reagent within 24 hr of collection, and 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 were conducted under low light in a fume hood. A sample aliquot sans phenanthroline reagent was used as a blank to correct the absorbance reading for the impact of dissolved organic matter. A standard curve was produced prior to each sampling using ammonium iron (II) sulfate hexahydrate (99.997%) and stored at room temperature in the dark until use. The remaining, acidified samples were then transferred to 20 mL scintillation vials for storage for future P determinations.

Subsamples for P determinations were submitted to the Environmental and Agricultural Testing Service at NC State University. Dissolved reactive phosphorus was analyzed using a multi-channel Quick Chem 8000 (Lachat Instruments, Milwaukee, WI, USA) using the method described by Prokopy and Wendt (1994). Dissolved total P was analyzed using an Inductively Coupled Plasma-Atomic Emission Spectrometer (Optima 2000, Perkin-Elmer, Waltham, MA, USA). Dissolved organic P was found by difference between DTP and DRP.

The frozen samples were thawed and allowed to return to room temperature. Solution pH was measured with a pH electrode. The sample solution was then immediately
analyzed for DOC using a Shimadzu TOC-5050 total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). The standard definition of dissolved organic carbon is organic C that passes through a 0.45 µm filter. Because the Rhizon samplers used in this study have a smaller pore size (0.15 µm), in this paper we define DOC as dissolved organic C that passes through a 0.15 µm filter.

3.7. Statistical Analysis
The experimental design was a split-split plot design with two soil treatments (mineral and organic), and two depth treatments (15 cm and 30 cm) for soil solution measurements. Three depths were used for root data (0-20 cm, 20-40 cm, and 40-60 cm). The two soil treatments were split among eight plots with four plots located on each soil type. Each plot was replicated with two instrumented trees per plot location. The data were analyzed in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) using the PROC MIXED procedure (SAS codes shown in Appendix D). Error bars shown in each figure depict the standard error of the mean. Multiple comparisons were adjusted using the Tukey method in SAS. Natural log transformations were used for DTP, DRP, DOP, and Fe$^{2+}$, and square root transformations were used for root counts and root length sum data to conform to the normality assumptions of PROC MIXED in SAS.

4. RESULTS
4.1 Rainfall and Water Table Depths
The daily rainfall and the water table depths from the manual wells recorded at the time of sampling are shown in Figure 3.3. Rainfall observed at Juniper Bay was below normal for both 2011 (956 mm) and 2012 (1020 mm) according to the USDA NRCS WETS
table for Robeson County (USDA-NRCS, 2013a), which reports a normal rainfall range of 1085-1290 mm. As a result, the average water table depth only went above the 15 cm depth (depth of the shallowest soil porewater sampler) of the mineral soil once during the two-year study. Shallow water tables did occur in the organic soils, however. This was likely due to the lower position of the organic soils on the relatively flat Carolina bay landscape. Because one of the primary objectives of this study was to observe changes in soil solution chemistry under saturated conditions in the field, the focus of this paper going further will be on the organic soil results, though mineral results are shown in Appendix A.

Saturated conditions occurred at the 30 cm depth from the beginning of the study in May through June 2011, February 2012 through September 2012, and again from January through May 2013. Saturated conditions occurred at the 15 cm depth for shorter durations than the 30 cm depth. At 15 cm the organic soils were saturated at the first sampling in May 2011, then from March 2012 through July 2012, then again from January 2013 through the end of the study in May 2013.

4.2 Redox Potential and pH

The redox potential of both the 15 and 30 cm depths over time are shown in Figure 3.4. The 30 cm depth, on average, was significantly more reduced (mean 158 mV, se ±34 mV) than the 15 cm depth (378 mV, se ±34 mV) (p<0.0001) across all sampling events. The lowest redox potentials occurred during times of saturation, particularly during late spring and early summer when the water table was still shallow and temperatures were increasing. The lowest redox potentials in both the 15 and 30 cm depths reached approximately 0 mV. The Eh was low enough for reduced Fe$^{2+}$ to occur, approximately +300 mV at pH 4 under
the assumptions listed by Vepraskas and Faulkner (2001), in the 30 cm depth from May to June 2011, and from January 2012 through the conclusion of the study. At the 15 cm depth \( \text{Fe}^{2+} \) would be expected from May to June 2011, March through November 2012, and from April through May 2013. The 15 cm depth reached a maximum redox potential of approximately +700 mV during the fall months of 2011 – the driest period of the study. The 30 cm depth reached +500 mV for the same period.

4.3 Tree and Root Measurements
The instrumented trees averaged 7.4 cm (se ± 0.2 cm,) DBH in January 2012, and increased to 10.9 cm (se ± 0.2 cm) DBH by July of 2013. Tree heights were 5.5 m (se ± 0.4 m) in January 2012, and increased to 5.7 m (se ± 0.4 m) by July 2013. Root counts from the minirhizotron images are shown in Figure 3.5 for the 0-20, 20-40, and 40-60 cm depth intervals over time with a square root-transformation. The net changes from month to month are shown in Figure 3.6. The highest number of roots occurred near the surface, while the lowest number of roots were found in the 40-60 cm depth interval. The largest increase in root growth occurred during the dry period in the summer months of 2011, followed by another smaller increase in root counts in the summer of 2012. Root death was prevalent in all depths from August 2012 to May 2013.

4.4 Dissolved Organic Carbon
Dissolved organic carbon (Figure 3.7) declined during the dry period of 2011 to minimums of 150 mg DOC L\(^{-1}\) in the 15 cm depth, and 250 mg DOC L\(^{-1}\) in the 30 cm depth. Following a rise in the water table and decreases in Eh at the two sampled depths in
February 2012, DOC rose to peaks of approximately 350 mg DOC L\(^{-1}\) by June 2012. There was no significant difference in DOC between depths.

4.5 Ferrous Iron
Reduced Fe concentrations (shown with a natural log-transformation in Figure 3.8) were highest after sustained periods of saturated conditions and low redox potentials. Concentrations in back-transformed values ranged from approximately 0 mg in the late summer of 2011 to 1.75 mg Fe\(^{2+}\) L\(^{-1}\). In August and September 2012 the water table was between the 15 and 30 cm depths creating oxidizing conditions in the 15 cm depth (Figure 3.4) and resulting in decreases in Fe\(^{2+}\) at that depth (Figure 3.8). In the 30 cm depth Fe\(^{2+}\) remained high with continued saturated and reduced conditions. Concentrations declined slightly in November 2012 at both depths, then increased again in May when water tables rose again.

4.6 Phosphorus
Dissolved total P concentrations (Figure 3.9) increased with increases in Fe\(^{2+}\) concentration and decreases in Eh. In addition, higher DTP concentrations (p=0.0008) were found at the 30 cm depth than at the 15 cm depth. This was likely due to the 30 cm depth having a lower Eh (Figure 3.4). The highest DTP concentrations occurred following sustained reduced conditions, particularly in May-July 2011, and again from August 2012 through February, 2013. Concentrations (in back-transformed values) of DTP ranged from 100 to 750 µg L\(^{-1}\). Concentrations of DRP largely followed the same trends as DTP, but differences were less pronounced (Figure 3.10). The DOP, found by the difference between DTP and DRP, is shown in Appendix C.
5. DISCUSSION

Dryer than normal conditions existed at Juniper Bay during this study, particularly during the late summer and fall of 2011 when the water table dropped below 80 cm in the organic soils. These dry conditions caused vigorous root growth from May through September of 2011 at all depths. Under dry conditions it is common for trees to drop some leaves and reallocate resources to the production of new roots (McDowell et al., 2008). Ewers at al. (2000) and Hacke et al. (2000) reported that increases in fine root density in response to dry conditions increases soil-to-root conductance of water when water tables are inaccessible. Megonigal and Day (1992) observed that bald cypress that experienced alternating flooding and dry soil conditions exhibited increased root production relative to continuously flooded sites, because intermittent flooding allowed the trees adequate moisture during times when the soil was not waterlogged.

The organic soil was saturated in the upper part (top 30 cm) from February 2012 through October 2012, and again from January 2013 through the completion of the study in May 2013 (Figure 3.3). As a result, Eh at the 30 cm depth remained below 300 mV – the Eh at which Fe would be expected to be reduced – from January 2012 through the completion of the study in May 2013 (Figure 3.4). The Eh at the 15 cm depth was also at or below 300 mV from January through November 2012, and again from April 2013 on. These sustained saturated and reduced conditions eventually led to root death at all depths from August 2012 through the completion of the study in May 2013 (Figure 3.5 and Figure 3.6). Under reduced conditions, anaerobic respiration cannot produce sufficient energy to sustain root growth in bald cypress (Pezeshki and DeLaune, 1990; Pezeshki, 1991), which in turn causes
root growth to cease and may result in root death (Pezeshki, 1991). This was likely the case here with Eh regularly below 300 mV, combined with a high concentration of existing roots present due to root growth during the preceding dry period.

In root-box rhizotron studies, several researchers have observed root growth in the soil surface concurrent with root death in deeper, more reduced soil layers (Schat, 1984; Slusher et al., 2013; Moorberg et al., 2013). That pattern of root redistribution was not observed in this minirhizotron-tube study. This could be due to the age of the trees studied. In root-box rhizotron studies, and other container studies, tree size is limited to seedlings and saplings which may be experiencing flooding for the first time and must develop adaptations or shift root distribution to adjust to anaerobic conditions. The trees studied here were planted during the restoration of the wetland in 2005, and had since experienced six wet seasons prior to the beginning of this study. These trees likely had already become adapted to flooded conditions and did not require a redistribution of roots.

Concentrations of DOC were lowest when the soil was oxidized and highest when the soil was reduced. Increases in DOC lagged behind rises in the water table. The peak DOC concentration of approximately 350 mg L⁻¹ was not reached until July 2012, while the soil had been saturated at both the 15 and 30 cm depths since March. However these rates of increases in DOC are in agreement with the root-box study presented in Chapter 2. There, DOC concentrations in reduced layers continued to rise throughout the 128 d study. Maximum concentrations of DOC in the rhizosphere treatment of the root-box rhizotrons study also correspond to concentrations observed here near 300 mg L⁻¹. Sources of DOC include decaying roots, root exudates, as well as accumulated organic matter that builds up
due to limited microbial oxidation in the reduced environment (Fiedler and Kalbitz, 2003). Accumulation of DOC may also occur due to oxygen constraints on phenol oxidase, allowing phenolic compounds to accumulate which results in inhibition of hydrolytic compounds responsible for the decomposition soil organic matter and DOC (Freeman et al., 2001).

Concentrations of Fe$^{2+}$ reached peaks approximately 1 to 2 months after each depth was saturated. Once that depth became oxidized following a drop in the water table concentrations of Fe$^{2+}$ immediately declined. These decreases in Fe$^{2+}$ occurred during the summer drawdown of the water table in both 2011 and 2012. During the summer drawdown in 2012, the drop in the water table was slow and started in June. This slow drop in the water table allowed for aerated conditions at 15 cm and reducing conditions at 30 cm. In response the Fe$^{2+}$ concentration at 15 cm decreased, while Fe$^{2+}$ concentrations remained at peak values in the 30 cm depth. Peak concentrations in Fe$^{2+}$ occurred where the soil was below a Eh of 300 mV (Figure 3.4) – the Eh at which Fe would be expected to be reduced for a soil pH of 4, according to the assumptions listed by Vepraskas and Faulkner (2001). Maximum concentrations of Fe$^{2+}$ matched the peak values observed in rhizotrons of approximately 1.7 mg L$^{-1}$, indicating that the root-box rhizotron study did accurately depict field observations for reduced iron.

Increases in DTP concentrations at both depths occurred following soil saturation, with peak concentrations of approximately 600 to 700 μg L$^{-1}$ occurring 3 to 4 months after the onset of saturation. Both the maximum concentration of DTP, and duration of soil saturation required to reach those concentrations, matched observations made in the root-
box rhizotrons. Patterns in DRP concentrations through time mirrored those of DTP, though at lower concentrations. Therefore, further discussion will focus on DTP.

The results presented in Chapter 2, which were in agreement with Moorberg et al. (2013), show that P dissolution, as well as the precipitation of dissolved P, was controlled primarily by Fe reduction and oxidation. In that chapter, it was concluded that the presence of living and/or dead roots did not cause additional dissolution of P above concentrations found in the matrix for the soil material having an organic C of 19.5% (based on C measurements by Abit, 2009). Further, P concentrations under sustained saturated and reduced conditions could be limited by the presence of living roots that contain aerenchyma. From those results, it can be inferred that the dissolution of P in this field study is the product of reduction processes occurring in the matrix, and that labile C was not limiting to soil reduction in the Histosol studied. Concentrations of DOC in this study exhibited minimal changes through the dry and wet seasons, while both DTP and Fe$^{2+}$ showed large variations in concentrations with changes through the seasons. Further, P concentrations increased with increases in Fe$^{2+}$ as the soil became more reduced. Upon the water table dropping below the 15 cm depth in August of 2012, and the resulting oxidizing conditions, Fe began to precipitate, and DTP concentrations immediately declined. Therefore, P dissolution is likely Fe-controlled in this restored, Carolina bay Histosol.

6. CONCLUSIONS

The research objectives of this study were to 1) determine if field measurements of soil solution chemistry reflects observations from a concurrent root-box rhizotron experiment, 2) determine if bald cypress root dynamics observed in-situ using minirhizotron
tubes under saturated conditions reflect observations made using root-box rhizotrons, 3) provide recommendations for further root studies in wetland soils, and 4) monitor soil reduction processes in-situ on a monthly basis over two years to elucidate the effects of high and low water tables on P dissolution.

Soil solution chemistry measurements did correspond well to observations made in the root-box rhizotron study in Chapter 2. Redox potentials, concentrations of Fe$^{2+}$, DOC, DTP, and DRP measured in the field all corresponded in both concentration magnitude, and the time it took to reach peak concentrations following saturation.

Root observations using the minirhizotron tubes showed root growth during a dry period in 2011. While root death did occur in the field under saturated conditions, death occurred at all depths. The minirhizotron results did not depict a “redistribution” of roots from deep in the profile to the surface, as was observed in the root-box rhizotron study in Chapter 2. This was likely due to the difference in tree age, and the respective number of wet seasons to which the trees had been exposed. The six-year-old trees at Juniper Bay had already experienced six periods of saturation, while the saplings used in the root-box rhizotron study were experiencing saturation for the first time. Root-box rhizotron studies may more accurately depict field root dynamics if the saplings are “conditioned” to flooded conditions for at least one season prior to beginning an experiment.

Dissolution of P did occur during extended periods of saturation, and was likely controlled by Fe reduction. Once oxidized conditions returned with a drop in the water table, P concentrations declined, making P less available for transport to nearby surface waters. Previous and concurrent root-box rhizotron studies showed that contributions of labile C
from the rhizospheres of bald cypress did not cause additional soil P dissolution, and that was assumed to be the case for this field study as well. However, the contribution of root dynamics on P dissolution in soils with low amounts of labile C is still unknown and needs further research.
7. SOURCES CITED


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8. FIGURES

Figure 3.1. Map of Juniper Bay and Study Sites.

Eight study sites were split between the two soil types, mineral and organic, at Juniper Bay.

Two trees were instrumented at each of the eight study sites, with four sites located on mineral soils, and four on organic soils.
As shown in picture A, each studied tree was instrumented with a (left to right) groundwater monitoring [manual] well, a rhizon soil porewater sampler and redox electrode station, and a minirhizotron tube. The redox electrodes and rhizon soil porewater samplers were installed at 15 cm and 30 cm depths (B) with one sampler and three redox electrodes at each depth. The minirhizotron tubes were installed at a 45 angle and imaged monthly (C).
Figure 3.3. Average water table depths for the mineral and organic soils and daily rainfall data for Juniper Bay.

The error bars depict standard error. Water table depths are shown for the duration of the field study. The solid black line at depth 0 depicts the soil surface, while the long-dashed line and the short-dashed line depict the location of the 15 cm and 30 cm depth samplers, respectively. Because these were abnormally dry years, the mineral site was rarely saturated at the depths studied, and thus depicted dryer conditions not representative of saturated wetland soils. Further discussion and results will instead focus on the organic soil that did become saturated for long periods of time.
Figure 3.4. Redox potential (Eh) for the 15 and 30 cm depths of the organic soil.
The error bars depict standard error. The Eh was lowest (most reduced) during periods of shallow water tables, and highest (most oxidized) during dry periods. The dashed line depicts where FeOOH would be expected to be reduced to Fe$^{2+}$ at the pH observed in the organic soil (pH=4) under the assumptions described by Vepraskas and Faulkner (2001).
Figure 3.5. Root counts (square root-transformed) from minirhizotron images for 0-20, 20-40, and 40-60 cm depths of the organic soil.

The y-axis is the square root of the number of roots observed for each of the three depths for a given sampling event. Error bars depict the standard error.
Figure 3.6. Change in root count by depth over time of the organic soil.

The values on the y-axes depict the change in overall root count for each depth from the count of the previous month. Positive numbers depict new root growth, while negative values show root death. The scale of the y-axis is different for each depth. The error bars depict standard error.
Figure 3.7. Concentration of dissolved organic C by depth over time.

Concentrations of DOC declined following a dry period in July-September 2011 and remained low during root growth through May 2012. Following May 2012 DOC increased during periods of shallow water tables and root death. The error bars depict standard error.
Figure 3.8. Concentration (natural log-transformed) of Fe$^{2+}$ by depth over time.

The y-axis shows natural log-transformed concentrations of Fe$^{2+}$, with time on the x-axis. The concentration of Fe$^{2+}$ increased with more reducing conditions during saturated, or near-saturated periods. The error bars depict standard error. The observed concentrations of Fe$^{2+}$ ranged, in back-transformed values, from approximately 0 to 1.7 mg Fe$^{2+}$ L$^{-1}$. 
Figure 3.9. Concentration (natural log-transformed) of DTP by depth over time.

The y-axis shows natural log-transformed concentrations of DTP concentration, with time on the x-axis. The concentration of DTP increased during periods of high Fe$^{2+}$ concentrations. The error bars depict standard error. The observed concentrations ranged in back-transformed values of approximately 100 μg L$^{-1}$ to 900 μg L$^{-1}$. 
Figure 3.10. Concentration (natural log-transformed) of DRP by depth over time.
The y-axis shows natural log-transformed concentrations of DRP concentration, with time on the x-axis. The concentration of DRP increased slightly during periods of high Fe$^{2+}$ concentrations. The error bars depict standard error. The observed concentrations ranged in back-transformed values of approximately 75 μg L$^{-1}$ to 500 μg L$^{-1}$. 
9. APPENDICES
Appendix A. Mineral Soil Results

Appendix A.1. Redox potential (Eh) for the 15 and 30 cm depths of the mineral soil. The error bars depict standard error. The Eh was lowest (most reduced) during periods of shallow water tables, and highest (most oxidized) during dry periods. The dashed line depicts where FeOOH would be expected to be reduced to Fe$^{2+}$ at the pH observed in the organic soil (pH=5) under the assumptions of Vepraskas and Faulkner (2001).
Appendix A.2. Root counts (square root-transformed) from minirhizotron images for 0-20, 20-40, and 40-60 cm depths of the mineral soil. The y-axis is the square root of the number of roots observed for each of the three depths for a given sampling event. Error bars depict the standard error.
Appendix A.3. Change in root count by depth over time for the mineral soil. The values on the y-axes depict the change in overall root count for each depth from the count of the previous month. Positive numbers depict new root growth, while negative values show root death. The scale of the y-axis is different for each depth.

Appendix A.4. Concentration of DOC by depth over time in the mineral soil. The mineral soil had minimal change with time throughout the duration of the experiment, likely due to mostly oxidizing conditions that kept concentrations low. Error bars depict standard error.
Appendix A.5. Concentration (natural log-transformed) of Fe$^{2+}$ by depth over time for the mineral soil. The y-axis shows natural log-transformed concentrations of Fe$^{2+}$, with time on the x-axis. The concentration of Fe$^{2+}$ increased with more reducing conditions during saturated, or near-saturated periods. The error bars depict standard error. The observed concentrations of Fe$^{2+}$ ranged, in back-transformed values, from approximately 0 to 1.7 mg Fe$^{2+}$ L$^{-1}$. 
Appendix A.6. Concentration (natural log-transformed) of DTP by depth over time for the mineral soil. The y-axis shows natural log-transformed concentrations of DTP concentration, with time on the x-axis. The concentration of DTP increased during periods of high Fe$^{2+}$ concentrations. The error bars depict standard error. The observed concentrations ranged in back-transformed values of approximately 50 μg L$^{-1}$ to 900 μg L$^{-1}$. 
Appendix A.6. Concentration (natural log-transformed) of DRP by depth over time. The y-axis shows natural log-transformed concentrations of DRP concentration, with time on the x-axis. Missing samples occurred in late 2012 due to small sample volume for which only DTP could be determined. The error bars depict standard error. The observed concentrations ranged in back-transformed values of approximately 25 μg L\textsuperscript{-1} to 150 μg L\textsuperscript{-1}. Sampling events that had too many missing values to calculate LSMeans were not reported.
Appendix B. Daily Water Table and Rainfall Depths.

Daily fluctuations in water table depth for each of the mineral (top) and organic (bottom) sites and daily rainfall for Juniper Bay. The black line depicts the soil surface.
Appendix C. Dissolved Organic P by Depth Over Time in the Organic Soil.

The y-axis shows the concentration (natural log-transformed) of DOP over time on the x-axis for the two samplers. The observed concentrations range, in back-transformed values, from approximately 0 to 400 μg DOP L⁻¹. Highest DOP concentrations occurred during periods of root death, as well as periods of saturation and reducing conditions.
Appendix D. SAS Programs.

Appendix D.1. SAS Program Macros for Ln(DOP), Ln(Fe\textsuperscript{2+}), and pH

```sas
%macro mixedATree1(depvar, id );
TITLE "1. Juniper Bay Tree - &depvar";
ods graphics on;
proc mixed data= a2 plots=all;
   class Soil Date Site Tree sdepth;
   model &depvar = Soil|sdepth| Date / ddfm=kr outpm=outmnx outp=outmx residual;
   random Site(Soil) Tree(Site*Soil) Tree*Sdepth(Site*Soil);
   lsmeans soil Sdepth Date / diff cl adjust=tukey;
   lsmeans soil*Sdepth/slice=(soil Sdepth) diff cl adjust=tukey;
   lsmeans date*soil /slice=(soil date) diff cl adjust=tukey;
   lsmeans date*Sdepth /slice=( Sdepth date) diff cl adjust=tukey;
   lsmeans date*soil*Sdepth /slice=(soil*Sdepth) diff cl adjust=tukey;
run;

title2 "Marginal Studentized residual";
proc print data= outmnx;
   where abs(StudentResid) gt 3.5;
run;

title2 "conditional Studentized residual";
proc print data= outmx;
   where abs(StudentResid) gt 3.5;
run;
quit;
%mend;
```

Appendix D.2. SAS Program Macros for Redox, DTP, and Ln(DTP)

```sas
%macro mixedATree2(depvar, id );
TITLE "2A. Juniper Bay Tree - &depvar";
proc mixed data= a2 plots=all;
   class Soil Date Site Tree sdepth;
   model &depvar = Soil|sdepth| Date / ddfm=kr outpm=outmnx outp=outmx residual;
   random Site(Soil) ;* Tree*Sdepth(Site*Soil);
   random Tree(Site*Soil) ;* /group=soil ;* Tree*Sdepth(Site*Soil);
   repeated date/subject=Tree*Sdepth(Site*Soil) type=sp(pow)(date);
   lsmeans soil Sdepth date / diff cl adjust=tukey;
   lsmeans soil*Sdepth/slice=(soil Sdepth) diff cl adjust=tukey;
   lsmeans soil*date/slice=(soil date) diff cl adjust=tukey;
   lsmeans Sdepth*date/slice=(sdepth date) diff cl adjust=tukey;
   lsmeans soil*Sdepth*date/slice=(soil date) diff cl adjust=tukey;
run;

title2 "Marginal Studentized residual";
proc print data= outmnx;
   where abs(StudentResid) gt 3.5;
run;

title2 "conditional Studentized residual";
proc print data= outmx;
   where abs(StudentResid) gt 3.5;
run;
quit;
%mend;
```
Appendix D.3. SAS Program Macros for Fe\(^{2+}\), DOC, and Ln(DOP)

```sas
%macro mixedATreeC1(depvar, id, grp);
TITLE "1. Juniper Bay Tree - &depvar";
proc mixed data= a2 plots=all;
   class Soil Date Site Tree sdepth;
   model &depvar = Soil*sdepth Date / ddfm=kr outpm=outmnx outp=outmx residual;
   random Site(Soil) Tree(Site*Soil) ;
   repeated /subject=Tree*Date(Site*Soil*Sdepth) group=&grp;
   lsmeans soil Sdepth date / diff cl adjust=tukey;
   lsmeans soil*Sdepth/slice=(soil Sdepth) diff cl adjust=tukey;
   lsmeans soil*date/slice=(soil date) diff cl adjust=tukey;
   lsmeans Sdepth*date/slice=(sdepth date) diff cl adjust=tukey;
   lsmeans soil*Sdepth*date/slice=(soil date) diff cl adjust=tukey;
run;

title2 "Marginal Studentized residual";
proc print data= outmnx;
   where abs(StudentResid) gt 3.5;
run;

title2 "conditional Studentized residual";
proc print data= outmx;
   where abs(StudentResid) gt 3.5;
run;
%mend;
```

Appendix D.4. SAS Program Macros for DRP, Ln(DRP)

```sas
%macro mixedATree2D(depvar, id, grp);
ods graphics on;
TITLE "2B. Juniper Bay Tree - &depvar";
proc mixed data= a2 plots=all;
   class Soil Date Site Tree sdepth;
   model &depvar = Soil*Sdepth Date(Soil*Sdepth ) / ddfm=kr outpm=outmnx outp=outmx residual;
   random Site(Soil) Tree(Site*Soil) ;
   repeated date/subject=Site*Soil*Sdepth*Tree type=sp(pow)(date) ;
   lsmeans soil Sdepth date / diff cl adjust=tukey;
   lsmeans soil*Sdepth/slice=(soil Sdepth) diff cl adjust=tukey;
   lsmeans soil*date/slice=(soil date) diff cl adjust=tukey;
   lsmeans soil*Sdepth*date/slice=(soil date) diff cl adjust-tukey;
run;

title2 "Marginal Studentized residual";
proc print data= outmnx;
   where abs(StudentResid) gt 3.5;
run;

title "conditional Studentized residual";
proc print data= outmx;
   where abs(StudentResid) gt 3.5;
run;
%mend;
```

Appendix D.5. SAS Program for Tree Diameter

```sas
TITLE "2. Juniper Bay Tree Diameter";
proc mixed data=mrhiztree plots=studentpanel;
   class Soil Date Site Tree;
   model DBH = Soil| Date/ ddfm=kr outpm=outmnx2 outp=outmx2 residual;
   random Site(Soil);
   repeated Date / subject = Tree(Site*Soil) type=csh rcorr=9 16 ;
   lsmeans Soil Date / diff cl adjust=tukey;
```

run;
title "Marginal Residual";
proc print data=outmnx2;
   where abs(StudentResid) gt 3.5;
run;

title "conditional residual";
proc print data=outmx2;
   where abs(StudentResid) gt 3.5;
run;

Appendix D.6. SAS Program for Tree Height

TITLE "1. Juniper Bay Tree Height";
proc mixed data=mrhiztree plots=studentpanel;
class Soil Date Site Tree;
model Height m = Soil| Date / ddfm=kr outpm=outmnx outp=outmx residual;
random Site(Soil) Tree(Site*Soil);
lsmeans Soil Date / diff cl adjust=tukey;
lsmeans Soil*Date / slice=(Soil Date Soil*Date) diff cl adjust=tukey;
run;
quit;
title "Marginal Residual";
proc print data=outmnx;
where abs(StudentResid) gt 3.5;
run;

title "conditional residual";
proc print data=outmx;
where abs(StudentResid) gt 3.5;
run;

Appendix D.7. SAS Program for Well Depth

PROC MIXED data=jbwell;
class Soil Date Site Tree;
model wDepth = Soil | Date / ddfm=kr outpm=outmnx outp=outmx residual;
random Site(Soil) Tree(Site*Soil);
lsmeans Soil Date / diff cl adjust=tukey;
lsmeans Soil*Date / slice=(Soil Date Soil*Date) diff cl adjust=tukey;
RUN;

Appendix D.8. SAS Program for Root Count

TITLE "4. Juniper Bay Roots nRoots";
proc mixed data=minrhizroot plots=studentpanel;; where site ne "23A";
class Soil Level Site Tree Date;
model sqrt_nRoots = Soil | Level | Date wDepth wDepth*soil wdepth*date wdepth*level/
ddfm=kr outpm=outmnx2 outp=outmx2 residual;
random Site(Soil) Tree(Site*Soil);
repeated Date / subject = Tree*Level(Site*Soil) type=sp(powa)(date) rcorr ;
lsmeans Soil Level Date ;
lsmeans Soil*Level / slice=(soil level);
lsmeans Level*Date / slice=(Level Date);
lsmeans Soil*Level*Date / slice=(Soil Level Date);
run;
title "Marginal Residual";
proc print data=outmnx2;
   where abs(StudentResid) gt 3.5;
run;
title "conditional residual";
proc print data=outmx2;
   where abs(StudentResid) gt 3.5;
run;
Appendix D.9. SAS Program for Root Length Sum

```sas
TITLE "2b. Juniper Bay Roots log_LengthSum";
proc mixed data=minrhizroot plots=studentpanel;;
    class Soil Level Site Tree Date; where site ne "23A" and nRoots gt 0;
    model sqrt_LengthSum = Soil | Level | Date wDepth wDepth*soil wdepth*date wdepth*level/
        ddfm=kr outpm=outmx4 outp=outmx4 residual;
    random Site(Soil);
    random Tree(Site*Soil) ;* group=soil;
    random Tree*Level(Site*Soil) /group=soil;
    repeated Date / subject = Tree (Site*Soil*level ) type= sp(pow)(date) ;
    lsmeans Soil Level Date ;
    lsmeans Level*Date / slice=(Level Date);
    lsmeans Soil*Level*Date / slice=(soil Level Date);
run;

    title "Marginal Residual";
    proc print data=outmx4;
        where abs(StudentResid) gt 3.5;
    run;

    title "conditional residual";
    proc print data= outmx4;
        where abs(StudentResid) gt 3.5;
    run;
```
CHAPTER 4. PHOSPHORUS FLUXES IN A RESTORED CAROLINA BAY WETLAND FOLLOWING EIGHT YEARS OF RESTORATION

1. ABSTRACT
Wetland restoration is done, in part, to improve water quality. However, P release to surface waters has been observed in many wetlands restored from agricultural land. Phosphorus is a limiting nutrient in many freshwater systems, so it is important to understand how wetland restoration on agricultural land may affect surface water quality. A P balance was used to examine if Juniper Bay, a Carolina bay wetland, is contributing P to surface waters. The change in soil P was evaluated between archived samples taken at restoration (2005), and eight years after restoration (2013). Measured P fluxes include atmospheric deposition, plant uptake, and loss to surface water outflow. The P pool at the time of restoration was 810 kg P ha\(^{-1}\). After eight years of restoration that P pool declined to 740 kg P ha\(^{-1}\), but that difference was not significant at the \(\alpha=0.05\) level. Atmospheric deposition contributed 7 kg P ha\(^{-1}\), plants extracted 30 kg P ha\(^{-1}\) and incorporated it into woody biomass, and 0.5 Mg P was lost to surface waters draining the site. Because the loss of P to surface waters was small, and that P concentrations were not high enough to cause eutrophication (< 0.1 mg/L), we concluded that Juniper Bay is not contributing to the degradation of surface water quality of nearby streams following restoration. This is due to little groundwater flowing either into or out of the site as a result of the small hydraulic gradient that exists in this large, flat, wetland system. Further, “isolated” wetlands such as this Carolina bay are ideal sites for future wetland mitigation projects due to limited impacts on surface water quality.
2. **INTRODUCTION**

Over 50% of the original wetlands in the lower 48 states of the U.S. were drained for food production between 1780 and 1980 (Dahl and Allord, 1996). Since 1977, federal and state programs have been enacted to reverse the loss by restoring drained areas to their original wetland condition, frequently by plugging or filling drainage ditches. Wetland restoration is accomplished, in part, to improve water quality. However, in cases where wetlands are restored from agricultural land high in P from years of fertilization, saturated and reduced soil conditions may cause P to be released from the newly flooded wetland causing eutrophication in surface waters. Between 1997 and 2001 there was an estimated annual net gain of 13,400 ha of wetlands nationally due to restoration of agricultural fields, while between 2001 and 2003 the annual net gain more than doubled from previous periods (USDA-NRCS, 2013b). Based on estimates from the North Carolina Ecosystem Enhancement Program, approximately two-thirds of restored wetlands in North Carolina originated from drained and fertilized agricultural lands – equivalent to approximately 1500 ha since 1999 (Smith, 2011, personal communication). Given that wetland restoration is increasing in the U.S., partially to improve water quality, it is critical to determine whether restoration will actually contribute to pollution of P-sensitive watersheds.

The problem of P dissolution in wetlands restored on agricultural fields has been observed around the world. Recent studies of P dissolution have been done on peat soils in the Netherlands (Van Dijk et al., 2004), soils used for dairy production in Florida (Pant and Reddy, 2003), restored lake fringe in Oregon (Aldous et al., 2007), and agricultural soils in the North Carolina Coastal Plain (Ardon et al., 2010a). These studies have shown that P
dissolution is largely driven by Fe reduction processes (Reddy and DeLaune, 2008), along with other mechanisms including ligand exchange (Earl et al., 1979; Lopez-Hernandez et al., 1986; Violante et al., 1991; Gerke, 1992b), P mineralization from drying and rewetting cycles (Song et al., 2007), changes in pH (Jackson, 1964; Ponnamperuma, 1972; Stumm and Morgan, 1981), and increased P diffusion (Turner and Gilliam, 1974a; b).

To ensure that wetland restoration and management practices do not contribute to pollution of nutrient sensitive streams, a better understanding of P fluxes within and out of wetlands restored from agricultural land is greatly needed in order to identify potential management strategies that will reduce P loss. This study focused on a previously cultivated Carolina bay wetland, known as Juniper Bay, that was recently restored and is currently releasing P into its drainage water (Vepraskas et al., 2010; Moorberg et al., 2013).

The objectives of this study were to: i) estimate the change in soil total P (TP$_{soil}$) over 8 years of a successful wetland restoration by comparing TP$_{soil}$ concentrations in archived samples collected prior to restoration, and present day samples taken from the same geo-referenced locations; ii) estimate P fluxes into and out of Juniper bay from atmospheric deposition, groundwater outflow, and plant uptake; iii) assess whether further studies of groundwater P fluxes are warranted, and iv) combine objectives i and ii to create a P balance for a Carolina bay restored from agricultural land.

3. METHODS

3.1 Site Description

Juniper Bay (Figure 4.1) is a Carolina Bay in Robeson County, NC that is located approximately 10 km south of Lumberton, NC (34°30′30″N 79°01′30″E). In 1999, the
North Carolina Department of Transportation (NCDOT) purchased this drained Carolina Bay wetland to mitigate the destruction of nearby wetlands caused by highway construction (Ewing, 2003). The bay is oval-shaped, oriented lengthwise along a northwest-southeast transect, and is virtually flat with an area of 291 ha. The NCDOT, with the aid of USDA-NRCS personnel, mapped Juniper Bay soils. Organic soils cover approximately 36% of Juniper Bay, largely in its center; mineral soils occupy the remainder. This bay was drained for agriculture beginning in 1971 by excavating a perimeter ditch around the edge of the bay, and installing primary and secondary ditches within the bay to facilitate drainage into a single surface water outlet on the southwestern edge of the wetland. Juniper Bay was fertilized and limed annually to meet soil-test recommendations. It remained in crop production until 2001. Preliminary restoration efforts began in June 2003, and wetland hydrology was restored in 2005 by filling primary ditches and plugging tertiary ditches, leaving only the perimeter ditch intact. That perimeter ditch drains into one outlet on the southwest side of the bay.

3.2 Phosphorus Balance

A P-balance was developed to better understand the nature and relationships of P fluxes into and out of Juniper Bay following restoration. The proposed P-balance in simplest form is shown in Equation 1:

$$\Delta P_{\text{soil}} = P_{\text{inputs}} - P_{\text{outputs}} \pm E$$  \hspace{1cm} [Equation 1]

where $\Delta P_{\text{soil}}$ is the change to the soil’s total P (TP) pool since restoration (2005-2013). The $P_{\text{inputs}}$ are sources of “new” P, while $P_{\text{outputs}}$ include all mechanisms that remove P from the
soil. The E is an error term that accounts for errors in the determination of the change of P in the soil, errors in P fluxes, and/or error or fluxes that have gone unrecognized.

We hypothesized that atmospheric deposition (P_{ATM}) is a major mechanism adding new P into the bay, but groundwater inflow (P_{GI}) could also be contributing P. Major ways for P to be removed from the soils in Juniper Bay include: plant uptake (P_{PL}), groundwater outflow (P_{GO}) and surface water outflow (P_{SO}). Previous work by Pati (2006) showed that the perimeter ditch would intercept most groundwater inflow into the bay, thus transforming P_{GI} into P_{SO} that drained out of the bay through the outflow structure. In addition, Pati (2006) showed that the groundwater outflow component would be intercepted by the perimeter ditch as well, as long as the water levels in the ditch are managed to stay below a critical elevation. Such management of the perimeter ditch is currently practiced so that groundwater outflow from the bay should be small. Huffman et al. (2007) estimated the net flow of ground- and surface water into the bay from the surrounding landscape was equivalent to 125 mm during the wet months of 2004, with inflows entering the perimeter ditch on the NW, NE, and SE sides of the bay, and groundwater outflows exiting on the SW side of the bay. The impact of the perimeter ditch is such that the terms for P_{GO}, P_{SO}, and P_{GI} were combined with the assumption that contributions from groundwater inflow are minimal, and that surface water outflow is primarily from drainage from Juniper Bay. This assumption was tested as part of this project.

The P balance for Juniper Bay can be written with the defined inputs and outputs as:

\[ \Delta P_{soil} = P_{ATM} - P_{PL} - [P_{GO} + P_{SO} - P_{GO}] \]

[Equation 2]
For simplicity, we will combine the components $P_{go}$, $P_{so}$ and $P_{gi}$ to one term called $P_{OUTFLOW}$ which will be measured collectively at the outflow structure. The modified P balance used for this study will be:

$$\Delta P_{\text{soil}} = P_{\text{ATM}} - P_{PL} - P_{\text{OUTFLOW}} \pm E$$  \hspace{1cm} \text{[Equation 3]}

3.2.1 Soil P-Pool Volume

The volume of soil considered for Juniper Bay has a horizontal area defined by the perimeter ditch (Figure 4.1), with the soil depth starting at the soil surface and extending to a depth of 1 m. The 1 m depth was selected because previous work showed that the P increases from agricultural applications in Juniper Bay were not observed below 1 m (Ewing, 2003).

3.2.2 Determining Change in the Soil P Pool ($\Delta P_{\text{soil}}$)

The change in the soil P pool was determined by measuring total phosphorus ($TP_{\text{soil}}$) in archived soil samples (2005) and comparing those values with $TP_{\text{soil}}$ found in present day samples extracted from the same locations. The $TP_{\text{soil}}$ concentrations for time-zero (2005) were determined from two groups of archived soil samples. Prior to restoration, soils were sampled in 2000 from 48 soil pit locations to a depth of 1 m by Ewing et al. (2012a), and in 2004 on a grid of 700 locations across the bay to depths of 0-0.15 m and 0.15-0.3 m using soil push probe. It was assumed that P concentrations in the subsoil (0.15-0.3 m) had not changed between 2000 and restoration in 2005 based on the observations made by Ewing et al. (2012a) which noted that subsoil P concentrations were low and did not exceed those found in nearby reference Carolina Bay wetlands. Since Juniper Bay remained artificially drained from 2000 to 2004, soil P should have remained immobile prior to restoration.
For each archived soil sample location, GPS coordinates were recorded in 2005 at the time of sampling allowing new samples to be extracted from the same locations. For the 0-0.15 m and 0.15-0.3 depths 138 locations of the 700 total were selected for re-sampling and analysis using an area-weighted, stratified random sampling scheme (Figure 4.2). The samples were separated into four strata based on the soil mapping units developed for restoration of Juniper Bay during the NCDOT soil survey (Figure 4.2). All archived surface and subsoil sites were located using GPS receivers with a wide area augmentation system (WAAS) correction and 2-5 m accuracy.

The study by Ewing et al. (2012a) included 48 soil pit locations. These locations consisted of 24 pairs of pits - one at the crest (middle) of the fieldlet, and one adjacent to the ditch. Ewing et al. (2012a) observed large amounts of disturbance in the ditch pits due to maintenance and dredging of the drainage ditches during agricultural production; therefore, only the crest pits were used in this study. Also, Ewing et al. (2012a) studied five pits from soils with histic epipedons at the transition from mineral to organic soils. Because these histic soils represent a small area of the bay, they were also omitted from this study. The remaining 19 soil pits used in this study are shown in Figure 4.3. The 2013 soil samples were extracted using a soil auger for all three depths.

All soil samples were submitted to the North Carolina Department of Agriculture Soil Testing Service for analysis of extractable P by the Mehlich III method. Soil TP analysis was performed on 25% of the re-sampled 0-15 cm and 15-30 cm depth soil samples that were selected at random within each stratum. Soil TP analysis was also performed on four representative horizons from each pit location. Soil TP was determined by nitric-
perchloric acid digestion (Carter, 1993). The TP_{soil} determined on a mass per mass basis were re-expressed as mass P per volume of soil for inclusion into the P-balance. Bulk densities reported by Ewing et al. (2012a) for the pre-restoration samples at all depths were used to convert the mass of soil to its equivalent volume. Bulk density was determined again for the 2013 samples for the 0-15 and 15-30 cm depths using the core method (Grossman and Reinsch, 2002). Samples were collected in triplicates at each of the 19 soil pit locations and averaged across the four soil mapping units for the 0-15 and 15-30 cm depths. Bulk densities for the 0.3 to 1.0 m depths were assumed to be the same as the pre-restoration values.

3.2.3 Atmospheric Deposition of P (P_{atm})

Atmospheric deposition of P was monitored from May 2012 through June 2013 at three locations within the wetland (Figure 4.4), as described by Kreiser (2003). Samplers were installed adjacent to existing rain gauges using a bulk rain water collection apparatus (Figure 4.5) modeled after Likens et al. (1967) and Johnson and Swank (1973). Samples were collected every 2 to 4 weeks and acidified for preservation. Samples were submitted to the North Carolina State University Environmental and Agricultural Testing Service (Raleigh, North Carolina, USA) for determination of dissolved reactive P (DRP) and dissolved total P (DTP). The average concentration of DTP for this time period, along with historic rainfall data collected on site were used to estimate P_{atm} from 2005 to 2013 over the entire area of the wetland.
3.2.4 Plant Uptake ($P_{plant}$)

Phosphorus uptake and accumulation by trees for the entire area of Juniper Bay was estimated using a tree survey of Juniper Bay. According to the N.C. Department of Environment, Health, and Natural Resources (DEHNR) wetland tree saplings were planted throughout Juniper Bay at the time of restoration (DEHNR, 2010). Between 2005 and 2010 DEHNR (2010) established and maintained 19 vegetation plots for wetland mitigation purposes at Juniper Bay. Those 10 m by 10 m plots were located and expanded by 20 m on all sides to create 30 m x 30 m plots for this tree survey, as shown in Figure 4.4 (plots not drawn to scale). Tree species, height, and diameter at breast height (DBH) were recorded for all trees greater than 10 cm DBH within each vegetation plot. Wood biomass was then estimated for each tree using allometric equations from Gonzalez-Benecke (2011) for loblolly pine ($Pinus taeda$ L.) and pond pine ($Pinus serotina$ Michx.), and allometric equations from Schroeder et al. (1997) and Jenkins et al. (2003) for all other species. Biomass P content was estimated for all species using P concentrations presented by Bedford et al. (1999). The total plot woody biomass per hectare and woody biomass P per hectare was determined by summing all of the tree biomass and biomass P within each plot, and dividing by the plot area. The total biomass and $P_{plant}$ for the entire bay was estimated by multiplying the woody biomass per hectare and the woody biomass P per hectare by the area of the bay.

3.2.5 Net Water Outflow ($P_{outflow}$)

The perimeter ditch surrounding Juniper Bay drains into a single surface water outflow structure at the edge of the bay (Figure 4.1). Samples from the drainage outlet were taken eight times daily from 2010 to 2013 using a Teledyne ISCO automatic water sampler.
(Teledyne ISCO, Lincoln, Nebraska, USA) and composited into one sample. From 2005 to 2010, grab samples were collected on a monthly basis. All water samples were acidified for preservation, and submitted to the North Carolina State University Environmental and Agricultural Testing Service (Raleigh, North Carolina, USA) for DRP and DTP analysis. Only DRP was measured on the grab samples (2005-2010), while both DRP and DTP were analyzed for the daily samples (2010-2013). Organic P (difference between DTP and DRP) was not determined from 2005-2010 and was assumed to contribute to the error term in the P balance as an un-accounted loss. A subset of samples were also analyzed for total P, but no significant difference between total P and DTP was observed. This indicated that particulate P concentrations were not present at this site in measurable amounts, so DTP was used for calculating $P_{\text{outflow}}$ instead of total P.

Surface outflow in the perimeter ditch was measured at the main outlet using two v-notch weirs that were installed in 2001. Discharge rates were measured from December 2010 through 2013. Discharge measurements were made using pressure transducers located upstream and downstream of the weir to determine the stage of the water. Discharge measurements were recorded using a Campbell Scientific CR-10X data logger.

Surface water discharge was estimated prior to December 2010 using a monthly water balance. Rainfall was measured on site at three rainfall stations (Figure 4.4). Evapotranspiration rates from the MODIS Land Subsets Oak Ridge National Laboratory Distributed Active Archive Center (ORNL DAAC, 2011) were used for January 2005 through December 2013. The MODIS Land Subset ET is remotely sensed ET that is determined from leaf area index (LAI) and radiation at the earth’s surface. That dataset
provides total ET over eight days. To estimate monthly ET, the eight-day ET values were divided by 8 to estimate the daily ET value on the day it was reported. Missing daily ET values were then interpolated using MatLab (MathWorks, Natick, MA, U.S.A.) and summed for each month to estimate total monthly ET from January 2005 to December 2009. Evapotranspiration from January 2010 to November 2010 was estimated using the Thornthwaite method (Thornthwaite, 1948) and monthly mean temperature data from the Lumberton Regional Airport (NOAA NCDC, 2013). The total amount of P lost in the drainage water was calculated by multiplying P concentrations in the drainage water by the volume of drainage water leaving through the outflow. This calculation was performed on a daily basis from December 2010 to 2013, and on a monthly basis for 2005 through November 2010.

3.2.6 Test of Groundwater P Flux Assumption

Measured runoff amounts at the outflow structure were compared with predicted runoff from a simple water balance to test whether larges fluxes of water were flowing into or out of Juniper Bay from the surrounding area. The years 2011 and 2012 had complete datasets for rainfall and runoff measured at the outflow structure. Predicted runoff was calculated as the difference between measured rainfall and estimated ET by the Thornthwaite method (Thornthwaite, 1948) using monthly temperature data from the nearby Lumberton Regional Airport (NOAA NCDC, 2013).

To further test the assumption that water and P fluxes into or out of Juniper Bay were minimal, P concentration gradients were evaluated across the four piezometer transects examined by Pati (2006), along with concurrent hydrologic head gradients. These
piezometer transects spanned the perimeter ditch, and included nests of piezometers at 25 and 75 m outside of the bay, and 5, 25, and 75 m inside the bay, relative to the perimeter ditch. Nests of piezometers were installed with screen-openings of one piezometer intersecting each of three layers of sand (identified as surface, middle, and deep by Pati, 2006) which were separated by two layers of clay. Heads in each piezometer in each nest were measured six times during this study, and TP and DRP were monitored in the nests located 25 m outside the bay at the time of each piezometer measurement. Phosphorus concentrations and heads were determined for all piezometers once on May 15, 2013 to evaluate P concentration gradients in each sand layer. Piezometers were sampled by purging each piezometer with three pore volumes of water with peristaltic pumps. Each sample was filtered and acidified for preservation using HCl acid, and submitted to the North Carolina State University Environmental and Agricultural Testing Service (Raleigh, North Carolina, USA) for analysis as previously described for the surface water outflow samples. Porewater velocity was determined in each layer of each transect using in-situ Ksat values reported by Pati (2006). Pati reported soil texture classes of each horizon in which piezometers were installed, but did not report bulk densities or porosities. Textural classes included sands, loamy sands, and sandy loams. Ewing reported an average bulk density of 1.40 g cm$^{-3}$ (se ±0.04 g cm$^{-3}$) for those same textures in three reference Carolina bays. That bulk density was used to estimate a porosity of 0.47 for further porewater velocity estimations.
3.2.7 Error Evaluation
The $\Delta P_{\text{soil}}$ was determined for an 8-year period, between time zero (date of wetland restoration, 2005) and 2013. The error (E) term in Equation 3, based on measured fluxes, was calculated as the remainder term between the soil $\Delta P_{\text{soil}}$ and flux $\Delta P$.

3.3 Statistical Analysis
Statistical analysis was performed on the soil total P data using the “glimmix” procedure in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) with a gamma distribution. The LSMeans presented were back-transformed using an “ilink” command. Mehlich III extractable P data were analyzed with a natural log transformation using the “proc mixed” procedure in SAS 9.3. The LSMeans reported were back-transformed using a procedure described by Jørgensen and Pederson (2013). Confidence intervals were corrected for multiple comparisons using a Tukey adjustment. A t-test was performed in SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA) to test for differences in woody biomass and biomass P content between the mineral and organic soils. Summary statistics were determined for $P_{\text{atm}}$ using SigmaPlot 12.5.

4. RESULTS

4.1. Phosphorus Balance

4.1.1. Change in the Soil P Pool ($\Delta P_{\text{soil}}$)
There was no difference in soil TP concentrations between 2005 and 2013 ($p=0.42$). The organic soils at Juniper Bay had higher ($p<0.0001$) concentrations of TP (0.131 kg m$^{-3}$, se 0.013) than the mineral soils (0.072 kg m$^{-3}$, se 0.005) across all three depths and both sampling years. The fixed effect of depth was highly significant ($p<0.0001$), and is summarized in Table 4.1. The concentrations were highest at the surface and declined with
depth. Total P concentrations between soil types in 2005 and 2013 are summarized in Table 4.2. There was no significant difference between years for any soil type-depth combination. Significantly higher TP concentrations were found in the 0-15 cm depth than in the 15-30 cm and 30-100 cm depths for both the mineral and organic soils for both years. There was no significant difference in TP concentration between the 15-30 cm depth and the 30-100 cm depth for either soil in 2005, or in the mineral soil in 2013. However, in 2013 the organic soil had significantly more TP in the 15-30 cm depth than in the 30-100 cm depth. Differences between mineral and organic soil TP concentrations were significant for the 0-15 cm depth and 15-30 cm depth, but not the 30-100 cm depth. This was consistent for both sampling years.

Although no significant difference in TP concentrations between years was observed, TP concentrations from Table 4.2 were used to estimate a change in TP over the eight years since restoration, as summarized in Table 4.3. The total TP held in the soils of Juniper Bay to a depth of 1 m was 234 Mg (800 kg ha\(^{-1}\)) in 2005, and 216 Mg (740 kg ha\(^{-1}\)) in 2013, yielding a net loss of 18.6 Mg (60 kg ha\(^{-1}\)). This is equivalent to a percentage loss in TP of 7.9%, relative to the 2005 TP content, or a loss of about 1% per year since restoration.

Figure 4.7 shows an interpolation of P concentration in Juniper Bay in 2005 at the three depths studied. In the 0-15 cm depth, the highest concentrations occurred primarily on the western two-thirds of the wetland, particularly in the center of the organic soils, and the southwestern side of the bay in the mineral soils. At the 30 cm depth, P concentrations were highest in the organic soil at the center of the bay, as well as on the eastern edge. The
concentrations of P_{soil} in the 30-100 cm depth were evenly distributed throughout the bay, and were much lower than the shallower depths. A map of the changes in the soil TP concentration is shown in Figure 4.8. The largest losses in the surface layer occurred in the same areas that had the highest concentrations in 2005. Most of the gains in TP concentration in the 15-30 cm depth occurred beneath zones of loss in the overlying 0-15 cm depth indicating downward leaching of P. This occurred primarily on the western two-thirds of the wetland. The 30-100 cm depth exhibited small changes overall, but generally had gains in the western portion of the bay, no change in the middle, and losses at the eastern edge. This may be caused by some movement of P into or out of the bay from/to the perimeter ditch, or possibly interaction with the surface sands of the surrounding landscape.

Mehlich III P was determined on all archived and resampled soil samples, however results and discussion will focus on nitric-perchloric P. Mehlich III P results are available in Appendix A.

4.1.3. Atmospheric Deposition of P (P_{atm})

During this study, P addition through rainfall averaged 0.11 mg DTP L$^{-1}$ (se 0.02). There was no significant difference in P between the three stations. The average concentration of rainfall DTP from 2005 to 2012 was assumed to be equal to the 0.11 mg DTP L$^{-1}$ observed in this study. That concentration and daily rainfall data for Juniper Bay were used, along with the total area of the bay, to estimate the P_{atm} following restoration in 2005 – a total of 2.4 Mg (8 kg ha$^{-1}$) over eight years, or 0.3 Mg (1 kg ha$^{-1}$) annually. Estimated monthly P_{atm} is summarized in Table 4.4. Monthly rainfall is summarized in Appendix A.
4.1.3. Plant Uptake ($P_{pl}$)

There was no significant difference in woody biomass or woody biomass P between soil types (mineral versus organic), and the average values across all plots were used for further calculations. The average woody biomass was 29,300 kg ha$^{-1}$ (se = 5,400), and the average woody biomass P was 26.6 kg P ha$^{-1}$ (se = 4.9). Extrapolated over the total 291.3 ha of Juniper Bay, only 7.7 Mg P was extracted by the trees over the eight years since restoration. Woody biomass across Juniper Bay totaled 8,500 Mg.

4.1.4. Net Water Outflow ($P_{outflow}$)

Discharge rates from Juniper Bay were estimated prior to December 2010 based on a simple water balance as the difference between monthly rainfall and monthly ET. Rainfall, measured on a daily basis, is summarized by month for 2005-2012 in Appendix A. Less than normal rainfall was observed for 2005, 2006, 2007, 2011 and 2012, while 2008-2010 had normal rainfall (USDA-NRCS, 2013a). Remotely measured ET (ORNL DAAC, 2011) for 2005-2009, and Thornthwaite-estimated ET rates from 2010-2012 are summarized in Appendix B. Discharge from the single outflow at Juniper Bay was measured directly starting in December 2010. Monthly discharge rates are summarized in Appendix C. For months where the estimated discharge was negative (ET>rainfall) the discharge was assumed to be 0 mm.

The concentration of DRP over time at the Juniper Bay outflow is shown in Figure 4.8. The concentration of DRP increased following restoration in 2005, and remained at elevated concentrations until 2010. Following 2010 the concentrations declined to pre-restoration levels. The P discharge, estimated on a monthly basis for 2005-2010 and a daily basis for 2011-2012, totaled 0.5 Mg P.
4.1.5. Phosphorus Balance Summary
The P balance for Juniper Bay is summarized in Figure 4.9. The main flux of P entering the bay was from the atmosphere, at 2.4 Mg P since restoration. Plant uptake into woody biomass was the largest P flux out of the wetland, and was estimated at 7.7 Mg P since restoration. Phosphorus leaving the site through the drainage water totaled 0.5 Mg P since restoration. This leaves the error term of -12.6 Mg P, which is larger than any flux, but is still reasonable considering that the difference in soil TP between 2005 and 2013 was not significant.

4.1.6. Test of Groundwater P Flux Assumption
Measured rainfall, runoff, and runoff ratios as well as predicted ET, runoff, and runoff ratios are shown in Table 4.5. For both 2011 and 2012 the measured runoff and the runoff predicted using a simple water balance were very close, indicating that water flux into or out of Juniper Bay from the surrounded landscape was likely minimal.

The distribution of hydraulic head and total P concentrations across four transects of the Juniper Bay perimeter are summarized in Appendix D. Groundwater gradients corresponded to gradients predicted by Pati (2006). The highest P concentrations were observed outside of the perimeter ditch, with the lowest concentrations occurring within Juniper Bay for the NW, NE, and SW transects. The P concentrations in the SE transect were mostly below the detection limit for all nests. Extractable P analysis of auger borings from Ewing (2012a) suggest that some of the high concentrations of P found here might originate from minerals in the underlying sediments, and are likely not from P loss from Juniper Bay or any of the nearby agricultural fields.
5. DISCUSSION

The primary focus of this study was to determine if Juniper Bay has been, is currently, or will be a source of P for downstream surface waters following wetland restoration of agricultural land. The increase in soil P over 30 years of agricultural fertilization did lead to soil P concentrations significantly higher than un-farmed reference Carolina bays (Ewing et al., 2012a) and a total P pool of 234 Mg P at the time of restoration in 2005. By 2013 the soil P pool had decreased by 8% to 216 Mg. However the difference in P between 2005 and 2013 was not significant.

A P balance was still constructed in order to better understand P fluxes at Juniper Bay, and to guide future management. The main flux of P into the bay was $P_{atm}$. Atmospheric deposition of P was small, at 2 Mg P over eight years. The concentration of DTP in the rainwater was also approximately the same concentration as the drainage water. Because the runoff ratio of Juniper Bay is very small, very little of that rain (and P) actually makes it to the outflow structure. The flux of P out of Juniper Bay in the drainage water was under 0.5 Mg P since restoration. The largest P loss was due to plant uptake. The trees at Juniper Bay acquired 8 Mg of P since restoration. This flux of P from the soil and into woody biomass should slow any potential release of P to drainage waters.

The error term of 13 Mg P of loss is larger than any of the fluxes, but is still reasonable considering that the difference in TP between 2005 and 2013 was not significant. One potential source of error was having only DRP and not DTP measurements of the drainage water prior to 2010. The organic P missed may account for a larger flux of P out of the wetland. However, the measured flux of P in the drainage water is still very small, and
even doubling that flux would only increase it to 1 Mg P over eight years. Plant uptake is likely larger than was predicted due to P taken up by trees <10 cm DBH, as well as by shrubs, herbaceous plants, and forest floor accumulation.

The agreement of measured runoff volumes at the outflow with runoff predicted with a water balance suggest that losses or gains of water from the surrounding landscape is minimal. This is further evidenced by the small hydrologic gradients in four transects around the bay, and the resulting relatively-slow porewater velocities. However, further study of the hydrology of Juniper Bay and the surrounding landscape, and of P concentrations in underlying aquifers may be warranted if a reduction of the P balance error term is desired.

While concentrations of P in the Juniper Bay drainage water did depict a small release of P out of the Juniper Bay, the concentrations have since declined to pre-restoration concentrations (≤ 0.1 mg P L⁻¹) within five years of restoration. Phosphorus concentrations above 0.1 mg L⁻¹ would be expected to contribute to eutrophication in freshwater systems (Correll, 1998). The concentration of P in the drainage water is also equal to P concentrations found in the rainwater. In addition, the 0.5 Mg P that has left Juniper Bay through the outflow since restoration accounts for approximately 0.2% of the total amount of P at the site in 2005. Because of the low concentrations of P in the drainage water, and the low magnitude of P losses to the drainage water relative to the total P pool, P export from Juniper Bay to surface waters is not expected to be a major concern in the future.

Ewing et al. (2012a) estimated that Juniper Bay had three times as much extractable P than in nearby reference Carolina bays. If that proportion of extractable P is also true for total P,
and if Juniper Bay continues to lose total P at the rate of approximately 1% per year, then it will take at least 60 years for Juniper Bay to return to “natural” P concentrations. Plant uptake may reduce the amount of plant available P – the P fraction most easily exported. However P loss to plant uptake alone will not be able to reduce P concentrations down to natural concentrations.

The results of this study indicate that while most of the residual soil P that was leftover from agricultural production is still in the Juniper Bay soils, it is not moving off site. In essence, it is remaining in place and doing little if any harm off site. This indicates that Carolina bays, like Juniper Bay, make excellent sites for wetland restoration. However, Carolina bays that are drained by streams may be exceptions to this rule. These wetlands would be expected to have more P leaving the site through surface outflow, because of the higher hydraulic gradients caused by the stream. The ideal areas for wetland restoration are the closed depressions that have precipitation as the main water source and evapotranspiration as the main water loss.

6. CONCLUSIONS

The objectives of this study were to estimate the change in soil total P over eight years of successful wetland restoration, estimate P fluxes into and out of Juniper Bay, assess whether further studies of groundwater P fluxes are warranted, and to create a P balance for a Carolina bay restored from agricultural land. The soil total P decreased from 234Mg in 2005 to 216 Mg in 2013, though that difference was not significant. Phosphorus fluxes into and out of Juniper Bay included a gain in P from atmospheric deposition, and losses of P to surface water outflow and plant uptake. The error term was large at an unaccounted loss of
12.8 Mg P, but is acceptable considering that the difference in soil total P was not significant. Phosphorus loss to surface waters was minimal both in magnitude (0.5 Mg P over 8 years), and in current concentrations (approximately 0.1 mg P L\(^{-1}\)). That concentration of P is approximately the same as was found in rainwater at Juniper Bay, and is not expected to contribute to eutrophication of downstream surface waters. Contributions of water and P to and from the surrounding landscape were determined to be minimal based on a comparison of observed runoff values with those predicted from a water balance, and from an evaluation of existing transects of P concentration and hydrologic gradients around the perimeter of Juniper Bay. A P balance for Juniper Bay was estimated based on measured changes in total P and fluxes of P. It will likely take 60 years or longer for Juniper Bay to return to “natural” concentrations of P.
7. SOURCES CITED


City of Raleigh. Public Utilities Reports - The Official City of Raleigh Portal. Available at http://www.raleighnc.gov/home/content/PubUtilAdmin/Articles/WaterQualityReports.html (verified 15 September 2013).


Smith, H. 2011. Personal communication.


### Tables

Table 4.1. Soil TP concentrations by depth for both mineral and organic soils combined.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>LSMean (kg TP m$^{-3}$)</th>
<th>$\pm$ (kg P m$^{-3}$)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.171 ± 0.014</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>30</td>
<td>0.093 ± 0.008</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>100</td>
<td>0.058 ± 0.007</td>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

Means with the same letter not significantly different ($\alpha=0.05$).
Table 4.2. Soil TP concentrations by soil type, depth, and year.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Depth</th>
<th>LS Mean</th>
<th>se</th>
<th>Significance</th>
<th>2005 (LS Mean ± se)</th>
<th>Significance</th>
<th>2013 (LS Mean ± se)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm</td>
<td>kg TP m(^3)</td>
<td>†</td>
<td>‡</td>
<td>§</td>
<td>kg TP m(^3)</td>
<td>†</td>
<td>‡</td>
</tr>
<tr>
<td>Mineral</td>
<td>15</td>
<td>0.122 ± 0.013</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>0.132 ± 0.015</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Mineral</td>
<td>30</td>
<td>0.069 ± 0.008</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>0.058 ± 0.007</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Mineral</td>
<td>100</td>
<td>0.051 ± 0.007</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>0.044 ± 0.006</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Organic</td>
<td>15</td>
<td>0.257 ± 0.039</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>0.207 ± 0.032</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Organic</td>
<td>30</td>
<td>0.118 ± 0.018</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>0.160 ± 0.025</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Organic</td>
<td>100</td>
<td>0.076 ± 0.017</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>0.065 ± 0.015</td>
<td>A</td>
<td>C</td>
</tr>
</tbody>
</table>

†Comparison of TP concentration between years for a given soil type and depth  
‡Comparison of TP concentration between depths within a given year and soil type  
§Comparison of TP concentration between soil types within a given year at a given depth  
Means with the same letter in a comparison are not significantly different
Table 4.3. Calculations for $\Delta P_{\text{soil}}$ by soil type and depth.

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Depth</th>
<th>Area</th>
<th>TP</th>
<th>se</th>
<th>TP</th>
<th>se</th>
<th>$\Delta P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral</td>
<td>15</td>
<td>186.2</td>
<td>34.2</td>
<td>3.7</td>
<td>36.8</td>
<td>4.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Mineral</td>
<td>30</td>
<td>186.2</td>
<td>19.2</td>
<td>2.1</td>
<td>16.2</td>
<td>1.9</td>
<td>-3.0</td>
</tr>
<tr>
<td>Mineral</td>
<td>100</td>
<td>186.2</td>
<td>66.1</td>
<td>9.4</td>
<td>57.1</td>
<td>8.1</td>
<td>-9.0</td>
</tr>
<tr>
<td>Organic</td>
<td>15</td>
<td>105.1</td>
<td>40.5</td>
<td>6.2</td>
<td>32.7</td>
<td>5.0</td>
<td>-7.9</td>
</tr>
<tr>
<td>Organic</td>
<td>30</td>
<td>105.1</td>
<td>18.6</td>
<td>2.9</td>
<td>25.2</td>
<td>3.9</td>
<td>6.6</td>
</tr>
<tr>
<td>Organic</td>
<td>100</td>
<td>105.1</td>
<td>55.8</td>
<td>12.5</td>
<td>47.9</td>
<td>10.7</td>
<td>-7.9</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td></td>
<td></td>
<td><strong>234.4</strong></td>
<td></td>
<td><strong>215.8</strong></td>
<td></td>
<td><strong>-18.6</strong></td>
</tr>
</tbody>
</table>
Table 4.4. Monthly $P_{atm}$ deposition (kg TP) between January 2005 and December 2012.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>16.3</td>
<td>22.3</td>
<td>5.2</td>
<td>23.0</td>
<td>10.3</td>
<td>26.3</td>
<td>10.9</td>
<td>22.4</td>
</tr>
<tr>
<td>Feb</td>
<td>13.4</td>
<td>22.6</td>
<td>14.1</td>
<td>33.8</td>
<td>13.6</td>
<td>33.7</td>
<td>38.6</td>
<td>22.4</td>
</tr>
<tr>
<td>Mar</td>
<td>15.7</td>
<td>4.5</td>
<td>11.5</td>
<td>30.2</td>
<td>27.1</td>
<td>26.0</td>
<td>32.6</td>
<td>29.4</td>
</tr>
<tr>
<td>Apr</td>
<td>16.1</td>
<td>13.6</td>
<td>20.5</td>
<td>29.4</td>
<td>8.8</td>
<td>5.5</td>
<td>19.6</td>
<td>17.1</td>
</tr>
<tr>
<td>May</td>
<td>11.9</td>
<td>40.0</td>
<td>13.1</td>
<td>23.7</td>
<td>76.1</td>
<td>24.7</td>
<td>29.8</td>
<td>49.2</td>
</tr>
<tr>
<td>Jun</td>
<td>15.3</td>
<td>42.6</td>
<td>26.9</td>
<td>22.6</td>
<td>36.9</td>
<td>54.2</td>
<td>12.0</td>
<td>33.3</td>
</tr>
<tr>
<td>Jul</td>
<td>36.7</td>
<td>35.6</td>
<td>4.3</td>
<td>28.0</td>
<td>32.4</td>
<td>80.5</td>
<td>28.7</td>
<td>28.8</td>
</tr>
<tr>
<td>Aug</td>
<td>4.4</td>
<td>44.8</td>
<td>19.3</td>
<td>52.1</td>
<td>47.1</td>
<td>23.9</td>
<td>57.6</td>
<td>68.8</td>
</tr>
<tr>
<td>Sep</td>
<td>10.9</td>
<td>8.3</td>
<td>3.5</td>
<td>66.7</td>
<td>2.6</td>
<td>61.7</td>
<td>29.5</td>
<td>26.3</td>
</tr>
<tr>
<td>Oct</td>
<td>19.5</td>
<td>5.6</td>
<td>14.0</td>
<td>6.5</td>
<td>21.8</td>
<td>9.8</td>
<td>17.7</td>
<td>9.7</td>
</tr>
<tr>
<td>Nov</td>
<td>26.2</td>
<td>28.3</td>
<td>0.7</td>
<td>37.3</td>
<td>56.5</td>
<td>10.3</td>
<td>26.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Dec</td>
<td>15.8</td>
<td>23.1</td>
<td>32.8</td>
<td>23.2</td>
<td>48.0</td>
<td>18.5</td>
<td>5.8</td>
<td>22.3</td>
</tr>
<tr>
<td>Total</td>
<td>202.1</td>
<td>291.2</td>
<td>165.8</td>
<td>376.6</td>
<td>381.2</td>
<td>375.2</td>
<td>309.0</td>
<td>329.8</td>
</tr>
</tbody>
</table>

*Rainfall data was acquired from a nearby weather station at the Lumberton, NC airport (NOAA NCDC, 2013).
Table 4.5. Water Balance Test of Hydrology Assumptions.

<table>
<thead>
<tr>
<th>Year</th>
<th>Rainfall</th>
<th>Runoff</th>
<th>Runoff Ratio</th>
<th>Thornthwaite ET</th>
<th>Estimated Runoff</th>
<th>Estimated Runoff Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>956</td>
<td>28</td>
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Figure 4.1. Map of Juniper Bay showing previous and existing drainage ditches, and mineral and organic soil distribution.

The Soils of Juniper Bay consist primarily of mineral soils (Aeric Alaquods) along the perimeter, and organic soils (Terric Haplosaprist) in the center of the wetland (soils outlined in black). The drainage system used during agricultural production is shown, along with the excavations used to fill the primary ditches during restoration. The perimeter ditch is still intact, and drains to one outlet on the southwest side of the bay.
Figure 4.2. Map of Juniper Bay showing all surface sample locations used in the 2004 sampling and 2013 re-sampling of the 0-15 and 15-30 cm depths.

An area-weighted, stratified random sampling was used to select 138 locations for resampling of the 700 total locations. The four soil mapping units (outlined in black) used during the restoration of Juniper Bay were used as the strata. The two letters in each mapping unit label represent the surface and subsoil textures, including SC (sand over clay), SS (sand over sand), OC (organic over clay) and OS (organic over sand). The organic soils are Terric Haplosaprists, and the mineral soils are Aeric Alaquods.
Figure 4.3. Map of Juniper Bay showing each of the soil pits and surface sample locations selected for TP analysis.

Total P analysis was performed on 19 soil pit locations for depths of 30-100 cm (shown as blue circles). Total P analysis was also performed on 25% of resampled surface samples (depths of 0-15 and 15-30 cm) (shown as orange circles).
The 19 tree survey plots (not drawn to scale) were centered on vegetation plots used by the NCEEP for mitigation monitoring, and are 30 m by 30 m in area. Atmospheric P deposition was collected adjacent to each of the three rain gauges.
Figure 4.5. The apparatus used for collection of atmospheric deposition of P, alongside a tipping bucket rain gauge and a traditional rain gauge.

The apparatus consisted of a plastic funnel, zip ties repel birds, a screen inside the funnel to keep bugs out of the tube, and a tube to direct the water to an acidified, brown, plastic bottle (not shown). The tube had a loop to act as an air lock to limit evaporation of samples.
Figure 4.6. Map of Juniper Bay TP concentrations prior to restoration (2005).

The 15 cm depth is a different color to signify a different scale of TP Concentrations at the surface. The maps were interpolated using the kriging tool in ArcGIS.
Figure 4.7. Map of the change in TP concentrations at Juniper bay between 2005 (restoration) and 2013.

The maps were interpolated using the kriging tool in ArcGIS. Blue and green show gains in TP, red and orange show losses, and lime green depicts no change.
Figure 4.8. Concentration of DRP at the Juniper Bay outflow over time.

The red line depicts the concentration at which eutrophication would be expected in freshwaters, assuming N is not limiting. The wetland was restored in 2005, after which an increase in DRP at the outflow was observed through approximately 2010. The DRP concentrations declined to pre-restoration levels thereafter.
Figure 4.9. Summary of the P balance for Juniper Bay from 2005-2013.

The fluxes include change in total P ($\Delta P$), atmospheric deposition ($P_{atm}$), plant uptake ($P_{pl}$), surface water outflow ($P_{outflow}$), and error ($E$).
10. APPENDICES
Appendix A. Changes in Mehlich III P

<table>
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<tr>
<th>Soil Type</th>
<th>Depth (cm)</th>
<th>2005 LSMean (kg P m⁻³)</th>
<th>se (kg P m⁻³)</th>
<th>Significance</th>
<th>2013 LSMean (kg P m⁻³)</th>
<th>se (kg P m⁻³)</th>
<th>Significance</th>
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Appendix B. Monthly Rainfall from January 2005 to December 2012

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†Below normal and ‡above normal rainfall according to the Robeson County, NC USDA NRCS WETS table (USDA-NRCS, 2013a).
Appendix C. Monthly ET between January 2005 and December 2012

Evapotranspiration was measured remotely for 2005-2009, and estimated using the Thornwaite equation in from 2010 to 2012.

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Appendix D. Monthly runoff between January 2005 and December 2012

Runoff was estimated using a simple water balance for 2005-2010, and measured directly for 2011 and 2012. For estimations of P, months with negative estimated runoff were assumed to have no runoff.

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Appendix E. The hydraulic head and total P concentrations across four piezometer transects around the Juniper Bay perimeter

The figures show elevation and head on the left y-axis, total P concentration on the right y-axis, and the distance from the ditch on the x-axis. The transects go (left to right) from outside the bay, across the ditch, and into the bay. Measurements shown were taken on May 15, 2013.

Appendix D.1. Piezometer Transect Across the Southwest Rim of Juniper Bay.
Appendix D.2. Piezometer Transect Across the Southeast Rim of Juniper Bay.

**Southeast Transect Surface Sands**

[Graph showing the relationship between elevation and head (m) vs. distance from the ditch (m) for Southeast Transect Surface Sands.

**Southeast Transect Middle Sands**

[Graph showing the relationship between elevation and head (m) vs. distance from the ditch (m) for Southeast Transect Middle Sands.

**Southeast Transect Deep Sands**

[Graph showing the relationship between elevation and head (m) vs. distance from the ditch (m) for Southeast Transect Deep Sands.
Appendix D.4. Piezometer Transect Across the Northwest Rim of Juniper Bay.
Appendix F. Estimations of Flow for Each Transect and Sand Layer

Porewater velocity was estimated from Hydraulic gradients and soil properties for the four piezometer transects at Juniper Bay. The saturated conductivities used were reported by Pati (2006). Porosity was estimated from average bulk densities reported by Ewing (2003). Hydraulic gradients were calculated from measurements made on May 15, 2013. Flow rates were small overall, due to both the small gradients present, and the low conductivities.

<table>
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<th>Outside H</th>
<th>Inside H</th>
<th>ΔH</th>
<th>Distance</th>
<th>Gradient</th>
<th>Porosity</th>
<th>q</th>
<th>v</th>
<th>Distance per Year</th>
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<td>0.67</td>
<td>80</td>
<td>8.37E-03</td>
<td>0.47</td>
<td>1.4E-07</td>
<td>3.0E-07</td>
<td>10</td>
<td>In</td>
</tr>
<tr>
<td>SE</td>
<td>Deep</td>
<td>4.9E-05</td>
<td>36.24</td>
<td>35.83</td>
<td>0.41</td>
<td>150</td>
<td>2.73E-03</td>
<td>0.47</td>
<td>1.3E-07</td>
<td>2.8E-07</td>
<td>9</td>
<td>In</td>
</tr>
</tbody>
</table>
Appendix G. SAS Code for Total P Analysis

Total P

title "4. Total P";
proc glimmix data=pbalancetp plots=all;
  class Year Soil MU Depth Loc;
  model TP = Year | Soil | Depth | MU(Soil)/dist=gamma ddfm=kr;
  random int/ subject=Loc(Mu*Soil);
  lsmeans Year Soil MU(Soil) Depth /diff cl ilink lines adjust=Tukey;
  lsmeans Year*Soil Year*MU(Soil) Year*Depth / slicediff=( Year Soil MU(Soil) Depth) diff cl ilink adjust=Tukey;
  lsmeans Year*Soil*Depth / slicediff=(Year Soil Depth) diff cl ilink adjust=Tukey;
  lsmeans Year*Soil*Depth / slicediff=(Year Soil Depth) diff cl ilink adjust=Tukey;
  lsmeans Year*Soil*Depth / slicediff=(Year Soil Depth) diff cl ilink adjust=Tukey;
  lsmeans Year*Soil*Depth / slicediff=(Year Soil Depth) diff cl ilink adjust=Tukey;
run;

Mehlich III P

title "1. Mehlich III M3P";
proc glimmix data=pbalancem3p plots=all;
  class Year Soil X_MU Depth Loc;
  model M3P = Year | Soil | Depth | X_MU(Soil)/dist=lognormal ddfm=kr;
  random int/ subject=Loc(X_Mu*Soil);
  *random _residual_/ subject=Loc*year(Mu*Soil) group=Mu*Soil;
  lsmeans Year Soil X_MU(Soil) Depth /diff cl ilink;
  lsmeans Year*Soil X_MU(Soil) Year*Depth / slice=( Year Soil X_MU(Soil) Depth) diff cl ilink;
  lsmeans Year*Soil*Depth /slice=(Year Soil Depth) diff cl ilink;
  lsmeans Year*Soil*Depth /slice=(Year Soil Depth) diff cl ilink;
  output out=out1 pred(blup noilink)=predicted pred(noblup noilink)=PredPA
    stderr(blup noilink)=Stderr PredPA
    Student(blup noilink)=Student Student(noblup noilink)=StudentPA
    lcl (blup noilink)=lower lcl (noblup noilink)=lowerPA
    Ucl (blup noilink)=upper Ucl (noblup noilink)=upperPA;
  ods output lsmeans= lsmnds_M3P CovParms= CovParm_M3P;
  ods exclude diffs LSMEANS;
run;

  title2 "Marginal Studentized residual";
  proc print data= out1;
    where abs(StudentPA) gt 3.5;
  run;

  title2 "conditional Studentized residual";
  proc print data= out1;
    where abs(Student) gt 3.5;
  run;

******************************;
proc contents data= lsmnds_M3P;
run;
proc print data=lsmnds_M3P(obs=10);
run;
proc print data=COVPARM_M3P;
run;
data _null_;  
set COVPARM_M3P;  
if CovParm="Residual" then do;  
call symput("Residvar", Estimate);  
end;  
run;  
data lsmnds_M3P2;  
set lsmnds_M3P;  
back_lsmean=exp(Estimate);  
back_sterr=STDERR*back_lsmean;  
back_lowerC= exp(lower);  
back_UpperC= exp(Upper);  
t_alpha= TINV(0.975, DF);  
new_lower= back_lsmean - t_alpha* back_sterr;  
new_upper= back_lsmean + t_alpha* back_sterr;  
run;  
title3 "backtransformed LSMEANS";  
proc print data= lsmnds_M3P2;  
run;  
**********************;  
quit;
CHAPTER 5. GENERAL CONCLUSIONS AND RECOMMENDATIONS

Phosphorus is a limiting nutrient in many freshwater ecosystems, and additional inputs of P from non-traditional sources such as restored wetlands should be of concern in nutrient-sensitive watersheds. Little is known about the impact of root dynamics on P dissolution in wetlands with fluctuating water tables. This highlights the need for studies examining root dynamics, P dissolution, and P loss from wetlands restored on agricultural land.

Soil reduction microsites in the vicinity of areas with high amounts of labile C and have been shown to be responsible for much of soil biogeochemical processes, such as denitrification and Fe reduction. We hypothesized that reduction microsites could also be the source of P dissolution in wetlands restored from agricultural soils. This idea was tested in soils from Juniper Bay, a Carolina bay wetland restored from agricultural land, using a root-box rhizotron study. Results from this study showed that despite conditions that favored additional P dissolution in the rhizosphere (for Fe reduction, higher DOC concentrations, etc.), no additional P was released into solution over concentrations found in the matrix. This was due to the high amounts of labile C already in the mineral (3.5% organic C) and organic (19.5% organic C) soils used in this study. Reduction microsites could still be a source of P dissolution in restored wetland soils with lower C content than the soils studied here.

The root-box rhizotron study also depicted a “redistribution” of roots from deep, more-reduced soil layers to less-reduced soil layers near the surface. The vigorous root
growth near the surface created zones of oxidized microsites where Fe could precipitate, and DOC could be decomposed. As a result, P concentrations near the surface declined with time. This redistribution of roots to the surface was not observed in a minirhizotron study conducted in-situ on the same Juniper Bay soils. Because the trees studied in the minirhizotron experiment had experienced six wet seasons by the start of the experiment, any adaption to saturated conditions via a redistribution of roots had already occurred. In other words, these trees had already moved their root systems to near the surface. In the root-box rhizotron study the saplings were being exposed to wet conditions for the first time, and had not yet been conditioned for saturated, reduced soil conditions.

While patterns of root growth near the soil surface in root-box rhizotron studies did not represent current observations of root growth and death in the field found for the older trees, soil solution chemistry results corresponded well with field conditions. Root-box rhizotron study patterns of increases in Fe$^{2+}$, DOC, and DTP matched closely with observations in the field both in magnitude and timing of increases in concentration. Further, both studies showed that the concentration of P in solution were largely controlled by Fe reduction and oxidation. In regard to methods, these studies show that root-box rhizotron studies can be useful tools in the examination of soil biogeochemical processes under saturated conditions. However, for simulating wetland conditions with tree species such as bald cypress, care should be taken to “condition” the trees to saturated, reduced conditions. This may be done by growing the trees in the root-box rhizotrons through multiple flooding and drainage cycles, which will require larger root-box rhizotrons to compensate for additional, root growth.
A P balance study was also conducted for Juniper Bay. Those results showed no significant change in the soil P pool for the entire area of Juniper Bay between wetland restoration (2005) and after eight years of successful wetland restoration (2013). The P balance showed that of the 234 Mg P that had accumulated in Juniper Bay by the end of 30 years of agriculture, there was no significant decrease in the soil P pool following eight years of successful restoration. There was a small total flux of P into the soil P pool through atmospheric deposition (2 Mg P), and total losses of P to plant uptake (8 Mg P) and surface water runoff (0.5 Mg P), with a total error of -13 Mg P (loss). While this error is large relative to the fluxes, it is reasonable considering that there was no significant change in the soil P pool. In short, fluxes of P into and out of Juniper Bay were very small.

The loss of 0.5 Mg P in the soil over the course of eight years is very small relative to the total P pool. The concentrations of P currently in the drainage water at Juniper Bay are approximately 0.1 mg P L^{-1}, which is at, or below the concentration at which eutrophication would be expected, and is also approximately the same concentration of P as was observed in the rainwater at Juniper Bay. Therefore, P release from Juniper Bay is unlikely to hinder surface water quality downstream.

The total (non-significant) loss of P over eight years of successful restoration was estimated to be 19 Mg P, or approximately 1% annually. Juniper Bay was estimated to have three-times the amount of P than nearby reference Carolina Bays that had not been farmed. A decline in P down to “natural” P concentrations would therefore take at least 60 years, assuming a constant rate of loss, and that the observed P loss was real.
While many wetlands restored from agricultural land have exhibited large releases of P, Juniper Bay did not. This was likely due to the low hydrologic gradient at the site. It is a very flat, large area with low gradients to drive water and P losses. The subsoil of Juniper Bay is also not saturated with P, and this may have slowed the movement of P as the subsoil could absorb some P moving through groundwater. Because Juniper Bay has such little runoff relative to the rainfall it receives, it acts similar to an isolated wetland, despite being physically connected to a stream through a drainage ditch. This isolation and related limited contribution of nutrients to nearby streams makes Juniper Bay, and other disturbed Carolina bays ideal for wetland restoration and mitigation despite having large pools of legacy P from prior agricultural use.

These studies have helped identify several future research directions. First, additional “field-truthing” of root-box rhizotron methods is needed to better match greenhouse growing conditions with field conditions in flooded wetland soils. Bald cypress was the focus of the experiments presented here. However, pond pine (*Pinus serotina* Michx.) is also very prevalent at Juniper Bay. Future root-box rhizotron and minirhizotron studies should include that species for a better understanding of the root dynamics at Juniper Bay. While the rhizosphere of bald cypress did not contribute to more P dissolution, root dynamics will play a major role in C cycling within Juniper Bay. Minirhizotron studies in wetland ecosystems are limited, yet a better understanding of tree root dynamics and related C cycling within wetland systems with fluctuating water tables is needed. Lastly, the P balance at Juniper Bay did not show any significant change over eight years of restoration, but will likely depict changes in P over longer time scales. A repeat of this study in 10-20
years would valuable since there are currently no long-term studies examining P loss from restored wetlands.