CTE / NCDOT
Joint Environmental Research Program

Final Report

METHODOLOGY TO ASSESS VEGETATION, HYDROLOGIC, AND SOIL PARAMETERS THAT AFFECT WETLAND RESTORATION SUCCESS

Prepared By

M.J. Vepraskas, A. Amoozegar, J.G. White, S.W. Broome, C. Conk, C.J. Moorberg,

and S. Abit,

North Carolina State University
Department of Soil Science
Box 7619
Raleigh, NC 27695-7619

1. Report No. **FHWA/NC/2008-16**	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle Methodology to Assess Vegetation, Hydrologic, and Soil Parameters that Affect Wetland Restoration Success		5. Report Date 3 February 2010
		6. Performing Organization Code
7. Author(s) M. J. Vepraskas et al.		8. Performing Organization Report No.
9. Performing Organization Name and Address North Carolina State University Dep. of Soil Science Box 7619 Raleigh, NC 27695-7619		10. Work Unit No. (TRAIS)
		11. Contract or Grant No.
12. Sponsoring Agency Name and Address North Carolina Department of Transportation Research and Analysis Group 1 South Wilmington Street Raleigh, North Carolina 27601		13. Type of Report and Period Covered Final Report July 1, 2007 to June 30, 2009
		14. Sponsoring Agency Code HWY-2008-16
Supplementary Notes:		

16. Abstract

Juniper Bay is a 750 acre Carolina Bay previously used for crop production that was purchased by the NC Department of Transportation for wetland restoration. The experimental results reported here were completed at Juniper Bay between 2007 and 2009, and represent the third and final phase of a study begun in 2001. Research for this period focused on identifying optimum soil and hydrologic requirements of trees that were selected for planting, evaluating tree planting techniques, evaluating amounts of P leaving the Bay in drainage water, and in documenting the occurrence of hydrologic trespass in the area surrounding the Bay as a result of the restoration. Greenhouse and field studies showed that bald cypress and pond pine were the two species best adapted to conditions at Juniper Bay. These trees grew better in the original topsoil compared to where topsoil was removed to fill ditches due to greater amounts of P and K in the topsoil. Planting trees on ridges provided small benefit over planting on smooth ground. Soil P is leaving the site in drainage water at concentrations that could contribute to eutrophication in downstream freshwaters. Plant uptake will help remove some of this P, and it appears to be the best management option at this time. Soils on the southwest side of the Bay appear to have higher water tables after restoration than before. It is not clear that this is due to water from Juniper Bay. However, the perimeter ditch must be kept free of obstructions (including beaver dams) if hydrologic trespass is to be avoided in the future.

17. Key Words Natural resources, ecosystems, environmental quality, land use	18. Distribution Statement		
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 148	22. Price

Form DOT F 1700.7 (8-72) **Reproduction of completed page authorized**

DISCLAIMER

ACKNOWLEGEMENTS

We would like to thank the Technical Advisory Committee for assisting in this research. The committee members include: James Hauser (chair), Gordon Cashin, Matt Flint, David Henderson, Leilani Paugh, Jason Elliott, Randy Griffin, Phil Harris, Elizabeth Lusk, Moy Biswas, and Dennis Pipkin.

From NC State University, this project was conducted by a number of graduate students and technicians. Chris Niewoehner was the technician in charge of field and laboratory efforts. Without their leadership and service this project would not have been completed.

EXECUTIVE SUMMARY

Juniper Bay is a 750 acre restored wetland in Robeson County NC. The Bay is a Carolina Bay wetland that was drained in the 1970's and used for agriculture until 2002. The NCDOT purchased the property and initiated wetland restoration in 2003 by planting trees. Restoration was completed in 2005 when ditches were plugged to restore the hydrology. Research conducted by NC State University has been conducted at the bay from 2002 through 2009 in part to improve wetland restoration techniques and identify potential problems. This report covers research conducted in 2008 and 2009 and summarizes the completion of the research program.

This research for this report had three principle objectives: 1) to identify soil chemical and physical properties and hydrologic requirements for optimum growth of Carolina Bay vegetation, 2) to test different planting methodologies for establishing tree species in Juniper Bay, 3) to assess the potential for P loss from the restored wetland into surface waters, and 4) to determine whether hydrologic trespass is occurring onto neighboring lands.

Research was conducted in both the field and greenhouse. Principle findings include the following. Bald cypress, a member of the Non-riverine swamp forest community, was the most tolerant of continuously (300 days) ponded conditions of the four tree species studied in greenhouse experiments. Pond pine seedlings subjected to 300 total days of ponding had a 75% death rate, but those subjected to 84 total days of ponding had a 100% survival rate, indicating that pond pine is better adapted to the drier locations in Juniper Bay.

Bald cypress trees grown in ponded organic soils in the greenhouse had the greatest overall growth as measured by height, diameter, and shoot biomass of the species examined. Bald cypress grew better in continuously ponded conditions than in drier soils, and grew best in organic soils compared to mineral soils. Based on our experiments, bald cypress would grow best in very wet organic soils, especially organic soils which were once in agricultural production that received higher inputs of N and P. It is well suited to the organic soil areas in Juniper Bay.

The more morphological and anatomical adaptations that are utilized by a species, the greater the likelihood that the species will survive extended periods of ponding. Continuously-ponded bald cypress seedlings adapted the best through a variety of morphological adaptations, and ponded sweet bay seedlings produced an extensive network of adventitious roots. The only adaptation of pond pine and swamp chestnut oak seedlings were hypertrophied lenticels on the submerged portion of the stem, which was insufficient and led to a 75% and 87% death rate, respectively. Pond pine trees should be used in the drier, mineral soil locations at Juniper Bay.

Our field studies confirmed these findings and showed that bald cypress and pond pine were well adapted to the soil and hydrologic conditions at the restored Juniper Bay. Sweet bay and Atlantic white cedar did not do well in the restored mineral soils at Juniper Bay. Survival rates for these two species ranged from 1 to 21%.

Removing topsoil to fill ditches will affect the growth and survival of some tree species such as pond pine. When topsoil is removed, the scalped lands should be fertilized to restore some of the lost P and K. Doing so will have small benefits for bald cypress, but could have large benefits on the survival and growth of pond pine. Benefits to planting trees on ridges as opposed to smooth ground were small.

Dissolved P is leaving the restored Juniper Bay in surface drainage water exiting the Bay at the perimeter ditch outflow point. Concentrations of total P have exceeded 0.2 mg/L after large storm events, and management methods should be used to reduce these levels to <0.1 mg/L, which were observed prior to restoration, in order to avoid causing excessive weed and algal growth in downstream waters. The source of the P is residual fertilizer left in the soil after agriculture had ceased. Mechanisms causing the release occur under anaerobic conditions which are common in wetland soils. Results suggest that it is not possible to prevent the dissolution of P in restored wetlands, so methods to retain the P onsite must be employed. At present, plant uptake appears to be the most viable method of P control.

Varying water flow velocities through soils in laboratory experiments did not result in a significant decrease in the amount of P dissolved and leached out of the soil systems. The concentrations detected at the outlet of experimental flow cells were consistently above the water quality limits and may cause eutrophication in adjacent surface water bodies. These findings matched field observations at the Bay, and suggest that hydrologic modifications cannot be used to lessen the P output from the Bay.

Water table levels in the area outside the southwest section of Juniper Bay appear to be higher after restoration than before. The water table in this area was observed to be in the 0 to 25 cm (0 to 10 in.) depth range for periods up to 103 days after restoration, compared to 26 to 38 days before the Bay's drainage ditches were plugged. Identification of the actual source of this water was beyond the scope of the study. However, previous work indicated that should water levels in the perimeter ditch rise to within or above the estimated critical range, then the water would move from the ditch into the surrounding land area. Water levels were observed to be within the critical range along three of the four ditch locations evaluated, but were below the critical range in the southwest sector where the high water tables were observed outside the Bay.

TABLE OF CONTENTS

Chapter 1

INTRODUCTION

M.J. Vepraskas

Many wetland restoration efforts in North Carolina have failed to meet the relatively limited restoration goals imposed by US Army Corps of Engineers (Corps) permits. On such sites that have been reviewed by the principal investigators, it is obvious that failures result from multiple shortcomings that include: planting of vegetation that is poorly adapted to survive under the restored hydrology, nutrient contamination of surface and ground waters, and raising of water tables in adjacent private lands. The research proposed here is designed to evaluate such problems in Juniper Bay, a converted Carolina Bay depressional wetland in Robeson County, NC. The Bay was purchased by NCDOT for the purpose of restoring it to a wetland, such that permits for road construction could be secured.

Juniper Bay was developed for agriculture several decades ago and up to the year 2000, approximately 750 acres had been drained and intensively managed as agricultural land. In 2005, the ditches were plugged to restore wetland hydrology. Wetland vegetation (trees) were planted on flat ground in approximately 2004, to allow the vegetation to establish for at least one year before hydrology was restored.

The site today appears to be wetter than originally anticipated such that a pond occurs in the central portion of the restored bay, and the pond persists for much if not all year. A perimeter ditch that completely surrounds the property could drain some excess water, but the ditch has not been consistently maintained, and at the time of this writing is not effective. Prior research has suggested that under the conditions that currently exist at Juniper Bay, the following problems could reasonably be expected to occur:

1. Restored vegetation may die in portions of the bay due to persistent ponded conditions.

2. Phosphorus, applied originally to the soil as fertilizer, remains in the soil in large amounts and may dissolve into groundwater and leave the site, eventually contaminating surface waters downstream.

3. With the perimeter ditch plugged, groundwater will exit the Bay (hydrologic trespass) and raise water tables in adjacent privately owned lands, some of which is used for agriculture.

The overall goal of the research is to determine if these problems are occurring, and if so, then to propose solutions to remedy each.

BACKGROUND

A. *Wetland Restoration Requirements* – Wetland mitigation as practiced by the North Carolina Department of Transportation (NCDOT) is the restoration of wetlands to replace those altered or destroyed in the course of road construction and maintenance. The type of wetland ecosystem that must be replaced is specified by the Corps permit that authorized wetland alteration in a particular road project in accordance with Section 404 of the Clean Water Act. To get full credit for wetland restoration efforts, Corps permits usually specify that wetland hydrology, hydric soils, and a plant community similar to the reference ecosystem be restored. The reference ecosystem is a functioning wetland located in the vicinity of the restoration site that has minimal alteration and that is judged to represent the prior natural condition of the restoration site. However, most wetlands have key hydrologic and soil characteristics, such that if the hydrology is restored to cause key soil processes to occur, then it is likely that the most important wetland functions will be restored.

B. ***Reasons Restoration Efforts at Juniper Bay could Fail –*** While restoration is simple in concept, it can be difficult to implement for a variety of reasons. Potential problems that appear likely to occur at the restored Juniper Bay include:

1. Restored Hydrology not Suitable for Restored Vegetation: While it seems obvious that wetland trees should be able to grow under saturated conditions, a site can actually be too wet for the plants to survive. Caldwell (2005) evaluated the hydrologic needs of four wetland plant communities found in natural wetlands in Bladen County, NC. Pond Pine Woodland was found where the soil surface was ponded for fairly short periods of about 15 days per year over a 40-year period. Bay Forest and Nonriverine Swamp Forest communities were found in areas where surface ponding occurred for over 100 days per year over a 40-year period. The restored vegetation at Juniper Bay was intended to recreate a Pond Pine Woodland, yet the restored hydrology appears closer to that of a Bay Forest community. Therefore, it is possible that a large number of trees planed for the Pond Pine Woodland community will die or grow very slowly.

2. Alteration of Hydrology Creates Wetter Conditions than Anticipated: Ewing (2003) showed that the surface of the organic soils in Juniper Bay have subsided by approximately 30 inches due to natural processes that occur in drained organic soils. Organic soils originally develop in areas where the water table remains at the soil surface for several months each year. Therefore, simply plugging ditches at Juniper Bay would be expected to raise the water table to a level above the soil surface of the organic soils to create a pond that could be up to 30 inches deep. In addition, Pati (2006) modeled the groundwater hydrology at the restored Juniper Bay, and showed that movement of groundwater offsite is likely if the perimeter ditch is not maintained to remove excess water from Juniper Bay. Visual observations of the central portion of the Bay today, clearly show that a pond has formed in the organic soil area.

3. Excessive Levels of Soil Nutrients: Wetlands that have been restored using field previously used for agriculture have high levels of P and Ca, among other nutrients, because of annual additions of fertilizer and lime that were necessary for attaining economic yields of agronomic crops. High levels of P can pose particular problems because the P can be dissolved in the groundwater through wetland chemical reactions. Once in the groundwater, the P can leach offsite and eventually reach rivers and streams where the P will serve as food for algae and other aquatic plants. Excessive growth of aquatic plants has been associated with fish kills in some NC waters. Brownfield (2007) has recently shown that P could be expected to be released from the organic soils in Juniper Bay if high levels of dissolved organic C occur. The critical levels of dissolved organic C needed for excessive P loss were not identified.

Successful restoration will require that potential limiting conditions such as those described above not occur. Should these problems develop and not be corrected, then the wetland permits could be in jeopardy.

PROBLEM DEFINITION

The purpose of this research will be to determine if the potential causes of restoration failure noted above, are actually occurring in Juniper Bay. If they are, then the research will also propose viable solutions to correct the problems before lands and waters offsite are impacted, or the Juniper Bay vegetation begins to die.

RESEARCH OBJECTIVES

1. Identify soil chemical and physical properties and hydrologic requirements for optimum growth of Carolina Bay vegetation.

2. Test different planting methodologies for establishing tree species in Juniper Bay.

3. Assess the potential for P loss from the restored wetland into surface waters.

4. Determine whether hydrologic trespass is occurring onto neighboring lands.

LITERATURE REVIEW

A. Geographic Extent

Carolina Bays are oval, NW-SE oriented depressions with sand rims that are located in upland landscapes in the southeastern Coastal Plain and occasionally in the lower Piedmont in certain areas. They range in size from a few acres to more than 7000 acres. Though most numerous in North and South Carolina, Carolina Bays have been identified as far south as north Georgia and as far north as Maryland and Delaware (Melton, 1938; Frey, 1950; Prouty, 1952; and Bliley and Pettry, 1979). Prouty (1952) estimated the total number of Carolina Bays at 500,000 with about 80% of that number occurring in the Carolinas. In North Carolina, bays are most numerous in the southern portion of the middle Coastal Plain, including the counties of Bladen, Columbus, Cumberland, Hoke, Robeson, Sampson, and Scotland. However, they occur in most of the counties of the southern and central Coastal Plain and occasionally in the northeastern Coastal Plain.

B. Hydrology

The hydroperiod of Carolina Bays ranges from permanently flooded to seasonally saturated. Due to the topographic gradient in bays, there is a soil drainage class gradient from excessively drained on the highest portions of the sandy rims to poorly drained or very poorly drained in the lowest elevation portions. Most have significant areas of jurisdictional wetlands, though some of the driest bays may have wetland in the lower elevation portion surrounded by nonwetland area. Many Carolina Bays contain natural lakes, the largest located in Columbus and Bladen Counties, NC. Bays vary significantly in types of connections to surface waters. Few have surface flow input, a notable exception being Lake Waccamaw in Columbus County. Some bays have surface runoff outlets, but the majority likely do not. The types of surface outlets range from dispersed overland flow during large rainfall events to well-developed stream channels.

Hydrologists have long theorized that the hydrology of Carolina Bays is influenced by subsurface flow inputs and fine-textured soil or parent material layers that restrict downward flux of stored water in the bay. Early limited studies of Carolina Bay hydroperiods showed that the hydroperiod was dominated by rainfall inputs and evaporation outputs (Sharitz and Gibbons, 1982). Only relatively recently, however, have detailed hydrology studies begun to elucidate the complex hydrology of Carolina Bays and shown the complex subsurface interactions with the surrounding area (Knight et al., 1989; Newman and Schalles, 1990; Lide et al., 1995; O'ney et al., 1999). In the bays studied by these authors, there was local depressional hydrology superimposed on the regional subsuface hydraulic gradients of the landscape in which the bay occurred. Both Lide et al. (1995) and O'ney et al. (1999) found that the topography of subsurface layers was similar to the surface topography. Sedimentary layers sloped downward from the surrounding uplands to lows under the lower elevation portions of the bay. Hydraulic gradients into the bays resulted in subsurface flows along sandy layers overlying fine-textured layers with upward gradients into the bays during the wet season. In both bays, water accumulated in the bay during the wet season of the year and then was depleted during the dry season. Lide et al. (1995) concluded that Thunder Bay in Barnwell County, SC provided significant ground water recharge during the drying period of late spring/early summer. O'ney et al. (1999) concluded that Chapel Bay in Bamberg County, SC likely provided some recharge but that drying was dominated by evaporation losses.

C. Vegetation

The vegetative communities of Carolina Bays in the Carolinas are diverse among bays and usually complex within bays (Sharitz and Gibbons, 1982; Schafale and Weakley, 1990). That diversity and complexity is related to topography, soils, hydrology, and disturbance history. Schafale and Weakley recognize nine natural community types that commonly occur in Carolina Bays in North Carolina: low pocosin, high pocosin, small depression pocosin, pond pine woodland, peatland Atlantic white cedar forest, bay forest, cypress savanna, small depression pond, and natural lake shoreline. However, examples of most common forest or emergent wetland vegetation types that occur in the Coastal Plain may be found in the complex vegetation mixes of Carolina Bays. All bays have a vegetation gradient from the xeric communities of the sandy rims to the wetland or aquatic communities of the lowest elevation area of the bay, usually in the southeastern quadrant (Sharitz and Gibbons, 1982).

The long-term success of the Juniper Bay restoration project will be determined partly by the similarity of the species composition and structure of its future stable plant community to that at reference Carolina bay sites. Certain key wetland functions at Juniper Bay should also be compared with functions at reference sites. In the early years following restoration at Juniper Bay, before a stable community is attained, successional changes in the community structure and wetland functions should be monitored to try to determine if they are progressing toward their desired future states. Clearly, determining the success of the Juniper Bay restoration requires characterization of reference sites.

Carolina bay lengths range from 50 m to 8 km (Frey, 1949). There appears to be no consistent relationship with particular geological formations or topography (Prouty, 1952). Some bays remain dry nearly all of the time; some contain permanent water; others are seasonally inundated (Sharitz and Gibbons, 1982). Many bays have been disturbed by forestry or agricultural practices while some remain in relatively pristine condition. Fire is a natural disturbance that has probably affected all bays, but to varying extents.

Against the background of extremely heterogeneous environmental influences, it is not surprising that plant community types vary widely among Carolina bays. Major community types previously reported at bays include pine forests, herbaceous marshes, shrub bogs, deciduous forests, evergreen bay forests, pond cypress swamps, prairies, and submerged aquatic beds (Buell, 1946; Penfound, 1952; Whitehead and Tan, 1969; Porcher, 1966; Wharton, 1978; Schalles and Shure, 1989). This vegetation variation suggests that the stable community type that will eventually develop at Juniper Bay is difficult to predict, and that several reference sites should be selected. These sites should be located close to Juniper Bay and have soils, hydrology, and disturbance histories similar to those at Juniper Bay prior to its conversion to agriculture. It is expected that the plant community types and wetland functions will vary among these reference sites. The range of structural and functional variability in the plant communities of these reference sites is the target for Juniper Bay.

D. Characterizing Selected Properties of Carolina Bays

Hydric soils are defined as those that are saturated, flooded, or ponded long enough during the growing season to develop anaerobic conditions in the upper part. Hydric soils must be anaerobic. Many water quality functions that wetlands perform occur because the soils have become anaerobic and chemically reduced.

Wetland restoration efforts are successful when the soils in the restored wetlands become anaerobic for extended periods of time. Development of anaerobic conditions can be monitored by analyzing water chemistry or by measuring the oxidation-reduction potential, which is also called the redox potential (Ponnamperuma, 1972). Water chemistry measurements are used to determine concentrations of reduced chemical species such as NH_4^+, and Fe(II). The presence of Fe(II) is considered proof that the soils are anaerobic.

Redox potential measurements are electrical measurements that determine the voltage developed between a Pt wire and a reference electrode buried in the soil. (Patrick et al., 1996). They are used to determine whether soils are developing the anaerobic conditions necessary for them to be considered as hydric soils. Redox potential measurements are probably the single most important measurements to be made on soils to confirm that a wetland restoration has been successful.

The time required to restore wetland functions is site specific. However, it has been found that some soils regain their hydric soil processes within years of construction (Vepraskas et al., 1999). In a study of created wetlands in the Midwestern U.S., Vepraskas et al. found that hydric soil field indicators developed within 3 yrs of wetland creation, and appeared to reach full development within 5 yrs. To develop the necessary low redox potential required for anaerobic conditions, soil organic matter levels apparently had to exceed 4% in the Midwestern U.S. The hydric soil field indicators adopted for use throughout the U.S. have been presented in USDA-NRCS (1998).

ORGANIZATION OF THE REPORT

The following chapters summarize the principal findings of the research done. Each chapter covers a separate topic and was written by the researchers involved. The chapters were written to be "self-contained" and can be read without referring to other chapters. This format was selected to make reading of the report easier.

PREVIOUS WORK

This report summarizes the work done at Juniper Bay and three reference bays from 2007 to 2007. Two previous reports documented work done from 2001 to 2003 (report no. FHWA/NC/2003-06) and 2003 to 2007 (report no. FHWA/NC/2007-06) by our research group. Wetland trees were planted in 2004 at Juniper Bay prior to the time hydrology was restored. Ditches were filled in 2005 and hydrology restored in 2006 at Juniper Bay.

REFERENCES

Bliley, D. J. and D. E. Pettry. 1979. Carolina bays on the eastern shore of Virginia. Soil Sci. Soc. Am. Jour. 43:558-564.

Buell, M.F. 1946. Jerome Bog, a peat-filled Carolina bay. Bull. Torrey Bot. Club 73: 24-33.

Caldwell, P.V. 2005. Characterization of the hydrologic regime of four Carolina Bay plant communities. Ph.D. dissertation, NC State Univ., Raleigh.

Ewing, J.M. 2003. Characterization of soils in a drained Carolina Bay wetland prior to restoration. Ph.D. dissertation, NC State Univ., Raleigh.

Frey, D.G. 1950. Carolina bays in relation to the North Carolina Coastal Plain. J. Elisha Mitchell Sci. Soc. 66:44-52.

Knight, R.L., J.S. Bays, and F.R. Richardson. 1989. Floral composition, soil relations, and hydrology of a Carolina bay in South Carolina. p. 219-234, *In* R.R. Sharitz and J.W. Gibbons (eds.). Freshwater wetlands and wildlife symposium: perspectives on natural, managed, and degraded ecosystems. US Dept. of Energy, Off. of Sci. and Tech. Info., Oak Ridge, TN. Conf. 8603101, DOE Symposium Series No. 61.

Lide, R. F., V. G. Meentemeyer, J. E. Pinder, III, and L. M. Beatty. 1995. Hydrology of a Carolina bay located on the upper Coastal Plain of western South Carolina. Wetlands 15(1):47-57.

Melton, F.A. 1938. Possible late Cretaceous origin of the Carolina "bays." Geol. Soc. Am. Bull. 49:1954.

Newman, M.C. and J.F. Schalles. 1990. The water chemistry of Carolina bays. Archiv für Hydrobiologie 118:147-168.

O'ney, S.E., M.H. Eisenbies, and M. Miwa. 1999. Hydrologic processes in the vicinity of a Carolina bay affecting water quality: an assessment in association with a hardwood fiber farm. Unpublished progress report. USDA Forest Service, Center for Forested Wetlands Research, Charleston, SC.

Pati, S. 2006. Effects of subsurface flows on wetland restoration at Juniper Bay and surrounding area. Ph.D. dissertation, NC State Univ., Raleigh.

Patrick, W.H., R.P. Gambrell, and S.P. Faulkner. 1996. Redox measurements of soils. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3: Chemical methods. SSSA Book Series 5, SSSA, Madison, WI.

Penfound, W.T. 1952. Southern swamps and marshes. Bot. Rev. 18: 413-446.

Porcher, R.D., Jr. 1966. A floristic study of the vascular plants in nine selected Carolina Bays in Berkeley County, S.C. MS thesis. Univ. SC, Columbia.
Ponnamperuma, F.N. 1972. The chemistry of submerged soils. Adv. Agron. 24:29-96.

Prouty, W. F. 1952. Carolina bays and their origin. Bull. Geol. Soc. Am. 63:167-224.

Schafale, M. P. and A. S. Weakley. 1990. Classification of the natural communities of North Carolina: Third approximation. North Carolina Natural Heritage Program, NC Dept. of Environment, Health, and Natural Resources, Raleigh, NC.

Schalles, J.F. and D.J. Shure. 1989. Hydrology, community structure, and productivity patterns of a dystrophic Carolina bay wetland. Ecol. Monog. 59(4): 365-385.

Sharitz, R. R. and J. W. Gibbons. 1982. The ecology of southeastern shrub bogs (pocosins) and Carolina bays: a community profile. FWS/OBS-82/04. Division of Biological Services, US Fish and Wildlife Service, Washington, DC.

USDA-NRCS. 1998. Field indicators of hydric soils in the United States. Version 4.0. G.W. Hurt, P.M. Whited, and R.F. Pringle (eds.) USDA, NRCS, Fort Worth, TX.

Vepraskas, M. J., J. L. Richardson, J. P. Tandarich, and S. J. Teets. 1999. Dynamics of hydric soil formation across of the edge of a created deep marsh. Wetlands 19(1):78-89.

Wharton, C.H. 1978. The natural environments of Georgia. Geol. And Water Res. Div. Res. Planning Sect., Office of Planning and Research, Ga. Dept. of Nat. Res., Atlanta, Ga. 227 pp.

Whitehead, D.R. and K.W. Tan. 1969. Modern vegetation and pollen rain in Bladen County, North Carolina. Ecology 50:235-248.

Chapter 2

ASSESSING THE RELIABILITY OF HYDROLOGIC CHARACTERIZATIONS OF FOUR WETLAND PLANT COMMUNITIES

C.E. Conk, M.J. Vepraskas, and S.W. Broome

INTRODUCTION

Important wetland functions include wildlife habitat, filtering water, acting as storage basins during flooding events, protecting shorelines, and serving as recreational areas (Mitsch and Gosselink 2000). However, wetlands have not always been recognized as economically or aesthetically valuable. Legislation passed during the late 1970's gave several governmental agencies the authority to begin protecting wetlands, followed by a policy of "no net loss" of wetlands in the late 1980's. These policies raised public awareness and created interest in restoring wetlands.

Typical wetland restoration projects include: choosing a reference wetland as a project target, establishing hydrology, establishing vegetation or target plant communities, and monitoring for three to five years to gauge the project's "success". Regulators, consultants, and wetland researchers use a variety of guidelines to assess wetland restoration "success". However, success is thought to be the viable establishment of a biologically and ecologically sound wetland ecosystem (Mitsch and Wilson 1996).

Wetland restoration is a complex process that requires extensive knowledge about the interaction of soil, hydrology, and plant communities. Through the use of hydrologic models, such as DRAINMOD (Skaggs 1978), those working on wetland restoration projects may be able to better understand the hydrology of reference wetlands as well as predict the hydrology of restored wetlands. Although DRAINMOD was originally designed to predict the effects of drainage and water management practices on water table depths, it has been used to determine the hydrology of natural wetlands (He et al. 2002) as well as to determine whether the wetland hydrologic standard could be met at specific sites (Wright, 2006).

Caldwell (2005) characterized the hydrologic regime of four plant communities, from three reference wetlands using DRAINMOD. The three undrained and naturally vegetated wetlands, from Bladen County, NC, were chosen as reference wetlands for a 256-ha Carolina Bay wetland restoration project. DRAINMOD was calibrated using measured rainfall, as well as water table depths and soil data from three wetlands. A 40-year simulation of water table levels was completed, using historic rainfall data, and correlated to the following plant communities: Non-riverine Swamp Forest (NRSF), Bay Forest (BF), High Pocosin (HP), and Pond Pine Woodland (PPW) (Table 1). The longest consecutive ponding events occurred during the winter months when evapotranspiration rates were the lowest. Non-riverine Swamp Forest and Bay Forest exhibited similar hydrological characteristics. These two communities had water levels above the soil surface, whereas Pond Pine Woodland had a water table depth of 15 cm below the soil surface for the 40-year simulation average. Overall, strong differences between the Non-riverine Swamp Forest/Bay Forest communities and Pond Pine Woodland communities were apparent with respect to hydrology.

While the hydrology of Non-riverine Swamp Forest community and Bay Forest plant community were similar, the Non-riverine Swamp Forest grew in soils with higher levels of available phosphorus. These small differences in soil fertility could explain differences in dominant vegetation types. The vegetation of NRSF is dominated by bald cypress [*Taxodium distichum* (L.) Rich], pond cypress (*Taxodium ascendens* Brogn.), swamp tupelo (*Nyssa biflora* Walter), loblolly pine (*Pinus taeda* L.), Atlantic white cedar [*Chamaecyparis thyoides* (L.) Britton, Sterns & Poggenb], pond pine (*Pinus serotina* L.), and tulip tree (*Liriodendron tulipifera* L.) (Schafale and Weakley 1990). Non-riverine Swamp Forest can be easily distinguished from other peatland communities by the dominance of bald cypress and swamp tupelo. Bay Forest, however, is dominated by sweet bay (*Magnolia virginiana* L.), loblolly bay [*Gordonia lasianthus* (L.) Ellis], red bay [*Persea borbonia* (L.) Spreng.], pond pine (*Pinus serotina* Michx.), and Atlantic white cedar [*Chamaecyparis thyoides* (L.) Britton, Sterns & Poggenb] (Schafale and Weakley 1990). Bay Forest is easily discerned from other plant communities by the dominance of the three bay species: loblolly bay, sweet bay, and red bay. Pond Pine Woodland is also easily distinguished from other plant communities due to the dominance of pond pine.

The objective of this study was to determine if growth of plant species from three wetland communities responded to hydrologic regimes as predicted by calibrating and simulating 40 years of hydrologic data using the DRAINMOD model (Caldwell 2005). High Pocosin (HP) was not evaluated in this study because it is considered to have a similar hydrologic regime as the Non-riverine Swamp Forest and Bay Forest plant communities (Caldwell 2005). Swamp chestnut oak, which is one of the dominant oak species of the Non-riverine Wet Hardwood Forest was included in order to study a range of flood tolerant tree species. If hydrologic regimes can be correctly predicted for target plant communities using modeling approaches, before wetland restorations begin, then higher success rates for these projects could most likely be achieved.

MATERIALS AND METHODS

Soil Collection

This study was conducted in a greenhouse using organic and mineral soils collected from Juniper Bay, a 256 ha Carolina bay wetland, located in Robeson County, near Lumberton, NC (34°30’30”N 79°01’30”E). Juniper Bay was drained in 1972 and used for agricultural production until 2006 when it was restored back to a wetland. During the years in agriculture, the soils were fertilized and limed to soil test recommendations for crop production (Ewing 2003).

Soil samples were collected from two sites that were selected for ease of access and soil type. Mineral soil was collected from the Ap and E horizons of a Leon sand (sandy, siliceous, thermic Aeric Haplaquod). Organic soil material was collected from the Oa horizon of a Ponzer muck (loamy, mixed, dysic, thermic Terric Haplosaprist). Small pits were dug by hand in each soil area to a depth of 25 cm. Soil was collected from the upper 25 cm of the wall of each pit, placed in buckets, transported to the greenhouse, air-dried for 3 days, and then passed through a 1.25-cm mesh sieve. Particle size distribution was determined by the hydrometer method after passing the material through a 2-mm mesh sieve (Gee and Bauder 1986). Undisturbed cores were collected in triplicate from the 15 to 25 cm depth using metal cylinders (10 cm in diameter by 10 cm in height). In the laboratory, soil cores were weighed, oven-dried at 105°C for 24 hours and weighed again for bulk density determination.

Soil samples were analyzed at North Carolina Department of Agriculture, Soil Testing Services, Raleigh, North Carolina for extractable P, K, Ca, Mg, Mn, Cu, Zn, and S using the Mehlich-3 extractant (Mehlich 1984) and an inductively coupled plasma emission spectograph (ICP). Buffer acidity, cation exchange capacity, and the sum of base cations were also determined (Mehlich 1976). The pH was determined using a 1:1 soil to water ratio. Two separate bulk samples were air-dried and ground to pass through a 2 mm sieve for the determination of organic carbon and total nitrogen with a Perkin-Elmer PE2400 CHN Elemental Analyzer (Culmo 1988) in the North Carolina State University Soil Science Analytical Services Lab, Raleigh, North Carolina.

Tree Seedlings

Pond pine (*Pinus serotina* Michx.), bald cypress [*Taxodium distichum* (L.) Rich.], and swamp chestnut oak (*Quercus michauxii* Nutt.) seedlings were purchased from North Carolina Division of Forest Resources, Goldsboro, North Carolina. Sweet bay (*Magnolia virginiana* L.) seedlings were purchased from SuperTree Nursery, Blenheim, South Carolina. Prior to planting, the seedlings were kept in the cold room at 7°C with adequate moisture.

Thirty seedlings, from each species, were chosen for the study based on their similar heights and overall health. Fifteen seedlings were potted in each soil type using 7.65-liter tree containers (Stuewe & Sons, Inc., Corvallis, OR, model TPOT3). Drain holes in the pots were covered with fiberglass mesh to minimize the soil loss from the bottom of the pots. The pots were filled with an appropriate amount of soil and the soil was packed to the desired field bulk density. The final bulk density for containers filled with mineral soil was 1.28 g/cm^3 and 0.47 g/cm^3 for organic soils. The tree seedlings were acclimated to greenhouse conditions by allowing them to grow under unsaturated conditions for 79 days. Twenty-four of the original 30 potted seedlings from each species were then selected for the study based on similar heights and overall appearance of health.

Three hydroperiods were utilized and included: control (C), intermittently ponded (IP), and continuously ponded (CP). Control treatments were watered to maintain a soil water pressure potential of -300 cm which was approximately field (or container) capacity. The intermittently ponded treatment was imposed by saturating pots from the base upward and maintaining a water level above the surface. After 14 days, the soil was drained and kept unsaturated for 42 days. This saturation and drainage cycle was repeated 6 times, totaling 84 days of ponding and 282 days of unsaturated conditions. The intermittently ponded hydroperiod treatment was intended to represent the 40-year average hydrologic characterization of the Pond Pine Woodland (PPW) plant community (Caldwell 2005). The continuously ponded treatment was saturated and kept ponded for a total of 300 days and allowed to drain for 65 total days over the 49-week experiment. The continuously ponded treatment was initially inundated for 110 days and then two subsequent 95-day periods of saturation were utilized. The continuously ponded hydroperiod treatment was intended to represent the 40-year average hydrologic characterization of the Non-riverine Swamp Forest (NRSF) and Bay Forest (BF) plant communities (Caldwell 2005). The water level was maintained 10 to 12 cm above the soil surface for the continuously ponded and intermittently ponded treatments, during their ponded phases. During the unponded cycles, soil moisture content was maintained close to field capacity. Tap water was used for all treatments. Experiments conformed to a split-plot design, containing four replications per treatment combination, after 3 months of treatment initiation.

Each plant container was equipped with two platinum electrodes: one placed at 10 cm below the soil surface and the second probe placed at 20 cm below the soil surface. The two redox probes were placed approximately 13 cm apart, on either side of the tree seedling, in the containers. The redox platinum electrodes were constructed and tested similar to the techniques of Wafer et al. (2004). Accurate redox probes were gently inserted by hand into the soil of the rhizotrons. A small amount of soil was mixed with water to create a soil slurry and any gaps around the redox probe were filled with the slurry.

The containers were equipped with a tensiometer to determine the soil water pressure head for watering purposes. Tensiometers were constructed similar to the methods used by Cassel and Klute (1986). The tensiometers were inserted to a depth of 7.5 cm below the soil surface in all plant containers before the treatments were begun. The septum stoppers were periodically replaced to maintain pressure inside the tensiometer. The tensiometers were read using a tensimeter (Soil Measurement Systems, Tucson, AZ).

Measurements

Redox potential measurements were made weekly using an auto-range digital multimeter (Radio Shack, Fort Worth, TX, model 22-163) and a calomel reference electrode (Thermo Fisher Scientific, Waltham, MA, model 13-620-259). The pH measurements of the organic and mineral soils were made bi-monthly using a portable pH/mV meter (Thermo Fisher Scientific, Waltham, MA, model 13-636-62A) in order to correctly interpret the redox measurements. Height and diameter were measured on a monthly basis using a meter stick and caliper. At the end of the study, the total growth of the 49-week experiment was determined by subtracting the initial height/diameter from the final height/diameter.

Chlorophyll fluorescence measurements were conducted using a hand-held OS-30p fluorometer (Opti-sciences, Hudson, NH, model OS-30p) using the 'Fv/Fm Kinetics' option. Two measurements were taken on each plant including: the second leaf from the bottom and the second fully developed leaf from the tallest shoot apex. However, leaves with obvious signs of senescence or poor overall health were not measured. Measurements were conducted in the pre-dawn hours on leaves, which had been adapted to the dark for at least 6 hours. The relative quantum yield, or quantum efficiency, of Photosystem II was calculated by the hand-held fluorometer as:

$$\Phi_{PS2} = (F_m - F_o)/F_m \qquad [1]$$

Stomatal conductance measurements were conducted in week 16 and again in week 29 with a Delta-T Porometer (Delta-T Devices Ltd, Burwell, Cambridge, England, model AP4-UM-3). Stomatal conductance was measured between the hours of 11:00 a.m. and 1:00 p.m. on a sunny day. One leaf per plant was measured per measurement day. The second fully developed leaf from the tallest shoot apex was measured and recorded. Relative humidity (RH) of the air entering the chamber was fixed, while RH in the greenhouse ranged from 45 to 50%. Photon flux density (PPFD) was also measured with the Delta-T Porometer and reached a maximum of 680 mmol m^{-2} s^{-1}.

Water samples were taken in week 22 to test for N and P concentrations in the soil solution of the continuously ponded seedlings. Phosphate and ammonium were determined using the colorimetric method with a lachat flow injection analyzer. Soil samples were taken at the end of the study. Soil samples were analyzed for extractable P using the Mehlich-3 extractant (Mehlich 1984) and an inductively coupled plasma emission spectrograph (ICP). Soil samples were also analyzed for phosphate (PO_4) using the colorimetric method with a lachat flow injection analyzer. Final measurements were conducted and the containers were dismantled 3 days later. Biomass was divided into shoot (stems and leaves) and roots. Stems and roots were washed, oven-dried for 72 hours at 70ºC, and weighed to calculate shoot, root, and total biomass.

Data Analysis

Statistical analyses were performed using SAS version 9.1 software (SAS 2005). The PROC MIXED function was used to test for significance at the $p \leq 0.05$ level for measurements including: changes in height, diameter, biomass, and root:shoot. The Tukey procedure was used to determine all pair-wise differences between treatment combinations. The data were transformed, as needed, to fulfill the ANOVA assumptions, especially the assumption of equal variances. Biomass data (i.e. shoot, root, total, root: shoot) was log transformed and growth data (i.e. height, diameter, root counts) received a square root transformation. Repeated measures analysis of variance was used to test for differences in means of chlorophyll fluorescence, stomatal conductance, and redox potential data. Redox potential was measured weekly, chlorophyll fluorescence was measured every two weeks, and stomatal conductance was measured twice during the experiment.

RESULTS

Redox Potential

For redox potential, there was a significant effect due to hydroperiod and also an interaction between hydroperiod and time after imposition of treatments for the two depths measured (Table 2). Because redox potential measured 10 and 20 cm below the soil surface did not vary significantly among species, the data were combined for all species for each depth (Fig. 1). The redox potential measured in the control and intermittently ponded organic soils tended to be slightly higher when compared to redox potential measured in mineral soils. The average redox potential for seedlings in the control moisture treatment was +425 mV for seedlings grown in mineral soils, and +500 mV for seedlings grown in organic soils. Well-drained soils typically have a redox potential range of +400 to +700 mV but redox potentials measured below +350 mV are considered characteristic of anaerobic soils (Patrick and DeLaune 1977).

Seedlings subjected to the intermittently ponded hydroperiod treatment experienced 6 cycles of saturation. After the 4^{th} period of inundation, redox potentials stabilized near -25 mV during the saturated cycle (Fig. 1). During the drained cycles for seedlings subjected to intermittently ponded conditions, the redox potentials closely followed the redox potentials observed for seedlings subjected to the control moisture treatment. The first saturated cycle for continuously ponded seedlings lasted 110 days. During this first inundation cycle, redox potentials fell to -100 mV for both mineral and organic soils. The second two cycles consisted of 95 days of consecutive ponding and the redox potentials were slightly elevated at approximately 0 mV during saturation for both soils. Redox potential measured 20 cm below the soil surface followed the same general trend as redox potential measured 10 cm below the soil surface (Fig. 1). More variability in redox potential was observed at the lower depth, especially for seedlings subjected to control and intermittently ponded hydroperiod treatments.

Survival Rates

Survival rates were 100% for all treatment combinations of bald cypress. Survival was also 100% for sweet bay in the control and intermittently ponded hydroperiod treatments. However, survival was 75% for continuously ponded sweet bay in organic soils but survival was 100% for continuously ponded sweet bay in mineral soils. One continuously ponded sweet bay in organic soil died after 50 days of ponding. This particular seedling was the smallest of the sweet bays used in the study.

Pond pine in the control and intermittently ponded hydroperiod treatments had 100% survival. After 110 days of consecutively ponded conditions, 75% of pond pine in organic soils had died. The last surviving continuously ponded pond pine in organic soil died after 150 days of saturation. Survival was 50% for continuously ponded pond pine in mineral soils. The first continuously ponded pond pine in mineral soil died after 191 days of ponding and the second pond pine died after 205 days of ponding. The remaining two continuously ponded pond pine in mineral soil maintained healthy chlorophyll fluorescence levels until the end of the 338-day experiment.

Like sweet bay and pond pine, swamp chestnut oak had 100% survival in the control and intermittently ponded hydroperiod treatments. All of the continuously ponded swamp chestnut oak in organic soil had died within 99 consecutive days of ponding. Continuously ponded swamp chestnut oak in mineral soil had a 75% survival rate. The death of continuously ponded swamp chestnut oak in mineral soil was more sporadic than the death observed in continuously ponded organic soil. The first continuously ponded swamp chestnut oak in mineral soil died within 29 days of ponding, the second seedling within 50 days of ponding, and the final seedling died within 110 days of ponding. However, the remaining swamp chestnut oak in this treatment combination survived the 300 total days of ponding, possibly due to the formation of hypertrophied lenticels.

Chlorophyll Fluorescence

When the fluorescence data were analyzed together, there were no significant main effects or interactions, which may have been due to the large amount of missing values for swamp chestnut oak due to deaths (Table 2). However, when the fluorescence data were analyzed separately, by species, there was a significant interaction between hydroperiod and the time of measurements for all species, except swamp chestnut oak (Table 2). There was also a main effect from hydroperiod in all species, except bald cypress, which showed similar fluorescence levels for all hydroperiod treatments (Table 2).

A plant is considered healthy, or unstressed, when the Fv/Fm ratio is between 0.75 and 0.85 (Bolhar-Nordenkampf and Oquist 1993) or above 0.78 (Adams et al. 1990). Bald cypress seedlings subjected to the three hydroperiod treatments maintained Fv/Fm ratio above 0.78 throughout the experiment indicating that all bald cypress were healthy throughout the 49-week experiment (Fig. 2). The three remaining species maintained Fv/Fm ratio of 0.78 or higher for most of the experiment in the intermittently ponded and control moisture treatments. Marked declines in Fv/Fm ratios were observed in the continuously ponded treatment with the lowest Fv/Fm ratio found for swamp chestnut oak (Fig. 2). The steep declines in Fv/Fm ratios indicate deterioration in the health of the seedlings.

During week 29, there was a significant peak in Fv/Fm for all of the seedlings subjected to intermittently ponded and control moisture conditions (Fig. 2), which was most likely due to the declining temperatures in the greenhouses. Temperatures in the greenhouse, during July and August, frequently exceeded 46ºC.

Stomatal Conductance

For stomatal conductance, all possible interactions between and among the treatment factors were significant (Table 2). There were significant main effects for species and soil (Table 2). As shown in Fig. 3, continuously ponded bald cypress had greater stomatal conductance than the two other hydroperiod treatments while pond pine and swamp chestnut oak showed decreased stomatal conductance in the continuously ponded hydroperiod. Continuously ponded bald cypress had the greatest stomatal conductance compared to the two other hydroperiod treatments and stomatal conductance increased from week 16 to week 29 (Fig. 3). Continuously ponded and intermittently ponded sweet bay showed increased stomatal conductance from week 16 to week 19 (Fig. 3). The increase in stomatal conductance indicates photosynthetic recovery after several weeks of inundation and drainage. All species, except pond pine, had higher stomatal conductance when grown in organic soil. Overall, bald cypress and sweet bay typically had higher stomatal conductance when compared to the less flood-tolerant species (i.e. pond pine, swamp chestnut oak).

Changes in Height and Diameter

For growth in height, there were significant interactions between species and hydroperiod as well as a three-way interaction between species, soil, and hydroperiod. For growth in diameter, there were significant interactions between species and hydroperiod, species and soil, and a three-way interaction between species, soil, and hydroperiod. The three main effects (i.e. species, soil, and hydroperiod) were also significant for growth in height and diameter.

Continuously ponded bald cypress had twice as much growth in height as intermittently ponded bald cypress and nearly 2.5 times the growth of bald cypress in the control moisture treatment (Fig. 4). Likewise, continuously ponded bald cypress had two times the growth in diameter of intermittently ponded bald cypress and five times the growth in diameter of bald cypress under well-drained conditions. Continuously ponded sweet bay exhibited the best growth in height when compared to sweet bay in the intermittently ponded and control moisture treatments (Fig. 4). Continuously ponded sweet bay also experienced the greatest growth in diameter due to the growth of adventitious roots at the base of the stem when compared to sweet bay in the intermittently ponded and control hydroperiod treatments. However, there was only a significant difference in diameter between continuously ponded and intermittently ponded sweet bay. Hydroperiod treatments did not significantly affect height in pond pine and swamp chestnut oak. However, intermittently ponded pond pine had the best growth in diameter when compared to pond pine in the control and intermittently ponded hydroperiod treatments. The growth in height of continuously ponded swamp chestnut oak was the least of any species and hydroperiod combination.

Bald cypress and sweet bay in organic soil exhibited more than 1.5 times more growth in diameter than bald cypress and sweet bay in mineral soil. Bald cypress had the greatest overall growth in height and diameter for all species. The change in height for bald cypress was four times larger than pond pine and sweet bay and 34 times greater than swamp chestnut oak. When the growth in height and diameter was averaged across species, seedlings grown in organic soil had greater growth in height and diameter than seedlings grown in mineral soil. Likewise, continuously ponded seedlings had the greatest growth in height and diameter, followed by seedlings subjected to intermittently ponded conditions. Most of the significant effect caused by hydroperiod was due to the tremendous growth exhibited by continuously ponded bald cypress.

Biomass

For shoot biomass, there was a significant interaction between species and hydroperiod as well as significant main effects from species and soil (Table 3). Continuously ponded bald cypress had greater shoot biomass accumulation than bald cypress in the intermittently ponded and control moisture treatment (Fig. 5). However, continuously ponded pond pine produced less shoot biomass than pond pine in the intermittently ponded or control moisture treatments. There were no significant differences within sweet bay or swamp chestnut oak. Overall, swamp chestnut oak had the greatest shoot biomass and sweet bay had the least accumulation of shoot biomass. Seedlings grown in organic soil had greater shoot biomass than seedlings grown in mineral soil.

For root biomass, there were significant interactions between species and soil, as well as species and hydroperiod (Table 3). There were also significant main effects from species, soil, and hydroperiod (Table 3). Continuously ponded bald cypress accumulated the most root biomass of the three moisture treatments (Fig. 5). Although continuously ponded sweet bay exhibited tremendous adventitious root growth above the soil surface, there were no significant differences within the three hydroperiod treatments. Ponded conditions significantly reduced the amount of root biomass for both pond pine and swamp chestnut oak. Bald cypress and sweet bay grown in organic soil had greater root biomass accumulation than those grown in mineral soil. There were no significant differences within pond pine and swamp chestnut oak grown in the two soil types. Overall, bald cypress accumulated the most root biomass, followed by swamp chestnut oak, pond pine, and sweet bay. Seedlings grown in organic soil accumulated 1.5 times more root biomass when compared to seedlings grown in mineral soil. Continuously ponded seedlings accumulated the least amount of root biomass and seedlings grown in the intermittently ponded and control moisture treatments accumulated similar root biomasses.

For total biomass accumulation, there were significant interactions between species and soil, as well as species and hydroperiod (Table 3). There were significant main effects from species, soil, and hydroperiod (Table 3). Continuously ponded bald cypress accumulated more total biomass than bald cypress in the control moisture treatment (Fig. 6). On the other hand, continuously ponded pond pine and swamp chestnut oak accumulated significantly less total biomass than those grown in the other two hydroperiod treatments. Three out of four species accumulated more total biomass when grown in organic soil than those grown in mineral soil; although pond pine accumulated more total biomass in organic soil, the difference was not significant when compared to pond pine grown in mineral soil (Fig. 6). Overall, bald cypress accumulated the most total biomass, followed by swamp chestnut oak, pond pine, and finally, sweet bay seedlings.

For root:shoot ratios, there were significant interactions between species and soil and species and hydroperiod (Table 3). There were also two significant effects from species and hydroperiod (Table 3). Bald cypress seedlings in the control moisture treatment allocated 3.5 times more biomass into root production than shoot production, whereas continuously ponded bald cypress only allocated 2.5 times more biomass into root production (Fig. 5). Continuously ponded pond pine and swamp chestnut oak allocated significantly less biomass into root production when compared to the control and intermittently ponded hydroperiod treatments (Fig. 5). Bald cypress and sweet bay grown in organic soil allocated significantly more biomass to their roots than those grown in mineral soil. There were no significant differences in biomass allocation for pond pine and swamp chestnut oak grown in the two soil types. Overall, bald cypress had the largest root:shoot ratios (2.95 g/g), whereas the other three species had root:shoot ratios between 1.64 g/g and 1.89 g/g. Continuously ponded seedlings allocated less of their biomass to root production whereas seedlings grown in the control moisture treatment allocated the most biomass to root production.

DISCUSSION

Bald cypress is one of the dominant species of the Non-riverine Swamp Forest (NRSF) plant community that is adapted to wet, very poorly drained peat deposits of the outer Coastal Plain, according to Schafale and Weakley (1990). Caldwell (2005) predicted that NRSF communities averaged 307 total days of ponding per year and 124 consecutive days of ponding over the 40-year hydrologic simulation. This prediction was verified in this study as all continuously ponded bald cypress survived the initial 110-days inundation with two subsequent 95-day periods of saturation. The 100% survival rates found in this study related well to the findings of other studies (Shanklin and Kozlowski 1985; Megonigal and Day 1992; Pezeshki and Anderson 1997; Anderson and Pezeshki 1999). It was also shown that bald cypress subjected to 300 total days of ponding in a 49-week period experienced significantly greater growth in height and diameter, as well as accumulated significantly more shoot, root, and total biomass when compared to bald cypress grown in the control moisture treatment (Figs. 4 and 5). Although bald cypress is considered to be a very flood-tolerant woody species (Hook 1984), previous studies have shown that prolonged saturation has caused decreased shoot biomass (Shanklin and Kozlowski 1985; McLeod et al. 1986; Conner et al. 1998; Anderson and Pezeshki 1999), decreased root biomass (Dickson and Broyer 1972; Shanklin and Kozlowski 1985; McLeod et al. 1986; Conner et al. 1998; Anderson and Pezeshki 1999), as well as decreased growth in height (Shanklin and Kozlowski 1985; Conner 1994). However, increases in stem diameter of continuously ponded bald cypress have been found to occur (Conner 1994; Pezeshki and Anderson 1997).

Previous research has also shown that bald cypresses grown in anaerobic, saturated soils have had initial decreases in stomatal conductance but regained stomatal function within a few weeks (Pezeshki 1991; Anderson and Pezeshki 1999). Although we measured stomatal conductance in week 16, the stomatal conductance for continuously ponded bald cypress was higher when compared to the other hydroperiod treatments (Fig. 3). Thirteen weeks later, stomatal conductance doubled for continuously ponded bald cypress, as well as for intermittently ponded bald cypress (Fig. 3). Furthermore, chlorophyll fluorescence was not reduced due to the various hydroperiod and soil treatments used in the current study (Fig. 2). This indicates that photosynthesis and respiration were not reduced in bald cypress due to the saturated and ponded conditions.

Sweet bay is one of the three dominant bay species of the Bay Forest plant community, which was estimated by Caldwell (2005) to experience an average 299 total days of ponding per year and 104 consecutive days of ponding. It was hypothesized that 100% survival would also be observed for continuously ponded sweet bay. However, survival rate was slightly greater than 87% due to one seedling dying within 50 days of the experiment initiation. This seedling was the smallest of the sweet bay seedlings used in the study and may have been naturally weaker than the rest of the continuously ponded sweet bays. Continuously ponded sweet bay exhibited the greatest growth in height, which was significantly different from the intermittently ponded treatment as well as the greatest growth in diameter, which was significantly different from the two other hydroperiod treatments (Fig. 4). Continuously ponded sweet bay produced the most new shoots; however, total shoot biomass in continuously ponded conditions was not significantly different from the other hydroperiod treatments. Furthermore, the physiological measurements conducted did not indicate that sweet bay was significantly harmed due to the intermittently ponded or continuously ponded hydroperiod treatments. Although chlorophyll fluorescence decreased initially in sweet bay subjected to intermittently ponded and continuously ponded conditions, the fluorescence remained close to the “healthy” fluorescence level of 0.78 for the remainder of the experiment. Stomatal conductance measured in sweet bay was unique from the other species (Fig. 3). Stomatal conductance of sweet bay subjected to intermittently ponded and continuously ponded conditions increased by 100-140 $mmol/m^2/s$, while stomatal conductance of seedlings subjected to the control moisture conditions decreased from week 16 to week 29. Sweet bay, like bald cypress, recovered well after 13 weeks to the continuously ponded and intermittently ponded conditions.

Caldwell (2005) hypothesized that the main difference between Bay Forest and Non-riverine Swamp Forest plant communities was found in higher plant available phosphorus in the soils of Non-riverine Swamp Forest compared to Bay Forest soils. There was a significant interaction between soil and hydroperiod for PO_4 extracted from the soils at the end of this study. Continuously ponded organic soils contained the greatest concentrations of $PO_{4,}$ (2.2 mg/L). The increase in PO_4 in continuously ponded organic soils may be due to the action of dissolved organic carbon (DOC) displacing phosphorus from the soil mineral surfaces (Hutchison and Hesterberg 2004; Brownfield 2007). Dissolved organic carbon are molecules that contain carboxylic acid functional groups, which gives DOC a net negative charge and allows it to compete with PO_4 for binding sites on colloid surfaces (Violante et al. 1991; Bolan et al. 1994). Overall, the soils where bald cypress seedlings grew contained the least amount of PO_4 and total P by the end of the study which suggests that bald cypress, one of the dominant tree species of Non-riverine Swamp Forest, is efficient in taking up available P from the soil, especially in continuously ponded organic soils. Caldwell (2005) may have been correct in suggesting that the differences in species of the Bay Forest and Non-riverine Swamp Forest communities are due to differences in available P in the soils and not due to difference in flood-tolerance.

Pond pine is the dominant species of the Pond Pine Woodland plant community, which Caldwell (2005) characterized as typically occurring in soils that were ponded for <15 days consecutively over the 40-year simulation period. Seventy-five percent of pond pine subjected to three periods of >95 days of consecutive ponding had died by the end of the study. If the study had continued longer, the remaining two pond pine most likely would have died as the needles were turning brown and senescing. Continuously ponded pond pine had the least amount of shoot, root, and total biomass accumulation compared to the other two hydroperiod treatments. Hunt (1950) found reduced root and shoot growth in pond pine that were continuously saturated with stagnant water for 3 months. Saturation can negatively impact the shoot growth of trees by suppressing leaf formation, expansion of leaves, and premature senescence and abscission of leaves (Kozlowski 1997). Although pond pine is considered a moderately tolerant species to saturated conditions (Hook 1984), pond pine produce less shoot and root biomass under prolonged ponded, or anaerobic, conditions. For intermittently ponded pond pine, survival was 100%. Intermittently ponded pond pine had greater stomatal conductance than pond pine in the control moisture treatment and continuously ponded conditions, although the difference between intermittently ponded and the control were not significant (Fig. 3). The intermittently ponded treatment in the current study was intended to mimic the hydrology of the Pond Pine Woodland plant community characterized by Caldwell (2005). Caldwell (2005) determined that the Pond Pine Woodland plant community averaged 89 total days of ponding per year and <15 consecutive days of ponding. Based on the results of this study, we would agree with the hydrologic characterization of the dominant species of the Pond Pine Woodland plant community.

The Non-riverine Wet Hardwood Forest community occurs on the margins of peatland areas in the outer parts of the Coastal Plain and grades into the Pond Pine Woodland (Schafale and Weakley 1990). Swamp chestnut oak is one of the three dominant bottomland oak species of the Non-riverine Wet Hardwood Forest. Although Caldwell (2005) did not describe the hydrologic profile of this community, we included this species to determine if swamp chestnut oak is, in fact, weakly tolerant to ponding as described by Hook (1984). Most (87%) swamp chestnut oak subjected to 300 total days of ponding died, but all lived that were subjected to 84 total days of ponding. Angelov et al. (1996) found that after one year of continuously ponded conditions, 100% of the swamp chestnut oak had died. Furthermore, continuously ponded swamp chestnut oak had a significantly lower root and total biomass accumulation when compared to swamp chestnut oak subjected to the intermittently ponded and control moisture treatments in this study. Although intermittently ponded swamp chestnut oak had the greatest shoot, root, and total biomass accumulation, the differences were not significantly different from the swamp chestnut oak in the control moisture treatment. Based on the findings of this experiment, we confirmed the assessment of Hook (1984) that swamp chestnut oak, one of the dominant species of the Non-riverine Wet Hardwood Forest, is weakly tolerant to ponded conditions.

CONCLUSIONS

The objective of this study was to determine whether hydrologic simulations can be used to correctly assess the hydrologic profile of wetland plant communities. Caldwell (2005) characterized the hydrologic regime of Non-riverine Swamp Forest, Bay Forest, and Pond Pine Woodland. Using similar durations and frequency of ponding, the accuracy of the hydrologic profiles was assessed by utilizing one of the dominant tree species of the respective plant community.

Caldwell (2005) determined that Non-riverine Swamp Forest could withstand the longest durations and total days of ponding per year over the 40-year simulation period of the plant communities described. Bald cypress, the dominant tree species of the Non-riverine Swamp Forest plant community, was also found to be the most tolerant of continuously ponded conditions of the four tree species studied in this 49-week experiment. Sweet bay, one of the dominant trees of the Bay Forest community, had close to 100% survival in the continuously ponded treatment, but growth was not significantly different when compared to the other hydroperiods studied. Bay Forest was characterized as averaging 299 total days of ponding per year and 104 consecutive days of ponding. The ability of sweet bay to withstand 300 total days of ponding in >95-day increments indicated that Caldwell's (2005) characterization of Bay Forest was correct. The major difference between the dominant species of Bay Forest and Non-riverine Swamp Forest appears to be the utilization of increased available phosphorus in continuously ponded organic soils. Pond Pine Woodland was characterized as experiencing 89 total days of ponding per year, on average, as well as <15 days of consecutive ponding. Pond pine seedlings subjected to 300 total days of ponding had a 75% death rate. However, pond pine seedlings subjected to 84 total days of ponding had a 100% survival rate. The biomass accumulation of pond pine subjected to 84 total days of ponding was similar to pond pine seedlings maintained close to field capacity supporting Caldwell's (2005) hydrologic characterization of the Pond Pine Woodland. The dominant species of Pone Pine Woodland can withstand 84 total days of ponding in <15 days of consecutive ponding.

It is common for success rates of wetland restoration projects to be less than 100%. Utilizing a hydrologic model, such as DRAINMOD, to more fully understand the target wetland hydrology and the hydrologic regime of the target plant community, higher success rates for these projects could most likely be achieved.

ACKNOWLEDGEMENTS

This research was sponsored by the North Carolina Department of Transportation (contract no. HWY-2008-16), as part of a large wetland restoration effort in the Coastal Plain of North Carolina. The authors would like to thank C.P. Niewoehner for assistance with the greenhouse and laboratory work, Dr. W.P. Hoffmann for his plant expertise, and Dr. Arrelano for statistical advice.

REFERENCES

Adams, W.W., III, B. Demmig-Adams, K. Winter, and U. Schreiber. 1990. The ratio of variable to maximum chlorophyll fluorescence from photosystem II, measured in leaves at ambient temperatures and at 77 K, as an indicator of photon yield of photosynthesis. Planta 180:166-174.

Anderson, P.H. and S.R. Pezeshki. 1999. The effects of intermittent flooding on seedlings of three forest species. Photosynthetica 37(4):543-552.

Angelov, M.N., S.S. Sung, R.L. Doong, W.R. Harms, P.P. Kormanik, and C.C. Black. 1996. Long- and short-term flooding effects on survival and sink-source relationships of swamp-adapted tree species. Tree Physiology 16:477-484.

Bolan, N.S., R. Naidu, S. Mahimairaja, and S. Baskaran. 1994. Influence of low-molecular-weight organic acids on the solubilization of phosphates. Biology and Fertility of Soils 18:311-319.

Bolhar-Nordenkampf, H.R. and G. Oquist. 1993. Chlorophyll fluorescence as a tool in photosynthesis research. p. 193-206. *In* D.O. Hall, J.M.O. Scurlock, H.R. Bolhar-Nordemkampf, R.C. Leegood, and S.P. Long (eds.). Photosynthesis and Production in a Changing Environment. Chapman & Hall, London, UK.

Brownfield, C.S. 2007. Phosphorus dissolution in soil material from a Carolina Bay as affected by reducing conditions. M.S. Thesis. North Carolina State University, Raleigh, NC, USA.

Caldwell, P.V. 2005. Characterization of the hydrologic regime of four Carolina Bay plant communities. Ph.D. Dissertation. North Carolina State University, Raleigh, NC, USA.

Cassel, D.K., and A. Klute. 1986. Water potential: Tensiometry. p. 563-596. *In* A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agronomy Mongraph 9, ASA and SSSA, Madison, WI, USA.

Conner, W.H. 1994. The effect of salinity and waterlogging on growth and survival of baldcypress and Chinese tallow seedlings. Journal of Coastal Research 10(4):1045-1049.

Conner, W.H., K.W. McLeod, and J.K. McCarron. 1998. Survival and growth of seedlings of four bomttomland oak species in response to increases in flooding and salinity. Forest Science 44(4):618-624.

Culmo, R.F. 1988. Princintermittently pondedle of Operation – The Perkin-Elmer PE 2400 CHN Elemental Analyzer. Perkin Elmer Corportion, Norwalk, CT, USA.

Dickson, R.E., and T.C. Broyer. 1972. Effects of aeration, water supply, and nitrogen source on growth and development of tupelo gum and bald cypress. Ecology 53: 626-634.

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

Gee, G.W. and J.W. Bauder. 1986. Particle-size analysis. p. 383-411. *In* A. Klute (ed.). Methods of soil analysis. Part 1. 2nd ed. American Society of Agronomy and Soil Science Society of America, Madison, WI, USA.

He, X., M.J. Vepraskas, R.W. Skaggs, D.L. Lindbo. 2002. Adapting a drainage model to simulate water table levels in coastal plain soils. Soil Science Society of America Journal 66:1722-1731.

Hook, D.D. 1984. Waterlogging tolerance of lowland tree species of the south. Southern Journal of Applied Forestry 8:136-149.

Hunt, F.M. 1950. Effects of flooded soil on growth of pine seedlings. Plant Physiology 26: 363-368.

Hutchison, K.J., and D. Hesterberg. 2004. Dissolution of phosphate in a phosphorus-enriched ultisol as affected by microbial reduction. Journal of Environmental Quality 33:1793-1802.

Kozlowski, T.T. 1997. Responses of woody plants to flooding and salinity. Tree Physiology Monograph No. 1:1-29.

Megonigal, J.P. and F.P. Day. 1992. Effects of flooding on root and shoot production of bald cypress in large experimental enclosures. Ecology 73(4):1182-1193.

Mehlich, A. 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. Communications in Soil Science and Plant Analysis 15(12):1409- 1416.

Mehlich A. 1976. New buffer method for rapid estimation of exchangeable acidity and lime requirement. Communications in Soil Science and Plant Analysis 7(7):637- 652.

McLeod, K.W., J.K. McCarron, and W.H. Connor. 1999. Photosynthesis and water relations of four oak species: impact of flooding and salinity. Trees 13:178-187.

Mitsch, W.J., and J.G. Gosselink. 2000. Wetlands, third edition. John Wiley & Sons, Inc., New York, NY,USA.

Mitsch, W.F. and R.F. Wilson. 1996. Improving the success of wetland creation and restoration with know-how, time, and self-design. Ecological Applications 6(1): 77-83.

Patrick, W.H., and R.D. DeLaune. 1977. Chemical and biological redox systems affecting nutrient availability in coastal wetlands. Geoscience and Man 18: 131-137.

Pezeshki, S.R. 1991. Root responses of flood-tolerant and flood-sensitive tree species to soil redox conditions. Trees 5:180-186.

Pezeshki, S.R. and P.H. Anderson. 1997. Responses of three bottomland species with different flood tolerance capabilities to various flooding regimes. Wetlands Ecology and Management 4:245-256.

SAS Institute. 2005. SAS Version 9.1. SAS Institute, Cary, NC, USA.

Schafale, M.P., and A.S. Weakley. 1990. Classification of the natural communities of North Carolina: Third approximation. North Carolina Natural Heritage Program, Division of Parks and Recreation, Department of Environment, Health, and Natural Resources. Raleigh, NC, USA.

Shanklin, J., and T.T. Kozlowski. 1985. Effect of flooding of soil on growth and subsequent responses of *Taxodium distichum* seedlings to SO_2. Environmental Pollution 38:199-212.

Skaggs, R. W. 1978. A water management model for shallow water table soils. Technical Report 134. Water Resources Institute, Raleigh, NC, USA.

Topa, M.A. and K.W. McLeod. 1986. Effects of anaerobic growth conditions on phosphorus tissue concentrations and absorption rates of southern pine seedlings. Tree Physiology 2:327-340.

Violante, A., C. Colombo, and A. Buondonno. 1991. Competitive adsorption of phosphate and oxalate by aluminum oxides. Soil Science Society of America Journal 55:65-70.

Wafer, C.C., J.B. Richards, and D.L. Osmond. 2004. Construction of platinum-tipped redox probes for the determination of soil redox potential. Journal of Environmental Quality 32:565-575.

Wright, J.D., M.R. Burchell II, R.O. Evans, and J.D. Shelby. 2006. Hydrologic effect of three restoration techniques on a prior converted wetland in eastern NC. Hydrology and Management of Forested Wetlands Proceedings of the International Conference 8-12 April 2006.

Table 1. Ponding parameters (mean ± SE) for four wetland plant communities. Data represent averages determined over a 40 year period. Adapted from Caldwell (2005).

Plant Community	Total Days of Ponding ($d\ yr^{-1}$)	Consecutive Days of Ponding ($d\ yr^{-1}$)	Ponding Depth (cm)
Non-riverine Swamp Forest	307 ± 6	124 ± 19	10 ± 1
Bay Forest	299 ± 9	104 ± 6	8 ± 1
High Pocosin	291 ± 12	111 ± 21	7 ± 2
Pond Pine Woodland	89 ± 45	15 ± 9	1 ± 1

Table 2. Statistical significances for repeated measures of stomatal conductance and fluorescence. S=significant. NS=not significant. Significances reported at the $p<0.05$ level.

Repeated Effects	Redox Potential	Stomatal Conductance	Fluorescence				
			All Species	Bald cypress	Sweet bay	Pond pine	Swamp c. oak
Species	NS	S	NS	-	-	-	-
Soil	NS	S	NS	NS	S	NS	NS
Hydro	S	NS	NS	NS	S	S	S
Period	NS	S	NS	S	S	S	NS
Species*Soil	NS	S	NS	-	-	-	-
Species*Hydro	NS	S	NS	-	-	-	-
Soil*Hydro	NS	NS	NS	NS	NS	NS	NS
Species*Soil*Hydro	NS	NS	NS	-	-	-	-
Species*Period	NS	S	NS	-	-	-	-
Soil*Period	NS	S	NS	S	NS	NS	NS
Hydro*Period	S	S	NS	S	S	S	NS
Species*Soil*Period	NS	NS	NS	-	-	-	-
Species*Hydro*Period	NS	S	NS	-	-	-	-
Soil*Hydro*Period	NS	NS	NS	S	NS	NS	NS
Species*Soil*Hydro*Period	NS	NS	NS	NS	NS	NS	NS

Table 3. Statistical significances of change in height and diameter and biomass accumulation. Significances reported at the $p<0.05$ level. S=significant. NS=not significant.

Effect	Growth		Biomass			
	Δ Height	Δ Diameter	Shoot	Root	Total	Root: Shoot
Species	S	S	S	S	S	S
Soil	S	S	S	S	S	NS
Hydroperiod	S	S	NS	S	S	S
Species*Soil	NS	S	NS	S	S	S
Species*Hydroperiod	S	S	S	S	S	S
Soil*Hydro	NS	NS	NS	NS	NS	NS
Species*Soil*Hydroperiod	S	S	NS	NS	NS	NS

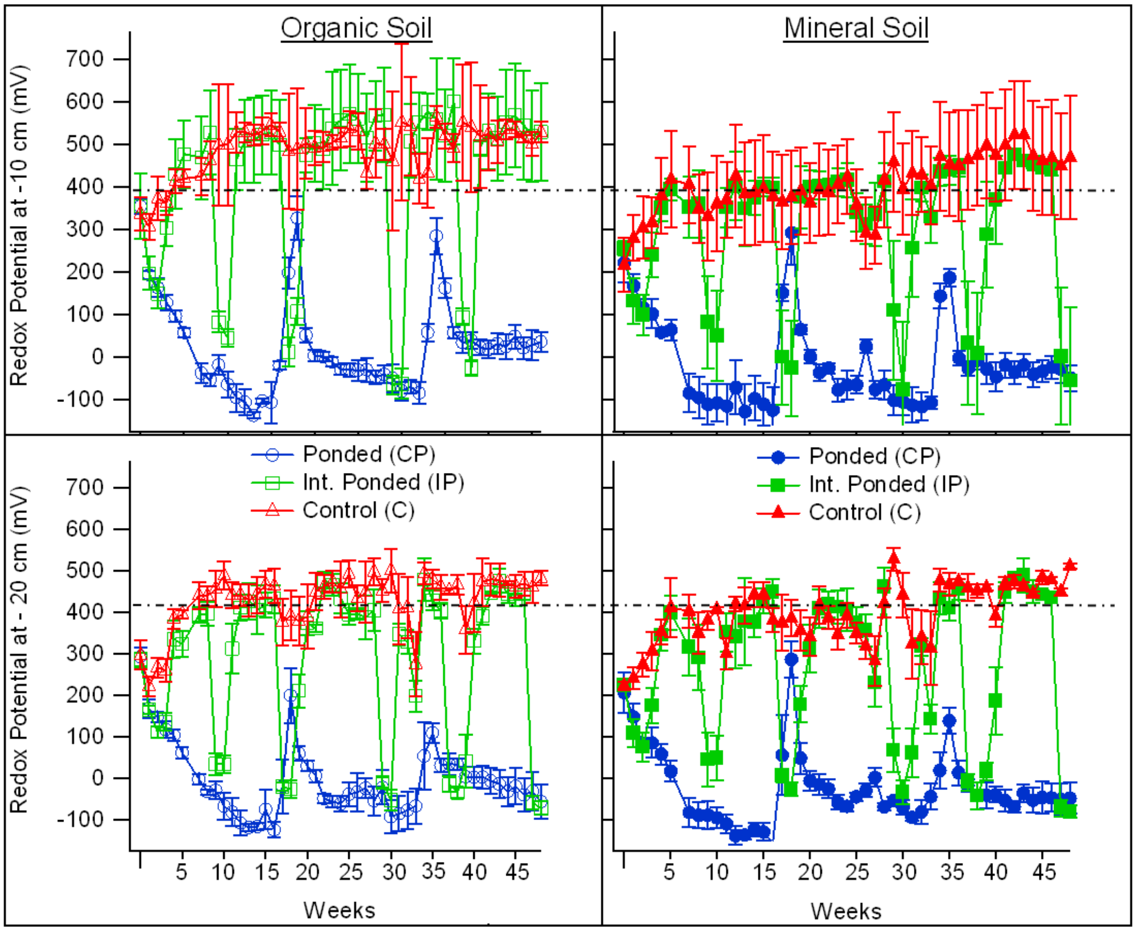

Figure 1. Redox potential measured 20 cm below the soil surface for all seedlings averaged together. The line indicates the boundary between aerobic (>400 mV) and anaerobic (<400 mV) conditions. Standard error bars are shown.

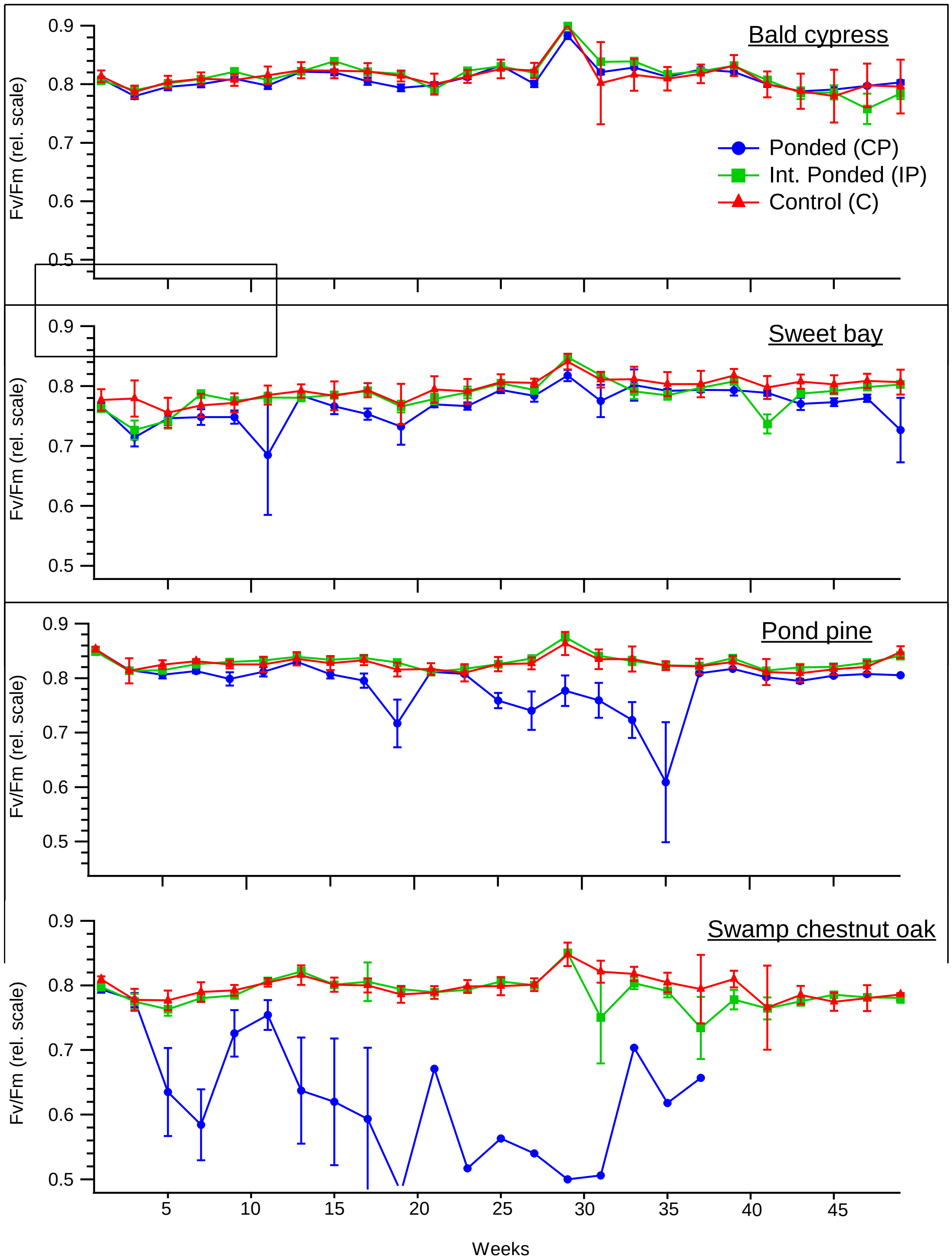

Figure 2. Fluorescence (F_v/F_m) measured on the top leaves of four wetland tree species over the 49-week experiment. Fluorescence ratios greater than 0.78 represent unstressed, or healthy, plants. Standard error bars are shown.

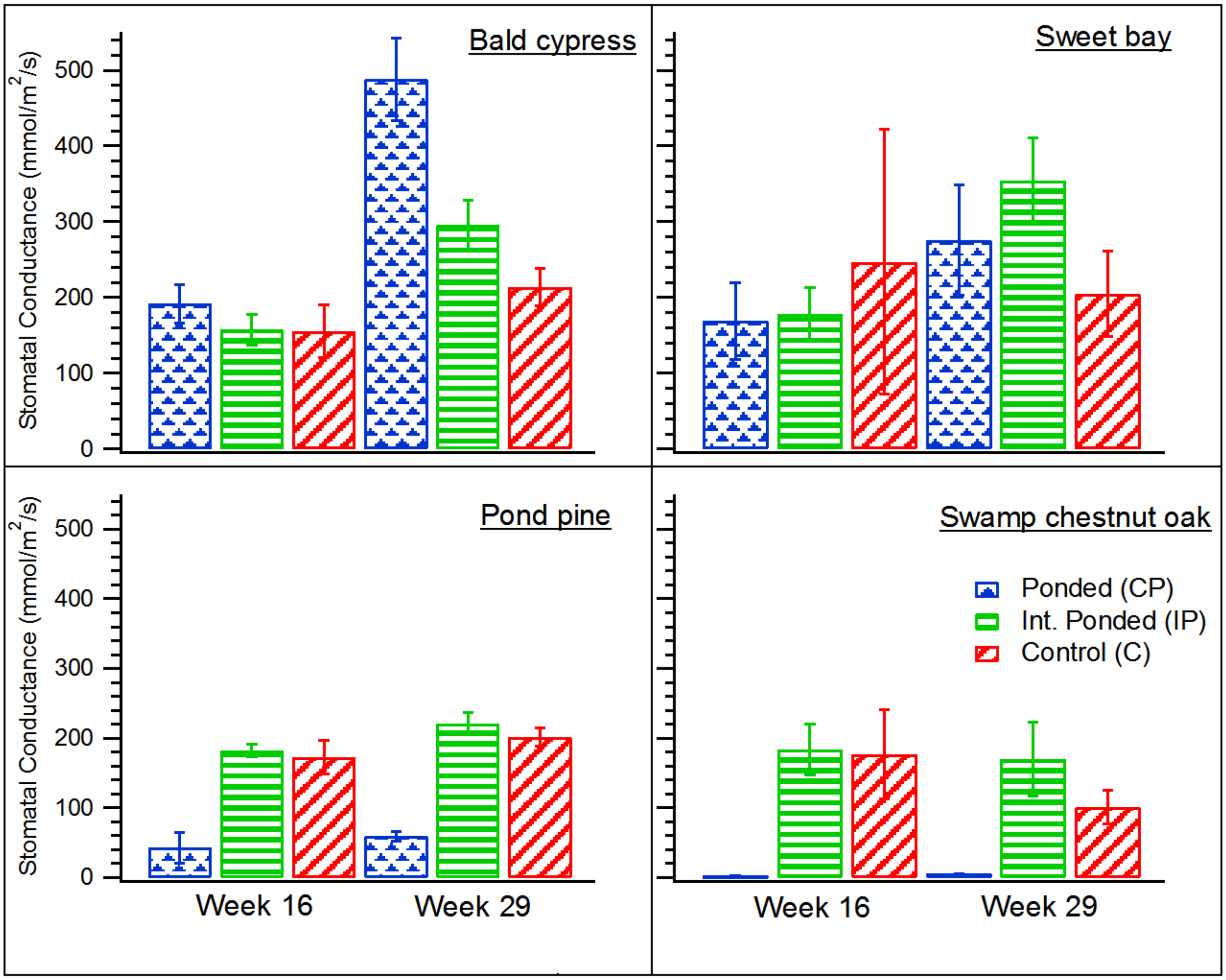

Figure 3. Stomatal conductance measured in week 16 and week 29 for four wetland tree species. Standard error bars are shown.

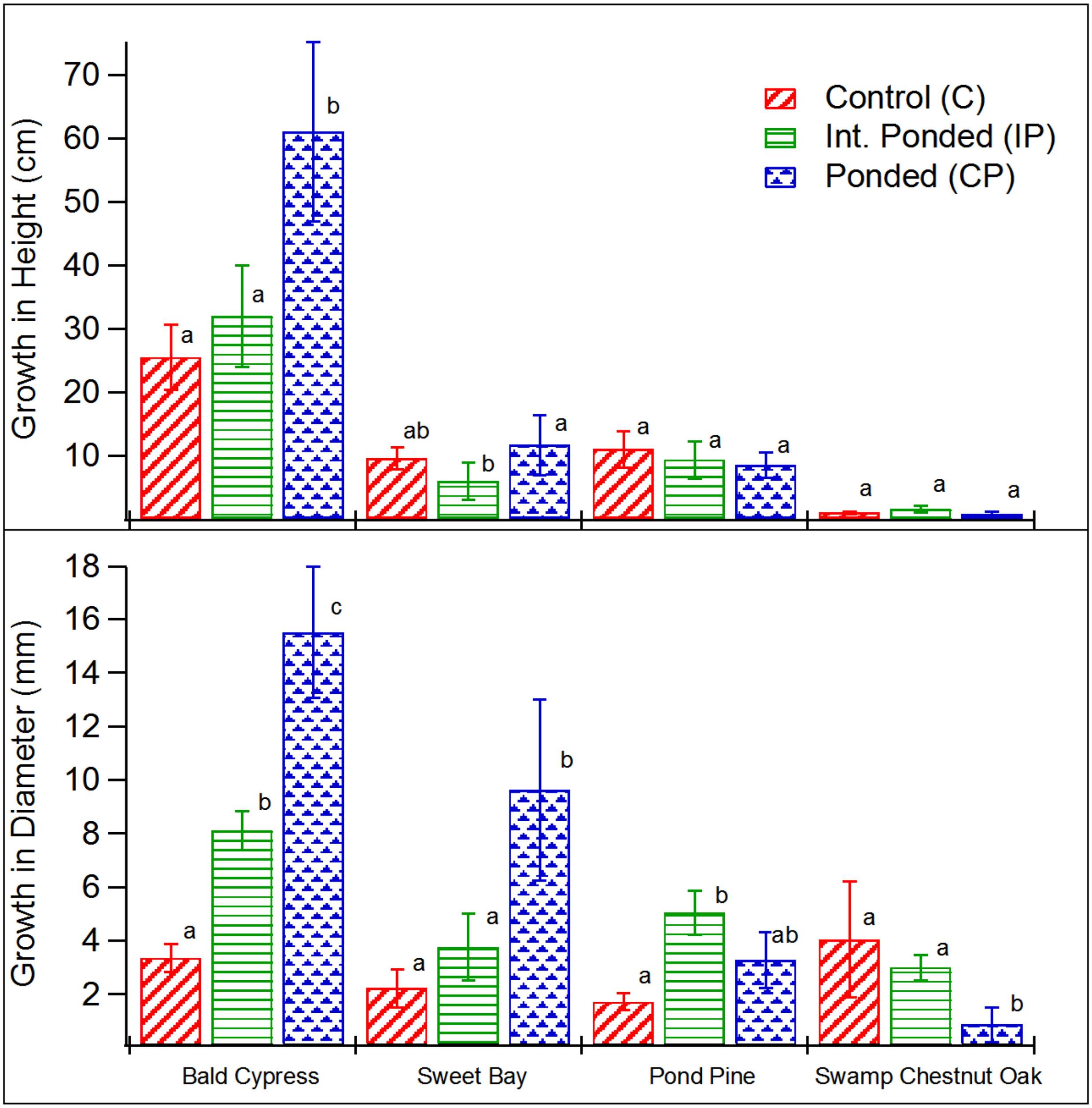

Figure 4. Effect of species and hydroperiod on change (final-initial) in height (cm) and diameter (mm). Standard error bars are shown. Dissimilar letters denote statistical differences within each species.

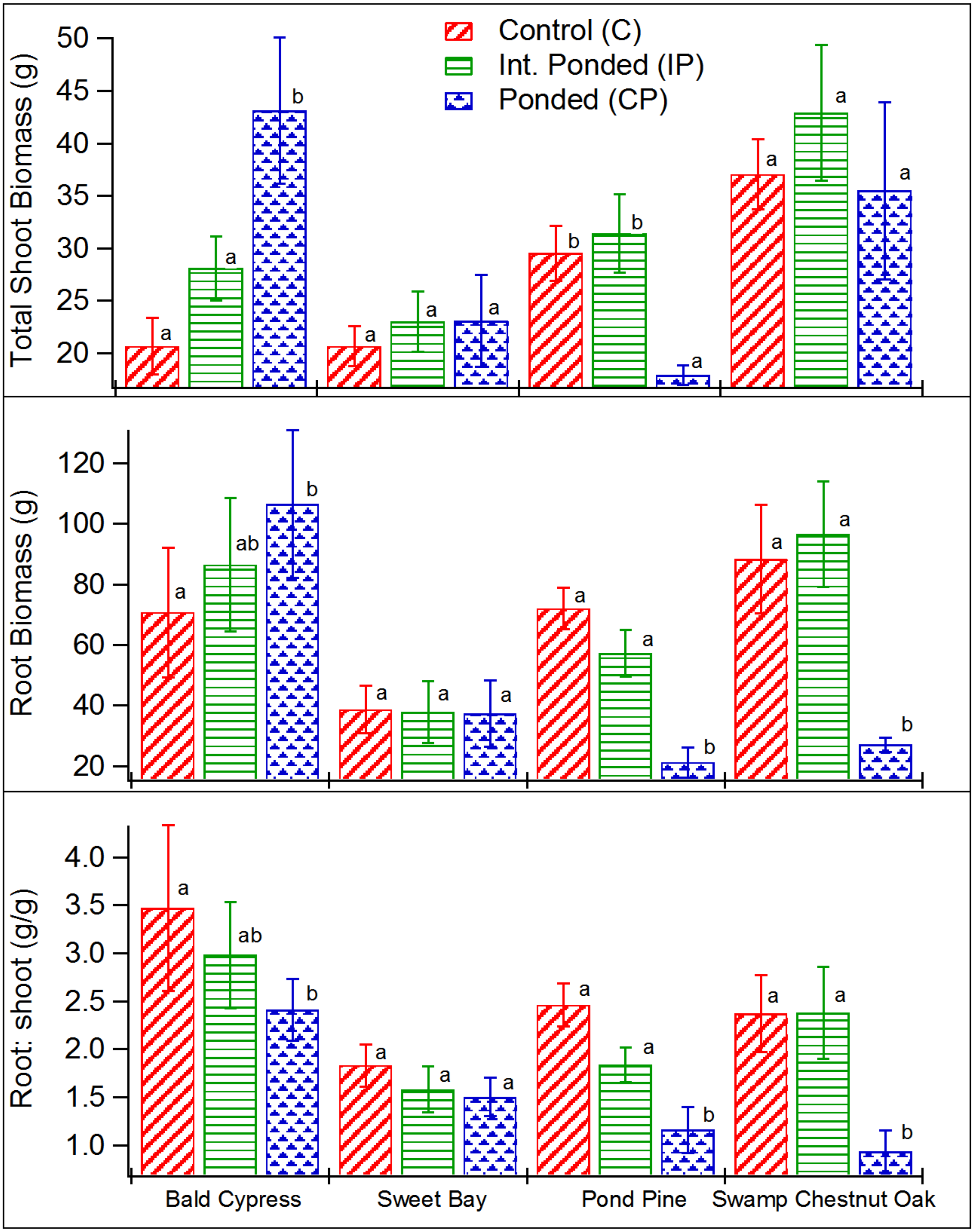

Figure 5. Effect of species and hydroperiod on shoot and root biomass (g) and root: shoot ratio (g/g). Standard error bars are shown. Dissimilar letters denote statistical differences within each species.

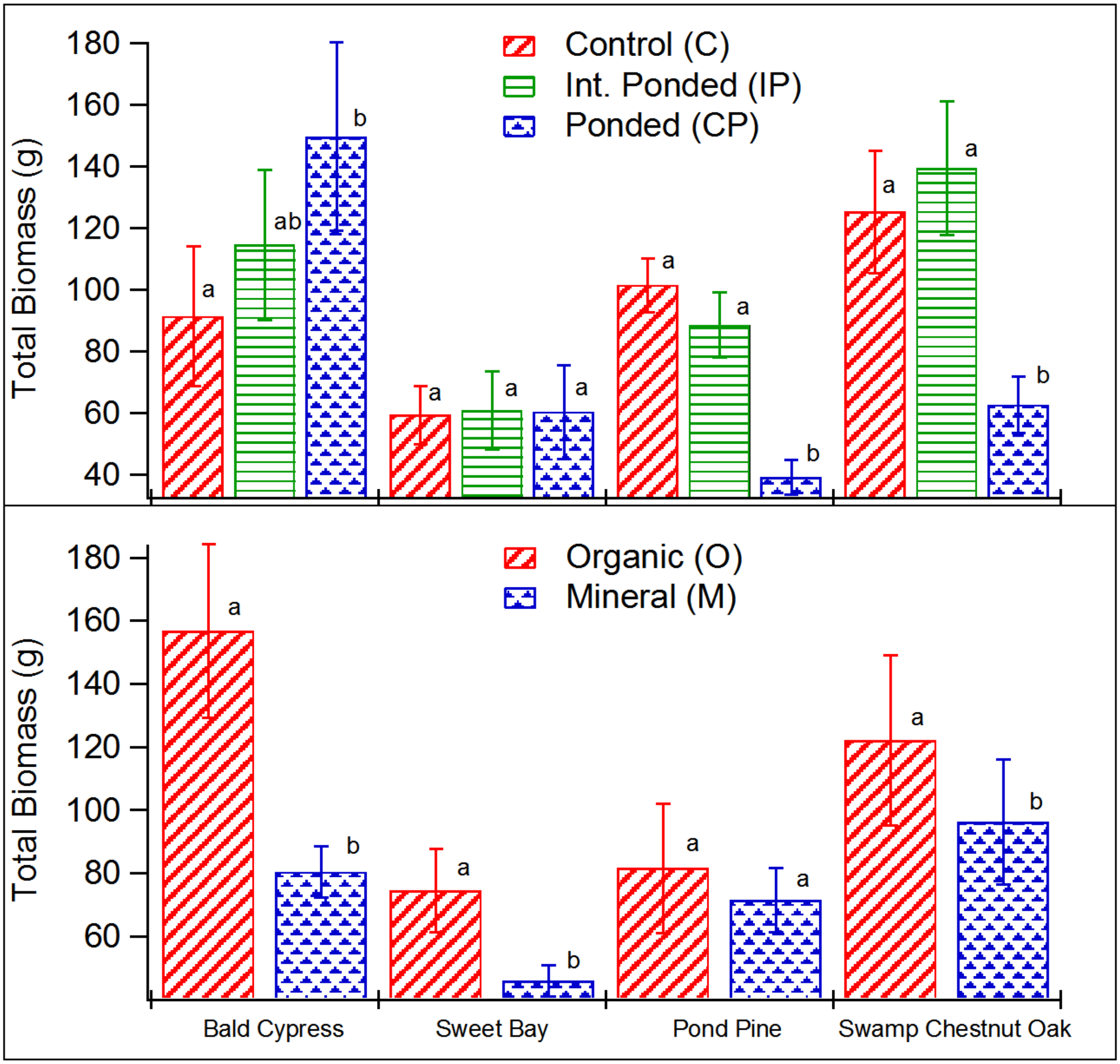

Figure 6. Effect of species and hydroperiod and species and soil type on total biomass (g) accumulation. Standard error bars are shown. Dissimilar letters denote statistical differences within each species.

Chapter 3

BALD CYPRESS AND SWAMP CHESTNUT OAK RESPONSE MECHANISMS TO SATURATED AND ANAEROBIC CONDITIONS

C.E. Conk, M.J. Vepraskas, and S.W. Broome

INTRODUCTION

Successful wetland restoration requires matching the hydrologic needs of the planted vegetation to the restored hydrology. The depth, duration, frequency, and the timing of alternating periods of saturated and unsaturated conditions define the water regime. Plant responses to flooding or saturation differ with the species, duration of saturation, depth of saturation, and with timing of the flooded conditions (Kozlowski 1984b).

Caldwell et al. (2007) characterized the hydrologic regime of four plant communities from three reference wetlands in three Carolina Bay wetlands in the NC Coastal Plain: Non-riverine Swamp Forest (NRSF), Bay Forest (BF), High Pocosin (HP), and Pond Pine Woodland (PPW). Using measured water table levels and rainfall data, DRAINMOD, a hydrologic simulation model, was calibrated for each well sampled, and a 40-year simulation of water table levels was determined for the soils in each plant community. Mean water table levels were defined for the four plant communities. Non-riverine Swamp Forest (NRSF) was found to be the most flood-tolerant of the plant communities with an average of 307 total days of ponding per year and 123 days of consecutive ponding (Caldwell et al. 2007). Bay Forest (BF) had a similar hydrologic regime to NRSF with 299 total days of ponding per year (Caldwell et al. 2007). A major difference between BF and NRSF was the estimated level of available phosphorus (P) in the soils from these two plant communities. These results show that appropriate target plant communities can be selected when the hydrology of the restored site is known.

Conk et al. (2008, unpublished) conducted a container study to test Caldwell's (2007) characterization of the hydrologic regime of plant communities. The container study utilized the hydrologic regimes and the dominant tree species from three of four of the plant communities described previously. In conducting the container study, it was observed that within several weeks of ponding that there were significant differences in the responses between the most flood-tolerant species, bald cypress [*Taxodium distichum* (L.) Rich], and the least flood-tolerant species, swamp chestnut oak [*Quercus michauxii* (Nutt.)]. It was hypothesized that the differences in flood tolerance of these two species were due to differences in root growth and anatomy.

The exact reasons why some plant communities are better adapted to some hydrologies more than others is unclear. Anaerobic conditions affect flood-tolerant and flood-intolerant species differently. Ponding inhibits the formation of new roots, growth of existing roots, and lessens root branching in most species (Kozlowski 1984a; Kozlowski1984b). Megonigal and Day (1992) determined that 28% of total dry mass of bald cypress was allocated to root production in continuously ponded soils, whereas, partially saturated treatments allocated 48% of their dry mass to roots. Anaerobic and reduced soil conditions can lead to development of root rot in some susceptible, flood-sensitive tree species because of increasing soil fungi activity. However, some flood-tolerant species are able to adapt to saturated conditions by producing aerenchyma tissue. Aerenchyma are enlarged gas-filled cavities or lacunae that create exceptionally porous plant tissue. Aerenchyma tissue forms in the aboveground and belowground portions of many wetland species, as well as some dryland species during periods of stress (Evans 2003).

Root systems can be studied by growing plants in a controlled environment. The use of containers allows researchers to study the interaction of specific environmental factors and root growth. Root boxes, or rhizotrons, are a common container approach to studying root systems. These root boxes are glass- (or Plexiglass) walled to make observation of root growth and development easier. The root boxes are kept at a 3-45° angle from the vertical to force roots to make contact with the glass plate. The use of a glass-faced root box allows direct observation of root morphology. Quantifying roots can be very difficult and time consuming. Measuring root biomass is practical but root length is the characteristic responsible for rates of water and nutrient uptake. Böhm (1979) reviewed most of the systematic procedures for studying roots systems in the field. In the trench-profile method, roots are mapped or counted using a square grid, which is placed against the soil profile. The counting method works well for quantifying the root distribution in a soil profile. Using root counts, root data are expressed as numbers of roots per unit area. Many researchers prefer to use the core method, which quantifies roots based on root length density. However, this practice is much more labor intensive and time consuming. Vepraskas and Hoyt (1988) compared the trench-profile method (root counts) to the core method (root length density) and determined that the trench-profile method produced similar results to the core method, which required five times as much labor to quantify root distributions.

Differences in physiological responses also may occur during ponding. The first signs of stress in any plant experiencing harsh environmental conditions are likely to be found in the photosynthetic electron transport system. Chlorophyll fluorescence has the potential of providing an important diagnostic test for the efficiency of photosynthesis (Bjorkman and Demmig 1987). Chlorophyll fluorescence has often been used as an indicator of physiological stress in plants (Lichtenthaler and Rinderle 1988). The ratio of variable fluorescence (Fv) to the maximum fluorescence measured (Fm) indicates the maximum efficiency of the photosystem center (PSII) and should be between 0.75 and 0.85 for healthy leaves (Bolhar-Nordenkampf and Oquist 1993).

Another physiological measure of stress is stomatal conductance. When stomata are not functioning properly, the efficiency of photosynthesis and respiration are significantly reduced and the health of the plant declines. Stomatal conductance, which is a measure of leaf exchange rates of water vapor, measured in mmol $m^{-2} s^{-1}$, is an effective way to measure the rate of stomatal function. A decrease in stomatal conductance is a common response to ponded conditions in both flood-tolerant and flood-intolerant tree species (Kozlowski 1984b; Kozlowski 1997; Pezeshki and Anderson, 1997).

The purpose of this study was to determine, in a controlled environment using rhizotrons, the effects of saturated conditions and soil type on: 1) morphological responses (e.g. formation of aerenchyma tissue, lateral root growth near surface) 2) physiological responses (e.g. chlorophyll fluorescence, stomatal conductance), and 3) biological responses (e.g. changes in height, diameter, biomass partitioning) in seedlings of bald cypress, considered a flood-tolerant species, and swamp chestnut oak, considered a weakly flood-tolerant species (McKnight et al. 1981).

MATERIALS AND METHODS

Soil Collection

Soil material was collected from Juniper Bay, a 256 ha Carolina bay, located in Robeson county, near Lumberton, NC (34°30’30”N 79°01’30”E). Juniper Bay was drained in 1972 and used for agricultural production until 2006 when it was restored back to a wetland. During the years in agriculture, the soils were fertilized and limed to soil test recommendations.

Soil samples were collected in December 2006. Two sites were located to collect soil samples based on ease of access and soil type. Mineral soil was collected from the Ap and E horizons of a Leon sand (sandy, siliceous, thermic Aeric Haplaquod). Organic soil material was collected from the Oa horizon of a Ponzer muck (loamy, mixed, dysic, thermic Terric Haplosaprist). Soil was collected from the upper 25 cm of the soil, placed in buckets, transported to the greenhouse, air-dried for three days, and then passed through a 1.25-cm mesh sieve. Undisturbed cores were collected in triplicate from the 15 to 25 cm depth using metal cylinders (10 cm in diameter by 10 cm in height). The undisturbed soil cores were weighed, oven-dried at 105°C for 24 hours and weighed again to determine bulk density. Particle size distribution was determined by the hydrometer method after passing the material through a 2-mm mesh sieve (Gee and Bauder, 1986).

Soil samples were analyzed at North Carolina Department of Agriculture, Soil Testing Services, Raleigh, North Carolina for extractable P, K, Ca, Mg, Mn, Cu, Zn, and S using the Mehlich-3 extractant (Mehlich 1984) and an inductively coupled plasma emission spectograph (ICP). Buffer acidity, cation exchange capacity, and the sum of base cations was also determined (Mehlich 1976). The pH was determined using a 1:1 soil to water ratio. Organic carbon and total nitrogen were determined with a Perkin-Elmer PE2400 CHN Elemental Analyzer (Culmo 1988) in the North Carolina State University Soil Science Analytical Services Lab, Raleigh, North Carolina.

Rhizotron Construction

Sixteen rhizotrons were constructed similar to the design described by James et al. (1985). However, two 61.5 × 35.5 × 1 cm sheets of transparent polycarbonate were utilized that were held apart by a 2.5 × 2.5 × 0.3 cm steel channel and rubber gaskets. Three small drainage ports were added, to insure proper drainage, at the bottom of each rhizotron. Two wooden legs were attached using wingnuts to ensure that rhizotrons were held at a 30º angle. Each rhizotron was equipped with three platinum electrodes to record redox potential. Electrodes were placed 10 cm, 20 cm, and 30 cm below the soil surface. The redox platinum electrodes were constructed and tested similar to the techniques of Wafer et al. (2004). Accurate redox probes were gently inserted by hand into the soil of the rhizotrons. A small amount of soil was mixed with water to create a soil slurry and any gaps around the redox probe were filled with the slurry. The rhizotrons were also equipped with a tensiometer to determine the soil water pressure head for watering purposes. Tensiometers were constructed similar to the methods used by Cassel and Klute (1986). The tensiometers were inserted to a depth of 7.5 cm below the soil surface in all plant containers before the treatments were begun. The septum stoppers were periodically replaced to maintain pressure inside the tensiometer. The tensiometers were read using a tensimeter (Soil Measurement Systems, Tucson, AZ).

Tree Seedlings

Bald cypress and swamp chestnut oak seedlings were purchased from the North Carolina Division of Forest Resources, Goldsboro, North Carolina. Fifteen seedlings from each species were potted in temporary 2.83 liter containers (Stuewe & Sons, Inc., Corvallis, OR, model Tall One), containing a potting soil medium, for approximately 14 days before being transferred to the rhizotrons. Eight tree seedlings from each species were selected based on similar root lengths, height, and overall appearance of health. Four seedlings from each species were planted in rhizotrons, one tree per rhizotron, containing mineral soil and the remaining four seedlings were planted in rhizotrons containing organic soil. The seedlings were then transplanted to the rhizotrons with the root collars of the seedlings at the soil surface, which was at a similar level in all rhizotrons. The seedlings were maintained under well-watered and well-drained conditions for 78 days until the treatments were initiated. During the 78-day acclimation period, the rhizotrons were kept at a 30° angle to allow for gravitropic response of the roots.

Two hydroperiod treatments were imposed: 1) control (C) where water content was maintained at -300 cm or close to field capacity and 2) continuously ponded (CP) where seedlings were saturated and water was ponded on the surface to a depth of 6 cm for 106 consecutive days. Eight rhizotrons were ponded and included four rhizotrons from the mineral soil and four rhizotrons from the organic soil. A total of two replications per treatment combination (2 reps x 2 hydroperiods x 2 soils x 2 tree species) were achieved by using 16 total rhizotrons in the experiment. The root boxes were placed vertically in a completely randomized design along a rack that was constructed to hold the rhizotrons securely in the vertical position. The rhizotrons were randomly rearranged every month to account for variable conditions in the greenhouse.

Measurements

Redox measurements were conducted on a weekly basis using an auto-range digital multimeter (Radio Shack, Fort Worth, TX, model 22-163) and a calomel reference electrode (Thermo Fisher Scientific, Waltham, MA, model 13-620-259). The pH of the organic and mineral soils were measured every other month using a portable pH/mV meter (Thermo Fisher Scientific, Waltham, MA, model 13-636-62A) in order to correctly interpret the redox measurements. Height and diameter were measured on a monthly basis using a meter stick and caliper. At the end of the study, the total growth of the 106-day experiment was determined by subtracting the initial height and diameter from the final height and diameter. Roots were counted every two weeks for all rhizotrons using a grid that was created by measuring and drawing two hundred and fifty-two 2.5 x 2.5 cm squares on a 2-mm wide sheet of transparent Plexiglass. The number of live roots were counted and recorded for each 2.5 x 2.5 cm square. The increases in the number of roots were determined by subtracting the initial root counts for each grid from the final root counts for each grid. The root boxes were divided into 3 depth increments: 1) top depth increment (0-18 cm below the soil surface), 2) middle depth increment (18-36 cm below the soil surface), and 3) bottom depth increment (36-54 cm below the soil surface). Each depth increment had an area of 542 cm^2. The increases in root counts reported for each depth increment represent the number of roots proliferated in the study in each 540 cm^2 area. Photographs were also taken bi-weekly.

Chlorophyll fluorescence measurements were conducted using a hand-held OS-30p fluorometer (Opti-sciences, Hudson, NH, model OS-30p) using the ‘Fv/Fm Kinetics’ option. Two measurements were taken on each plant including: the second leaf from the bottom and the second fully developed leaf from the tallest shoot apex. However, leaves with obvious signs of senescence or poor overall health were not measured. Measurements were conducted in the pre-dawn hours on leaves, which had been adapted to the dark for at least 6 hours. The relative quantum yield, or quantum efficiency, of Photosystem II was calculated by the hand-held fluorometer as:

$$\Phi_{PS2} = (F_m - F_o)/F_m \qquad [1]$$

Stomatal conductance measurements were measured once in week 12 with a Delta-T Porometer (Delta-T Devices Ltd, Burwell, Cambridge, England, model AP4-UM-3). Stomatal conductance was measured between the hours of 11:00 and 1:00 on a sunny day. One leaf per plant was measured per measurement day. The second fully developed leaf from the tallest shoot apex was measured and recorded. Relative humidity (RH) of the air entering the chamber was fixed. RH in the greenhouse was also measured during this time and ranged from 45 to 50%. Photon flux density (PPFD) was also measured with the Delta-T Porometer and reached a maximum of 680 $\mu mol\ m^{-2}\ s^{-1}$.

Final measurements were made on October 15th and 16th, 2007. The rhizotrons were dismantled 2 days later. The leaves and stems were separated and oven-dried for 48 hours at 70ºC, or until a constant weight was reached. Leaves were ground and analyzed by the North Carolina State University Soil Science Analytical Services Lab, Raleigh, North Carolina. Nitrogen was measured with a CHN analyzer and P, Ca, Mg, Mn, Fe, and Zn were measured with ICP-emission spectrometer. The root zone was divided into three depth increments (0-18 cm, 18-36 cm, and 36-54 cm). Soil samples from each depth increment were collected and air-dried for 7 days. On October 18, root and stem tissue samples were taken for SEM analysis. The root tissue was sampled between 0-18 cm and 36-54 cm and fixed in a 3% glutaraldehyde in a 0.05M sodium phosphate buffer at pH 7.2. The stem tissue samples were sampled in approximately 2 cm diameters and fixed in a 3% glutaraldehyde in a 0.05M sodium phosphate buffer at pH 7.2. These tissue samples were sent to Center for Electron Microscopy at North Carolina State University for SEM analysis.

Data Analysis

Statistical analyses were performed using SAS version 9.1 software (SAS Institute, Cary, NC, 2005). The PROC MIXED function was used to test for significance at the $p \leq 0.05$ level for measurements including: changes in height, diameter, root counts, biomass, root/shoot ratio, and stomatal conductance data. The Tukey procedure was used to determine all pair-wise differences between treatment combinations. The data was transformed, as needed, to fulfill the ANOVA assumptions, especially the assumption of equal variances. Biomass data (i.e. shoot, root, root: shoot) was log transformed and growth data (i.e. height, diameter, root counts) was square root transformed. Repeated measures analysis of variance was used to test for differences in means of fluorescence and redox potential data. Fluorescence was measured every two weeks after treatment initiation and redox potential was measured weekly.

RESULTS

Redox Potential

Tree species had no significant effect on redox potential at the three depths measured. However, there was a significant effect due to hydroperiod and also an interaction between hydroperiod and time after imposition of treatments for the three depths measured. Redox potential measured 10 cm below the soil surface averaged +300 mV in mineral soil and above +400 mV in organic soil in the control treatment. In the control moisture treatment, redox potential was +350 mV at 20 cm below the soil surface (Fig. 1), but decreased to an average of +275 mV at 30 cm below the soil surface. An Eh range of +400 to +700 mV is characteristic of aerobic soils (Pezeshki 1991). The root boxes that were maintained at field capacity were close to aerobic conditions for the duration of the experiment. Redox potential 10 cm below the soil surface stabilized at -100 mV after 12 wk of saturation for both mineral and organic soils. Redox potential 20 cm below the soil surface stabilized after nine wk of saturation and after eight wk for redox potential at 30 cm below the soil surface. Anaerobic soils typically have redox potentials below +350 mV (Patrick and DeLaune 1977). Therefore, the continuously ponded root boxes were anaerobic for the duration of the experiment.

Survival Rates

Survival rates were 100% for all treatment combinations of bald cypress. Survival rates were 100% for swamp chestnut oak maintained at field capacity (control) in both mineral and organic soils. By week eight, 100% of swamp chestnut oak in ponded organic soils had died. Swamp chestnut oak in ponded mineral soils had a 100% survival rate at the conclusion of the experiment. The onset of root death was noted in swamp chestnut oak in this treatment in wk 10 and gradually continued until the conclusion of the experiment. By the end of the experiment, hypertrophied lenticel development was observed for swamp chestnut oak in ponded mineral soils.

Chlorophyll Fluorescence

For chlorophyll fluorescence, there were no significant interactions between the treatment factors; however, the main effects of both species and hyroperiod were significant. Values of Fv/Fm, measured in the top leaves of bald cypress, remained above 0.8 for all treatment combinations (Fig. 2). In week 11 there was a substantial increase in Fv/Fm in bald cypress, especially those under ponded conditions, which peaked close to 0.9. Swamp chestnut oak in the control moisture treatment maintained Fv/Fm at or above 0.8. A plant is considered healthy when the Fv/Fm ratio is between 0.75 and 0.85 (Bolhar-Nordenkampf and Oquist 1993) or above 0.78 (Adams et al. 1990). Thus based on chlorophyll fluorescence, bald cypress and swamp chestnut oak grown in moist soil conditions close to field capacity did not exhibit stress for the entire experiment. However, swamp chestnut oak in ponded organic soils maintained an Fv/Fm of 0.8 before abruptly dying in week 8. A decrease in Fv/Fm was observed in swamp chestnut oak in ponded mineral soils during week 12; from week 12 until the end of the experiment, Fv/Fm steadily decreased by 14% to 0.72 (Fig. 2). Declining Fv/Fm in ponded swamp chestnut oak grown in mineral soils suggests that some physiological stress was occurring at the end of the experiment. Chlorophyll fluorescence measured using the bottom leaves of seedlings followed the same general trends and are not reported here.

Stomatal Conductance

Stomatal conductance was measured once during the experiment after 78 days of ponding. Although there were no significant interactions at the $p \leq 0.05$ level (species and hydroperiod were significant at the $p \leq 0.10$ level), there was a significant main effect from tree species. Bald cypress had a significantly higher stomatal conductance compared to swamp chestnut oak when all treatment combinations were averaged together for each species. Ponded bald cypress had the highest rate of stomatal conductance (425 mmol (H_20) $m^{-2}s^{-1}$) while ponded swamp chestnut oak had the lowest stomatal conductance (29 mmol (H_20) $m^{-2}s^{-1}$). Swamp chestnut oak in ponded organic soils was not included in the measure of stomatal conductance because 100% of the seedlings had died by week 12.

Changes in Height and Diameter

For growth in height, all possible interactions between and among the treatment factors were significant (Table 1). Growth in height was also significantly affected by the main effects of species and soil (Table 1). Bald cypress and swamp chestnut oak had higher average total growth in organic soil (17 cm) than in mineral soil (4 cm). Bald cypress in organic soil had the greatest increase in height for all species and soil combinations (Tables 2 and 3); exhibiting four times greater growth than bald cypress seedlings in mineral soils (Table 2). Ponded bald cypress seedlings had the best overall growth in height for all species and hydroperiod combinations, more than twice that of bald cypress in the control hydroperiod (Tables 2 and 3). Overall, bald cypress had the greater change in height with a mean of 20 cm, compared to an average growth of 1.6 cm in swamp chestnut oak, when all treatment combinations were averaged together for each species, which may be due to natural differences in relative growth rates.

For growth in diameter, there were significant interactions between species and soil and species and hydroperiod (Table 1). Significant main effects included species, soil, and hydroperiod (Table 1). Increase in diameter for ponded bald cypress was five-fold greater than bald cypress grown in the control moisture treatment (Table 2). Bald cypress seedlings grown in organic soils had the greatest increase in diameter when compared to all other species and soil combinations (Tables 2 and 3). Similar to the change in height, growth in diameter of bald cypress (6.0 mm) was greater than swamp chestnut oak (1.3 mm), when all treatment combinations were averaged together for each species. Seedlings in organic soils (4.7 mm) had greater growth in diameter than in mineral soils (2.6 mm), while ponded seedlings (5.5 mm) had greater growth compared to the control hydroperiod (1.8 mm).

Biomass

For shoot biomass, there were significant interactions between species and soil, as well as species and hydroperiod (Table 1). There was also a main effect from hydroperiod on shoot biomass (Table 1). Bald cypress grown in organic soil (37 g) had greater shoot biomass than when grown in mineral soil (20 g). Ponded bald cypress shoot biomass (39 g) was significantly greater than bald cypress in the controlled moisture treatment (14 g), which had the least shoot biomass of all species and hydroperiod combinations (Tables 2 and 3). Total shoot biomass was significantly greater for ponded seedlings compared to the control. On average, ponded seedlings had a shoot biomass of 36 g, compared to 25 g, for the control moisture treatment.

For root biomass in the surficial depth increment (0-18 cm), there were significant interactions between species and soil and species and hydroperiod, as well as a main effect from species. Swamp chestnut oak in mineral soil had significantly greater root biomass (36 g) in the 0-18 cm depth than all other species and soil treatment combinations (Tables 2 and 3). Swamp chestnut oak in the control moisture treatment (34 g) also had significantly greater root biomass than all other species and hydroperiod combinations (Tables 2 and 3). Overall swamp chestnut oak had a greater root biomass than bald cypress. This difference was most likely due to the differences in the tap roots of these two species. Swamp chestnut oak has a woodier tap root than bald cypress. For root biomass at the second depth increment (18-36 cm), there was a significant interaction between species and hydroperiod, as well as a significant main effect from species. Ponded bald cypress had the greatest root biomass in this depth increment of all species and hydroperiod combinations (Tables 2 and 3). Overall at this depth increment, bald cypress had a greater root biomass (27 g) than swamp chestnut oak (14 g). In the lowest depth increment (36-54 cm), there was a significant main effect from hydroperiod. Seedlings in the control moisture treatment had greater root biomass (11 g) than ponded seedlings (1 g). Total belowground biomass, summed from the three depth increments, had a significant interaction between species and hydroperiod (Table 1). Ponded bald cypress had the greatest total root biomass (64 g) while ponded swamp chestnut had the lowest total root biomass (28 g) of all species and hydroperiod combinations (Tables 2 and 3).

For root: shoot ratios, there was a main effect from species and hydroperiod (Table 1). Overall, bald cypress (2.24 g/g) allocated more biomass into root production than swamp chestnut oak (1.39 g/g), when all treatment combinations were averaged together for each species. Ponded seedlings (1.36 g/g) allocated less biomass to root production than seedlings in the control moisture treatment (2.27 g/g).

Soil solutions from the continuously ponded containers of bald cypress and swamp chestnut oak were sampled and measured for PO_4-P concentrations (Table 4). There were significant main effects of soil type and species on the PO_4-P concentrations in the soil solutions sampled. Organic soils (0.32 mg/L) had greater concentrations of PO_4-P in the soil solution than mineral soils (0.068 mg/L PO_4-P). Bald cypress seedlings in organic soils had an average soil solution concentration of 0.19 mg PO_4-P/L, which was lower than the average soil solution concentration for swamp chestnut oak seedlings grown in organic soils. This is most likely due to the higher biomass accumulation for bald cypress seedlings, which created a greater need for macronutrients. Soil water analysis was also conducted for NH_4-N but no significant differences were observed (values ranged from 0.21 to 4.41 mg NH_4-N/L).

Leaf Tissue Analysis

Leaf tissue samples were analyzed for percentage of N, P, and Ca, as well as the concentrations of Mg, Fe, Mn, and Zn. For the percentage of N in leaf tissue, there were significant main effects from species and soil. Swamp chestnut oak (2.03%) had greater N than bald cypress seedlings (1.69%). Seedlings grown in organic soil (2.01%) had a greater percentage of N than seedlings grown in mineral soil (1.71%). For percentage of P accumulated in leaf tissue, there was a significant main effect from soil and no significant interactions between factors. Seedlings grown in organic soils (0.11%) had greater accumulation of P than seedlings grown in mineral soils (0.08%) (Table 4). For percentage of Mg in leaf tissue, there was a significant main effect from soil. Seedlings grown in organic soils (0.40%) accumulated more Mg than seedlings grown in mineral soils (0.33%). Interestingly, seedlings grown in organic soils had a significantly greater amount of N, P, and Mg in leaf tissue samples, when compared to leaf tissue samples from those grown in mineral soils. The soils used in this study were analyzed for nutrient concentrations prior to the start of the experiment. Organic soils contained 10 times more total N and 4 times more Mg than mineral soils.

For Mn, there was a significant interaction between species and soil as well as a main effect from species. Swamp chestnut oak in organic soil (690 ppm) accumulated significantly greater Mn than swamp chestnut oak in mineral soil (426 ppm). However, bald cypress in mineral soils (124 ppm) accumulated more Mn than bald cypress in organic soils (98 ppm). Mineral soil used in this study contained nearly twice as much Mn as organic soil. There were no significant effects or differences to report for % Ca or for concentrations of Fe and Zn.

Root Counts

For increases in root counts in the top depth increment (0-18 cm), there was a significant interaction between species and hydoperiod as well as two significant main effects from species and hydroperiod (Table 1). Ponded bald cypress had the greatest increase of roots in the top depth increment (952 roots/540 cm^2) while ponded swamp chestnut oak produced the least amount of roots near the surface (32 roots/540 cm^2) (Fig. 3). A photograph illustrating the proliferation of roots in a ponded bald cypress seedling near the soil surface is included (Fig. 4). Overall bald cypress had greater proliferation of roots near the soil surface than swamp chestnut oak seedlings. Likewise, ponded seedlings increased their root production near the surface of the soil compared to seedlings grown under the control moisture treatment.

For increases in root counts in the middle depth increment (18-36 cm) there was a significant interaction between soil and hydroperiod as well as a significant main effect from hydroperiod. Seedlings grown in continuously ponded organic soils produced 10 times more roots than seedlings grown in organic soils of the control moisture treatment at this depth increment. Like root proliferation in the top 18 cm of the soil surface, ponded seedlings produced more roots in the middle depth increment. There were no significant interactions or main effects in root production in the bottom third (36-54 cm) of the rhizotrons to report.

Aerenchyma Tissue Development

Ponded bald cypress developed a greater amount of aerenchyma tissue in the roots than bald cypress in the control moisture treatment (Fig. 5 A-D). In the mineral soil, aerenchyma tissue development was more pronounced at the shallower depth, 0-18 cm, but aerenchyma tissue development was more visible in the deeper depth, 36-54 cm, for bald cypress in organic soils. It was difficult to sample bald cypress roots in the organic soil, at both depths, because the root tissue was extremely “spongy” and prone to fall apart (Fig. 5 C-D). Overall, ponded bald cypress in organic had a greater amount of aerenchyma tissue development than ponded bald cypress in mineral soils. For ponded swamp chestnut oak, there was minimal aerenchyma tissue development in the roots (Fig. 5 E-F). Flood-sensitive species are less able to develop extensive, enlarged air spaces in their roots and shoots, in response to saturation, and eventually, root and plant death is observed. Aerenchyma tissue was also observed in the basal stems of ponded bald cypress seedlings in both organic and mineral soils (Fig. 6). The development of aerenchyma tissues is a significant morphological adaptation for species frequently subjected to saturated and anaerobic soil conditions.

DISCUSSION

Bald cypress was more flood-tolerant than swamp chestnut oak, as was expected according to the flood tolerance rankings of Hook (1984). All ponded bald cypress seedlings survived that were saturated for 106 days consecutively. This survival rate is in accordance with several other studies (Shanklin and Kozlowski 1985; Megonigal and Day 1992; Pezeshki and Anderson 1997; Anderson and Pezeshki 1999). Overall, ponded bald cypress in organic soils had the best growth in height, diameter, and in shoot and total biomass. This may be due to the increased availability of P in the soil solution of ponded organic soils. In this study, we found higher PO_4-P concentrations in ponded organic soils for both bald cypress and swamp chestnut oak (Table 4). Brownfield (2007, unpublished data) found that dissolved phosphorus was greater in organic soils than mineral soils from Juniper Bay in Robeson County, N.C. This was due to the action of dissolved organic carbon (DOC) displacing phosphorus from the soil colloids in organic soils similar to the organic soils used here. We observed the highest percentages of N and P, as well as the highest accumulation of Fe in plant tissues of ponded bald cypress in organic soils. The increased uptake of these nutrients may have aided in the increased growth in height and diameter, as well as overall biomass.

In addition to the increased uptake of P from ponded organic soils, bald cypress in ponded organic soils adapted well by producing the most lateral roots near the soil surface when compared to bald cypress and swamp chestnut oak grown in other treatment combinations. Soil inundation typically results in the decay of roots due to the increased activity of root-rotting fungi. Woody tree species that are intolerant to ponding will lose part of their original root system and fail the regenerate new roots. However, flood-tolerant species will lose part of that original root system and subsequently regenerate new roots on the original root system, on the submerged stem, or both (Kozlowski 1984). Root death was observed within a few weeks of soil inundation in bald cypress in organic soils. However, new roots, which appeared to be more succulent than original roots, quickly emerged near the soil surface. It is thought that adventitious roots compensate for the loss of portions of the original root system through increasing the water absorption by roots, as well as oxidizing the soil rhizosphere and transforming toxic compounds into less harmful compounds (Kozlowski 1997).

In addition to the presence of lateral surface roots, ponded bald cypress produced aerenchyma tissue in the roots and stems after 106 days of flooding (Fig. 5 and 6). Flood-tolerant species, such as bald cypress, are known to develop aerenchyma tissue in response to saturated conditions. Aerenchyma tissue provides the plant an extensive, low-resistance internal aeration system, which can transfer oxygen, via diffusion and convection, to anaerobic rhizospheres (Evans 2003). Plants with interconnected gas spaces are able to transport oxygen from stems and leaves to the roots, while toxic gases, such as carbon dioxide, ethylene, and methane may move through these spaces, or tissues, to the shoots to the atmosphere (Colmer 2003). Bald cypress have produced aerenchyma tissue in saturated conditions, and to a lesser extent, in aerated conditions (Kludze et al. 1994). However, Pezeshki (1991) found that roots of bald cypress subjected to low soil Eh conditions had increased amounts of aerenchyma tissue due to cell separation in newly generated cortical cells. Bald cypress from the control moisture treatment had formed no aerenchyma tissue during the 14-day experiment (Pezeshki 1991). The formation of aerenchyma is an invaluable asset to flood-tolerant tree species surviving prolonged periods of soil inundation.

Swamp chestnut oaks are considered to be weakly tolerant to saturated conditions (Hook, 1984) and it was expected that 100% survival would not be observed for this species. By the end of the study (15 weeks), 50% of ponded swamp chestnut oak had died. Conner et al. (1998) observed 10% death for swamp chestnut oaks saturated for 17 weeks. Ponded swamp chestnut oaks did not produce lateral roots near the surface as roots died, like bald cypress. There was also minimal development of enlarged cavities in continuously ponded swamp chestnut oak, unlike ponded bald cypress.

CONCLUSIONS

The objective of this study was to observe and quantify the responses of two wetland tree species, bald cypress and swamp chestnut oak, to saturated and anaerobic conditions using rhizotrons to observe the changes in root morphology. Bald cypress adapted to saturated and anaerobic conditions by producing aerenchyma tissue and lateral roots near the surface of the soil, as roots in lower depths began senescing. Ponded bald cypress in organic soils had the greatest overall growth as measured by height, diameter, and shoot biomass. The increase in diameter was most likely due to the increase in aerenchyma tissue development in the basal portion of the stem, which was more developed than in the stems of ponded bald cypress in mineral soils. Aerenchyma tissue development in the roots of ponded bald cypress was greater in organic soils than mineral soils. The results of this study showed that bald cypress adapted well continuously ponded conditions when compared to the control moisture treatment. Bald cypress grown in the control moisture treatment showed less growth in height, diameter, as well as root and shoot biomass. Stomatal conductance and Fv/Fm for bald cypress was lower in the control moisture treatment than the continuously ponded hydroperiod, indicating greater photosynthetic efficiency in ponded seedlings. Based on the information gathered in this experiment, bald cypress would grow best in very wet, organic soils, especially organic soils which were once in agricultural production that received higher inputs of N and P. The ability of dissolved organic carbon to displace P on Fe- and Al-complexes may aid in the superior growth of ponded bald cypress in organic soils.

Swamp chestnut oak grew far less than bald cypress, in this 106-day experiment. Although there was less growth, many important results were found. Although swamp chestnut oak is considered to be one of the more flood-tolerant *Quercus* species (McKnight et al. 1981), plant death was observed within 40 days for ponded swamp chestnut oak in organic soils. Plant death was not observed for ponded swamp chestnut oak in mineral soils for 106 days. The rapidly declining Fv/Fm observed in these seedlings was evidence of plant stress and an indication that the ponded swamp chestnut oak may have died if the experiment had continued. There were no significant differences in growth of height, diameter, or shoot biomass of swamp chestnut oak due to hydroperiod or soil treatments. Based on the results of this experiment, swamp chestnut oak should be planted on the outer fringes of wetlands where prolonged periods of saturation rarely occur. Swamp chestnut oak is not tolerant of saturated and anaerobic conditions. This research reinforces the necessity of understanding the interaction between soil, hydroperiod, and vegetation type before selecting the target plant community during wetland restoration projects.

ACKNOWLEDGEMENTS

The authors would like to thank C.P. Niewoehner for assistance with the greenhouse and laboratory work, Dr. W.P. Hoffmann for his plant expertise, and Dr. C. Arrelano for statistical advice.

REFERENCES

Adams, W.W., Demmig-Adams, B., Winter, K., and Schreiber, U. 1990. The ratio of variable to maximum chlorophyll fluorescence from photosystem II, measured in leaves at ambient temperatures and at 77 K, as an indicator of photon yield of photosynthesis. Planta 180:166-174.

Anderson, P.H., Pezeshki, S.R. 1999. The effects of intermittent flooding on seedlings of three forest species. Photosynthetica 37(4):543-552.

Bjorkman, O., and Demmig, B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170:489-504.

Böhm, W. 1979. Methods of studying root systems. Spring-Verlag, Berlin, Germany.

Bolhar-Nordenkampf, H.R., and Oquist, G. 1993. Chlorophyll fluorescence as a tool in photosynthesis research. In: Hall, D.O., Scurlock, J.M.O., Bolhar-Nordemkampf H.R., Leegood, R.C., and Long, S.P. (eds.) Photosynthesis and production in a changing environment. Chapman & Hall, London, pp. 193-206.

Caldwell, P.V., Vepraskas, M.J., Skaggs, R.W., and Gregory, J.D. 2007. Simulating the water budgets of natural Carolina bay wetlands. Wetlands 27(4): 1112-1123.

Cassel, D.K., and Klute, A. 1986. Water potential: tensiometry. In: Klute, A. (ed.) Methods of soil analysis, part 1, 2nd ed. Agronomy Mongraph 9, ASA and SSSA, Madison, Wisconsin, pp. 563-596.

Colmer, T.D. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant, Cell, and Environ. 26:17-36.

Conner, W.H., McLeod, K.W., and McCarron, J.K. 1998. Survival and growth of seedlings of four bomttomland oak species in response to increases in flooding and salinity. For. Sci. 44(4):618-624.

Culmo, R.F. 1988. Principle of Operation – The Perkin-Elmer PE 2400 CHN Elemental Analyzer. Perkin Elmer Corporation, Norwalk, Connecticut.

Evans, D.E. 2003. Aerenchyma formation. New Phytol 161:35-49.

Gee, G.W., and Bauder, J.W. 1986. Particle-size analysis. In: Klute, A. (ed.) Methods of soil analysis, part 1, 2nd ed. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, pp. 383-411.

Hook, D.D. 1984. Waterlogging tolerance of lowland tree species of the south. South J. Appl. For. 8:136-149.

James, B.R., Bartlett, R.J., and Amadon, J.F. 1985. A root observation and sampling chamber (rhizotron) for pot studies. Plant and Soil 85: 291-293.

Kludze, H.K., Pezeshki, S.R., and DeLaune, R.D. 1994. Evaluation of root oxygenation and growth in bald cypress in response to short-term hypoxia. Can. J. For. Resour. 24:804-809.

Kozlowski, T.T. 1984a. Extent, causes, and impacts of flooding. In: Kozlowski, T.T. (ed.) Flooding and plant growth. Academic Press, New York, pp. 1-5.

Kozlowski,T.T. 1984b. Plant responses to flooding of soil. BioScience 34:162-167.

Kozlowski, T.T. 1997. Responses of woody plants to flooding and salinity. Tree Physiol. Monog.r No. 1:1-29.

Lichtenthaler, H.K., and Rinderle, U. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Crit. Rev. Anal. Chem. 19:29-85.

McKnight, J.S., Hook O.O., Landon O.G., and Johnson, R.L. 1981. Flood toleranceand related characteristics of trees of the bottomland hardwood forests of the southern United States. In: Clark, J.R., and Benforado, J. (eds.) Wetlands of bottomland hardwood forests. Elsevier Scientific, Amsterdam, pp. 29-69.

Megonigal, J.P., and Day, F.P. 1992. Effects of flooding on root and shoot production of bald cypress in large experimental enclosures. Ecol. 73(4):1182-1193.

Mehlich, A. 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. Comm. Soil Sci. and Plant Anal. 15(12):1409-1416.

Mehlich, A. 1976. New buffer method for rapid estimation of exchangeable acidity and lime requirement. Comm Soil Sci and Plant Anal 7(7):637-652.

Patrick, W.H., and DeLaune, R.D. 1977. Chemical and biological redox systems affecting nutrient availability in coastal wetlands. Geosci. and Man. 18:131-137.

Pezeshki , S.R. 1991. Root responses of flood-tolerant and flood-sensitive tree species to soil redox conditions. Trees 5:180-186.

Pezeshki, S.R., and Anderson, P.H. 1997. Responses of three bottomland species with different flood tolerance capabilities to various flooding regimes. Wetl. Ecol. and Manage. 4:245-256.

SAS Institute (2005) SAS Version 9.1. SAS Institute, Cary, NC.

Shanklin, J., and Kozlowski, T.T. 1985. Effect of flooding of soil on growth and subsequent responses of *Taxodium distichum* seedlings to SO_2. Environ. Poll. 38:199-212.

Vepraskas, M.J., and Hoyt, G.D. 1988. Comparison of the trench-profile and core methods for evaluating root distributions in tillage studies. Agron. J. 80(2):166-172.

Wafer, C.C., Richards, J.B., and Osmond, D.L. 2004. Construction of platinum-tipped redox probes for the determination of soil redox potential. J Environ Qual 32:565-575.

Table 1. Statistical significances for selected measurements.

Effect	Growth		Biomass				Root Counts		
	Δ Height	Δ Diam.	Shoot	Root	Total	Root: Shoot	0-18 cm	18-36 cm	36-54 cm
Species	S	S	NS	S	NS	S	S	S	NS
Soil	S	S	NS	NS	NS	S	NS	NS	NS
Hydroperiod	NS	S	S	NS	NS	S	S	S	NS
Species*Soil	S	S	S	NS	NS	NS	NS	NS	NS
Species*Hydroperiod	S	S	S	S	S	NS	S	NS	NS
Soil*Hydro	S	NS	NS	NS	NS	NS	NS	S	NS
Species*Soil*Hydroperiod	S	NS	NS	NS	NS	NS	NS	NS	NS

***S= significant at the p≤0.05 level. *NS=not significant at the p≤0.05 level.**

Table 2. Change in height, diameter, and final biomass (roots and shoots) accumulation of bald cypress after 106 days. Standard errors are shown. Dissimilar letters denote statistical differences across the row.

	Bald cypress			
	Organic		Mineral	
	Control	Ponded	Control	Ponded
Δ Height	12.6±3.1 a	53.2±3.3 b	10.8±2.8 a	2.1±0.5 c
Δ Diameter	2.7±0.6 a	12.9±2.2 b	0.7±0.3 ac	7.7±1.3 ad
Shoot Biomass	23.6±1.2 ab	50.9±11.7 a	13.9±1.7 b	27.5±0.3 ab
Root Biomass:				
0-18 cm	12.0±0.6 ab	31.0±6.3 a	11.7±3.9 b	25.1±2.7 ab
18-36 cm	19.9±1.6 a	24.3±5.2 a	21.3±4.5 a	43.8±17.2 a
36-54 cm	21.9±0.8 a	3.1±0.9 a	8.6±4.0 a	1.6±1.3 a
Total Root Biomass	53.7±3.0 a	58.4±10.7 a	41.5±8.4 a	70.4±21.4 a
Total Biomass	77.3±4.2 a	109.3±22.3 a	55.5±10.1 a	97.9±21.1 a
Root:shoot	2.28±0.01 a	1.16±0.06 a	2.95±0.25 a	2.57±0.80 a

Table 3. Change in height, diameter, and final biomass (roots and shoots) accumulation of swamp chestnut oak after 106 days. Standard errors are shown. Dissimilar letters denote statistical differences across the row.

	Swamp chestnut oak			
	Organic		Mineral	
	Control	Ponded	Control	Ponded
Δ Height	1.8±0.0 a	2.1±1.0 a	1.7±0.1 a	0.8±0.5 a
Δ Diameter	2.7±1.2 a	0.4±0.4 a	1.0±0.2 a	1.2±0.1 a
Shoot Biomass	28.9±2.1 a	29.8±8.6 a	35.4±3.3 a	36.9±2.7 a
Root Biomass:				
0-18 cm	25.8±0.2 ab	13.5±5.4 a	42.3±2.6 b	29.8±6.1 ab
18-36 cm	19.9±10.7 a	8.0±2.1 a	22.3±10.1 a	4.4±0.2 a
36-54 cm	8.2±4.0 a	0.3±0.3 a	5.6±1.1 a	0.3±0.1 a
Total Root Biomass	53.8±18.5 a	21.8±5.1a	70.1±8.6 a	34.5±6.2 a
Total Biomass	82.7±20.6 a	51.6±13.7 a	105.5±5.3 a	71.4±3.5 a
Root:shoot	1.82±0.51 a	0.75±0.05 a	2.02±0.43 a	0.95±0.23 a

Table 4. Phosphorus concentrations in soil solution (mg/L) and in dry leaf tissue samples (%) of continuously ponded tree seedlings. Standard errors are shown. Dissimilar letters denote statistical differences across the row.

	Bald cypress		Swamp chestnut oak	
	Organic	Mineral	Organic	Mineral
Soil Solution PO4-P (mg/L)	0.19±0.019 a	0.0033±0.03 a	0.52±0.14 b	0.11±0.05 a
P in Dry Leaf Tissue (%)	0.11±0.0057 a	0.080±0.0080 b	0.10±0.010 ab	0.080±0.0016 b
Average Dry Weight of Leaves (g)	14.08±3.28	7.25±0.30	9.75±1.95	7.63±1.58
P Content of Leaves (g/plant)	1.5	0.58	0.98	0.61

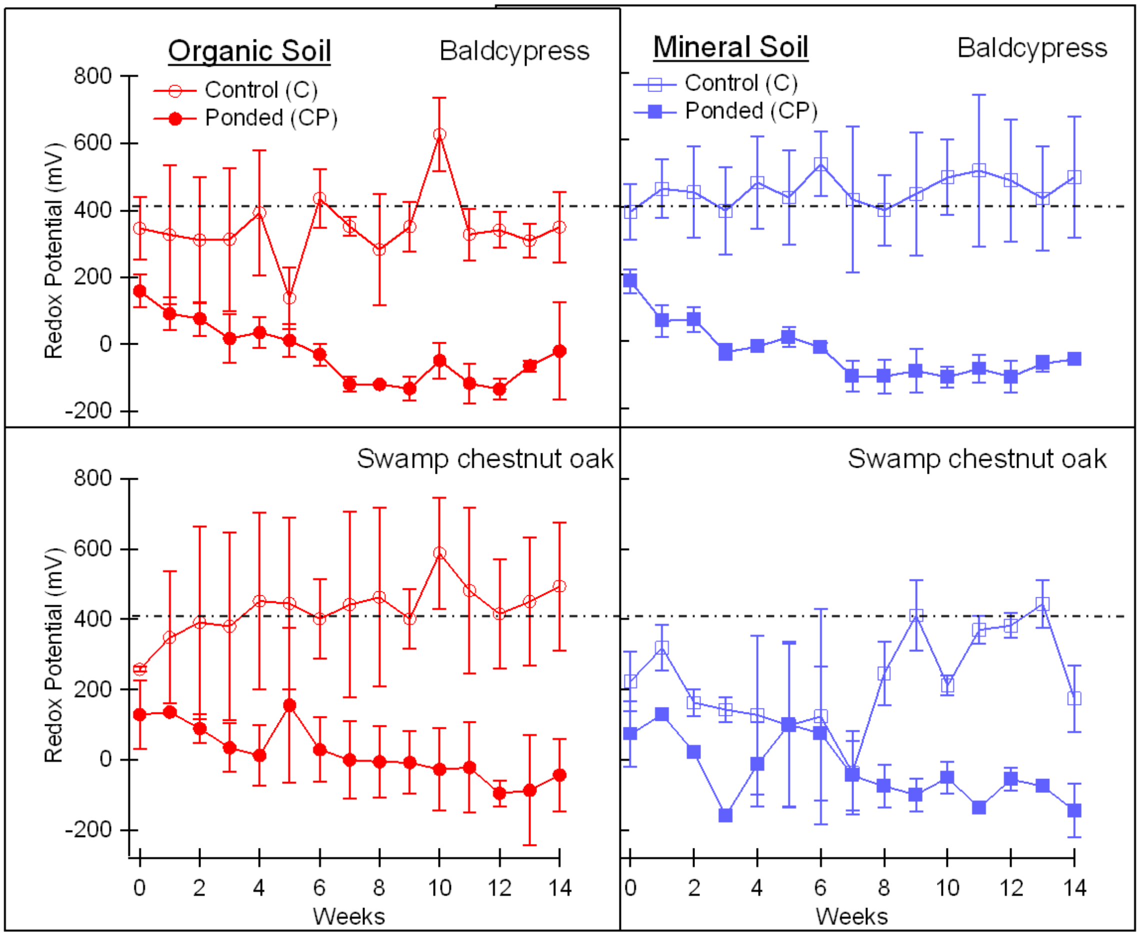

Figure 1. Redox potential (mV) conditions for two wetland tree species, bald cypress and swamp chestnut oak, measured 20 cm below the soil surface. Redox potentials for organic soils are shown on the left and mineral soils are shown on the right. Dashed line indicates the boundary between aerobic (>400 mV) and anaerobic (<400 mV) conditions. Standard errors are shown.

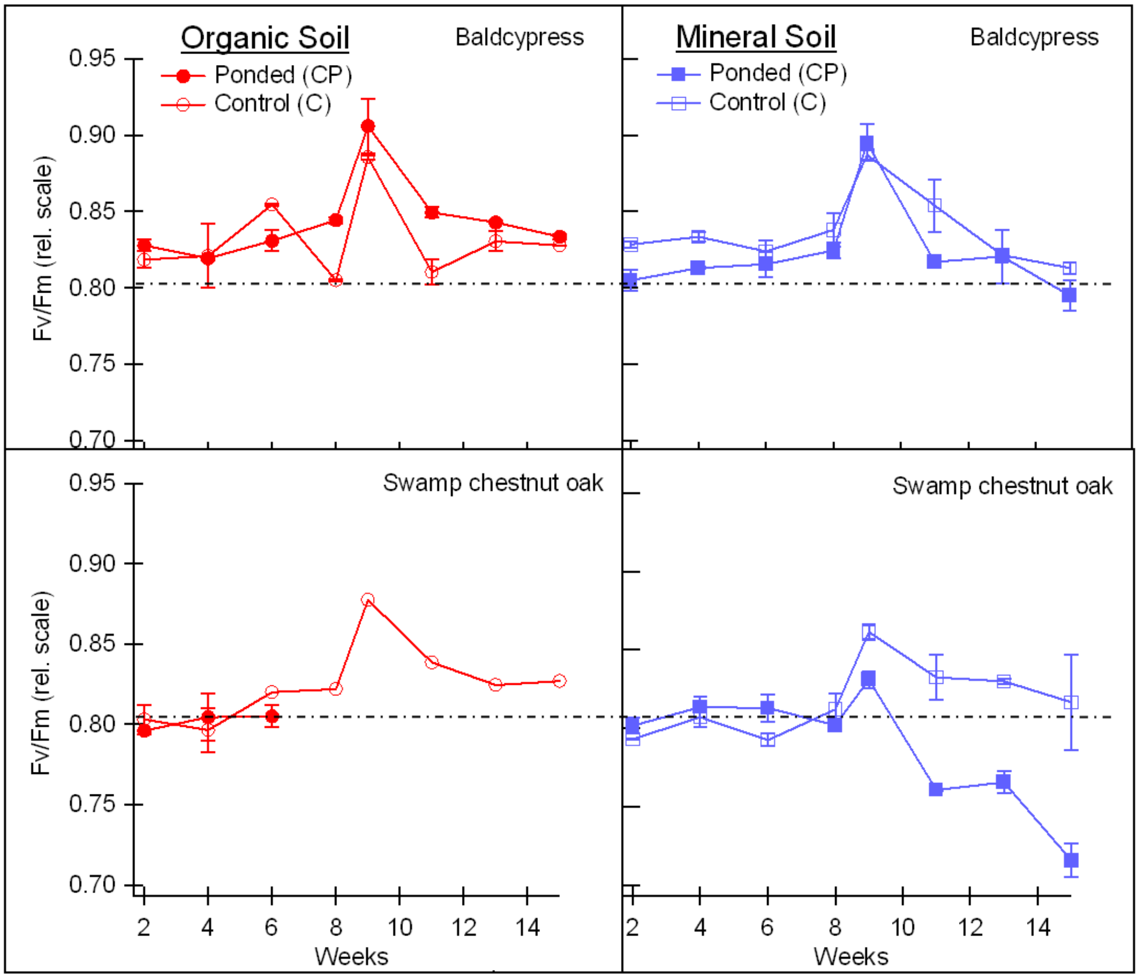

Figure 2. Daily mean fluorescence measured at the top of bald cypress and swamp chestnut oak seedlings. Fluorescence for organic soils are shown on the left and mineral soils are shown on the right. Standard error bars are shown.

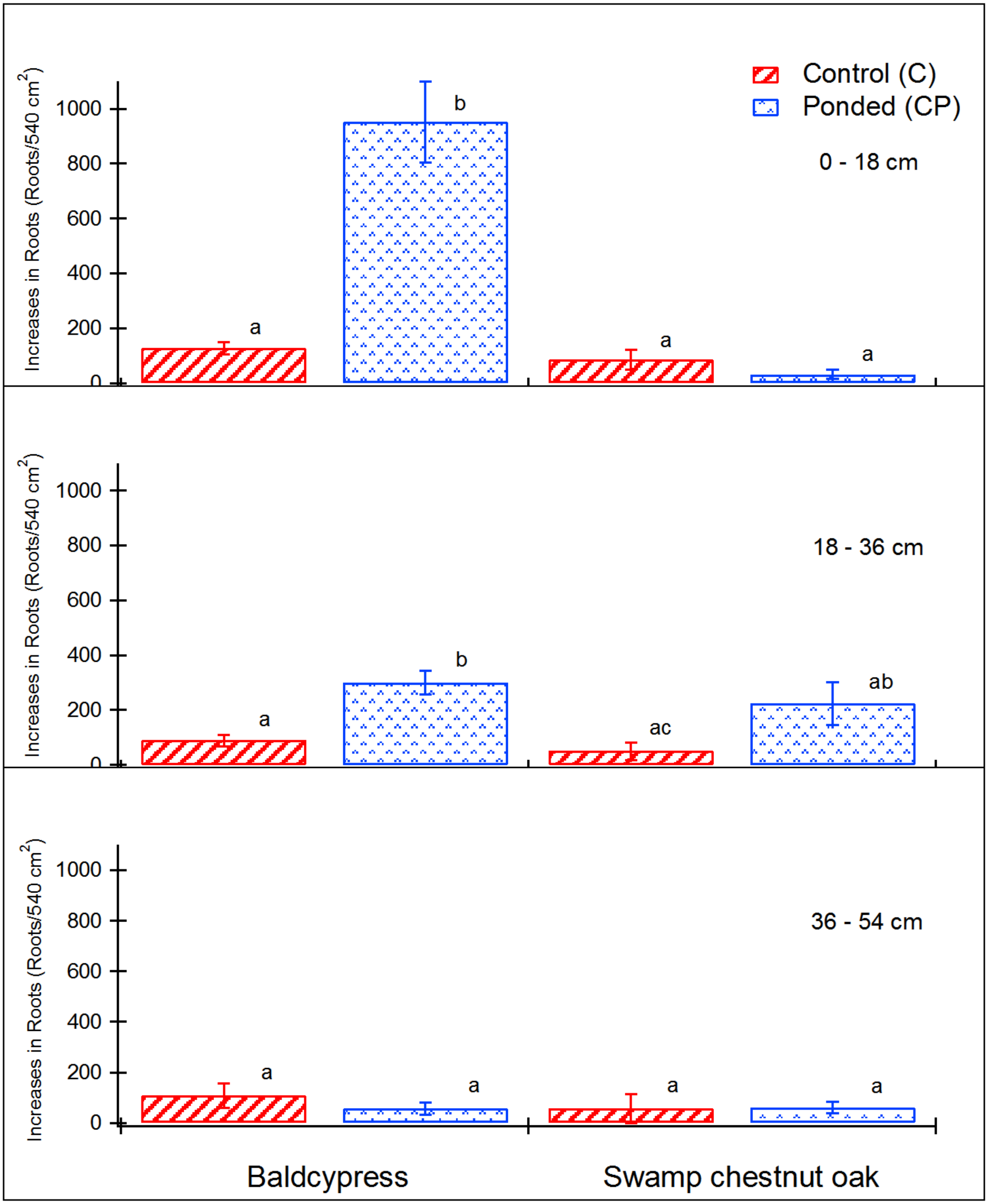

Figure 3. Average increases in the number of roots counted for two wetland tree species, bald cypress and swamp chestnut oak, at three root depths (0-18, 18-36, 36-54 cm). Measured as the increase in the number of roots per 540 cm^2. Standard error bars are shown.

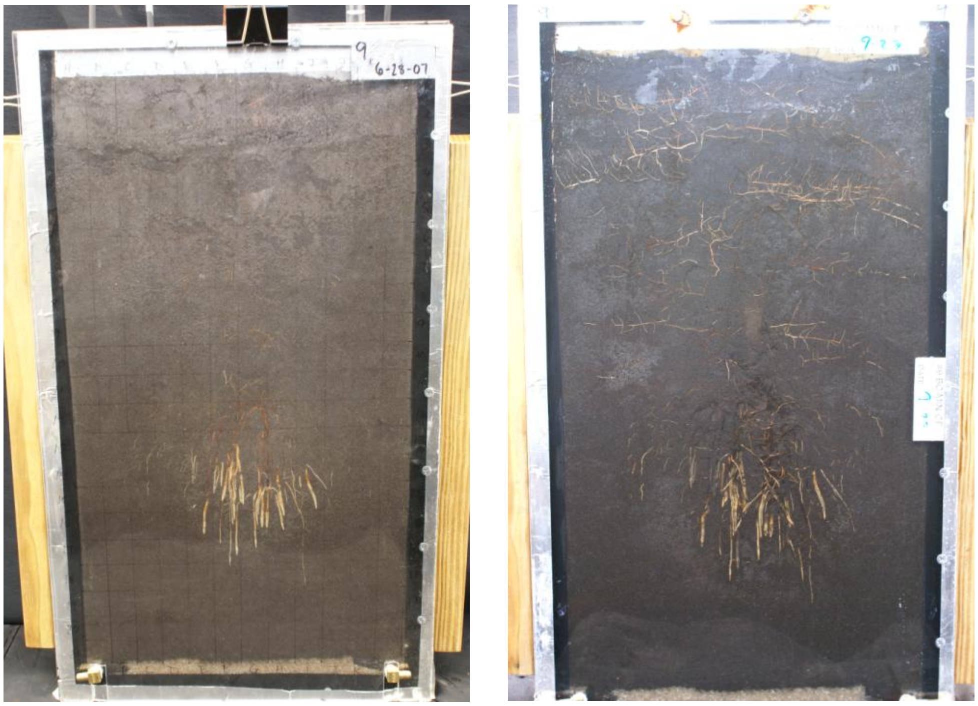

Figure 4. Root growth of baldcypress seedlings before (left) and after (right) continuously ponded conditions for 106 days. Root proliferation near the surface of the soil was observed for continuously ponded bald cypress seedlings in both organic and mineral soils.

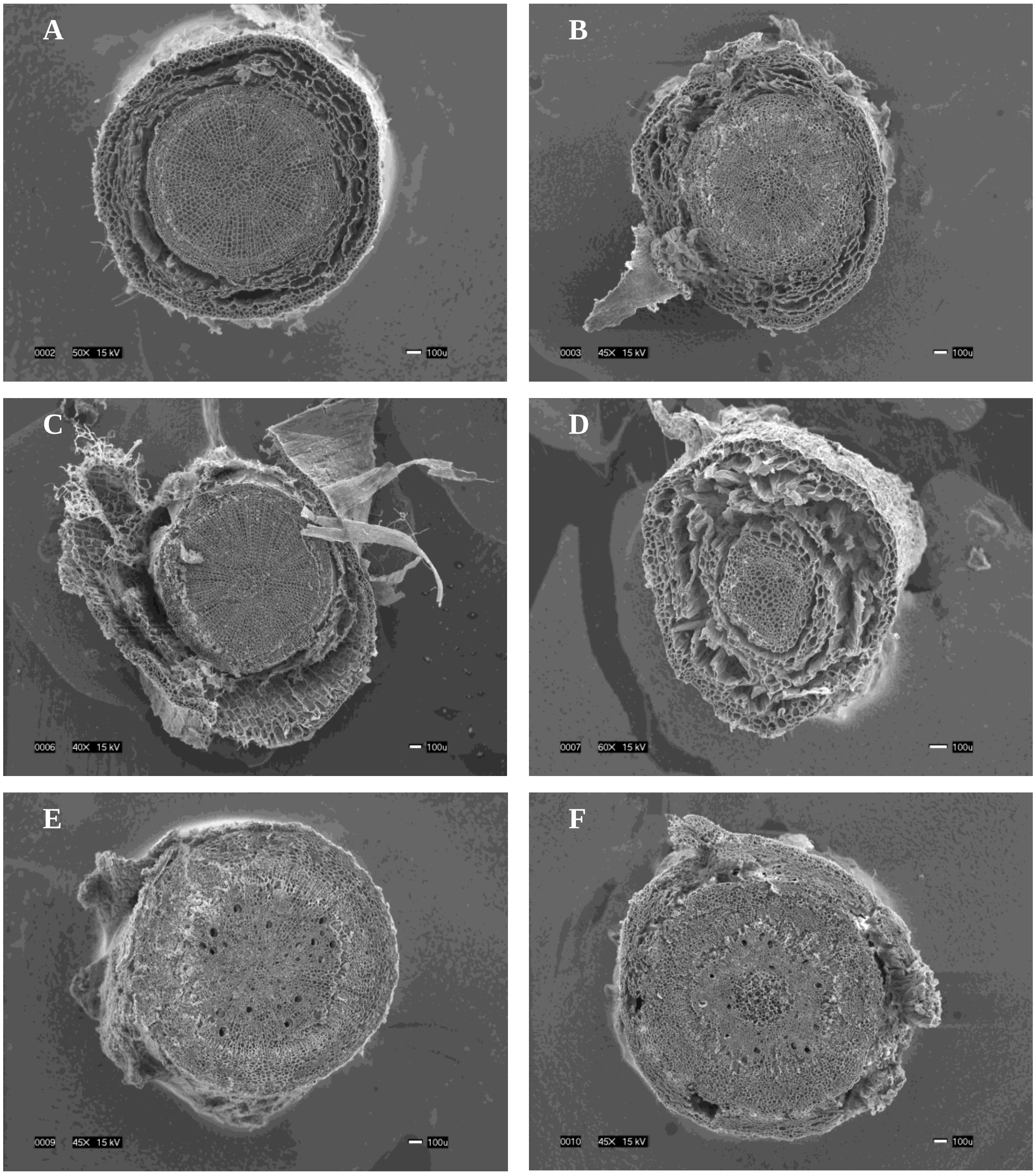

Figure 5. SEM micrographs of cross-sections of roots from two wetland tree species. Figs A-B of bald cypress roots from continuously ponded, mineral soils at a depth < 18cm (Fig. A) and > 36cm (Fig. B). Figs C-D of baldcypress roots from continuously ponded, organic soils at a depth of < 18cm (Fig. C) and > 36cm (Fig. D). Figs. E-F of swamp chestnut oak roots from continuously ponded, mineral soils (Fig. E) and continuously ponded, organic soils (Fig. F) at a depth > 36cm.

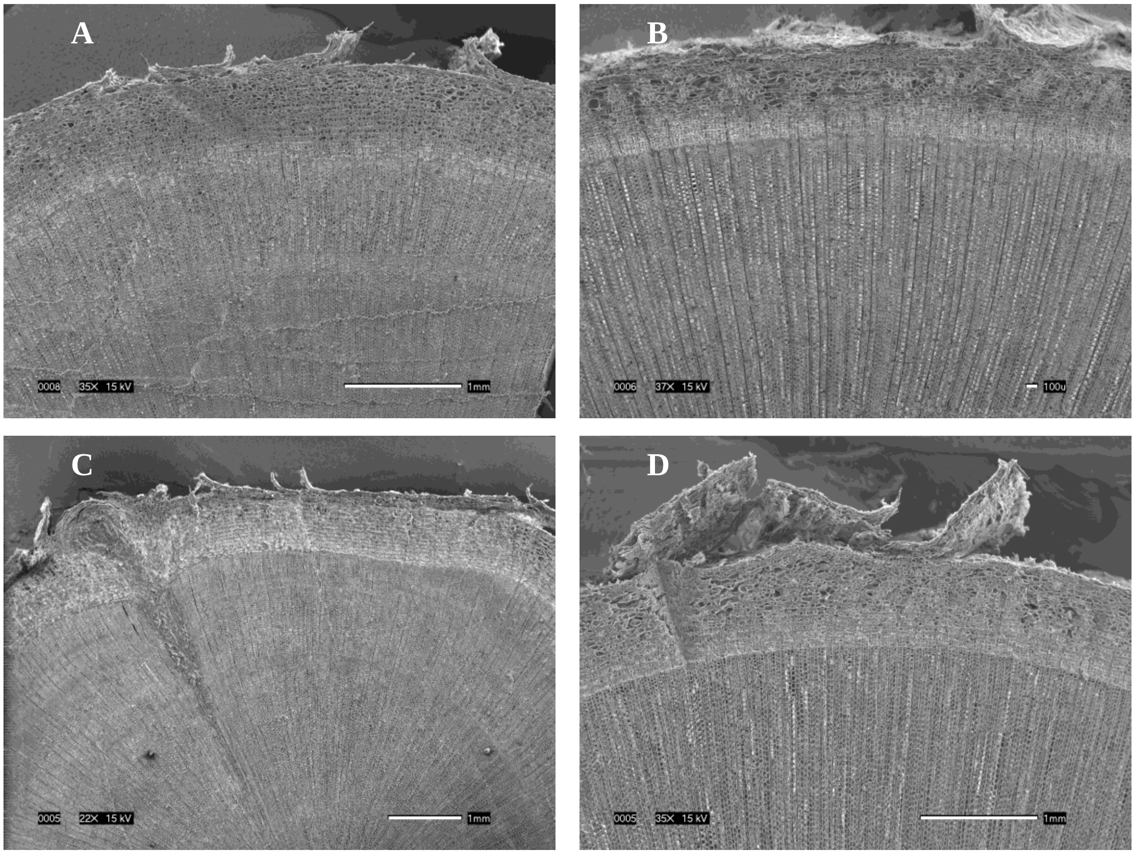

Figure 6. SEM micrographs of cross-sections of stems from baldcypress seedlings. Fig. A is a stem cross-section from a seedling grown in organic soil with no ponding. Fig. B is a stem cross-section from an organic soil and ponded conditions. Fig. C is a stem cross-section from a seedling grown in a mineral soil with no ponding. Fig. D is a stem cross-section from a seedling grown in mineral soil with ponded conditions.

Chapter 4

MORPHOLOGICAL RESPONSES OF FOUR WETLAND TREE SPECIES TO SATURATED AND ANAEROBIC SOILS

Carlin E. Conk, Michael J. Vepraskas, and Stephen W. Broome

INTRODUCTION

When soils become saturated, the water displaces air from soil pores. Through the activity of microorganisms and plants, all remaining oxygen in the soil system is depleted and the soil becomes anaerobic. Anaerobic conditions affect flood-tolerant and flood-intolerant species differently. In flood-intolerant plant species, prolonged anaerobic conditions reduce growth and increase mortality due to changes in metabolic functions (Kozlowski 1984). Plants, which are tolerant of saturated conditions are able to switch from aerobic to anaerobic metabolic pathways, although the anaerobic pathways are less efficient (Pezeshki 1994).

Morphological adaptations are another type of mechanism that are utilized by flood-tolerant woody species to adapt to anaerobic soils. These include the emergence of hypertrophied lenticels, adventitious root production, the formation of aerenchyma tissue, and stem hypertrophy (Hook 1984). Lenticels are openings located on stems and roots that act as the major pathway through which gases, especially oxygen, travel to the living cells of the cambium. Oxygen enters herbaceous species through the leaf but it has been found that lenticels at the base of the stem are considered more important for oxygen entering woody plants (Coutts and Armstrong 1976). Lenticels are connected through intercellular spaces in the cortex and phloem (Hook 1984). Hypertrophied lenticels are enlarged lenticels that provide a greater surface area for gas exchange, especially the uptake of dissolved oxygen in flood waters, and enhance internal aeration. Gill (1970) used a vacuum on the submerged portion of a stem to show that air in the stem could be continuously pulled out of submersed lenticels if lenticels were present above the submerged portion to allow air into the stem. Hypertrophied lenticels also provide an exit path for potentially toxic compounds (such as ethanol, acetaldehyde, and ethylene) in some species (Chirkova and Gutman 1972). Kozlowski (1982) found that the enlargement of lenticels was due to ethylene formation in response to flooding. The time needed for the formation of hypertrophied lenticels in the submerged portion of the stem depends on the flood-sensitivity of the species being inundated with water.

Although part of the original root system of trees may die during submergence, flood-tolerant species adapt by generating new roots, either on the original root system or on the submerged portions of the stem, or both. Adventitious roots grow during saturation and are thought to increase the absorption of water and minerals (Vartapetian and Jackson 1997) but may also oxidize the rhizosphere and detoxify harmful compounds (Hook et al. 1970, Hook and Brown 1973). Megonigal and Day (1992) determined that adventitious roots of bald cypress seedlings became the primary root system after years of saturation. Adventitious roots are often more succulent and permeable than roots of the original root system (Hook et al. 1970). Furthermore, they also tend to be larger in diameter and less branched than original roots (Megonigal and Day 1992). Adventitious roots have been found to initiate from either beneath or within the cork cambium (phellogen) and then erupt through the center of the lenticel, in the cork cambium within a lenticel, or originate deep within the vascular cambium independent of lenticels (Hook et al. 1970). However, it is thought that adventitious roots typically form in conjunction with hypertrophied lenticels.

Some flood-tolerant woody species will increase the proportion of parenchymatous (i.e. air-filled) tissue in the xylem and phloem of their stems in response to prolonged saturated conditions (Kozlowski 1997). Aerenchyma tissues are a specialized form of parenchyma tissue. Aerenchyma contain enlarged gas-filled cavities, or lacunae, that form through the differentiation and separation of cells (schizogenous) or through the death of some cells (lysigenous), leading to a less ordered cell pattern (Kawase 1981). Aerenchyma provide the plant with an alternative mechanism for obtaining oxygen. The interconnected, enlarged pore spaces provide the plant an extensive, low-resistance internal aeration system that can transfer oxygen via diffusion and convection to anaerobic rhizospheres (Evans 2003). Toxic gases such as carbon dioxide, ethylene, and methane also move through aerenchyma to the shoots, and are then released to the atmosphere (Colmer 2003). In flood-tolerant plants that produce these specialized intercellular spaces for greater aeration, stem diameters are sometimes increased in response to aerenchyma formation. The term for the increase in diameter is stem hypertrophy or stem buttressing. Stem hypertrophy has been found to occur in bald cypress, water tupelo, and black willow (McKevlin et al. 1998), all very flood-tolerant tree species.

Although much is known about the types of morphological adaptations that occur due to saturated conditions, very little is known about the specific hydrology and soil conditions that cause woody species produce these adaptations. With present knowledge, field observation of morphological adaptations is not used for estimating the duration and frequency of inundations of the soil (Environmental Laboratory, 1987). A better understanding of the hydrologic and soil combinations required for morphological adaptations to occur could possibly be developed into a useful tool for field assessment of the hydrologic regime in several important wetland tree species. For example, if hypertrophied lenticels were found to develop within 2 weeks of ponding, while adventitious roots developed after 10 weeks, it would be possible to estimate relative durations of ponding in a wetland by looking for these adaptations on a particular species of tree.

The objectives of this study were to experimentally determine the effects of periodic and prolonged ponding on: 1) hypertrophied lenticel development, 2) adventitious root growth, 3) aerenchyma tissue formation, and 4) stem hypertrophy in four wetland tree species. The four wetland tree species used in this study are listed from the most flood-tolerant to the least flood-tolerant (Hook 1984): bald cypress (*Taxodium distichum* L. Rich), sweet bay (*Magnolia virginiana* L.), pond pine (*Pinus serotina* Michx.), and swamp chestnut oak (*Quercus michauxii* Nutt.).

MATERIALS AND METHODS

This study was conducted in a greenhouse using organic and mineral soils collected from Juniper Bay, a 256 ha Carolina bay, located in Robeson County, near Lumberton, NC (34°30’30”N 79°01’30”E). Juniper Bay was drained in 1972 and used for agricultural production until 2006 when it was restored to a wetland. During the years in crop production, the soils were fertilized and limed according to soil test recommendations for the production of soybeans (*Glycine max* L. Merr.), oats (*Avena sativa* L.), cotton (*Gossypium hisutum* L.), corn (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.), wheat (*Triticum aestivum* L.), and other crops (Ewing 2003). Soil sampling sites were selected based on ease of access and soil type. Mineral soil was collected from the Ap and E horizons of a Leon sand (sandy, siliceous, thermic Aeric Haplaquod). Organic soil material was collected from the Oa horizon of a Ponzer muck (loamy, mixed, dysic, thermic Terric Haplosaprist). Small pits were dug by hand in each soil area to a depth of 25 cm. Undisturbed cores were collected in triplicate from the 15 to 25 cm depth using metal cylinders (10 cm in diameter by 10 cm in height) for bulk density determination. Soil was collected from the upper 25 cm, placed in buckets, transported to the greenhouse, air-dried for 3 days, and then passed through a 1.25-cm mesh sieve. Particle size distribution was determined by the hydrometer method after passing the material through a 2-mm mesh sieve (Gee and Bauder 1986). In the laboratory, soil cores were weighed, oven-dried at 105°C for 24 hours and weighed again. Before potting, sub-samples of organic and mineral soils were taken to determine gravimetric water content, which was used to estimate the amount of soil needed to achieve the bulk densities measured in the field.

Pond pine, bald cypress, and swamp chestnut oak seedlings were purchased from North Carolina Division of Forest Resources, Goldsboro, North Carolina. Sweet bay seedlings were purchased from SuperTree Nursery, Blenheim, South Carolina. Prior to planting, the seedlings were kept in a cold room at 7°C with adequate moisture. Roots were rinsed with tap water before planting to remove any attached soil.

Thirty seedlings from each species were chosen for the study based on similar heights and overall appearance of health. Fifteen seedlings were potted in each soil type using 7.65-liter tree containers (Stuewe & Sons, Inc., Corvallis, OR, model TPOT3). Drain holes in the pots were covered with fiberglass mesh to minimize the soil loss from the bottom of the pots. The pots were filled with an appropriate amount of soil and the soil was packed to the desired field bulk density. The final bulk density for containers filled with mineral soil was 1.28 g/cm^3 and 0.47 g/cm^3 for organic soils, which were similar to field bulk densities. Tree seedlings were planted in containers and acclimated to greenhouse conditions for 79 days under unsaturated conditions. Twenty-four of the original 30 potted seedlings from each species were then selected for the study based on similar heights and overall appearance of health.

All containers were equipped with a tensiometer to determine the soil water pressure head for watering purposes in the control and intermittently ponded hydroperiod treatments. Tensiometers were constructed similar to the methods used by Cassel and Klute (1986). The tensiometers were inserted to a depth of 7.5 cm below the soil surface in all plant containers before the treatments were begun. The septum stoppers were periodically replaced to maintain pressure inside the tensiometer. The tensiometers were read using a tensimeter (Soil Measurement Systems, Tucson, AZ).

Each container was equipped with two redox probes: one placed at 10 cm below the soil surface and another probe placed at 20 cm below the soil surface. The redox probes were constructed and tested according to the techniques utilized by Wafer et al. (2004). A calomel reference electrode was used and all subsequent redox potential measurements were adjusted as necessary. The redox probes were gently inserted by hand into the soil of the containers. A small amount of soil was mixed with water to create soil slurry and any gaps around the redox probe were filled with the slurry. The redox probes were approximately 40 cm to 75 cm in length and placed approximately 13 cm apart, on either side of the tree seedling, in each container.

Three hydroperiods were imposed to simulate average ponding durations of bald cypress, sweet bay, and pond pine as determined by Caldwell (2005). Hydroperiods selected were: control (C), intermittently ponded (IP), and continuously ponded (CP). Control treatments were watered to maintain a soil water pressure potential of -300 cm which was considered to be near field (or container) capacity. The intermittently ponded treatment was saturated from the base upward to maintain a water level above the surface for 14 days and then the soil was drained and kept unsaturated for 42 days. This saturation and drainage cycle was repeated 6 times, totaling 84 days of ponding and 282 days of unsaturated conditions. The continuously ponded treatment was initially inundated for 110 days and then 2 subsequent 95-day periods of saturation were utilized for a total of 300 days of ponded conditions and 65 total days of drained conditions over the experiment. When ponded, the water level was maintained 10 to 12 cm above the soil surface for the continuously ponded and intermittently ponded treatments. During the unponded cycles, soil moisture content was maintained close to field capacity, or near 300 cm of tension. Tap water was used for all treatments. Experiments conformed to a split-plot design, containing four replications per treatment combination.

Redox measurements were conducted on a weekly basis using an auto-range digital multimeter (Radio Shack, Fort Worth, TX, model 22-163) and a calomel reference electrode (Thermo Fisher Scientific, Waltham, MA, model 13-620-259). Soil pH was measured bi-monthly using a portable pH/mV meter (Thermo Fisher Scientific, Waltham, MA, model 13-636-62A) to correctly interpret the redox measurements. Plant observations were noted on a weekly basis. Diameters of adventitious roots were measured with a caliper. Destructive sampling of bald cypress and swamp chestnut oak seedlings occurred after 100 days of saturation. The root tissue was sampled between 0-18 cm and 36-54 cm and fixed in a 3% glutaraldehyde in a 0.05M sodium phosphate buffer at pH 7.2. The stem tissue samples were sampled in approximately 2 cm diameters and fixed in a 3% glutaraldehyde in a 0.05M sodium phosphate buffer at pH 7.2. These tissue samples were sent to Center for Electron Microscopy at North Carolina State University for SEM analysis.

RESULTS

Redox potential data are shown in Fig. 1. Redox potential, for the continuosly ponded treatment, at 10 and 20 cm below the soil surface was approximately -100 mV for the first saturated period and increased to approximately 0 mV for the final saturated period. The initial cycle of saturation for the intermittently ponded hydroperiod, averaged 125 mV, when measured 10 and 20 cm below the soil surface. After the first cycle of saturation, redox potential decreased for the next three subsequent cycles to -100 mV. However, the last cycle of saturation in the intermittently ponded treatment averaged 0 mV, when measured 10 and 20 cm below the soil surface. Anaerobic soils typically have redox potentials below +350 mV (Patrick and DeLaune 1977). Therefore, both mineral and organic soils were anaerobic during their saturated cycles.

During the unsaturated phases of the continuously ponded conditions, the soils were anaerobic. An Eh range of +400 to +700 mV is characteristic of aerobic soils (Pezeshki 1991). During unsaturated phases of the intermittently ponded conditions, the soils were aerobic with their redox potential averaging +425 mV. The control treatments, which were maintained close to field capacity, were aerobic during the entire experiment with an average redox potential of +450 mV.

The development of morphological adaptations observed over time for each tree species grown in continuously ponded treatment in the two soils is shown in Table 1. The formation of morphological adaptations was less prominent in seedlings subjected to intermittently ponded conditions. Hypertrophied lenticels were observed during the first saturated period for intermittently ponded sweet bay, pond pine, and swamp chestnut oak seedlings. Slight stem hypertrophy was observed for intermittently ponded bald cypress seedlings, especially intermittently ponded bald cypress seedlings grown in organic soils. Timing of the appearance of hypertrophied lenticels, adventitious roots, and stem hypertrophy differed between mineral and organic soils for continuously ponded and intermittently ponded bald cypress and sweet bay seedlings; morphological adaptations typically appeared first with seedlings subjected to ponded, organic soils.

Bald cypress seedlings subjected to continuously ponded conditions developed stem hypertrophy, adventitious roots below the soil surface, and aerenchyma tissue in the basal portion of the stem (Table 1). In continuously ponded organic soils, stem hypertrophy was greater than bald cypress seedlings in continuously ponded mineral soils (Fig. 2). The increase in stem diameter for intermittently ponded bald cypress seedlings was less pronounced when visually compared to continuously ponded bald cypress seedlings. The increase in stem diameter of flood-tolerant seedlings has been attributed to aerenchyma formation in the xylem and phloem of the stems (Kozlowski 1997). In this study, the increases in air spaces seemed to be limited to the cortex of the stem, near the bark cambium (Fig. 3). The outer surface of the bark on the lower portion of continuously ponded bald cypress seedlings split due to stem hypertrophy.

After 15 weeks of inundation, adventitious roots were visible near the soil surface on bald cypress seedlings growing in continuously ponded conditions and organic soils. Some of these adventitious roots were protruding from the soil surface and growing vertically against the container. These succulent roots were approximately 1 mm in diameter and contained very few branches. Adventitious roots were minimal for continuously ponded bald cypress seedlings grown in mineral soils, but the roots that were visible were approximately 0.5 mm in diameter (Fig. 2). Overall, root growth was visibly greater in bald cypress seedlings subjected to continuously ponded organic soils compared to continuously ponded mineral soils (Fig. 2). The survival rate for continuously ponded and intermittently ponded bald cypress seedlings was 100% in both mineral and organic soils.

Within 3 weeks of inundation, leaves from sweet bay seedlings began to senesce. The bottom-most leaves quickly turned yellow and fell from the stem. This occurred for intermittently ponded and continuously ponded hydroperiod treatments, which were both initially inundated on the first day of the experiment. However, seven out of eight sweet bay seedlings quickly recovered after producing hypertrophied lenticels within 2 weeks of saturation.

One sweet bay seedling, grown in organic soils and continuously ponded, did not recover and died within 13 weeks of saturation.

Within 5 weeks of inundation, continuously ponded sweet bay seedlings grown in organic soils began producing adventitious roots from the submerged, hypertrophied lenticels. It appeared that the adventitious roots erupted through the center of the hypertrophied lenticels (Fig. 4), as was proposed by Hook et al. (1970). Hypertrophied lenticels were also visible on the largest adventitious roots, as well. The adventitious roots extended directly from the stems and grew long enough to reach and penetrate the soil surface. The adventitious roots of continuously ponded sweet bay seedlings that extended from the topmost, submerged portion of the stem were thick, sparsely branched roots (Fig. 5). The adventitious root diameters ranged from 1 to 6 mm. The formation of adventitious roots in continuously ponded sweet bay seedlings, grown in organic soils, was followed by a significant amount of shoot growth from the main stem but also the emergence of new side stems. Due to the increased root growth from the stem, stem diameters were greater than the control and intermittently ponded hydroperiod treatments, for continuously ponded seedlings grown in organic soils.

Continuously ponded sweet bay seedlings grown in mineral soils experienced some hypertrophied lenticel development, but it was much less than found for continuously ponded seedlings grown in organic soils. Furthermore, formation of hypertrophied lenticels took longer to develop in continuously ponded sweet bay seedlings grown in mineral soils compared to continuously ponded organic soils (Fig. 4). Within 21 weeks of saturation, continuously ponded sweet bay seedlings grown in mineral soils began to produce adventitious roots from the center of lenticels. However, the adventitious roots formed in mineral soils were unlike those formed in organic soils. Adventitious roots of mineral soils were produced on the basal portion of the submerged portion of the stem and were approximately 1 to 3 mm in diameter (Fig. 5). The roots were still very succulent and contained few branches from the main adventitious root. Sweet bay seedlings grown in intermittently ponded hydroperiod treatments produced hypertrophied lenticels but did not produce any adventitious roots from the lenticels. At the conclusion of the study, hypertrophied lenticel development was still visible in intermittently ponded sweet bay seedlings of both soil treatments.

Pond pine seedlings, grown under continuously ponded conditions, produced hypertrophied lenticels within 9 weeks of inundation (Fig. 6). These hypertrophied lenticels were large (1 cm diameter), white, globular lenticels that became enlarged following saturation. However, during the first period of unsaturated conditions, these hypertrophied lenticels decreased in size. Hypertrophied lenticel development was minimal on continuously ponded pond pine seedlings after the first drained cycle. This may have played a part in the increased death of pond pine seedlings, which were subjected to continuously ponded conditions. After 29 weeks of saturation, six out of eight pond pine seedlings had died in both mineral and organic soils, although continuously ponded seedlings grown in organic soils died first. Hypertrophied lenticels appeared desiccated on the two surviving continuously ponded pond pine seedlings, at the conclusion of the experiment (Fig. 6). There were very few hypertrophied lenticels at the conclusion of the experiment on pond pine seedlings, which were subjected to intermittently ponded conditions.

Several, but not all, swamp chestnut oak seedlings that were under continuously ponded and intermittently ponded conditions produced hypertrophied lenticels on the submerged portion of the stem (Fig. 7). The development of hypertrophied lenticels of swamp chestnut oak seedlings was visibly less than that of the hypertrophied lenticels of sweet bay and pond pine seedlings. The size and shape of hypertrophied lenticels of swamp chestnut oak seedlings was more uniform, as well. During periods of unsaturated conditions, most of these lenticels desiccated and were not produced again. However, at the conclusion of the experiment, the one remaining continuously ponded swamp chestnut oak seedlings had significant hypertrophied lenticel development (Fig. 7). This morphological adaptation may have caused a greater ability to survive in ponded conditions, compared to the other continuously ponded swamp chestnut oak seedlings, which had died within 14 weeks of the experiment.

DISCUSSION & CONCLUSIONS

The objective of this study was to determine the timing for development of morphological adaptations in four wetland tree species subjected to differing durations of saturated and anaerobic conditions. Bald cypress was the most flood-tolerant of the four species studied, and produced multiple morphological adaptations. One of those adaptations was the development of air-filled cavities, or aerenchyma, which led to stem hypertrophy of continuously ponded bald cypress seedlings (Figs. 2 and 3). Similar results were found by Shanklin and Kozlowski (1985) who showed that after 8 weeks of ponding, bald cypress trees had an 18% increase in stem diameters due to swelling of the lower stems. Megonigal and Day (1992) found that bald cypress seedlings produced the greatest amount of aerenchyma in the stems and roots of continuously ponded seedlings when compared to seedlings kept in a control moisture treatment. Another important morphological feature in continuously ponded bald cypress seedlings was the production of adventitious roots, especially in continuously ponded bald cypress seedlings grown in organic soils. Megonigal and Day (1992) found that the roots of continuously ponded bald cypress seedlings were negatively geotropic, or grew upwards toward the soil surface, producing adventitious roots directly below the soil surface. Pezeshki and Anderson (1997) found that adventitious roots formed on the submerged portion of the stem within three weeks of saturation and also observed the formation of hypertrophied lenticels on submerged portions of bald cypress stems after four weeks of ponding. Hypertrophied lenticels were not observed in this experiment. Furthermore, it is thought that bald cypress seedlings are capable of oxidizing their root rhizosphere through the translocation of oxygen (Hook 1984), but this was not determined in this experiment. However, the formation of aerenchyma tissue in the stems of continuously ponded bald cypress seedlings suggests that the translocation of oxygen from the shoot to the root may have occurred.

Sweet bay seedlings were also tolerant to saturated and anaerobic conditions due to the development of an extensive network of hypertrophied lenticels and adventitious roots, especially in organic soils (Figs. 4 and 5). Hypertrophied lenticels were observed within five weeks of saturation in continuously ponded sweet bay seedlings grown in organic soils but in mineral soils, 21 weeks of saturation were required to produce hypertrophied lenticels (Table 1). Likewise, continuously ponded sweet bay seedlings grown in organic soils had visibly higher amount of shoot growth compared to the continuously ponded seedlings grown in mineral soils. This increase in shoot growth was likely related to the superior development of adventitious roots and hypertrophied lenticels, which would allow an increase in the uptake of nutrients and dissolved oxygen and purging of toxic compounds such as ethylene and ethanol. No studies of the impact of continuous ponding on sweet bay growth and development could be found in the literature.

Pond pine is considered to be moderately tolerant to saturated and ponded conditions (Hook 1984). It is thought that mature pond pine trees are able to tolerate prolonged soil saturation (Hook 1984). This study showed, however, that young seedlings may not be as tolerant as more established trees. Pond pine seedlings initially adapted to continuously ponded conditions by producing a significant amount of hypertrophied lenticels on the submerged portion of the stem (Fig. 6). Topa and McLeod (1986) also observed hypertrophied lenticel development on the stems and root collars of pond pine seedlings, which accounted for the majority of oxygen entry for the roots of seedlings subjected to continuously ponded conditions. However, once saturated conditions were suspended for four to six weeks in the current study, hypertrophied lenticels essentially dried up and returned in much smaller numbers when saturated again (Fig. 6). The inability to reproduce these lenticels may have had a negative impact on continuously ponded pond pine seedlings since six out of eight continuously ponded seedlings died.

Swamp chestnut oak seedlings are considered to be weakly tolerant to saturated conditions (Hook 1984), and in this experiment, it was the species least tolerant to saturated and ponded conditions. The only morphological adaptation observed in swamp chestnut oak was production of a few hypertrophied lenticels on the submerged portion of the stem. These hypertrophied lenticels were small and sparse when compared to the other two species that produced hypertrophied lenticels. The only swamp chestnut oak to survive saturated conditions for an extended amount of time developed an extensive network of hypertrophied lenticels (Fig. 7). Anderson and Pezeshki (2001) observed the development of hypertrophied lenticels in 83% of continuously ponded swamp chestnut oak seedlings after 10 weeks of ponding. Anderson and Pezeshki (2001) also found the formation of adventitious roots in 67% of continuously ponded swamp chestnut oak seedlings, which was not observed in this experiment. The appearance of sparse hypertrophied lenticels was the only morphological adaptation produced by this species in this 49-week study.

For bald cypress and sweet bay, there was a difference in the appearance of specific morphological adaptations between seedlings grown in mineral and organic soils (Table 1). Bald cypress seedlings grown in continuously ponded organic soils showed the formation of adventitious roots and stem hypertrophy within 17 weeks of the start of the experiment. However, the appearance of the same features took an additional 12 weeks for continuously ponded bald cypress seedlings grown in mineral soils. Likewise, adventitious roots and hypertrophied lenticels appeared within five weeks for sweet bay seedlings grown in continuously ponded organic soils but appeared later for seedlings grown in continuously ponded mineral soils. We first hypothesized that differences in redox potential between the two soils could have caused the differences in timing of the formation of morphological adaptations. However, there were no differences in redox potentials measured between organic and mineral soils (Fig. 1).

Plants that undergo stressful environmental conditions, such as soil anaerobiosis, increase the production of 1-aminocyclopropane-1-carboxylic acid (ACC), which is the precursor of ethylene, in plant tissues (Grichko and Glick 2001). Ethylene can also be produced by anaerobic or facultative anaerobic microorganisms (Goodlass and Smith 1978). Ethylene becomes trapped and continues to accumulate in plant tissues and in the soil, due to slow diffusion rates of the gas into water. Elevated levels of ethylene cause distinct responses in the tissues experiencing the stress directly. The production of adventitious roots has been associated with accumulations of ethylene, as well as, the formation of aerenchyma in the roots and shoots in flood-tolerant species (Jackson 1990). The accumulation of ethylene in flood-sensitive species may damage original roots and inhibit the growth of new roots. Smith and Restall (1971) determined the concentrations of several hydrocarbons in six soils under anaerobic conditions for 10 days. An organic soil used in their study, which contained 38% organic matter (OM), accumulated 24 µg ethylene/kg soil, which was 56 times the amount of ethylene accumulated by a sandy soil with 1.4% OM. They concluded that total evolution of ethylene was correlated with OM content of the soils - the higher the OM content, the higher the ethylene concentrations. Soils used in the current study are similar to the mineral and organic soils used in Smith and Restall (1971). Although ethylene levels were not measured in this study, higher amounts of ethylene may have been produced and trapped in the organic soils than the mineral soils. The flood-tolerant species, like bald cypress and sweet bay, responded to ponding by producing adventitious roots, hypertrophied lenticels, and aerenchyma tissue that may have allowed venting of the harmful gas away from plant tissues. The less flood-tolerant species, like pond pine and swamp chestnut oak, may have been slower in their response to the build-up of ethylene; therefore, ponded pond pine and swamp chestnut oak grown in organic soils died before seedlings grown in continuously ponded mineral soils (Table 1).

Results from this study show that the more morphological and anatomical adaptations that are utilized by a species, the greater the likelihood that the species will survive extended periods of ponding. Continuously ponded bald cypress seedlings adapted the best through a variety of morphological adaptations and ponded sweet bay seedlings produced an extensive network of adventitious roots. The only adaptation of pond pine and swamp chestnut oak seedlings were hypertrophied lenticels on the submerged portion of the stem, which was insufficient and led to a 75% and 87% death rate, respectively. This short-term greenhouse experiment indicated that the timing of the appearance of adaptations to flooding varies among species, as well as between organic and mineral soils. As a result, the use of morphological adaptations as indicators of ponding duration will be difficult and require additional research.

ACKNOWLEDGEMENTS

The authors would like to thank C.P. Niewoehner for assistance with the greenhouse and laboratory work, Dr. W.P. Hoffmann for his plant expertise, and Dr. C. Arrelano for statistical advice. The help provided by all these individuals, and the NCDOT, is greatly appreciated.

REFERENCES

Anderson, P.H., and S.R. Pezeshki. 2001. Effects of pre-conditioning on responses of three bottomland tree species to soil waterlogging. Journal of Plant Physiology 158:227-233.

Cassel, D.K., and A. Klute. 1986. Water potential: Tensiometry. p. 563-596. *In* A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. American Society of Agronomy and Soil Science Society of America, Madison, WI, USA.

Chirkova, T.V., and T.S. Gutman. 1972. Physiological role of branch lenticels in willow and poplar under conditions of anaerobiosis. Russian Journal of Plant Physiology 19:289-295.

Colmer, T.D. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant, Cell, and Environment 26:17-36.

Coutts, M.P. and P.W. Armstrong. 1976. Role of oxygen transport in the tolerance of trees to waterlogging. p. 361-365. *In* M.G.R. Cannell and F.T. Last (eds.) Tree Physiology and Yield Improvement. Academic Press, New York, NY, USA.

Environmental Laboratory. 1987. Corps of Engineers Wetlands Delineation Manual. Technical Report Y-87-1, U.S. Army Engineers Waterways Experiment Station, Vicksburg, MS, USA.

Evans, D.E. 2003. Aerenchyma formation. New Phytologist 161:35-49.

Gee, G.W. and J.W. Bauder. 1986. Particle-size analysis. p. 383-411. *In* A. Klute (ed.). Methods of soil analysis. Part 1. 2nd ed. American Society of Agronomy and Soil Science Society of America, Madison, WI, USA.

Gill, C.J. 1970. The flooding tolerance of woody species-a review. Forestry Abstracts 31:671-688.

Goodlass, G., and K. Smith. 1978. Effects of organic amendments on evolution of ethylene and other hydrocarbons from soil. Soil Biology & Biochemistry 10:201-205.

Grichko, V.P., and B.R. Glick. 2001. Ethylene and flooding stress in plants. Plant Physiology and Biochemistry 39:1-9.

Hook, D.D., C.L. Brown, and P.P. Kormanik. 1970. Lenticels and water root development of swamp tupelo under various flooding conditions. Botanical Gazette 131:217-224.

Hook, D.D., and C.L. Brown. 1973. Root adaptations and relative flood tolerance of five hardwood species. Forest Science 19(3):225-229.

Hook, D.D. 1984. Waterlogging tolerance of lowland tree species of the south. Southern Journal of Applied Forestry 8:136-149.

Jackson, M.B. 1990. Hormones and developmental change in plants subjected to submergence of soil waterlogging. Aquatic Botany 38:49-72.

Kawase, M. 1981. Anatomical and morphological adaptations of plants to waterlogging. HortScience 16:8-12.

Kozlowski, T.T. 1982. Water supply and tree growth. II. Flooding. Forestry Abstracts 43:145-161.

Kozlowski, T.T. 1984. Extent, causes, and impacts of flooding. p. 1-5. *In* T.T. Kozlowski (ed.). Flooding and Plant Growth. Academic Press, New York, NY, USA.

Kozlowski, T.T. 1997. Responses of woody plants to flooding and salinity. Tree Physiology Monograph No. 1:1-29.

McKevlin, M.R., Hook, D.D., and A.A. Rozelle. 1998. Adaptations of Plants to Flooding and Soil Waterlogging. p. 173-203. *In*: M.G. Messina and W.H. Conner (eds.). Southern Forested Wetlands: Ecology and Management. Lewis, London, UK.

Megonigal, J.P., and F.P. Day. 1992. Effects of flooding on root and shoot production of bald cypress in large experimental enclosures. Ecology 73(4):1182-1193.

Patrick, W.H., and R.D. DeLaune. 1977. Chemical and biological redox systems affecting nutrient availability in coastal wetlands. Geoscience and Man 18:131-137.

Pezeshki, S.R. 1991. Root responses of flood-tolerant and flood-sensitive tree species to soil redox conditions. Trees 5:180-186.

Pezeshki, S.R. 1994. Plant responses to flooding. p. 289-321. *In*: R.E. Wilkinson (ed.). Plant-Environment Interactions. Marcel Dekker, Inc., New York, NY, USA.

Pezeshki, S.R., and P.H. Anderson. 1997. Responses of three bottomland species with different flood tolerance capabilities to various flooding regimes. Wetlands Ecology and Management 4:245-256.

SAS Institute. 2005. SAS Version 9.1. SAS Institue, Cary, NC, USA.

Shanklin, J., and T.T. Kozlowski. 1985. Effect of flooding of soil on growth and subsequent responses of *Taxodium distichum* seedlings to SO_2. Environmental Pollution 38:199-212.

Smith, K.A., and W.F. Restall. 1971. The occurrence of ethylene in anaerobic soil. Journal of Soil Science 22(4):430-442.

Topa, M.A., and K.W. McLeod. 1986. Aerenchyma and lenticels formation in pine seedlings: a possible avoidance mechanism to anaerobic growth conditions. Physiologia Plantarum 68(3):540-550.

Vartapetian, B.B., and M.B. Jackson. 1997. Plant adaptations to anaerobic stress. Annals of Botany 79:3-20.

Wafer, C.C., J.B. Richards, and D.L. Osmond. 2004. Construction of platinum-tipped redox probes for the determination of soil redox potential. Journal of Environmental Quality 32:565-575.

Table 1: Timeline for morphological and anatomical adaptations for continuously ponded seedlings.

<table>
<tr><th rowspan="2">Ponding Duration (weeks)</th><th colspan="2">Bald Cypress</th><th colspan="2">Sweetbay</th><th colspan="2">Pond Pine</th><th colspan="2">Swamp Chestnut Oak</th></tr>
<tr><th>Organic</th><th>Mineral</th><th>Organic</th><th>Mineral</th><th>Organic</th><th>Mineral</th><th>Organic</th><th>Mineral</th></tr>
<tr><td>3</td><td></td><td></td><td colspan="2">Leaf Senescence</td><td></td><td></td><td></td><td></td></tr>
<tr><td>5</td><td></td><td></td><td>Adv. Roots & Hypertrophied Lenticels</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>9</td><td></td><td></td><td></td><td>Hypertrophied Lenticels</td><td colspan="2">Hypertrophied Lenticels</td><td colspan="2">Hypertrophied Lenticels</td></tr>
<tr><td>15</td><td colspan="2">Aerenchyma in stems and roots</td><td></td><td></td><td></td><td></td><td>100% Death</td><td>50% Death</td></tr>
<tr><td>17</td><td>Adv. Roots & Stem Hypertrophy</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>21</td><td></td><td></td><td>Stem Hypertrophy</td><td>Adventitious Roots</td><td>100% Death</td><td></td><td></td><td></td></tr>
<tr><td>29</td><td></td><td>Adventitious Roots</td><td></td><td></td><td></td><td>50% Death</td><td></td><td></td></tr>
<tr><td>31</td><td></td><td>Stem Hypertrophy</td><td></td><td>Stem Hypertrophy</td><td></td><td></td><td></td><td></td></tr>
</table>

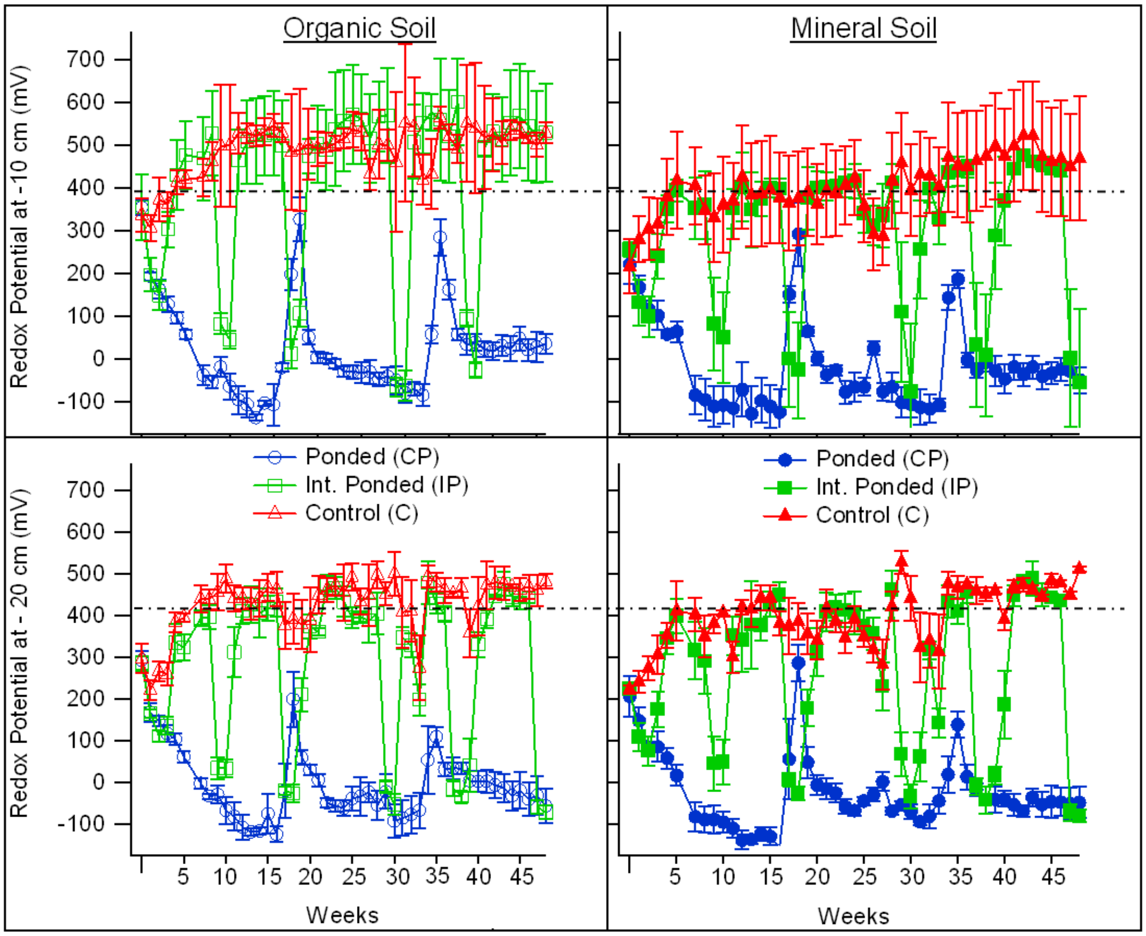

Figure 1. Redox potential (Eh) for organic (left) and mineral (right) soils for three hydroperiod treatments. Black line indicates the boundary between aerobic (>300 mV) and anaerobic (<300 mV) conditions. Standard error bars are shown.

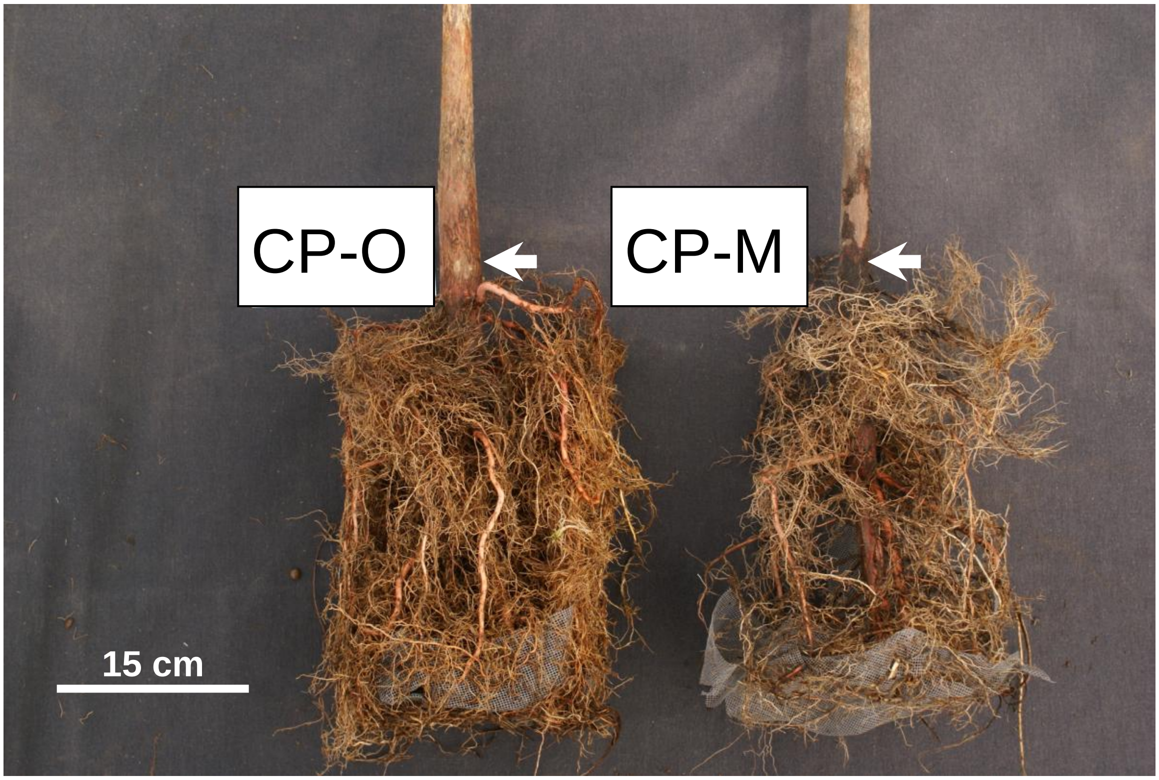

Figure 2: Differences in stem hypertrophy (arrows) and root growth between bald cypress grown in continuously ponded organic (CP-O) and continuously ponded mineral soils (CP-M). Stems of bald cypress seedlings subjected to continuously ponded conditions and grown in organic soils had the greatest growth in stem diameter. Surficial and overall root growth was greatest for bald cypress seedlings in continuously ponded organic soils.

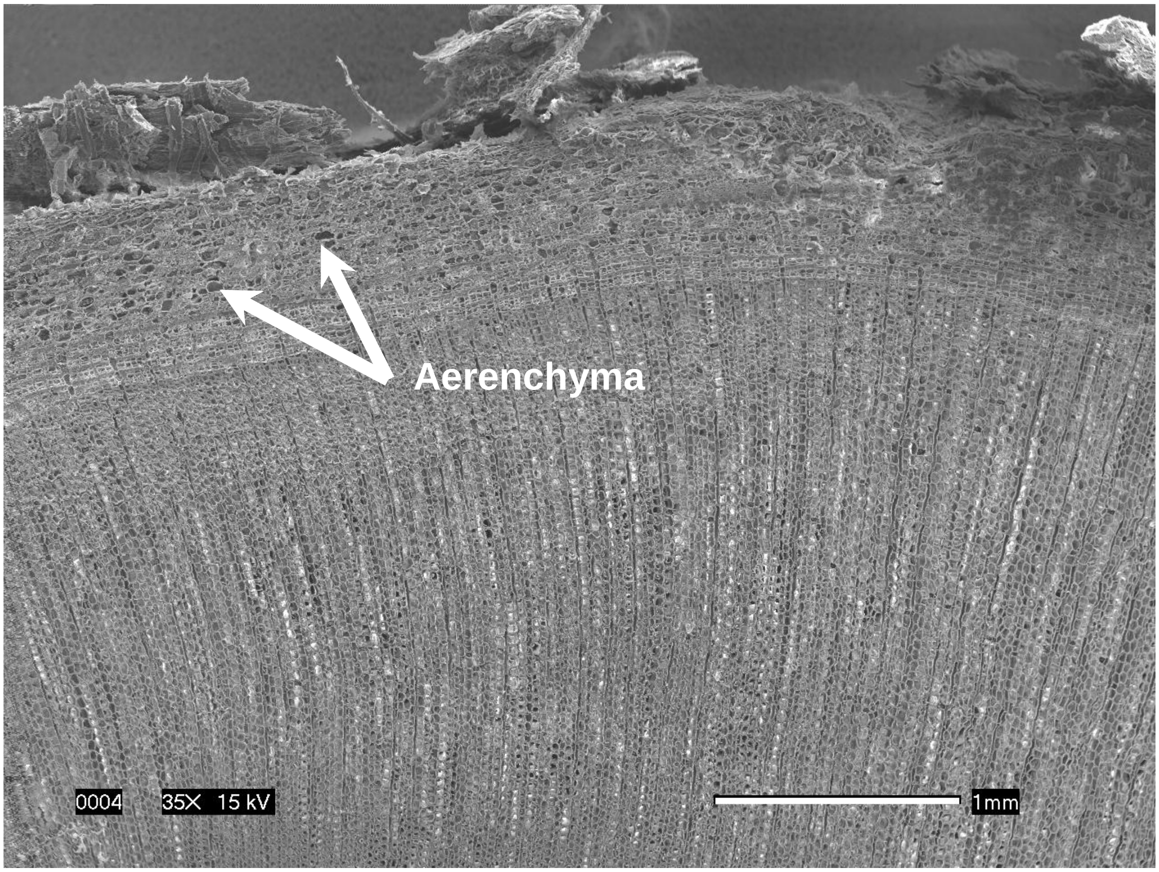

Figure 3: Scanning electron micrograph (SEM) of aerenchyma development in the basal portion of a bald cypress stem after 15 weeks of flooding.

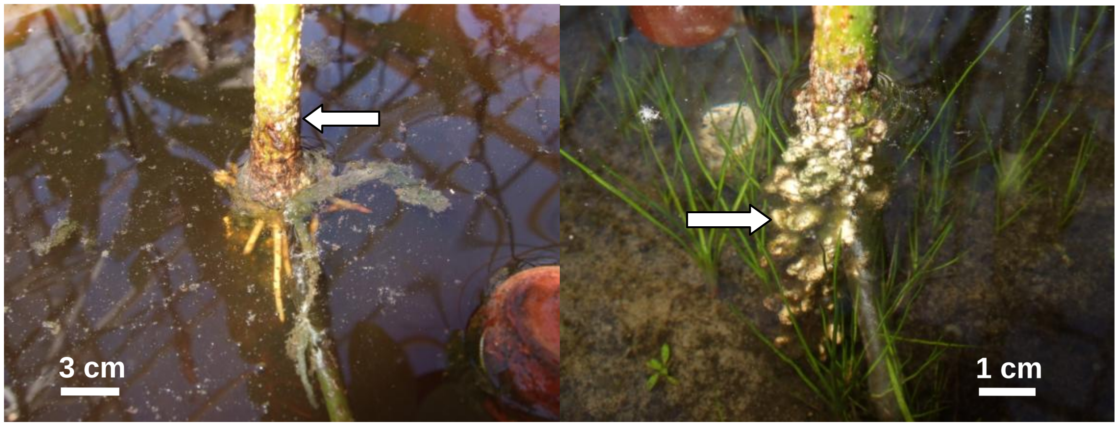

Figure 4: Initial formation of hypertrophied lenticels (arrow) and adventitious roots on sweet bay seedlings after four weeks of inundation in organic soils (left). Initial formation of hypertrophied lenticels (arrow) after nine weeks of inundation in mineral soils (right).

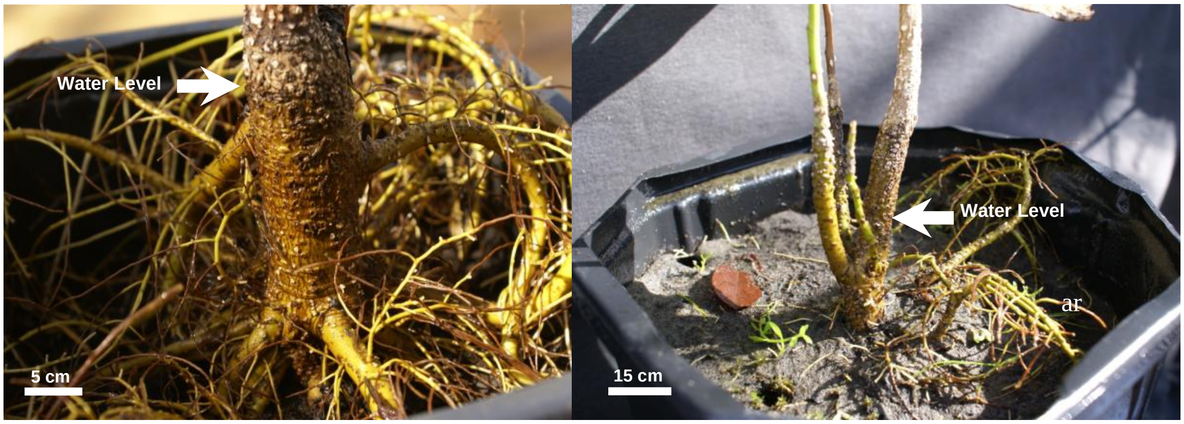

Figure 5: Adventitious roots (ar), hypertrophied lenticels, and stem hypertrophy of sweet bay seedlings grown in organic soils and saturated for 42 weeks (left). Stem diameter was greatest in sweet bay seedlings that were grown in organic soils and continuously ponded due to the prolific growth of adventitious roots. Adventitious roots of sweet bay seedlings grown in organic, continuously ponded soils occurred on the submerged portion of the stem. Adventitious roots and hypertrophied lenticel formation in sweet bays of mineral soils after 42 weeks of saturation (right). Adventitious roots occurred near the soil surface, at the basal portion of the stem.

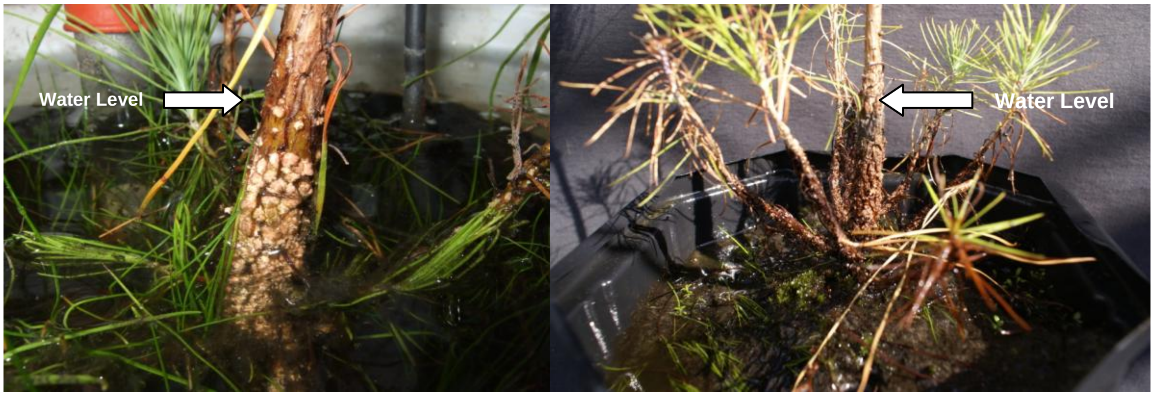

Figure 6: Hypertrophied lenticel development in pond pine seedlings in continuously ponded conditions after nine weeks of saturation (left). Dessicated hypertrophied lenticels on pond pine seedlings subjected to continuously ponded mineral soils after three cycles of saturation and drainage (right). Half of the pond pine seedlings subjected to continuously ponded mineral soils died. All of the pond pine seedlings subjected to continuously ponded organic soils died within 20 weeks of saturation.

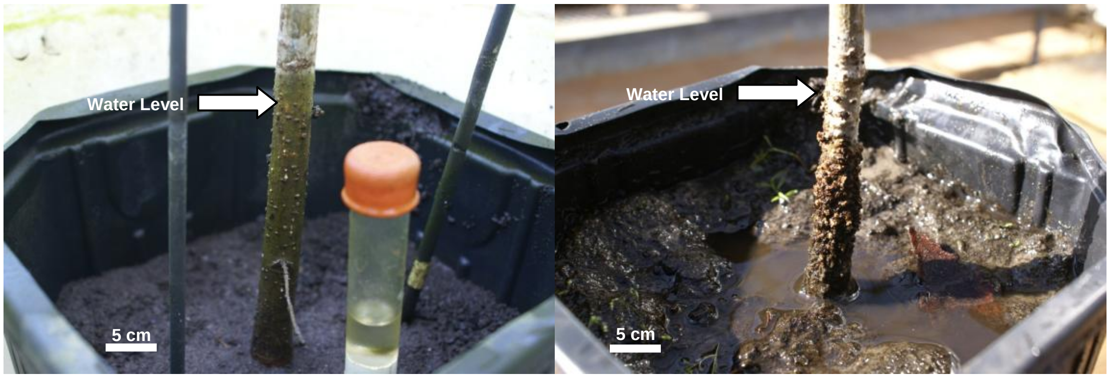

Figure 7: Hypertrophied lenticel development on swamp chestnut oak seedlings in continuously ponded organic soils after 15 weeks of saturation (left). Hypertrophied lenticel development after 42 weeks of saturation in the sole surviving swamp chestnut oak in continuously ponded mineral soils (right). Hypertrophied lenticels were small and sparse in swamp chestnut oak seedlings when compared to sweet bay and pond pine seedlings. Hypertrophied lenticels of swamp chestnut oak seedlings disappeared after the first draining cycle in most swamp chestnut oak seedlings.

Chapter 5

TREE-PLANTING METHODOLOGIES FOR WETLAND RESTORATION

M.J. Vepraskas, J.G. White, and S.W. Broome

INTRODUCTION

Restoration ecology manipulates altered lands to speed the recovery of pre-existing ecosystems to their natural state (Dobson et al., 1997). Wetland restoration, a subset of restoration ecology, modifies the soils, hydrology, and vegetative communities of disturbed wetland sites to approximate the conditions that prevailed prior to alteration. Soil modifications involve contouring the surface to slow runoff of water, while hydrologic modifications (e.g., ditch filling) raise the site's water table and increase periods of saturation. In most cases, a specific wetland plant community is selected as the target ecosystem, and plant species associated with that community are planted at the restoration site. Unfortunately, the hydrologic regime of restored wetlands frequently differs from that of natural wetlands, and the planted vegetation is not adapted to the hydrology of the site (Dennison and Schmid, 1997). This is sometimes a result of improper hydrologic design, but in many cases it is due to a lack of understanding of the hydrology required by wetland plant communities. To insure that at least some vegetation survives, wetland designers will frequently plant a variety of vegetation at a site with a wide range of hydrologic requirements in hopes that the hydrologic conditions of a restored wetland will be able to support some of the planted species (Morgan and Roberts, 2003). This practice, though effective in some cases, is inefficient and has no guarantee of success. Quantitative data describing the survival and growth of various tree species in restored wetlands would allow much more efficient and successful establishment of wetland vegetation at a restoration site.

The objectives of this study were: 1) to evaluate the survival and growth of four wetland tree species in Juniper Bay mineral soils, 2) to determine the impact of topsoil removal on tree growth and survival, 3) to determine if planting trees on ridges improved growth and survival over planting on smooth ground, and 4) to evaluate tree growth and survival in areas where a ditch had been filled.

MATERIALS AND METHODS

Field Site

The study was conducted in Juniper Bay, a 750-acre Carolina Bay near Lumberton, NC that was purchased by NCDOT for wetland mitigation credit. The site was a wetland that was drained in stages during the 1970s and used for crop production through 2003. Ditches were filled in 2005 to restore the original wetland hydrology. The restored bay consists of organic soils at its center, with mineral soil extending outward to a sandy perimeter rim. Experiments for this study were conducted in the mineral soils only, because the organic soils were too wet to impose our treatments when the study began.

The mineral soil series is of Leon loamy sand (sandy, siliceous, thermic, Aeric Alaquods). The main drainage ditches at the site were filled in 2005 by scraping topsoil from the soils adjacent to the ditch. The scraping process removed approximately 30 cm of topsoil from the soils next to the ditch, for a distance of at least 13 m (40 ft.) back from the ditch. This process created three basic mineral soil conditions at the site: a) soils with topsoil present (original condition), b) soils with topsoil removed, and c) soils in the filled ditch.

A 2 X 2 X4 factorial treatment structure (topsoil X ridge X tree species)) was implemented in a split-split-plot design in incompletely randomized complete blocks. The main-plot factor (Fig. 1) was topsoil (present or absent), which could not be randomized due to constraints inherent in the ditch filling that created the treatments. The split-plot factor was ridging (smooth vs. ridge), and the split-split-plot factor was tree species.

Eight experimental blocks were located around Juniper Bay as shown in Figure 2. Ridge and smooth treatments were installed in each of the main plots where topsoil was present or removed. The smooth treatment consisted of the natural ground surface without any modification. The ridges were created with a disk plow to be approximately 30 cm (12 inches) high, 60 cm (24 inches) wide, and 18 m (60 ft.) long. Eight ridged-rows were created in each of the ridged plots where topsoil was either present or removed.

For the filled-ditch study, a 2 X 4 factorial treatment structure (ridge X tree species) was installed in randomized complete blocks at three locations around the bay. One ridged row was placed in the ditch treatment, with the second row left smooth.

The four tree species planted were pond pine (*Pinus serotina* Michx.), bald cypress (*Taxodium distichum* (L.) Rich.), sweet bay (*Magnolia virginiana* L.), and Atlantic white cedar (*Chamaecyparis thyoides* L.) The first three species were selected because previous work by Caldwell (2005) in natural Carolina Bays indicated that those species are well adapted to conditions found in Carolina Bays. The fourth species (Atlantic White Cedar) is a valuable tree species native to some Carolina Bays. The place name "Juniper Bay" is derived from another common name for Atlantic white cedar (Laderman, 1989). In the topsoil-present and removed plots, trees were planted according to the plan shown in Figure 3. Twenty trees of each species were planted in each treatment in two rows of 10 trees each and spaced 1.5 m (5 ft.) apart. For the ditch treatment, all species were randomly planted along a single ridged and single smooth rows. Rows contained 60 trees, 15 of each species, which were randomized by species.

Planting was done on two dates, April 2006 and January 2007, due to difficulty in securing an adequate number of plants by the first planting date. In 2006, the topsoil-present and removed treatments were planted in Blocks 1 and 2. As shown in Figure 1, these blocks did not contain a ditch treatment. The remaining blocks, including the ditch study, were planted in 2007.

Following planting, rainfall was measured daily onsite. In August 2007, soil samples were collected from all plots to a depth of 0 to 15 cm (0 to 6 in.). These samples were sent to the NC Department of Agriculture and Consumer Services Agronomic Services Division Soil Testing laboratory for routine analysis. Tree survival and height were measured March and August, 2007, May 2008, and May 2009. Only data from 2009 will be reported.

RESULTS AND DISCUSSION

Monthly rainfall data for 2006 through 2009 are shown in Table 1. The monthly totals were classified as being dry, normal, or wet by using the USDA's WETS data tables (Sumner et al., 2009). The WETS tables show the 30^{th} and 70^{th} percentiles for monthly rainfall. Rainfall amounts that are considered "normal" fall within the 30^{th} and 70^{th} percentiles. Drier conditions have monthly rainfall that is less than the 30^{th} percentile, while wet conditions occur when the monthly values are greater than the 70^{th} percentiles. Total rainfall values for each year are also shown.

Both of years in which the trees were planted (2006 and 2007) were dry years. However, trees planted in April 2006 were planted during a month of normal rainfall, and the remaining months of the year that followed planting ranged from dry to wet, with five of those months being either normal or wet. In contrast, 2007 was a very dry year. The total amount of rain for the year was about half of the 2006 total. Trees were planted in January 2007, which was during a dry period, and nine of the following months were dry, one of the severest droughts in recent years.

Soil nutrient data are reported in Table 2. Removing topsoil resulted in lower soil P, K, Mg and Zn compared to where topsoil was present. Low amounts of soil P and K are likely to reduce plant growth . The soil material in the filled ditch had a higher pH, base saturation, Ca, and K levels than in the topsoil treatment. The reason for this appears to be the location of two of the three ditch treatments. The ditch treatments near plots 3, 4, 7, and 8 were along the road which ran parallel to the ditch that was filled. This road was heavily travelled because it led to the only entrance to the bay, and provided access to all other parts of the bay. A prior bay-wide grid soil sampling indicated areas of higher pH to the south of this road, which may have been due to lime having been stockpiled there, or the soil along this road may received lime and fertilizer falling from trucks/spreaders transporting it to the rest of the fields when the site was used for agriculture. Soil material from the road may have been used to fill the ditch, and this could account for the higher pH values seen in filled ditches.

The survival percentages of trees planted in the main treatments are shown in Table 3 for all four species. Survival percentages of trees in the topsoil treatments planted in 2006 were approximately twice those of trees planted in 2007, including those in the ditch study. These differences between years were attributed to the impact of the drought in

2007. The topsoil and ridging treatments had no effect on tree survival. Survival ($\leq$21%) of sweet bay and Atlantic white cedar were much less than bald cypress and pond pine. Nearly all Atlantic white cedar trees died. The data clearly show that sweet bay and Atlantic white cedar were not adapted to conditions at this site as were bald cypress and pond pine. For the remainder of the report, only data for bald cypress and pond pine planted in 2006 will be discussed.

The impacts of treatments on the height of trees surviving in May 2009 are shown in Table 4. For the trees planted outside ditches, there was a significant interaction between the topsoil and species treatments, meaning that the effect of topsoil removal varied between species, so simple effects of these treatments are shown in Table 5. Considering first the main effects, for trees planted in 2007, height was greater in plots containing topsoil, possibly because of the additional P and K in the topsoil; the same trend was seen for trees planted in 2006, but means were not statistically significantly different due to high variability relative to only two replicates. For trees planted in 2006, ridging had no effect on height. For trees planted in 2007, the height on ridges was about 15% greater than on the flat. For trees planted in 2006, height of pond pine was greater than bald cypress, while the reverse was true for trees planted in 2007.

As evident in the simple effects of topsoil and species shown in Table 5, for trees planted in 2006, the impact of topsoil removal was greater for pond pine, where removal decreased height by 66%, while bald cypress height decreased 25%. The same effects were apparent for trees planted in 2007, where topsoil removal decreased pond pine height by 43% and bald cypress height by 16%. Height differences due to topsoil removal may have been due to lower P and K relative to topsoil plots. Within the topsoil present treatment, the height of pond pine was always greater than that of bald cypress. When topsoil was removed, there were no significant differences between species.

In summary, planting on ridges had only a slight positive effect on height of trees planted in 2007, otherwise no effects were apparent. One reason for this may be that the ridges tended to collapse over time. One year after planting the ridges were approximately one half their original height) of the original ridge (15 vs. 30 cm, respectively), and this apparently was not sufficient to provide any benefit compared to the smooth ground.

CONCLUSIONS

The results of this study support the following conclusions:

1. Bald cypress and pond pine trees were well adapted to the fertility and hydrologic conditions found at this site. Sweet bay and Atlantic white cedar were not. Atlantic white cedar has specific soil and hydrologic requirements. It is usually found on organic soils that are saturated most of the growing season, conditions not met by the mineral soils used for this study.

2. Removing topsoil to fill ditches will likely have adverse effects on the growth of bald cypress and particularly pond pine. When topsoil is removed, the scalped lands probably

should be fertilized to restore some of the lost P and K. Doing so will have small benefits for bald cypress, but could have large benefits on the survival and growth of pond pine.

3. There was little benefit to planting trees on ridges as opposed to smooth ground.

4. Drought conditions likely prevented a full evaluation of tree survival and growth in the filled ditches, where treatments had no effect. However, for trees planted under more favorable moisture conditions, we expect that the results would be similar to those found in the plots containing topsoil, because the two areas were similar in quantities of major plant nutrients.

REFERENCES

Caldwell, P.V. 2005. Characterization of the hydrologic regime of four Carolina Bay plant communities. Ph.D. dissertation, N. C. State University, Raleigh.

Dennison, M. S., and J. A. Schmid. 1997. Wetland mitigation: Mitigation banking and other strategies for development and compliance. Government Institutes, Inc., Rockville, MD.

Dobson, A. P., A. D. Bradshaw, and A. J. M. Baker. 1997. Hopes for the future: restoration ecology and conservation biology. *Science* 277: 515-522.

Laderman, A.D. 1989. The ecology of Atlantic white cedar wetlands: a community profile. U.S. Fish and Wildlife Service Biological Report 85 (7.21). Washington, DC, 114 pp.

Morgan, K. L. and T. H. Roberts. 2003. Characterization of *wetland* mitigation projects in Tennessee, USA. Wetlands 23:65-69.

Sumner, J.P., M.J. Vepraskas, and R.J. Kolka. 2009. Methods to evaluate rainfall for short-term hydrology assessment. Wetlands 29(3):1049-1062.

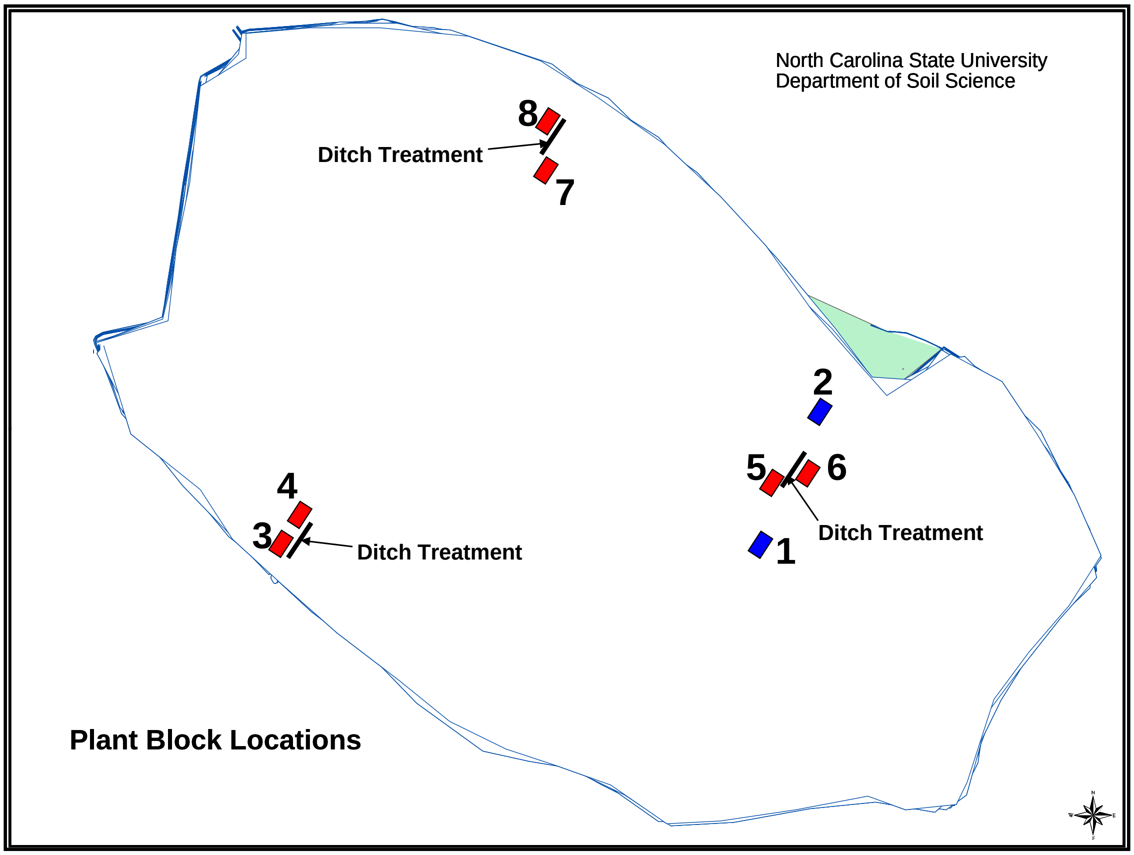

Figure 1. Plan view of experimental plots (numbered) in Juniper Bay. All blocks were placed adjacent to a ditch that had been filled with topsoil material scraped off the surface of the soil adjacent to the ditch. Three plots were in filled ditches (ditch treatments).

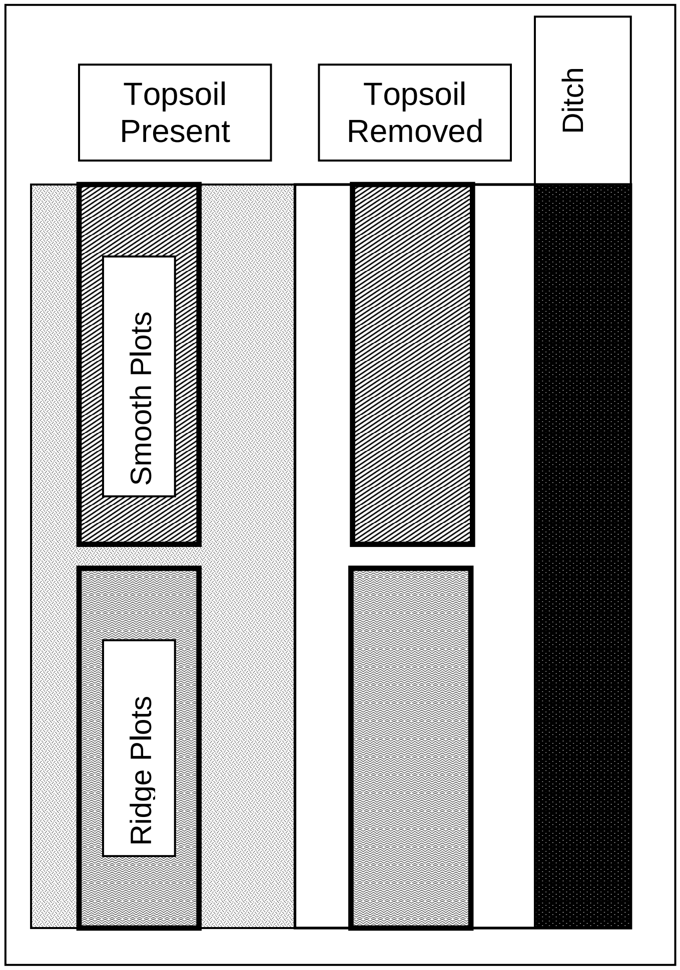

Figure 2. Plan view of experimental treatments that were placed in the main plots (numbered) in Figure 1. Plots with topsoil present represent the major soil condition at Juniper Bay. Plots with topsoil removed represent soil conditions along ditches that were filled by scraping topsoil off of soils adjacent to the ditch. The "ditch treatments" were placed in filled ditches. The topsoil present and topsoil removed plots were split in two, and two surface treatments were imposed: ridged and smooth. Smooth treatments were left in their natural condition, while ridged plots had raised beds formed for the plant rows. Ditch treatments also contained one ridged row and one smooth row.

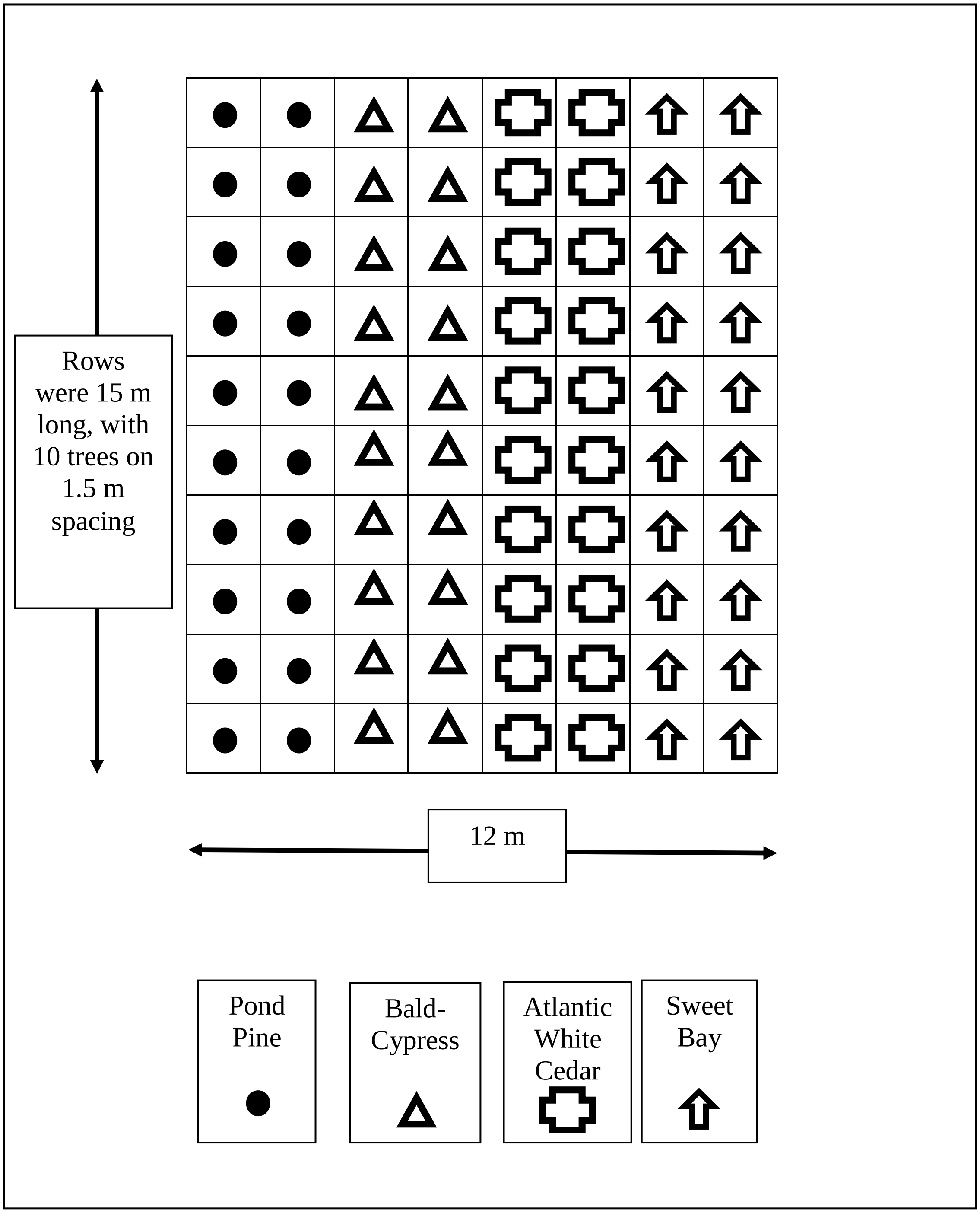

Figure 3. Planting plan used for the topsoil present and topsoil removed plots, in both the ridged and smooth subplots. For ditch treatments trees were planted in two rows with species randomized within the row.

Table 1. Rainfall data for the period from planting through 2009. Planting occurred in both 2006 and 2007. Rainfall condition was determined using the WETS data table (Sumner et al., 2009). Rainfall during 2006 was nearly twice as much as in 2007 which experienced a severe drought.

	Rainfall totals and WETS condition							
	-------------------------------------year-------------------------------------							
	2006		**2007**		**2008**		**2009**	
Month	cm	Condition	cm	Condition	cm	Condition	cm	Condition
Jan	6.9	Dry	1.6	Dry	7.1	Dry	3.2	Dry
Feb	7.0	Normal	4.4	Dry	10.5	Wet	4.2	Dry
Mar	1.4	Dry	3.6	Dry	9.3	Normal	8.4	Normal
Apr	4.2	Normal	6.3	Normal	9.1	Normal	2.7	Dry
May	12.4	Wet	4.0	Dry	7.3	Normal	23.5	Wet
Jun	13.2	Normal	8.3	Dry	7.0	Dry	11.4	Normal
Jul	11.0	Dry	1.3	Dry	8.7	Dry	10.0	Dry
Aug	13.8	Normal	6.0	Dry	16.1	Wet	14.6	Normal
Sep	2.6	Dry	1.1	Dry	20.6	Wet	0.8	Dry
Oct	1.7	Dry	4.3	Dry	2.0	Dry	6.8	Normal
Nov	8.8	Normal	0.2	Dry	11.5	Wet	17.5	Wet
Dec	7.1	Normal	10.1	Normal	7.2	Normal	14.8	Wet
Total/year	**90.1**	**Dry**	**51.3**	**Dry**	**116.5**	**Normal**	**117.9**	**Normal**

Table 2. Soil fertility comparisons among soil treatments.			
	Treatment		
Element	**Topsoil Present**	**Topsoil Removed**	**Ditch**
P (mg/dm^3)	76	50	67
pH (Buf AC)	5.4	5.3	6.4
Base Sat. (%)	64	56	83
Ca (meq/100cm^3)	4.1	3.4	7.3
Mg (meq/100cm^3)	0.87	0.60	0.87
K (meq/100cm^3)	0.08	0.04	0.15
Zn (mg/dm^3)	7.5	3.3	5.0
Cu (mg/dm^3)	0.69	0.63	0.77
Humic Matter (g/100cm^3)	3.5	3.3	2.8

Table 3. Main effects of topsoil, ridging, and tree species on survival as of May 2009 of the trees planted at Juniper Bay in 2006 and 2007. There were no interactions among treatments, so only main effects are shown. A drought occurred in 2007 that affected the survival of trees planted that year. Trees planted in 2006 apparently had developed deeper root systems that allowed them to extract water from subsoil layers that allowed a larger proportion of them to survive the drought.

	Topsoil & Ridge Treatments			Ditch Treatment
Treatment	Planted 2006 (n=32)	Planted 2007 (n=48)		Planted 2007 (n=24)
	---------------------------Survival (%)-------------------------			
Topsoil	NS†	NS		
Present	46	21		NA
Removed	36	23		NA
Ridging	NS	NS		NS
Smooth	45	23		24
Ridged	36	21		18
Tree species				
Bald Cypress	81a‡	41a		44a
Pond Pine	60b	38a		29ab
Sweet Bay	21c	7b		7bc
Atlantic White Cedar	1d	2b		4c
Mean	41	22		21
STD	16.3	19.5		18.6
CV	40%	89%		89%

†NS, not significant (α =0.05)

‡Means within a treatment and column followed by the same letter are not significantly different (α =0.05).

Table 4. Main effects of topsoil, ridging, and tree species on overall height of surviving trees as of May 2009. There were significant topsoil X species interactions for trees planted in 2006 and 2007, so simple effects for these are shown in Table 5.

	Topsoil & Ridge Treatments			Ditch Treatment
Treatment	Planted 2006 (n=16)	Planted 2007 (n=44)		Planted 2007 (n=24)
	---------------------------------Height (cm)---------------------------			
Topsoil †	NS			
Present	161	92a§		NA¶
Removed	79	62b		NA
Ridging	NS			NS
Smooth	125	72b		97
Ridged	115	83a		95
Tree species†				NS
Bald Cypress	99b‡	87a		91
Pond Pine	140a	69b		101
Mean	120	78		96
STD	20	12		18
CV	17	15		18

†N.B.: Topsoil X species significant ($p=0.006$), see Table 5 for simple effects.
‡NS, not significant ($\alpha = 0.05$)
§Means within a treatment and column followed by the same letter are not significantly different ($\alpha = 0.05$).
¶NA: Not applicable.

Table 5. Simple effects of topsoil and tree species (averaged over bedding) on height of surviving trees as of May 2009 . There were significant topsoil X species interactions for trees planted in 2006 and 2007, so simple effects for these are shown here; main effects are shown in Table 4.

	Planted 2006 (n= 16)			Planted 2007 (n=44)	
	Species			**Species**	
Treatment	Bald cypress	Pond pine		Bald cypress	Pond pine
	----------------------------Height (cm)----------------------				
Topsoil †					
Present	113aB	209aA		74aB	108aA
Removed	85aA	72bA		62bA	62bA

†N.B.: Topsoil X species significant (p=0.006).

‡Means within a treatment and column (effect of topsoil) followed by the same small letter and in a row (effect of species) followed by the same capital letter are not significantly different (α =0.05).

Chapter 6

PHOSPHORUS RELEASE TO SURFACE WATER FROM JUNIPER BAY

C.J. Moorberg and M.J. Vepraskas

INTRODUCTION

Wetland restoration is practiced in part to improve water quality. When wetlands are restored on agricultural lands, it is critical to determine whether residual plant nutrients will leave the restored site and contaminate downstream freshwater with nutrients such as P. Excessive amounts of plant nutrients, mainly phosphorus and nitrogen, are among the principal factors that may adversely affect surface water quality. Of the 1,126,300 km of rivers and streams assessed for water quality status in the U.S. in 2000, 8% or 90,000 km of rivers and streams were impaired by excess nutrients (USEPA, 2000). In addition, excessively high nutrient loading was the primary reason for the impairment of 22% of the 6.9 million ha of lakes that had been assessed (USEPA, 2000). Excess nutrients, particularly N and P, adversely affect humans, domestic animals and aquatic life (Dodds and Welch 2000) by causing eutrophication and severe degradation of water quality through the proliferation of algae (Correll, 1998).

The inadequacy of wetlands for mitigating P mobility to surface waters, and perhaps even enhancing P mobility, has received insufficient scientific attention. As recently as 1998, the US-EPA had not established criteria for phosphorus impacts on water quality (Parry, 1998), but as little as 10 to 20 μgP/L is suspected to cause eutrophication in P-limited freshwaters of the southeastern USA (Sawyer, 1947; Vollenweider, 1968; Correll, 1998, Osmond, 2006). Optimal growth of crops in nearby upland soils typically requires an order-of-magnitude greater P concentration (100 to 200 μg P/L) in soil solution (Fox and Kamprath, 1970). Since wetlands are commonly positioned between extensively fertilized agricultural lands and nutrient-sensitive surface waters (Reddy et al., 1999), immobilization of P to maintain water quality is an important wetland function. Moreover, if a wetland is created from an agricultural soil that contains nutrient levels optimized for crop growth, an additional several-fold increase in soil-solution P, as is often observed under reducing soil conditions, could be disastrous for nutrient-sensitive water bodies.

Because the hydrologic restoration of artificially-drained agricultural lands is often perceived to be straightforward, these areas make common wetland restoration targets (Ewing et al., 2004). Scientists are concerned, however, that reducing soil conditions established during restoration of high-P agricultural soils may result in excessive P discharge to nutrient sensitive surface waters (Sallade and Sims, 1997; Shenker et al., 2004; Young and Ross, 2001). This concern is especially important in states like North Carolina, a state that once had more natural wetland acreage than all but five other states (Mitch and Gosselink, 2000) and also has had an abundance of high-P agricultural soils (Cahoon and Ensign, 2004).

Soil reduction has been shown to increase P in soil solution, especially in soils high in organic matter (Hutchison and Hesterberg, 2004; Young and Ross, 2001). Many different mechanisms of P dissolution can occur in reduced soils (Hutchison and Hesterberg, 2004), but one of the most common is through Fe(III) reduction. In anaerobic soils, Fe(III) is reduced to more soluble Fe(II) and bound P also becomes subject to dissolution (Bartlett and James, 1993; Lindsay, 1979; Stumm and Morgan, 1996). The reductive dissolution of Fe(III) hydroxides was found to be mainly responsible for P release in restored wetland peat soils from Israel in 120 d incubation experiments (Shenker et al., 2004).

Hutchison and Hesterberg (2004) found up to sevenfold increases in dissolved reactive P (DRP, from 1.5 up to 10 mg/L) after 40 days of microbial reduction in continuously-stirred redox reactors containing soil material from the surface horizon of a Cape Fear sandy clay loam (Typic Umbraquult) from Plymouth, NC. In a separate batch experiment, Hutchison and Hesterberg (2004) used citrate additions to show that increased dissolved organic matter (DOM) produced under anaerobic conditions likely contributed to the increase in DRP, and they speculated that this effect might have been due to competitive adsorption between phosphate and DOM for iron and aluminum oxide minerals or to the formation of ternary DOM-Fe-PO_4 or DOM-Al-PO_4 complexes.

The goal of our research is to determine the extent of phosphorus dissolution from restored wetland soils in the rhizosphere. We hypothesize that P released in wetland soils will be greatest in the rhizospheres of roots that have recently died and are being decomposed by bacteria. Knowledge of the chemical processes controlling P dissolution in soils could be used to develop a screening process that identifies soils most prone to P-release by reductive dissolution. This screening process might be used to site wetland restoration projects in areas that will not impact quality of surface waters adjacent to the restored site.

The objectives of this work were to: 1) document whether P is being released to surface water draining from Juniper Bay, and 2) to identify mechanisms that are responsible for the P release.

MATERIALS AND METHODS

Site Description

The NCDOT with the aid of USDA-NRCS personnel mapped Juniper Bay soils. Organic soils cover 60% of Juniper Bay largely in its center. Mineral soils occupy the remainder. A perimeter ditch that drains to only one outlet surrounds the entire bay. Surface outflows at the outlet are measured by a weir.

Surface Water Sampling

From 2001 to 2002, surface outflow samples of 100 mL were taken with an ISCO 3700 sampler (ISCO Inc. Lincoln, NE, USA) at the weir located at the perimeter ditch outflow point (Figure 1). From 2003 through 2009, samples were collected by hand using a 30 cml syringe. All samples were passed through a 0.45 μm filter to remove particulate material that would interfere with the analysis. Two drops of concentrated sulfuric acid (H_2SO_4) were then added to each 60 mL of filtered sample reducing the pH to approximately 1 to eliminate microbial activity in the sample. Phosphate (PO_4^3-P) was measured using a Lachat Quickechem 8000 slow injection auto analyzer (Latchat Instruments, Loveland, CO, USA). Methods used in the analysis are outlined in Greenberg et al. (1992).

Rhizosphere Studies: Greenhouse-Rhizotron Experiments

Greenhouse experiments were conducted to measure P dissolution in microsites around both live and dead roots in soils from Juniper Bay (Fig. 2). Experiments were conducted using rhizotrons, or rootboxes, similar to those of Neufeld et al. (1989). Rhizotrons are boxes consisting of expanded polyvinyl chloride (PVC) sides and polycarbonate windows on the front and back (60 cm long x 30 cm wide x 6 cm deep). Drainage ports are fitted on the bottoms, and support stands are used to keep the boxes at a 30° angle from the vertical to encourage root growth against the lower (front) polycarbonate window.

Twenty-four rhizotrons were used in total. Each was filled Leon Ap (Aeric Haplaquod) mineral soil material collected from Juniper Bay. Soil material was collected from the upper 20 cm and sifted with machine cloth to remove rocks, roots, and other plant materials. The soil material was thoroughly mixed and then packed into the rhizotrons. Tensiometers were installed in each box to a depth of 10 cm to monitor soil water potential. Platinum microelectrodes will be installed at a depth of 30 cm to monitor redox potential (Fielder et al., 2007). Fifteen rhizotrons were planted and the rest were unplanted to be used as controls for each water treatment.

Bald cypress (*Taxodium distuchum*) seedlings (obtained from a tree farm) were grown in the rhizotrons because this species has been planted and grown successfully at Juniper Bay, and this species typically grows in the organic soils at the reference wetlands (Caldwell et al., 2007). The seedlings were grown for three months until roots reached the bottom of the boxes and were well distributed from side to side. Preliminary studies indicated that an appropriate root distribution would occur after approximately 2 to 3 months of growth. Polycarbonate windows were covered with metal plates to prevent light penetration before soil pore water samplers were installed. After the samplers were installed, aluminum foil was used to prevent light penetration into the front of the rhizotron.

During the initial 3-month growth phase, water was applied as needed to the root boxes to maintain the soil matric water potentials between –100 to –300 cm as determined from tensiometers. This was considered field capacity for these experiments. Root numbers were measured monthly by counting the number of roots within a grid of 4 x 4 cm squares covering the lower side of the polycarbonate windows. Only roots 2 cm or greater in length with at least 1 cm of root within that grid square were counted. Also, roots were classified as alive or dead based on their color and appearance. Black roots were considered dead, and brown and/or white roots were considered alive.

When roots were well distributed in the boxes the experimental water treatments were be imposed. Two water treatments over 110 days will be studied: 1) control (field capacity), and 2) saturated to surface. Under saturated conditions, the water level was be maintained at approximately 5 cm above the soil surface. For each treatment, five rhizotrons were planted, and three were unplanted (controls), yielding eight rhizotrons per water treatment. Root boxes were kept vertical during this phase of the experiment. Drainage of water was only allowed in the control treatment, which will be watered as described earlier. Redox potential was monitored weekly (20 cm depth) in all rootboxes. Caldwell et al. (2007) found that in the natural wetlands

110 d was the average annual ponding duration that occurred in organic soils of the reference wetlands. This ponding duration has also been found at the restored Juniper Bay as well.

After the water regimes are imposed, water samplers were installed horizontally through the polycarbonate of the saturated treatments of both soil groups. Preliminary work suggested roots die within approximately 1 month of saturation, and dead roots are most prevalent below a depth of 40 cm in the rhizotrons. Water samples were collected biweekly to analyze for pH, dissolved P, and DOC. For sampling, each rhizotron was divided into three levels; top (0-17 cm), middle (17-32 cm), and bottom (32-52 cm). A redox probe and a pore water sampler was installed in each level adjacent to a root (in planted rhizotrons), or in the matrix (in unplanted rhizotrons).

Pore water samples were taken under saturated conditions using Rhizon Flex Pore water samplers (Item # 1908D2.5, Soil Moisture Corporation, Santa Barbara, CA, USA). Embedded Rhizon samplers will be connected to evacuated serum bottles through lure lock valves and syringe needles in order to collect saturated pore water samples. Two samples were taken for each sampler. The first was extracted with a 30 ml serum bottle until approximately 15 ml of sample was collected and was frozen within 30 min of sampling for storage. The second, 30 ml sample, was collected in a 100 ml serum bottle which was previously acidified with sulfuric acid and stored at room temperature. To ensure proper soil moisture conditions to recover minimum sample volume in the drained treatments, all eight drained rhizotrons were saturated with tap water from the surface until water dripped freely out of the drainage ports. Then, the ports were plugged and the soil pore water was allowed to come to equilibrium with the soil for 4 hr prior to sampling.

Live and dead roots were distinguished from one another and counted during the treatments using a 4 x 4-cm grid of squares. When live roots adjacent to a sampler died during this phase, water samples continued to be collected. Photographs were also taken during the time of root counts. Weekly measurements included tree height, diameter, and soil redox potential.

At the end of the experiment (day 111), the boxes were drained, laid horizontal, and the polycarbonate panels will be removed one at a time. Soil samples were collected at distances within 3 cm of each rhizon samplers. Samples were analyzed for organic C, pH, Mehlich III extractable P, and microbial biomass C and P.

RESULTS AND DISCUSSION

P Exiting the Bay in Surface Water

Concentrations of dissolved P in drainage water exiting Juniper Bay are shown in Figure 3 for both pre- and post restoration periods. The amounts of P leaving the Bay following restoration were nearly three-fold higher than before restoration. The variation in P levels is high following restoration and is related to rainfall amounts.

P levels in excess of 0.1 mg/L are believed to contribute to eutrophication and fish kills. While the Juniper Bay drainage water will be diluted as it moves downstream, the loss of this P should be of some concern. The mechanisms causing this release were examined in greenhouse experiments in hopes of discovering a management method for capturing the released P and keeping it the Bay.

Greenhouse Experiments

Findings from the saturated treatment will be the only results reported, because few changes in phosphorus were observed in the moist treatment. Changes in total P over time in the saturated treatments are shown on Figure 4. The control treatment simulated what occurs in the soil matrix away from roots in restored wetland sites. Total P concentrations increased progressively up to 60 days in the control treatments and then experienced a gradual decline thereafter. In the planted treatments, total P levels remained low in the upper section of the rhizotrons (0-17 cm depth). In the lower section (32-52 cm depth) of the planted treatment, total P concentrations increased much like those in the control treatments, but showed a greater decline after 60 days than found in the control plots. These results showed that growing plants did not cause total P to increase over that in the soil away from the roots, but could actually reduce the P concentrations.

Changes in root concentrations over time (Figure 5) help explain why the P decreases occurred when they did. Roots in the top section remained alive throughout the study and their numbers increased approximately 100 fold over 100 days. Thus, the low P levels found in the upper section of the rhizotrons (Figure 4) were likely due to plant uptake. In the bottom section approximately one-third of the roots had died by day 60, but after this point roots began to proliferate and eventually reached a concentration that was about one-half that found in the upper section by the end of the study. In saturated soils, roots can remain alive by pumping oxygen to the root tissues through internal channels called arenchyma. Apparently the arenchyma were active in roots within the upper section, but developed after 60 days in roots in the lower section. Once the roots began to regrow in the lower section of the rhizotrons, the dissolved P levels declined.

Redox potential data (Figure 6) showed marked differences in the planted and control treatments. The unplanted controls had similar redox potentials at both depths throughout the study. Redox potential declined through day 60 and stabilized as microbial activity caused the soils to become anaerobic as the native organic materials in the matrix were utilized. In contrast, in the upper the redox potential remained stable and relatively high at 200 mV throughout the study. This points to the impact of the arenchyma in the roots oxidizing the rhizosphere. In the lower section of the rhizotrons, redox potentials declined during the first 30 days and then began a gradual increase through the duration of the study. The lower redox potentials reached in this treatment were enhanced by the death of the roots which supplied more organic materials for microbial respiration. Root death may have ceased when the roots developed arenchyma and began to oxidize the rhizosphere.

The decomposition of organic materials by microorganisms releases dissolved organic carbon (DOC) to the soil solution (Figure 7). The residual C in the soil matrix (control treatments) is used by the microbes, and DOC levels showed a gradual increase through day 60 followed by a decline. Reasons for the decline in DOC could be due to DOC being absorbed onto soil particles, or by it being decomposed by microorganisms. The impact of the rhizosperes on DOC varied with depth in the rhizotrons. In the upper section of the planted treatments, DOC concentrations remained low throughout the study. While microbial activity was occurring, the result in the upper section was that little DOC was being produced. The oxidizing conditions found in this layer apparently resulted in rapid removal of DOC from solution, either by microbial respiration of the DOC, absorption onto soil particles, or by plant uptake. In contrast, the DOC in the lower section increased sharply during the first 30 days as the roots died and were decomposed. Declines in DOC after this point occurred because root death ceased, slowing DOC production, and microbial oxidation, particle absorption, and plant uptake processes began to remove DOC from solution.

Ferrous Fe was also produced in both the planted and control treatments during the course of the study (Figure 8), and differences Fe concentrations among depths and treatments were related to redox potential. In the controls, Fe concentrations were similar between upper and lower sections and gradually rose over the first 80 days of the study. The Fe was reduced as respiring microorganisms used it as an electron acceptor as they oxidized organic C in the soil matrix. In contrast, the upper section of the planted treatments had low Fe concentrations in the rhizospheres because these areas were better oxidized and Fe was not used as the terminal electron acceptor by the microbes. In the lower section, high concentrations of Fe were produced because of the lack of oxidizing conditions in the rhizospheres, and the large amount of C made available by the dead roots. Concentrations of DOC and Fe peaked at approximately the same time, suggesting the amounts of both were related to death of the roots.

In order to identify the major mechanism producing the increases in dissolved P following wetland restoration (Figure 3), we correlated dissolved P with dissolved Fe and DOC for the first 60 days of the experiment. Results were best for the correlation between Fe and dissolved P (Figure 9) suggesting that the Fe reduction mechanisms may be the major mechanism releasing P in these soils. However, Fe and DOC were also correlated with each other and so the DOC mechanism cannot be ruled out. As discussed above, increases in Fe and DOC occur together because both are tied to microbial activity under anaerobic conditions.

CONCLUSIONS

Dissolved P is leaving the restored Juniper Bay in surface drainage water exiting the Bay at the perimeter ditch outflow point. Concentrations of TP have exceeded 0.2 mg/L after large storm events, and management methods should be used to reduce these levels to <0.1 mg/L, which were observed prior to restoration, in order to avoid causing excessive weed and algal growth in downstream waters.

The source of the P is residual fertilizer left in the soil after agriculture had ceased. Mechanisms causing the release occur under anaerobic conditions which are common in wetland soils. In this study, concentrations of TP of approximately 800 μg TP L^{-1} were observed in both the rhizosphere and the matrix. Rhizopshere concentrations of TP or ortho-P (not presented here) never exceeded the matrix; therefore, conditions in a flooded bald cypress rhizosphere do not release additional P from the soil into solution. However, once mature bald cypress tree's roots were allowed to proliferate and mature (after 60 d of continuous flooding), TP in solution decreased, presumably from plant uptake. Mechanism 1 (P-release through Fe reduction) appears to be occurring due to the strong correlation between TP and Fe (II) in solution. When roots died in the bottom level, no additional P was released into solution. Therefore, Mechanism 2 (DOC competition for cations) is not dominant. However, it is difficult to separate Mechanisms 1 and 2 because Fe (II) and DOC are highly correlated. The contribution of Mechanism 3 is unknown at this time. Future P fractionation may help estimate its potential contribution. These results suggest that it is not possible to prevent the dissolution of P in restored wetlands, but methods to retain the P onsite must be employed.

Possible management techniques that may help reduce P loss from Juniper Bay include planting hydrophytic plants, and managing the hydrology. Hydrophytic plants oxygenate the root zone, thus limiting the effects of all previously mentioned P-release mechanisms. Also, plant uptake of P will be highest in plants that are adapted to flooded soil conditions which will help remove P from the soil pore water solution. In this study, decreases in P were observed after a long, stable period of saturation in both the rhizosphere and the matrix. There may be an unknown P immobilization pathway occurring. Managing wetland hydrology to mimic long periods of saturation may help decrease the concentration of P in soil pore water, thus, limit how much P is drained from the system.

REFERENCES

Abit, S.M. 2005. Evaluation of subsurface solute transport and its contribution to nutrient load in the drainage ditches prior to restoration of a Carolina Bay. M.S. thesis, North Carolina State University, Raleigh.

Bartlett, R.J,. and B.R. James. 1993. Redox Chemistry of Soils. Adv. Agron. 50:151-208.

Brownfield, C.S. 2007. Phosphorus dissolution in soil material from a Carolina Bay as affected by reducing conditions. M.S. thesis, North Carolina State University, Raleigh.

Cahoon, L.B. and S.H. Ensign. 2004. Spatial and temporal variability in excessive soil phosphorus levels in eastern North Carolina. Nutrient Cycling In Agroecosystems 69(2): 111-125.

Caldwell, P.V., M.J. Vepraskas, R.W. Skaggs, and J.D. Gregory. 2007. Simulating the hydrology of natural Carolina Bay wetlands. Wetlands 27(4):1112-1123.

Correll, D. 1998. The role of phosphorus in the eutrophication of receiving waters: a review. J. Environ. Qual. 27:261-266.

Dahl, Thomas E. 1990. Wetlands losses in the United States 1780's to 1980's. U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C.

Dodds, W. K., and E. B. Welch, 2000, Establishing nutrient criteria in streams, J. N. Am. Benthol. Soc. 19(1):186-196.

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

Fiedler, S., M.J. Vepraskas, and J.L. Richardson. 2007. Soil redox potential: importance, field measurements, and observations. Advances Agronomy 94:1-54.

Fiedler, S., and K. Kalbitz. 2003. Concentrations and Properties of Dissolved Organic Matter in Forest Soils as Affected by the Redox Regime. Soil Sci. 168:793-801

Fimmen, R.L., D. deB. Richter, D. Vasudevan, L.T. West, and M. Williams. 2008. Rhizogenic Fe-C redox cycling: A hypothetical biogeochemical mechanism that drives crustal weathering in upland soils. Biogeochemistry (in press).

Fox, T.R. and N.B. Comerford. 1992. Rhizosphere Phosphotase Activity and Phosphatase Hydrolyzable Organic Phopshorus in Two Forested Soils. Soil Biol. Biochem. 24:579-583.

Greenberg , A.E., L.S. Clesceri and A.D. Eaton. 1992. Standard methods for examination of water and waste water. 18th Ed. APHA. Washington D.C.

Greaves, M.P. and D.M. Webley. 1965. A Study of the Breakdown of Organic Phosphates by Micro-organisms from the Root Region of Certain Pasture Grasses. J. Appl. Bact. 28:454-465

Hoagland, D.R. and D.I. Arnon. 1938. The water culture method for growing plants without soil. California Agr. Exp. Sta. Circ. 347.

Holford, I.C.R., and W.H. Patrick. 1981. Effects of Duration of Anaerobiosis and Reoxidation on Phosphate Sorption Characteristics of and Acid Soil. Aust. J. Soil Res. 19:69-78

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

Kaczorowski, R.T. 1977. The Carolina bays: a comparison with modern oriented lakes. Tech. Rep. 13-CRD, Coastal Research Division, Dept. Geo., Univ. South Carolina, Columbia.

Kreiser, G.S. 2003. Nutrient analysis of drainage waters from Juniper Bay. M.S. thesis, North Carolina State University, Raleigh.

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

Mitch, W.J., and J.G. Gosselink. 2000. Wetlands. John Wiley and Sons, New York.

Neufeld, H.S., D.M. Durall, P.M. Rich, and D.T. Tingey. 1989. A rootbox for quantitative observations on intact entire root systems. Plant and Soil 117:295-298.

North Carolina Dept. of Environment and Natural Resources. 2007. Div. Water Qual. "Redbook" Surface Waters and Wetlands Standards. North Carolina Admin. Code. 15A NCAC 02B .0223 Nutrient Sensitive Waters.

Osmond, D. 2006. Personal Communication. Raleigh, North Carolina.

Patrick, W.H., and R.A. Khalid. 1974. Phosphate Release and Sorption by Soils and Sediments: Effect of Aerobic and Anaerobic Conditions. Science 186:53-55

Ponnamperuma, F.N. 1972. The Chemistry of Submerged Soils. Advances in Agron. 24:29-96

Rea, K. 2001. Corps Regulators have large, difficult mission. In USACE Missions: Regulatory Program, Protecting our Water Resources. Engineer Update Vol. 25 No.7. U.S. Army Corps of Engin., Washington, D.C.

Reddy, K.R., R.H. Kadlec, E. Flaig, and P.M. Gale. 1999. Phosphorus retention in streams and wetlands: a review. Crit. Rev. in Environ. Sci. Tech. 29(1): 83-146.

Richter, D.D, H.L. Allen, J.W. Li, D. Markewitz, J. Raikes. 2006. Bioavailability of slowly cycling soil phosphorus: Major restructuring of soil-P fractions over four decades in an aggrading forest. *Oecologia* DOI 10.1007/s00442-006-0510-4.

Richter, D.D., N.H. Oh, R. Fimmen, J. Jackson. 2007. The rhizosphere and soil formation. In Zoe Cardon & Julie Whitbeck, eds. *The Rhizosphere - An Ecological Perspective*. Springer-Verlag, New York, USA.

Raghu, K., and I.C. MacRae. 1966. Occurrence of Phosphate-dissolving Micro-organisms in the Rhizosphere of Rice Plants and in Submerged Soils. J. Appl. Bact. 29:582-286

Sallade, Y.E., and J.T. Sims. 1997. Phosphorus transformations in the sediments of Delaware's agricultural drainageways: I. Effect of reducing conditions on phosphorus release. J. Environ. Qual. 26:1579-1588.

Sah, R.N., and D.S. Mikkelsen. 1985. Transformations of Inorganic Phosphorus During the Flooding and Draining Cycles of Soil. Soil Sci. Am. J. 50:62-67

Sawyer, C.N. 1947. Fertilization of lakes by agricultural and urban drainage. J. New England Water Works Assoc. 69:109-127.

Sharitz, R.R., and J.W. Gibbons. 1982. The ecology of southeastern shrub bogs (pocosins) and Carolina bays: a community profile. FWS/OBS-82/04. US Fish and Wildlife Service, Division of Biological Services. Washington, DC.

Shenker, M., S. Seitelbach, S. Brand, A. Haim, and M.I. Litaor. 2004. Redox reactions and phosphorus release in re-flooded soils of an altered wetland. Eur. J. Soil Sci. 56(4):515-525.

Song, K., K Zoh, and H. Kang. 2006. Release of phosphate in a wetland by changes in hydrological regime. Sce. Total Environ. 380:13-18

Stumm, W., and J.J. Morgan. 1996. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. 3rd Edition. John Wiley and Sons, New York.

Szember, A. 1960. Influence on Plant Growth of the Breakdown of Organic Phosphorus Compounds by Micro-organisms. Plant and Soil 13:147-158

Turner, F.T. and J.W. Gilliam. 1976a. Effect of Moisture and Oxidation Status of Alkaline Rice Soils on the Adsorption of Soil Phosphorus by an Anion Resin. Plant and Soil. 45:353-363

Turner, F.T. and J.W. Gilliam. 1976b. Increased P Diffusion as an Explenation of Increased P Availability in Flooded Rice Soils. Plant and Soil. 45:365-377

United States Congress. 1972. Federal Water Pollution Control Act (Clean Water Act). Public Law 92-500,33. 1251.

USDA-NRCS. 2003. National resources inventory. 2003 Annual NRI: Wetlands. Available at: http://www.nrcs.usda.gov/technical/NRI/2003/nri03wetlands.html

USEPA. 2000. National Water Quality Report. Available at: http://www.epa.gov/305b/2000report/

Vadas, P.A., and J.T. Sims. 1998. Redox Status, Poultry Litter, and Phosphorous Solubility in Atlantic Coastal Plain Soils. Soil Sci. Soc. Am. J 62:1025-1034

Vepraskas, M.J., J.G White, R.L Huffman, G.P Fernandez, R.W. Skaggs, C.W. Zanner, J.M. Stucky, J.D. Gregory, S.W. Broome, J.M. Ewing, B. Lees, S. Luginbuhl, G.S. Kreiser and R.P. Szuch. 2003. Methods to assess soil, hydrologic, and site parameters that affect wetland restoration. Project report to NCDOT, NCSU, NC.

Voteller, T.H. and T.A. Muir. 2002. Wetland Protection Legislation. In National Water Summary on Water Resources. WSP 2425. U.S. Geol. Surv. Washington, D.C.

Vollenweider, R.A. 1976. Advances in defining critical loading levels of phosphorus in lake eutrophication. Mem. 1st. Ital. Idrobiol. 33:53-83.

Young, E.O., and D.S. Ross. 2001. Phosphate Release from Seasonally Flooded Soils: A Laboratory Microcosm Study. J. Environ. Qual. 30:91-101.

Zelasko, A.J., D.L. Hesterberg, and M.J. Vepraskas. 2008. Soil reduction rates under water saturated conditions in relation to soil properties. Soil Sci. Soc. Am. (In review).

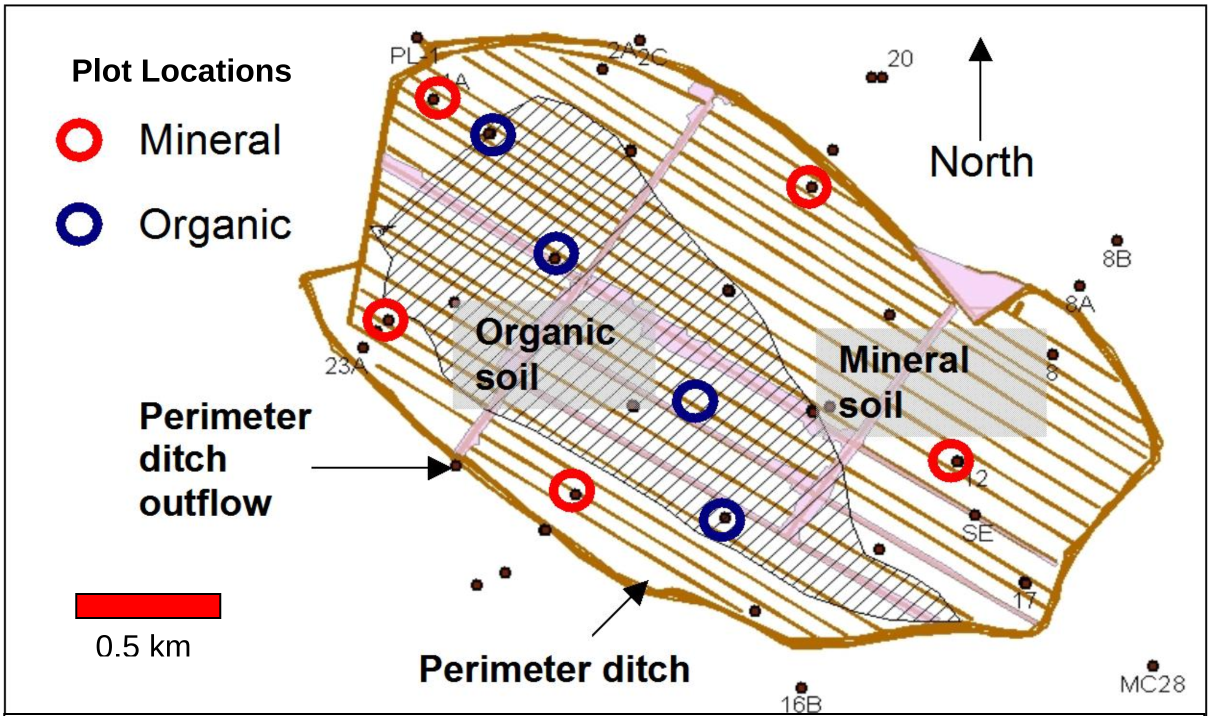

Figure 1. Schematic of Juniper Bay showing distribution of organic soils (center) and mineral soils (edge). Plot locations are shown for both soil groups where water tables will and drains to a single outlet where water samples will be collected.

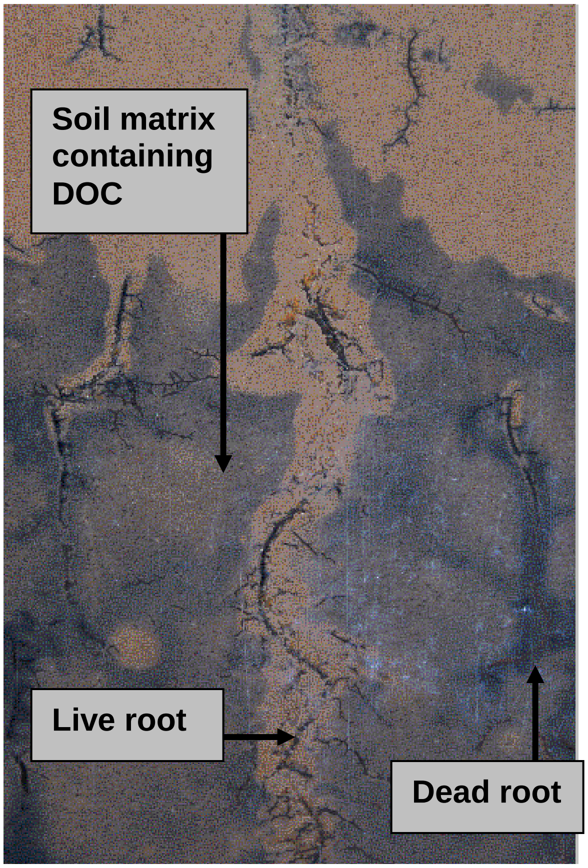

Figure 2. Photograph through a rhizotron "window" showing roots of a tree seedling that was grown for 120 days under saturated conditions in mineral soil. The ponding duration killed some roots, but not all. Dead roots (black color) produced dissolved organic C (DOC, gray color) which was being leached from top to bottom by water at the time of the photograph. Soil around live roots contained the least DOC.
For this research, we sampled soil and water from areas around live roots, dead roots, and the soil matrix without roots to determine if production of DOC was related to dissolved P.
Large roots are approximately 2 mm in diameter in photograph.

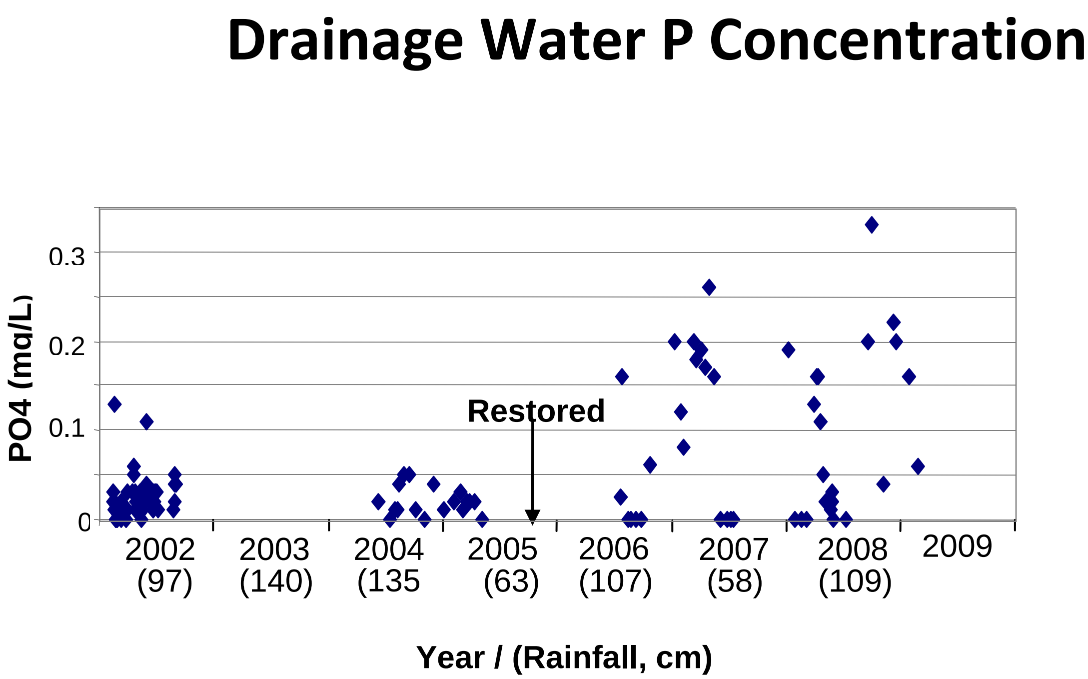

Figure 3. Phosphorus concentrations measured in Juniper Bay surface water outflow pre- and post wetland restoration. Results show that P concentrations have increased following restoration, raising concern that downstream waters will receive excessive P loads.

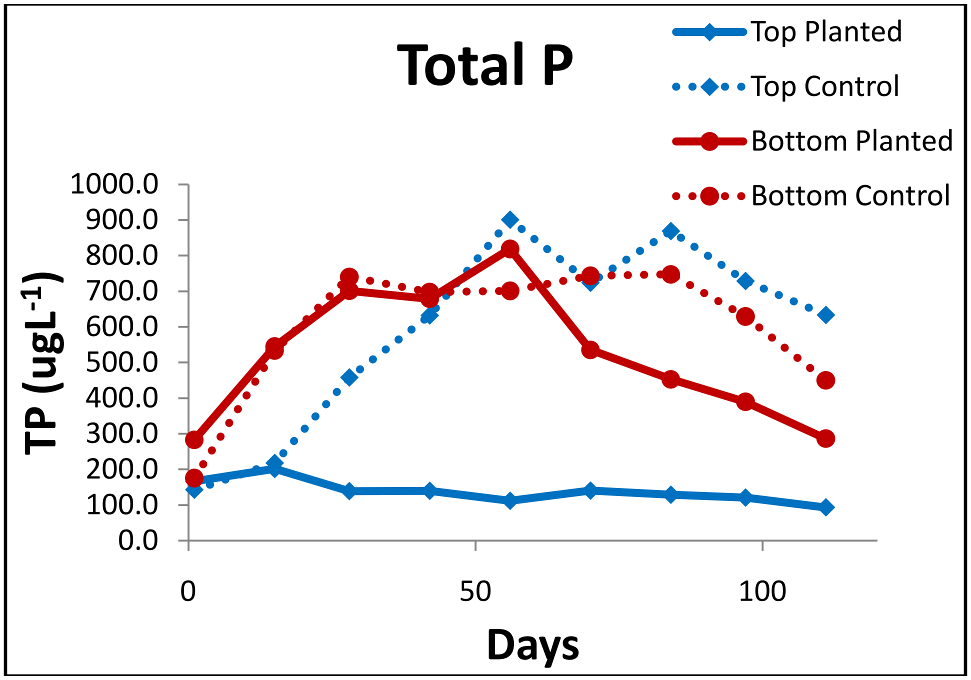

Figure 4. Concentrations of total P (TP) over time in rhizotrons that were kept saturated for 120 d to simulate wetland conditions. Mean concentrations (from eight replicates) from two depths are shown in the planted (rhizosphere samples) and unplanted controls that simulate the soil matrix. The TP was released during the first 60 d in the controls (both depths) and in the bottom layer of the planted treatment. Less TP was released in the top of the planted treatment. TP declined after 60 d possibly due to plant uptake, microbial immobilization, or by the P being absorbed onto soil particles.

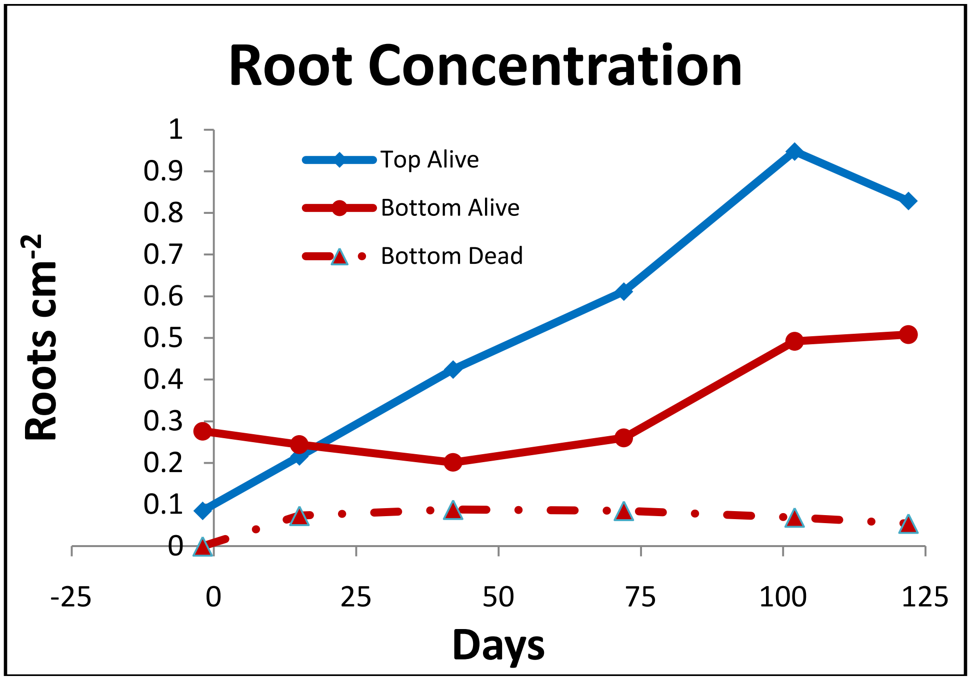

Figure 5. Root status (live or dead) and concentrations with time in the saturated rhizotrons that were planted with bald cypress trees. Data are means of four replicates. In the upper layer of the rhizotron, roots remained alive and proliferated. In the lower section, approximately one-third of the roots died during the first 60 d of saturation. However, after this point roots began to regrow.

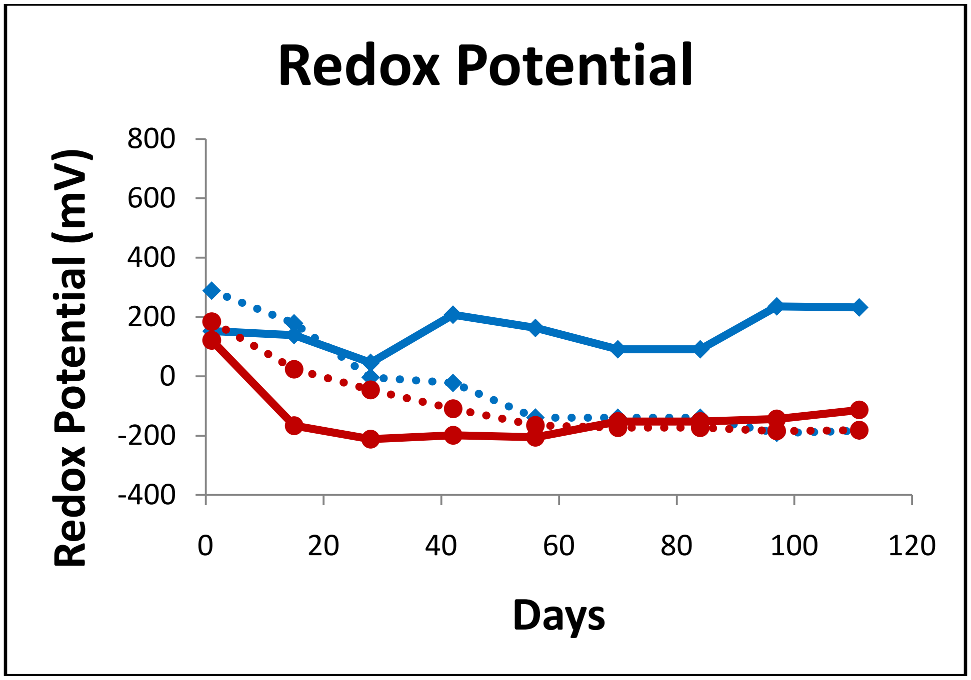

Figure 6. Soil redox potential over time in the saturated rhizotrons. In general, the redox potential declined after saturation indicating the soil layers were anaerobic in the controls and in the bottom of the planted treatment. The top of the planted treatment did not become as reduced, possibly because of the bald cypress trees ability to pump oxygen to its shallow roots.

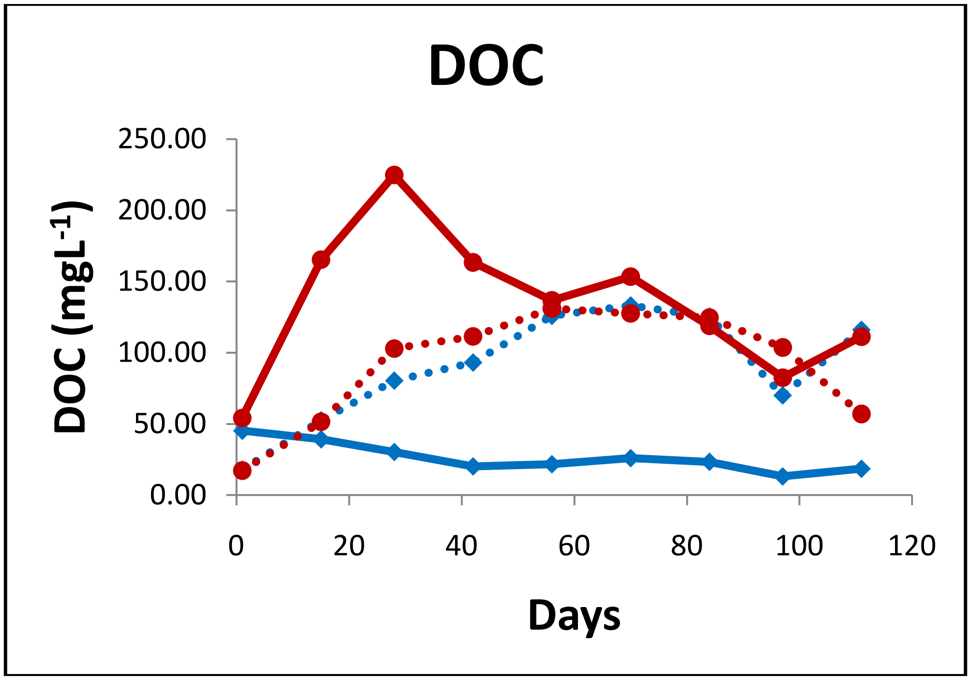

Figure 7. Dissolved organic C (DOC) concentrations over time in the saturated rhizotrons. The DOC concentrations in the controls increased up to 60 d due to microbial decomposition of the organic residues in the soil matrix. Higher DOC levels were observed in the lower layer of the planted treatment due to release of C from dead roots. DOC concentrations in the upper layer of the planted treatment remained relatively low, because there were no dead roots, and this zones oxidizing conditions allowed a more complete microbial oxidiation of the residual organic C.

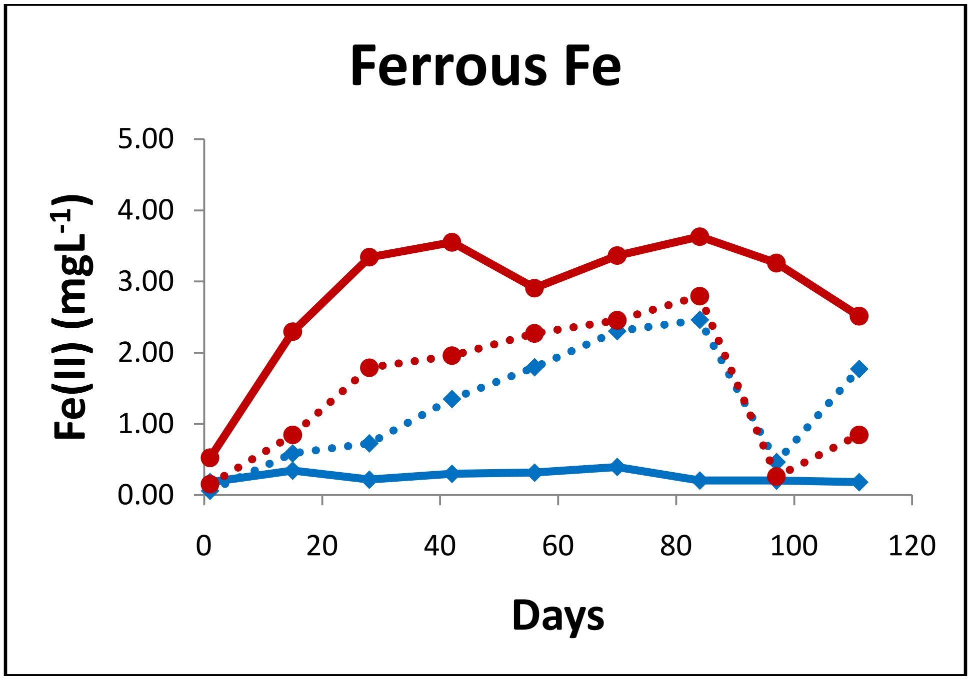

Figure 8. Dissolved Fe (II) concentrations over time in the saturated rhizotrons. Fe concentrations were related to the changes in redox potential shown in Figure 6. The Fe concentrations were highest in the lower layer of the planted treatment which developed the lowest redox potentials. Fe concentrations were lowest in the upper layer of the planted treatment due to it being more oxidized by the oxygen brought into the layer through the bald cypress roots.

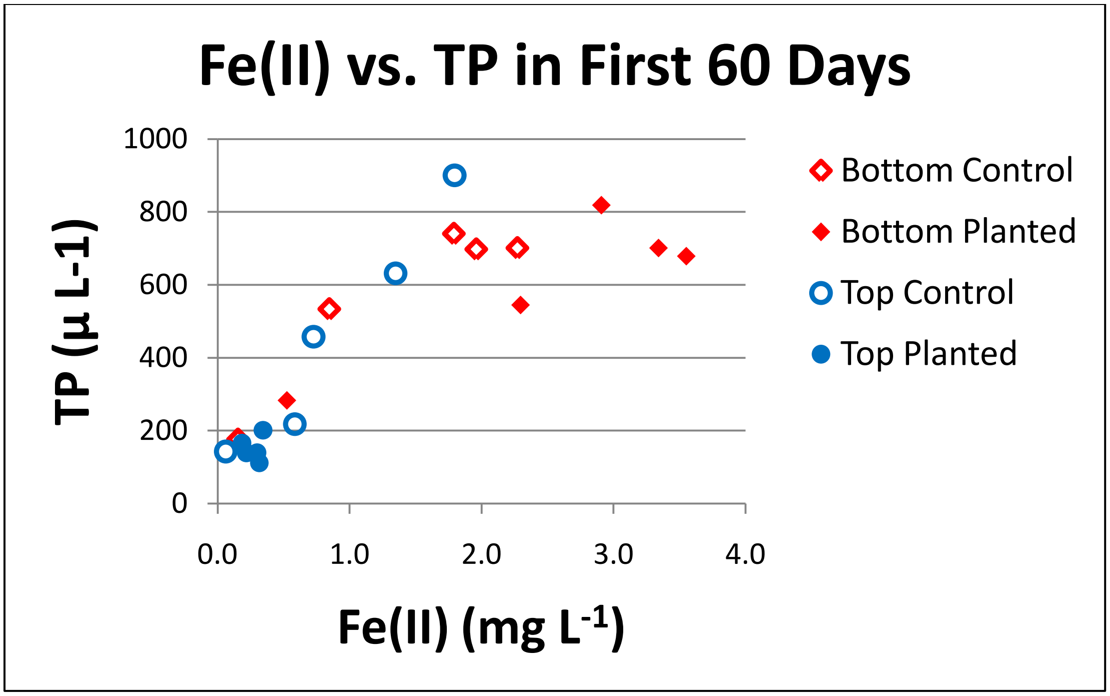

Figure 9. Correlation of reduced Fe and TP concentrations in the first 60 d of saturation. Mechanism 1 (P-release through Fe reduction) appears to be an important P-release pathway in this system due to the close relationship of Fe reduction and P-release. This graph shows the concentration of each in solution within the first 60 d of sampling at different levels.

Chapter 7

DISSOLUTION OF PHOSPHORUS INTO PORE-WATER FLOWING THROUGH AN ORGANIC SOIL

S. M. Abit, A. Amoozegar, and M.J. Vepraskas

INTRODUCTION

Wetlands are destroyed through highway or public works projects, as well as private developments. Restoration of a wetland at another site to compensate for the lost wetland function resulting from these projects is required for permitting any wetland destruction. Areas that are commonly utilized for wetland restoration in North Carolina (and perhaps other states) are previous wetland areas that had been drained and devoted for several years to agricultural production.

Years of cultivation and fertilizer application of a previous wetland area lead to nutrient accumulation in near-surface layers. Four decades of cultivation of Histosols soils in the Hula Valley, previously an Eastern Mediterranean wetland in Northern Israel, resulted in at least 50% higher total P in the top 20 cm of the soil compared to underlying layers (Shenker et al., 2005). At least 30 years of fertilizer application to a Histosols soil in a drained Carolina Bay in North Carolina resulted in 173% higher Mehlich-extractable P in the top 20 cm of the soil compared to that in an undisturbed reference wetland (Ewing, 2003). In addition, the Mehlich-extractable P in a Spodosols soil at the same drained Carolina Bay increased by 138% (compared to a reference wetland) after 20 years of cultivation (Ewing, 2003). Given the elevated nutrient status in these areas, wetland restoration activities should include nutrient management strategies because re-flooding during restoration of wetland hydrology had been known to increase dissolution of P (Shenker et al., 2005). An increase in soil solution P is a concern as it may affect water quality in adjacent streams, rivers and lakes through its effects on eutrophication (Correll, 1998).

Many studies have shown that flooding of soils results in P release into solution that was largely attributed to reduction processes and dissolution of Fe-P minerals or to Fe-hydroxide dissolution and release of the adsorbed P (Patrick and Khalid, 1974; Sah and Mikkelsen, 1986; Vadas and Sims, 1997; Turner and Haygrath, 2001, Shenker et al., 2005).

Oxidation of organic matter becomes less efficient under anaerobic conditions resulting in the accumulation of dissolved organic matter (DOM) in pore-water (Fiedler and Kalbitz, 2003). At elevated DOM concentrations, additional P-dissolution mechanisms had been reported to influence pore-water phosphate (PO_4) concentrations. These include competitive adsorption of DOM and PO_4 by ligand exchange on mineral surfaces, and DOM-enhanced dissolution of surface Fe or Al with concomitant release of PO_4 (Hutchison and Hesterberg, 2004, Brownfield, 2007). Increase in pH associated with the development of reduced conditions also tends to reduce sorption of PO_4 (Hutchison and Hesterberg, 2004).

The above mentioned P-dissolution mechanisms are triggered by the development of anaerobic and reducing conditions. Monitoring of reduction potential (Eh) above and below the WT in a column study by Stall (2008) revealed that while Eh measurements indicate reducing conditions below the water table (WT), conditions at 10 and 30 cm above the WT were consistently oxidizing. A field study by Abit et al. (2008a) showed that while nitrate was lost probably due to denitrification below the WT, nitrate persisted in zones that were within the capillary fringe (CF) (within 30 cm above the WT) for most of the 84-day study period. Their field study indicated that while conditions below the WT were reducing, the CF remained generally aerobic. In a separate study that evaluated redox dynamics in horizontal subsurface flow constructed wetlands, Dušek et al. (2008) reported a correlation between Eh and flow rate. This implied that pore-water velocity may be an important hydrologic parameter that influences the development of reducing conditions in the subsurface.

The jurisdictional wetland hydrology requirement in the United States is achieved if "saturation (i.e., condition below the WT) occurs within a major portion of the root zone (usually within 30 cm from the surface)" and this must occur "continuously for at least 5% of the growing season in most years" (US Army Corps of Engineers, 1987). This rule suggests that the WT does not have to be at the surface to meet the wetland hydrology requirement. This could also mean that the hydrology of the wetland restoration area could be managed to keep the WT from the phosphorus-enriched layers (i.e., the upper 20 cm of the soil) yet meet the wetland hydrology criteria. If this management strategy results in lesser P-dissolution in the P-enriched zones, then it could minimize the threat of P-exportation from the wetland restoration sites to their adjacent water bodies.

This study was conducted to test the hypothesis that P-dissolution in locations that are within 30 cm above the WT is significantly lower than in locations below it. Also of interest was the possible effect of pore-water velocity on the degree of P dissolution from a P-enriched organic wetland soil. The specific objective of this study was to evaluate the dissolution of phosphorus (P) in water flowing through the vadose zone-shallow ground water continuum of an organic soil.

MATERIALS AND METHODS

Soil Material

Soil material was collected from the surface layer (Oap horizon; 0-10 cm) of a site classified as the Ponzer series (Loamy, mixed, dysic, thermic, Terric Haplosaprists). The site was in Juniper Bay, a drained wetland area in Robeson County, NC, that had been devoted to crop production for at least 30 years prior to collection of soil material. The soil was air-dried and passed through a 2-mm mesh sieve. A representative bulk sample was collected for soil analysis. Saturated hydraulic conductivity (K_{sat}) was measured by the constant head procedure (Amoozegar and Wilson, 1999) using uniformly-packed soil material in cylindrical cores (7.6 cm in diameter and length). Soil water retention between 0 and 400 cm pressure was measured by the pressure cell procedure (Dane and Hopmans, 2002) and bulk density was determined by the core method (Grossman and Reinsch, 2002) using the same intact cores used in the K_{sat} determination. The pH was determined using a 1:1 soil to water (mass basis) suspension ratio. Total elemental C and N were measured using a Perkin Elmer CHNS Elemental Analyzer (Model PE 2400) after combustion of oven dried soil samples that were passed through a 2-mm sieve (Nelson and Sommers, 1996). Total elemental C was considered equivalent to TOC in these acidic, non-calcareous soil samples (Essington, 2004).

Experimental Set-up

Three flow cells having length, width and thickness dimensions of 90 cm, 50 cm and 8 cm, respectively, were used in this study. The front side of each flow cell was constructed using a 0.64-cm thick transparent polycarbonate sheet while the bottom and other sides were made of flat polyvinylchloride (PVC) sheets (Fig. 1; see photograph in Figure A1 in the Appendix). Two 2.5 cm-wide chambers, with perforated inner walls, were constructed on the two sides of the flow cell. The left and the right chambers were used as inlet and outlet chambers, respectively. The central portion of the flow cell (85-cm wide) that was bounded by perforated flat PVC sheets was packed with the soil material.

Packing was done by adding previously sieved air-dried soil in the flow cell and tamping uniformly with a flat-ended piece of wooden dowel. To minimize layering, the surface of the tamped soil was stirred before more air-dried soil was added on top of it. Packing was done in approximately 5 cm-thick sections at a time. As the packing progressed, two sets of solution samplers were installed at 32 cm and 53 cm from the inlet chamber. Each set had a solution sampler at 7, 17 and 32 cm above the bottom of the flow cell. These locations corresponded to 5 cm below a simulated WT and at 5 cm and 20 cm above the simulated WT as will be discussed later (see Fig. 1). This arrangement allowed collection of two samples from each of the three depths monitored in each flow cell. A blackened platinum-tipped redox electrode (Pt electrode) was also installed at the middle of the flow cell (42.5 cm from either the inlet or outlet chambers) at all depths that the solution samplers were installed. Locations where soil solution samples were collected are hereafter called “monitoring locations”.

The top 5 cm of the flow cell was packed with commercially-available coarse sand. The coarse sand served as a capillary barrier that kept the packed soil from being wetted (via capillary action) all the way to the top. Having the capillary barrier also reduced the likelihood of evaporative losses. The top of the flow cell was then covered with aluminum foil (with a few pinholes) to further discourage evaporative losses that could have encouraged upward flux of water in the flow cell.

Soil solution was collected using micro-samplers installed through holes at the front side of the flow cell (see Fig. 1). The hydrophilic porous polymer tube (Soilmoisture Equipment Corp, Sta. Barbara, CA) that were used for the construction of the micro-sampler in this study does not sorb P and has a bubbling pressure equivalent to approximately 200 kPa (2 atm). The micro-samplers were positioned horizontally inside the flow cell to collect water samples across the thickness of the packed soil (see Appendix Fig. 2 for an illustration of the micro-sampler). The holes on the flow cell through which the micro-samplers were installed were sealed with silicone rubber sealant.

Sealed 120-mL serum bottles were used for sampling. A drop of hydrochloric acid (HCl) was added to each bottle before it was covered with a rubber septum cap and clamped with an aluminum seal (Wheaton, Millville, NJ) to keep it air-tight. Air in the serum bottle was then evacuated using a pump to create a vacuum of 400 cm inside the bottle (hereafter referred to as "evacuated bottle"). To collect a sample, a dedicated evacuated bottle was attached to the sampler. This was accomplished by piercing a hypodermic needle that was attached to the micro-sampler (by a Luer-lock connector- see Fig. A2 in the Appendix) into the rubber cap of the evacuated bottle. A 20-mL solution sample was collected using this procedure for each sampling. After collecting a 20-mL sample using the pre-acidified serum bottles, a smaller non-acidified evacuated bottle (20-mL serum bottle with cap) was attached to a dedicated micro-sampler to collect 5 mL of sample that was used for determination of soil solution pH.

The Pt electrodes used in the experiment were built according to the specifications in Wafer et al. (2004) and blackened using a platinizing solution (chloroplatinic acid hexahydrate and lead acetate trihydrate – Ricca Chemicals, Arlington, TX) as described in Jackson (1975). Blackened electrodes have been recommended for use in measuring reduction potential (Whisler et al, 1974; Quispel, 1947). Each Pt electrode was connected to a CRX10 data logger (Campbell Scientific, Logan, Utah) that was programmed to measure and record voltage measurements every 15 minutes. Electrodes were standardized in a ferrous-ferric iron solution or Light's solution (Light, 1979) before installation.

Flow-through Experiment

Before introducing distilled water into each flow cell packed with soil material, the outlet chamber was connected to a plastic tubing with an open end fixed at 12 cm above the bottom of the flow cell. The four inlet/outlet ports at the bottom of the flow cell and the inlet and outlet chambers were connected via a plastic tubing manifold to an aeration reservoir (Fig. 1, see Fig. A1 in Appendix for a photograph of the manifold). The aeration reservoir was connected to a 25-L distilled water reservoir (Marriotte bottle). The tip of the air inlet tube in the water reservoir was also set at 12 cm above the bottom of the flow cell (Fig. 1). A gel-filled Calomel reference electrode (Fisher Scientific, Pittsburgh, PA, ID No. 13-620-258) and the 3 Pt electrodes already installed in the flow cell were connected to a dedicated data logger.

Distilled water from the Marriotte bottle reservoir was initially supplied to the aeration reservoir while clamping the tubing that connected the aeration reservoir to the flow cell through the manifold. Using an aerator, air was bubbled through the distilled water in the aeration reservoir to keep it uniformly aerated. Once a static water level was established in the aeration reservoir, the tubing connecting it to the flow cell was unclamped allowing delivery of water through the manifold to the bottom and sides of the flow cell to establish a simulated WT. The soil material in the flow cell was saturated from the bottom to prevent air entrapment as the flow cell was flooded to the desired level. The reference electrode was immediately installed at the outlet chamber (tip of reference electrode submerged in water) as soon as water started to flood in it. The data loggers collected voltage measurements (between reference and Pt electrodes) every 15 minutes.

Four hours after a static WT was established 12 cm above the bottom of the flow cell, background soil solution samples were collected from all micro-samplers. One hour after collection of the background samples, the tube connecting the aeration reservoir to the bottom and sides of the flow cell were clamped. Distilled water was then introduced into dedicated inlet chambers to bring about horizontal flow across the packed soil. A pre-determined number of peristaltic pump tubes of different diameters was installed in a variable rate peristaltic pump for each flow rate. These tubes supplied distilled water from the aeration reservoir to the respective inlet chambers at rates of 1.2, 2.4 and 3.6 L d^{-1} that were equivalent to horizontal pore-water flow velocities of approximately 6, 12 or 18 cm d^{-1} across a dedicated flow cell. The resulting average pore-water velocities (V_{ave}) in the experiment were computed using the formula:

$$V_{ave} = \text{Flux/Water-Filled Porosity}$$

where Flux is the Darcian velocity, which is the total application rate divided by the cross sectional area of the flow path. The cross sectional area of the flow path included both the 12-cm saturated zone and the 30-cm thick CF. Since the CF is nearly saturated, the water-filled porosity was taken to be the same as the total porosity of 0.63 cm^3 cm^{-3}.

Outlet samples were collected daily and frozen until analyzed for pH, dissolved reactive phosphorus (DRP) and dissolved organic carbon (DOC). Outflow samples were filtered using 2.5-μm particle retention filter paper (Whatman International Ltd, Kent, UK, Whatman no. 42) prior to chemical analysis. Solution samples were collected from the monitoring locations at 3, 7, 14, 21 and 28 days after initial saturation. Soil solution samples were analyzed for pH, DOC, DRP and total Fe. When not collecting samples, the front side of each flow cell was covered with aluminum foil to prevent any impact that light could have on chemical/microbial activities.

Analysis of DOC was carried out using the Total Organic Carbon (TOC) Auto-analyzer (Shimadzu Corp., Columbia, MD) while DRP was analyzed using the Lachat Quickechem 8000 slow injection auto analyzer (Greenberg et al., 1992). Dissolved Fe was measured using inductively coupled plasma optical emission spectrometry (Perkin-Elmer ICP-OES 2000DV, Elmer, Germany). After 28 days, the flow cells were emptied, cleaned and re-packed with the air-dried Ponzer soil material and subjected to the same mode of saturation, pore-water velocities and sampling schemes to duplicate the experiment.

RESULTS AND DISCUSSION

The soil material used in the study contained 0.08 g kg^{-1} of Mehlich-extractable P, an expectedly high soil TOC content of 195 g kg^{-1}, and an extractable Fe of 10 to 63 mmol kg^{-1} depending upon the extraction procedure (Table 1). The soil also contained 130 mmol kg^{-1} of extractable aluminum (Al).

Outflow Solution Chemistry

Intuitively, P dissolution is expected to be higher at a relatively low pore-water velocity than at higher pore-water velocities. This is because slower pore-water velocities indicate a longer residence time of the water in the system, resulting in more time that the water is in contact with the soil and exposed to P-dissolution reactions. However, within the time frame of the experiment, the degree of P dissolution was not affected by the pore-water velocities employed in various flow cells. This was indicated by the absence of a significant difference between the mass of DRP leached-out of the different flow cells subjected to various pore-water velocities (Fig. 2). In effect, within the timeframe of the experiment, passing the same volume of water through a given volume of soil at pore-water velocities of 6, 12 and 18 cm per d^{-1} dissolved and leached a statistically similar amount of DRP from the soil. The absence of any significant effect of the pore-water velocities employed in this experiment on the degree of P dissolution may indicate that the resulting differences in residence time were not wide enough to cause any significant difference in the degree of dissolution.

It should be noted that regardless of the pore-water velocity, DRP concentrations of the outflow solution ranged between 0.217 to 2.69 mg L^{-1}, which exceeded the 0.1 mg L^{-1} USEPA water quality limit (USEPA, 1986). This indicates that water passing through Ponzer soil (or other similar soils) has a high probability of triggering environmental problems when drained to surface water bodies.

Phosphorus Dissolution

Given that water flowing through the Ponzer soil material yielded eutrophic levels of P in solution, the next question to ask is where in a vadose zone and ground water continuum P dissolution largely occurs with flowing water? Knowing this information is important as it could aid in devising possible management strategies to limit the degree of P exportation off-site during wetland restoration or (agricultural) drainage practices.

In addition to horizontal solute transport in the ground water, horizontal solute transport in the capillary fringe (CF), which is a part of the vadose zone, has been demonstrated in laboratory (Amoozegar et al., 2006; Silliman et al., 200; Henry and Smith, 2002) and field (Abit et al., 2008a, b) experiments. This indicates that P dissolution both in the CF and in the ground water could influence the amount of P that is leached out of the system.

Figure 3 shows a general increasing trend (with time) in DRP concentration of soil solution samples collected from the flow cells. The trend was observed across different pore-water velocities and the observed increase in DRP concentration was more obvious at the monitoring locations 5 cm below and 5 cm above the WT. In addition, detected DRP concentrations at these two depths were consistently significantly higher than those detected (on the same day) at 20 cm above the WT. This suggests that much of the increase in DRP detected at the outlet was from the dissolution of P near the WT in both the saturated and vadose zones. The following discussions are largely focused on the results from the monitoring locations at 5 cm above and 5 cm below the WT as these were the zones where much of P dissolution occurred.

The observed increase in soil solution DRP concentration with time was coupled by a decrease in DOC concentration (Figure 4). This trend contradicts the information in the literature showing that an increase in dissolution of DRP is coupled by an increase in DOC (Hutchison and Hesterberg, 2004; Brownfield, 2007). The observed inverse relationship between DRP and DOC may be due to the fact that unlike the reactor/closed systems used in the above-mentioned experiments, wherein whatever carbon and P that were dissolved stayed and accumulated in the system, this study was conducted in a flowing system which presents the possibility that a fraction of the DOC and DRP could be leached with the flowing solution.

Results from the study of Hutchison and Hesterberg (2004) showed that an increase in DOC concentration from around 40 to 125 mg L^{-1} resulted in an 8 mg L^{-1} increase in dissolved DRP. Such observed increase in DRP was largely attributed to: i) competitive adsorption of DOM and PO_4 by ligand exchange, and ii) DOM-enhanced dissolution of surface Fe and Al with concomitant release of PO_4. These mechanisms are hereafter referred to as "DOM-triggered" mechanisms. Viewed differently, their results indicate that the DOM-triggered P-dissolution mechanisms could proceed at DOC concentrations between 40 to 125 mg L^{-1}. Results from the reactor study of Brownfield (2007) involving the Ponzer soil indicated that an increase in DOC concentration from around 20 to 60 mg L^{-1} resulted in roughly 0.8 mg L^{-1} increase in DRP.

Despite the observed decrease in DOC in the flow cells with flowing water used in this experiment, DOC concentrations were consistently above 40 mg L^{-1}(Fig. 4). In fact, only once was the DOC observed to be below 50 mg L^{-1} in soil solution samples from the flow cells. In effect, despite the drop in DOC concentration in soil solution in the flow cells, DOC was still present in quantities that could cause the above-mentioned DOC-triggered P-dissolution mechanisms.

The amount of DOC in the system proved to be sufficient to encourage the progressive development of reducing conditions at 5 cm above and 5 cm below the WT (Fig. 5). Figure 5 shows that the Eh dropped below the hydric standard reduction line (below which Fe(III) reduction is expected to be extensive – USDA-NRCS, 2007) and remained very low (reducing conditions) despite the continued decrease in DOC in solution. Figure 5 also shows that the observed drop in Eh below the hydric soil standard reduction line was coupled by an increase in soil solution total Fe concentration. The observed spike in total Fe as the Eh dropped is most likely due to the reduction of Fe(III) resulting in an increased Fe(II) concentration in solution. However, after the initial spike (observed on day 3) total Fe concentration either essentially leveled-off or decreased throughout the remainder of the experiment. Figure 5 also shows that, at 20 cm above the WT, where the Eh reflected a consistently oxidizing environment, no increase in Fe concentration was observed because conditions were not favorable for the reduction of Fe(III) to the soluble Fe(II) form.

In an Fe-P complex, one mole of Fe binds a mole of P. As shown in the idealized example reaction below, reductive dissolution of 1 mole of Fe^{2+} is accompanied by the dissolution of a mole of HPO_4^{2-}.

$$\text{Mineral--Fe--PO}_4 + e^- + H_2O \longrightarrow Fe^{2+} + HPO_4^{2-} + OH^-$$

This would mean that if P-dissolution was largely due to the reductive dissolution of Fe(III) minerals with associated PO_4 (hereafter referred to as “Eh-triggered” mechanism), then the number of moles of P that are released into solution should be similar to the increase in the number of moles of total Fe that gets dissolved. Figure 6 indicates that the increase in soil solution DRP with time was not largely due to the Eh-triggered mechanism. This was shown by the fact that solution DRP concentration (in mM) continued to increase at monitoring locations 5 cm above and 5 cm below the WT despite the fact that the total Fe concentration leveled-off or decreased after the 3rd day of the experiment. In other words, the increase in total Fe in solution under reducing conditions did not account for the increase in dissolved P as the experiment progressed.

It should be noted that the extractable Al content of the soil was at least double the extractable Fe. This suggests that much of the P in the soil was possibly initially complexed by Al rather than by Fe. This could also mean that much of the P that continued to be dissolved after total Fe concentration in solution stabilized or decreased resulted from the DOC-enhanced dissolution of Al with concomitant release of PO_4. Knowing that contributions of Fe reduction to P dissolution was minimal, and that DOC was present at concentrations reported in the literature to be sufficient to cause P-dissolution (despite the observed decrease in DOC concentration), we believe that the increase in dissolved PO_4-P in our flowing system was largely due to the DOC-triggered mechanisms which were: i) competitive adsorption of DOC and PO_4 by ligand exchange, and ii) DOC-enhanced dissolution of surface Fe and Al with concomitant release of PO_4.

CONCLUSIONS AND RECOMMENDATIONS

This study was conducted to evaluate the dissolution of phosphorus (P) in a vadose zone-shallow ground water continuum of an organic soil with flowing water. Passing equal amounts of water through a flow cell packed with Ponzer soil material at pore-water flow velocities of 6, 12 or 18 cm d^{-1} did not result in a significant difference in the amount of phosphorus dissolved and leached out of the system. However, regardless of the pore-water velocity, the concentrations detected at the outlet were consistently above the water quality limits and may cause eutrophication in adjacent surface water bodies. Dissolution of P was found to be significantly more extensive in monitored locations 5 cm above and 5 cm below the water table (WT) than at 20 cm above the WT. This indicated that much of the P leached out of the system was from 5 cm above the WT and below. The dominant mechanisms believed to contribute to P dissolution in the experimental system include: i) competitive adsorption of DOC and PO_4 by ligand exchange, and ii) DOC-enhanced dissolution of surface Fe and Al with concomitant release of PO_4. We recommend that Al concentration of soil solution be monitored if a similar study is conducted.

Phosphorus export had been reported when areas previously devoted to agriculture were restored into a wetland. Highest extractable P concentration in these agricultural areas usually occurs in the upper 10 cm of the soil. The wetland hydrology requirement is considered satisfied if the WT is within 30 cm from the surface for a required number of days during the growing season. Extensive dissolution and off-site exportation of P (especially in the first few years of wetland restoration when vegetation may not yet be fully established) can be prevented while meeting the wetland hydrology requirement by managing the hydrology of the system. This can be accomplished by keeping the WT within 30 cm from the surface but below the layer of high extractable P content. It should be noted, however, that our study showed that P dissolution occurs in parts of the CF that are close to the WT (within 5 cm). To prevent P dissolution, the WT should be at least 5 cm below the P-enriched layer.

REFERENCES

Abit, S.M., A. Amoozegar, M. J. Vepraskas and C.P. Niewoehner. 2008a. Fate of nitrate in the capillary fringe and shallow groundwater in a drained sandy soil. Geoderma. 146:209-215.

Abit, S. M., A. Amoozegar, M. J. Vepraskas and C. P. Niewoehner. 2008b. Solute transport in the capillary fringe and shallow groundwater: Field evaluation. Vadose Zone J. 7:890-898.

Amoozegar, A., C.P. Niewoehner and D.L. Lindbo. 2006. Lateral movement of water in the capillary fringe under drain fields. In: Proc. 2006 Tech. Educ. Conf., Denver Co. 27-31 Aug. 2006. Natl. Onsite Wastewater Recycling Assoc. Sta. Cruz, CA.

Amoozegar, A., and G. V. Wilson. 1999. Methods for measuring hydraulic conductivity and drainable porosity. In: R.W. Skaggs and J. van Schilfgaarde (eds). Agricultural Drainage. Agronomy Monograph No. 38. Soil Sci. Soc. Am, Madison, Wisconsin.

Brownfield, C.S. 2007. Phosphorus dissolution in soil material from a Carolina Bay as affected by reducing conditions. Master’s Thesis, Department of Soil Science, North Carolina State University, Raleigh, NC.

Correll, D. 1998. The role of phosphorus in the eutrophication of receiving waters: A review. J. Environ. Qual. 27:261–266.

Dane, J. H., and J. W. Hopmans. 2002. Water retention and storage. In: J. H. Dane, and G. C. Topp (eds). Methods of Soil Analysis Part 4: Physical Methods. No. 5 in Soil Sci. Soc. Am. Book Series. Soil Sci. Soc. Am., Madison, WI.

Dušek, J., T. Picek and H. Čížková. 2008. Redox potential dynamics in a horizontal subsurface flow constructed wetland for wastewater treatment: Diel, seasonal and spatial fluctuations. Ecological Engineering. 34(3):223-232.

Essington, M. E. 2004. Soil and Water Chemistry: An Integrative Approach. CRC Press. Boca Raton, FL.

Ewing, J. 2003. Characterization of soils in a drained Carolina Bay wetland prior to restoration. PhD Dissertation. Department of Soil Science. North Carolina State University, Raleigh, NC.

Fiedler, S. and K. Kalbitz. 2003. Concentrations and properties of dissolved organic matter in forest soils as affected by redox regimes. Soil Sci. 168:793-801.

Greenberg, A. E., L. S. Clesceri, and A. D. Eaton. 1992. Standard Methods for Examination of Water and Wastewater. 18th Ed. APHA. Washington, DC.

Grossman, R. B., and T. G. Reinsch. 2002. Bulk density. In: J. H. Dane, and G. C. Topp (eds). Methods of Soil Analysis Part 4: Physical Methods. No. 5 in Soil Sci. Soc. Am. Book Series. Soil Sc. Soc. Am., Madison, WI.

Henry, E. J. and J. E. Smith. 2002. The effect of surface-active solutes on water flow and contaminant transport in variably saturated porous media with capillary fringe effects. J. Contam. Hydrol. 56:247-270.

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

Jackson, M.L. 1975. Soil Chemical Analysis. Published by author. Madison, WI. Page 661.

Nelson, D. W., and L. E. Sommers. 1996. Total carbon, organic carbon and organic matter. p. 961-1010 In: D.L. Sparks (ed.) Methods of Soil Analysis: Part 3. Chemical Methods. ASA and SSSA, Madison, WI.

Patrick, W.H. Jr. and R.A. Khalid. 1974. Phosphate release and sorption by soils and sediments: Effect of aerobic and anaerobic conditions. Science 186:53-55.

Quispel, A. 1947. Measurement of oxidation-reduction potentials of normal and inundated soils. Soil Sci. 63:265-275.

Sah, R.H. and D.S. Mikkelsen. 1986. Transformations of inorganic phosphorus during flooding and drainage cycles of soils. Soil Sc. Soc. Am J. 50:62-67.

Shenker, M., S. Seitelbach, S. Brand, A. Haim and M.I. Litaor. 2005. Redox reactions and phosphorus release in re-flooded soils of an altered wetland. European Journal of Soil Science. 56:515-525.

Silliman, S. E., B. Berkowitz, J. Simunek, and M. Th. Van Genuchten. 2002. Fluid flow and solute migration within the capillary fringe. Ground Water. 40(1):76-84.

Stall, C.J. 2008. Microbial fate and transport in a seasonally saturated North Carolina coastal plain soil. Master’s Thesis. Department of Soil Science. North Carolina State University, Raleigh, NC.

Turner, B.L. and P.M. Haygrath. 2001. Phosphorus dissolution in re-wetted soils. Nature. 411: 258.

USEPA. 1986. Quality Criteria for Water. USEPA Bulletin 440/5-86-001. pp 240-243.

USDA-NRCS. 2007. Technical standards for hydric soils. Hydric Soils Technical Notes 11. USDA-Nat. Resour. Conser. Ser., at: http://soils.usda.gov/use/hydric/ntchs/tech_notes/index.html. Accessed: April 2009.

US Army Corps of Engineers. 1987. Corps of Engineers Wetlands Delineation Manual. Technical Report Y-87-1. Vicksburg, MS. pp. 30-32.

Vadas, P.A. and J.T. Sims. 1998. Redox status, poultry litter, and phosphorus solubility in Atlantic coastal plain soils. Soil Sci. Soc. Am. J. 62:1025-1034.

Wafer, C. C., J. B. Richards, and D. L. Osmond. 2004. Construction of platinum-tipped redox probes for determining soil redox potential. J. Environ. Qual. 33:2375-2379.

Whisler, F.D., J.C. Lance, and R.S. Leinebarger. 1974. Redox potentials in soil columns intermittently flooded with sewage water. J. Environ. Qual. 3(1):68-74.

Zelasko, A. J. 2007. Soil reduction rates under water saturated conditions in relation to soil properties. MS Thesis. Department of Soil Science. North Carolina State University, Raleigh, NC.

Table 1. Selected soil properties of Ponzer soil material used in study.

Soil Properties	Measurements
Mehlich 3-Extractable P (g kg^{-1})	0.08
Total Organic Carbon (g kg^{-1})	195.3 $\pm$ 9.7
Total Nitrogen (g kg^{-1})	5.7 $\pm$ 0.1
Iron * (mmol kg^{-1})	
Citrate Bicarbonate Dithionate-extractable	63 $\pm$ 3
Na Pyrophosphate-extractable	11 $\pm$ 0.8
Oxalate-extractable	10 $\pm$ 2.0
Aluminum (Citrate Bicarbonate Dithionate-extractable; mmol kg^{-1})	130 $\pm$ 3
pH	4.5 $\pm$ 0.2
Bulk Density (Mg m^3)	0.62 $\pm$ 0
Porosity (cm3 cm^{-3})	0.63 $\pm$ 0.01
Saturated Hydrauic Conductivity (K_{sat}) (m d^{-1})	1.17 + 0.1

*- Extractable Fe an Al values from Zelasko (2007)
Note: Bulk density, porosity and K_{sat} were measured from packed core samples

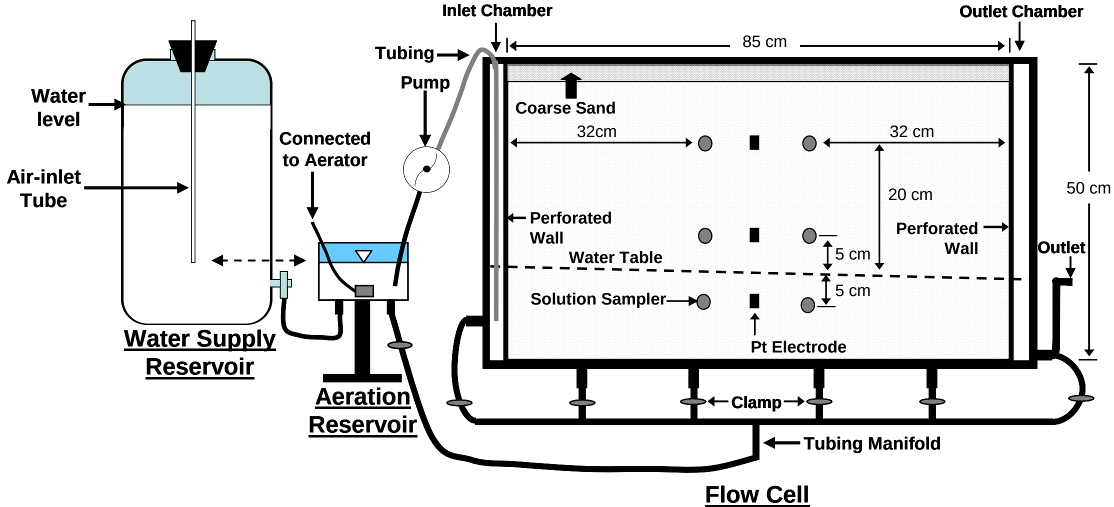

Figure 1. Two-dimensional representation of the set-up used in the experiment.
Note: The flow cell is 8 cm thick and illustration is not to scale.

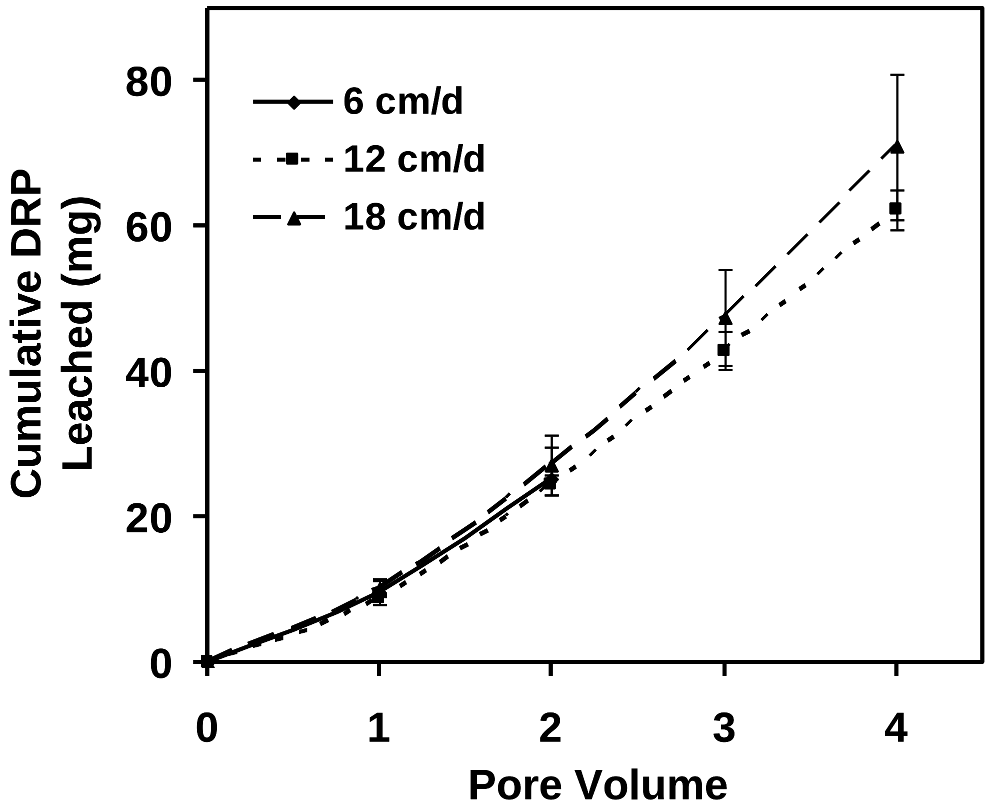

Figure 2. Cumulative amount of dissolved reactive phosphorus (DRP) leached out of the flow cell (pore volume basis) at various pore-water velocities.

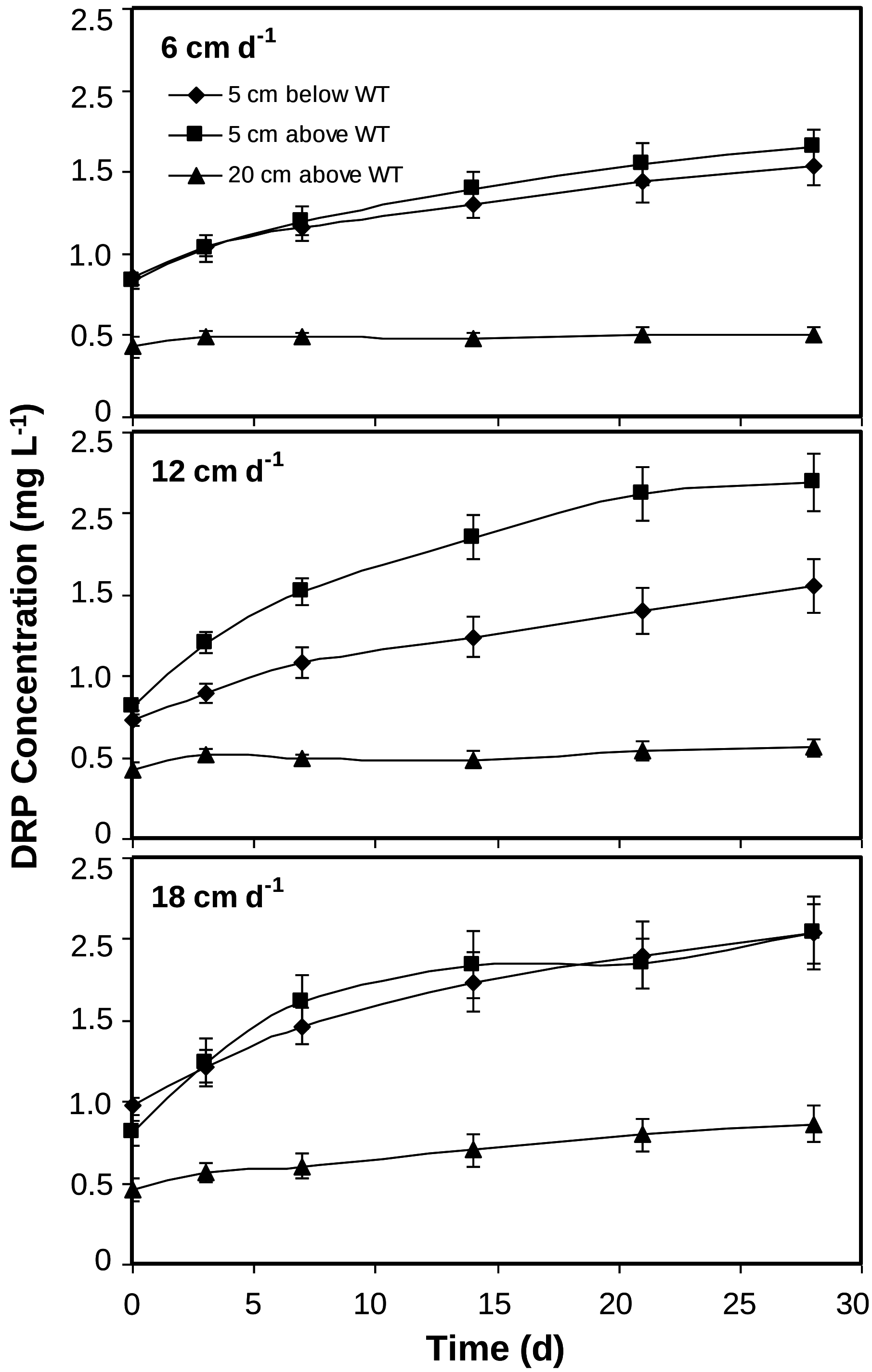

Figure 3. Soil solution DRP concentrations at various monitoring locations in flow cells subjected to various pore-water velocities.

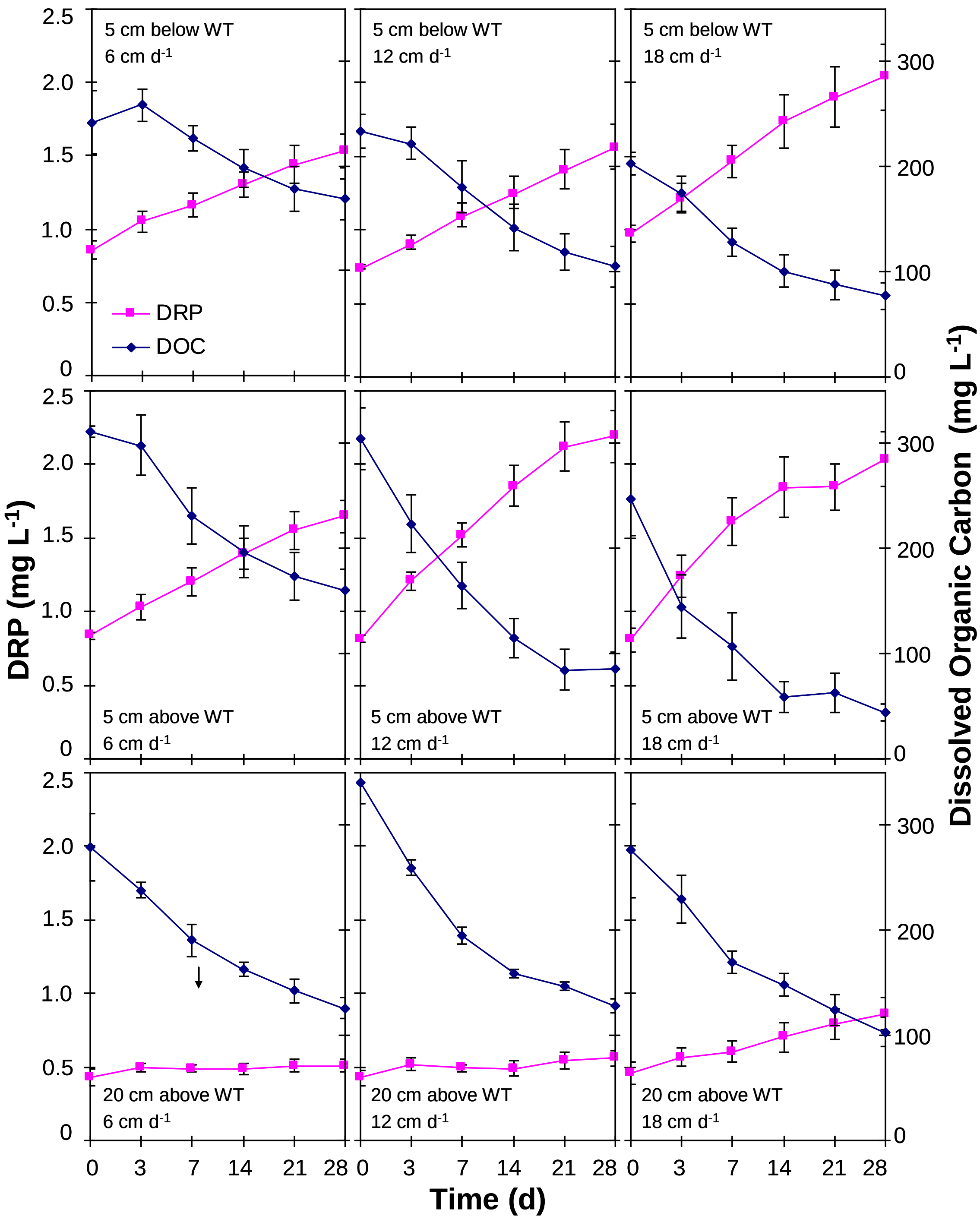

Figure 4. Changes in DRP and dissolved organic carbon (DOC) concentrations at various locations in the flow cells that were subjected to different pore-water velocities.

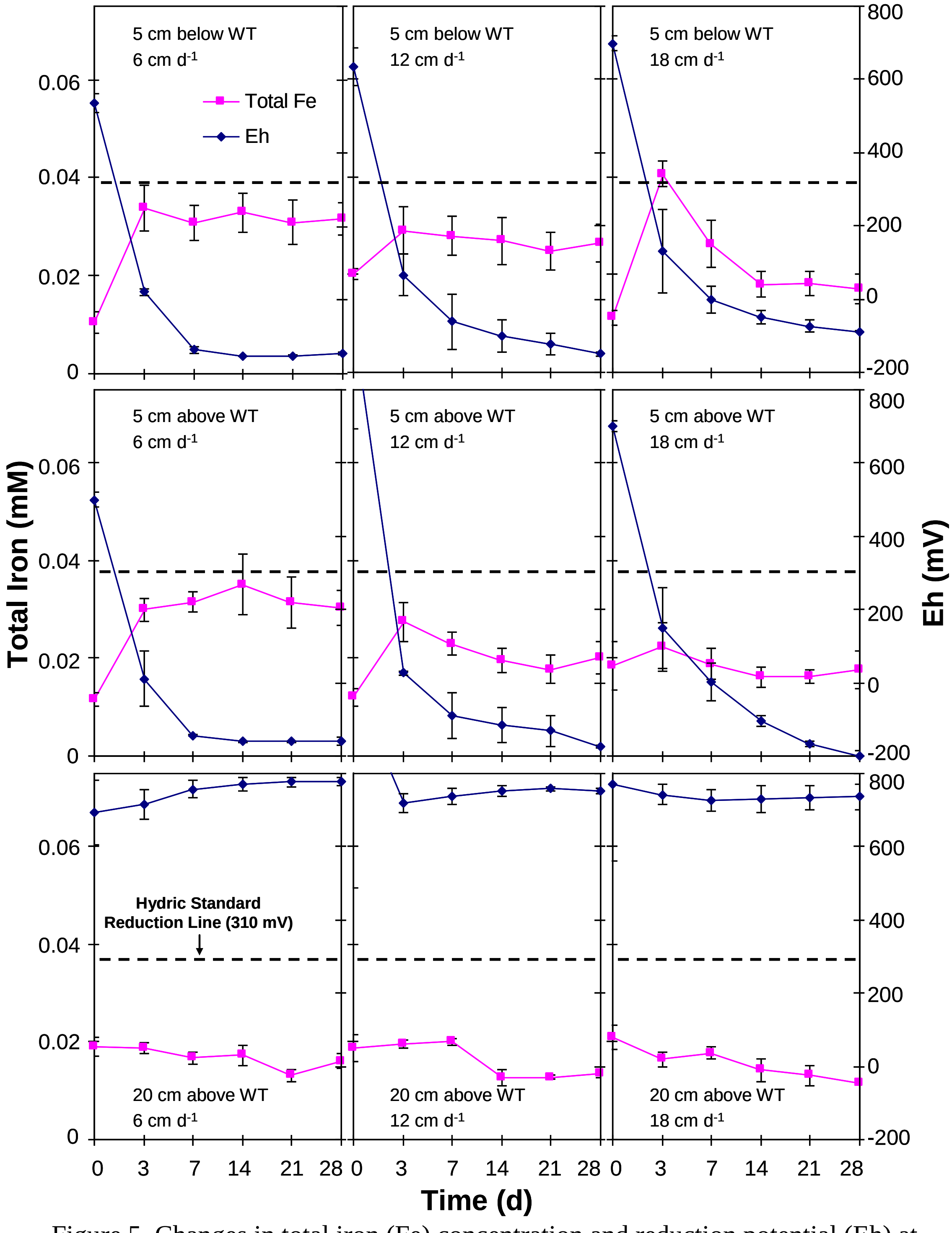

Figure 5. Changes in total iron (Fe) concentration and reduction potential (Eh) at various locations in the flow cells subjected to different pore-water velocities.

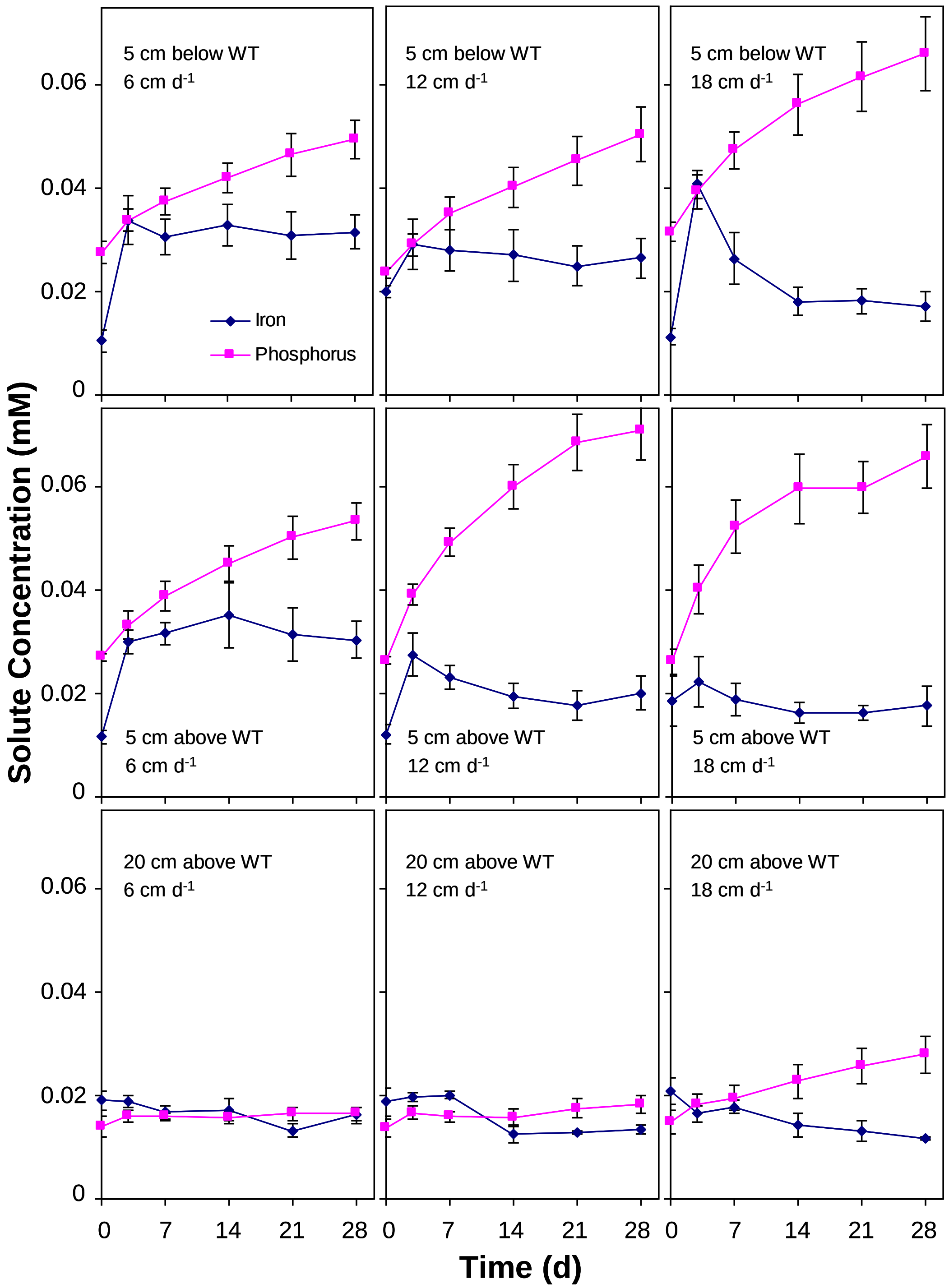

Figure 6: Comparison of DRP and total iron concentrations at various locations in the flow cells subjected to different pore-water velocities.

7. Appendix

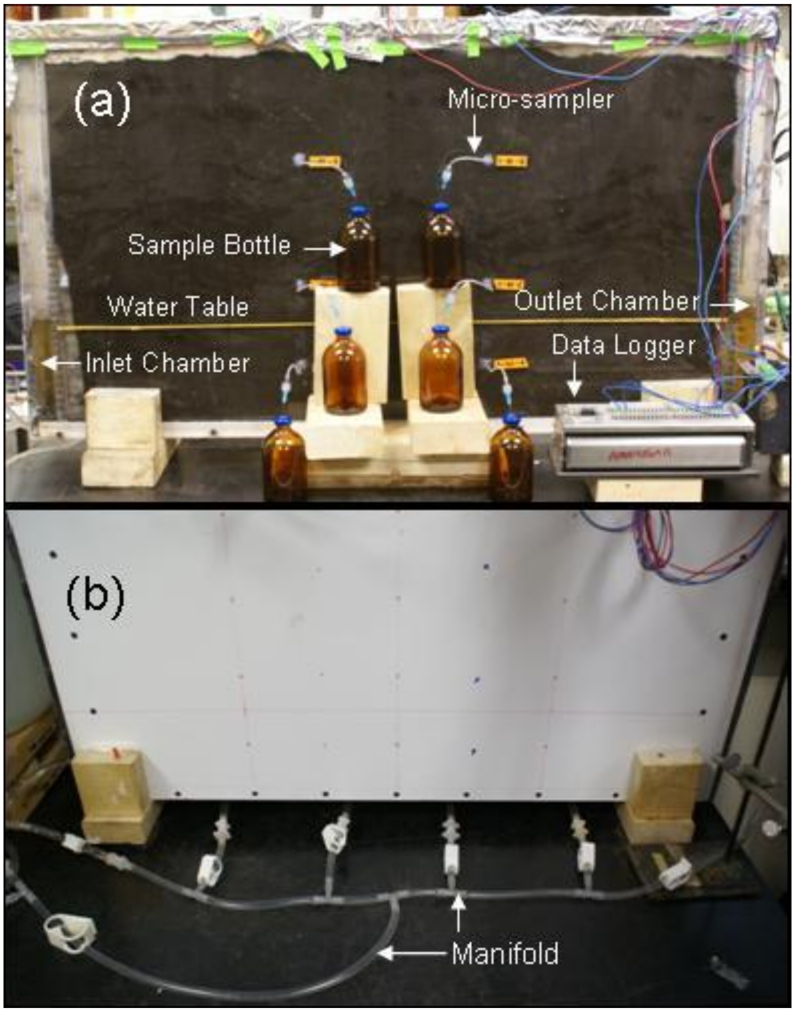

Figure A1. Photographs showing: a) flow cell with attached serum bottles. b) back-view of the flow cell showing the manifold used to supply water to establish a water table. Transparent side of flow cell was covered with aluminum foil when not sampling.

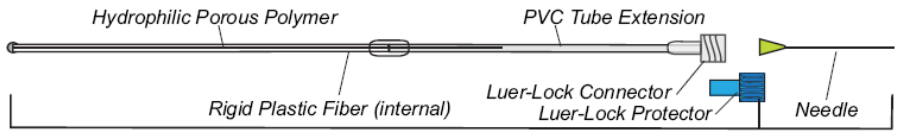

Figure A2. Illustration of the micro-sampler used in the experiment. Illustrations from Soilmoisture Equipment Corp.

Chapter 8

EVALUATION OF HYDROLOGIC TRESPASS RESULTING FROM THE WETLAND RESTORATION OF JUNIPER BAY

M. J. Vepraskas

INTRODUCTION

Wetland restoration at Juniper Bay has included filling most of the major or primary drainage ditches that were installed when the Bay was used for agriculture. The only drainage ditch that functions to drain water from the bay is the "perimeter ditch" that surrounds the restored Bay (Fig. 1). This ditch has been kept open to remove excess water from the bay, as well as to catch groundwater flowing into the bay from surrounding upland areas. If the perimeter ditch were to be filled in or plugged, then groundwater would enter the bay, and raise the water table to the point that a pond was created in the central portion of the bay. Such ponding should be minimized to avoid killing the planted trees. Previous work has also shown that if the water levels rise too high within the perimeter ditch itself, then groundwater would flow out of the bay into the surrounding land on the southwest side (Huffman et al., 2007) creating the condition known as "hydrologic trespass".

A previous modeling study has suggested that water levels in the perimeter must be kept below specific elevations to avoid hydrologic trespass from occurring (Huffman et al., 2007). Those levels are shown in Table 1. These elevations are estimates based on a hydrologic model that was developed for the bay (Huffman et al., 2005). Results of that study showed that the southwest portion of the Bay is the region most susceptible to having hydrologic trespass occur because it is in the lowest position.

Because the data in Table 1 were based on modeling estimates, Huffman et al., (2007) recommended that the findings be validated with on-site measurements, particularly in the southwest sector. The objective of this study was to monitor water table levels outside the perimeter ditch on the southwestern side of the bay to determine if hydrologic trespass is likely to be occurring.

MATERIALS AND METHODS

Groundwater monitoring wells were installed at three locations outside the Bay in the southwest sector Figure 1. These have been in use since 2003, and have made daily measurements of water table levels to a depth of approximately 2 m. Rainfall data have also been collected since 2003. In addition, manual wells were installed in the ditches at the four locations shown in Figure 1 to monitor ditch elevations. Measurements were made periodically in 2008, 2009, and 2010.

The assessment of hydrologic trespass in the southwest section of the site was performed by comparing water table depths before and after restoration for the three well locations shown in Fig. 1. This was done for the same monthly periods during 2003 (pre-restoration) and 2009 (post-restoration). The time intervals selected for comparison where based on when similar rainfall amounts were found in both 2003 and 2009, and were: 8 Janurary to 14 October for wells 25A and 25B, and 16 April to 25 June for well 16B.

RESULTS AND DISCUSSION

Results of the water table level comparisons for the pre- and post-restoration periods are shown in Table 2. The most striking differences occurred in the 0 to 25 cm depth range. Following restoration (in 2009), the water table at wells 25A and B were within the 0 to 25 cm depth range for 103 days, compared to 26 to 38 days that occurred prior to restoration. Rainfall was actually less for the post restoration period indicating that rainfall amounts cannot explain the differences observed between the years. Similar results were found for well 16B. The results from the three wells show that the land outside Juniper Bay on the southwest side of the Bay, was wetter near the surface following restoration than before. The increased durations of saturation are close to the surface, and occur during the growing season such that crop growth on adjacent fields may be impacted if similar water table levels occur in the fields.

Water levels in the perimeter ditch were measured on the dates shown in Table 3, and were compared to the critical ditch levels identified by Huffman et al. (2007) that were not to be exceeded if hydrologic trespass was to be avoided. The two critical levels shown represent the range from dry to wet conditions. It can be seen that the water levels in the perimeter ditch on the southwest section were slightly less than the critical values, while for the other sections the water levels were within the critical range. These data suggest that the perimeter ditch on the southwest side of the Bay was most likely not the cause of the higher water table levels found at the 0 to 25 cm depth (Table 2), although outflow from the perimeter ditch in the other sectors may be occurring.

Water levels in the perimeter ditch at the southwest section were lower than found in the other locations of the ditch (Table 3). On 3/18/2009 the water levels in the southwest section were 1 m lower than found in the southeast portion of the ditch. One reason for this is that a beaver dam occurred between the two ditch locations at a point that was approximately northeast of well 16B. The dam backed water up into the southeast section, and prevented it from reaching the southwestern portion of the ditch. Beaver dams were identified in approximately five locations around the perimeter ditch, and account for the water levels in the perimeter ditch at the southeast, northeast, and northwest locations to be within the critical range where some hydrologic trespass was expected to occur.

CONCLUSIONS

Water table levels in the area outside the southwest section of the Juniper Bay perimeter ditch appear to be higher after restoration than before. The water table was observed in the 0 to 25 cm (0 to 10 in.) depth range for periods up to 103 days after restoration, compared to 26 to 38 days before the Bay was restored. Identification of the actual source of this water was beyond the scope of the study. However, previous work indicated that should water levels in the perimeter ditch rise within or above the estimated critical range, then water would move from the ditch into the surrounding land area. Water levels were observed to be within the critical range along three of the four ditch locations evaluated, but were below the critical range in the southwest sector where the high water tables were observed outside the Bay.

Beaver dams were observed at a number of locations in the perimeter ditch, and we believe these are responsible for the water levels in some portion of the ditch being within the critical range. Such dams should be removed periodically to prevent ditch water from causing hydrologic trespass.

REFERENCE

Huffman, R.L., M.J. Vepraskas, and S. Pati. 2007. Assessment of groundwater flows at Juniper Bay and their impacts on the surrounding area. Final Report for NCDOT Project 2002-19, FHWA/NC/2006-19, NC Dep. of Transportation, Raleigh.

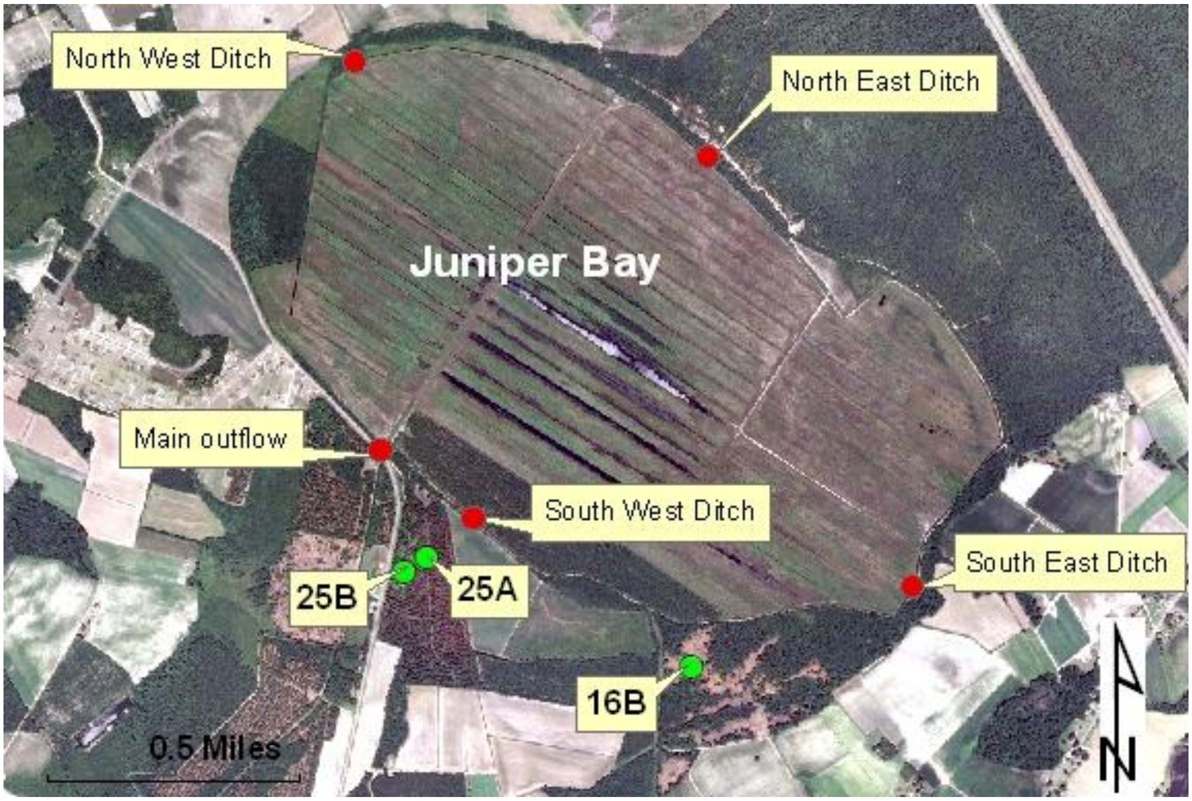

Figure 1. Aerial view of Juniper Bay. The positions along the perimeter ditch of the locations studied by Huffman et al. (2007) (northwest, northeast, southeast, and southwest) are shown. Wells for the current study were placed at the points marked 16B, 25A, and 25B. Water from the perimeter ditch leaves the site at the main outflow point indicated.

Table. 1. Summary of critical ditch control levels as estimated by Huffman et al. (2007). When ditch water levels were above the levels shown, water will flow out of the ditch into the surrounding soil, possibly causing hydrologic trespass on adjacent lands.			
Location along Ditch	Wet Conditions	Dry Conditions	Ditch Top Elevation
	-------------------------------m (ft.) above MSL---------------------------------		
Southwest	35.9 (117.8)	36.1 (118.4)	37.0 (121.4)
Southeast	35.9 (117.8)	36.3 (119.1)	36.7 (120.4)
Northeast	36.3 (119.1)	36.5 (119.8)	36.5 (119.8)
Northwest	36.1 (118.4)	36.3 (119.1)	36.6 (120.2)

Table 2. Comparison of the number of days the water table was within the depth range shown before (2003) and after restoration (2009). Data for the 0 to 25 cm depths suggests that the water table was within this depth range for longer periods after restoration than before.

			Water Table Depths			
Well no.	Dates	Rainfall	Above surface	0 to 25 cm	20 to 50 cm	50 cm
		cm (inches)	-------------------------days-------------------------			
25A	8 Jan. to 14 Oct., 2003	118 (46.4)	12	38	61	169
“	8 Jan. to 14 Oct., 2009	83 (32.6)	0	103	47	130
25B	8 Jan. to 14 Oct., 2003	118 (46.4)	2	26	49	203
“	8 Jan. to 14 Oct., 2009	83 (32.6)	16	103	39	122
16B	16 April to 23 June, 2003	23 (8.9)	0	1	23	45
“	16 April to 23 June, 2009	32 (12.5)	0	20	30	19

Table 3. Water levels in four locations of the perimeter ditch on five dates. Critical water levels (dry-wet conditions) of Huffman et al. (2007) are also shown for comparison.						
Location	**Dates of Measurements**					**Critical Levels**
	3/11/2008	8/12/2008	12/9/2008	3/18/2009	1/6/2010	
	-----------------------------------m above MSL---------------------------------------					
Southwest	35.4	35.2	35.3	35.2	35.5	35.9-36.1
Southeast	36.2	35.6	36.1	36.2	36.2	35.9-36.3
Northeast	36.4	no water	36.4	36.4	36.4	36.3-36.5
Northwest	36.2	no water	36.2	36.2	36.2	36.1-36.3

Chapter 9

PRINCIPLE FINDINGS AND CONCLUSIONS

M.J. Vepraskas

1. **Preferred Hydrology for Tree Species**: Bald cypress, a member of the Non-riverine swamp forest community, was the most tolerant of continuously (300 days) ponded conditions of the four tree species studied. Sweet bay, one of the dominant trees of the Bay Forest community, had close to 100% survival in the continuously ponded treatment, but growth was not significantly different when compared to the other hydroperiods studied. Pond pine seedlings subjected to 300 total days of ponding had a 75% death rate, but those subjected to 84 total days of ponding had a 100% survival rate, indicating that pond pine is better adapted to the drier locations in Juniper Bay.

2. **Best Trees for Organic Soils:** Bald cypress trees grown in ponded organic soils had the greatest overall growth as measured by height, diameter, and shoot biomass of the species examined. The increase in diameter was most likely due to the increase in aerenchyma tissue development in the basal portion of the stem. Bald cypress grew better in continuously ponded conditions than in drier soils, and grew best in organic soils compared to mineral soils. Based on our experiments, bald cypress would grow best in very wet organic soils, especially organic soils which were once in agricultural production that received higher inputs of N and P. It is well suited to the organic soil areas in Juniper Bay.

3. **Trees Best Suited to Ponded Conditions:** The more morphological and anatomical adaptations that are utilized by a species, the greater the likelihood that the species will survive extended periods of ponding. Continuously ponded bald cypress seedlings adapted the best through a variety of morphological adaptations, and ponded sweet bay seedlings produced an extensive network of adventitious roots. The only adaptation of pond pine and swamp chestnut oak seedlings were hypertrophied lenticels on the submerged portion of the stem, which was insufficient and led to a 75% and 87% death rate, respectively. These trees should be used in drier locations. Atlantic white cedar did not survive in some experiments with ponded soils, and should be used cautiously for wetland restoration.

4. **Preferred Tree Planting Methods:** Removing topsoil to fill ditches will affect the growth and survival of some tree species such as pond pine. When topsoil is removed, the scalped lands probably should be fertilized to restore some of the lost P and K. Doing so will have small benefits for bald cypress, but could have large benefits on the survival and growth of pond pine. There was no benefit to planting trees on ridges as opposed to smooth ground.

5. **Loss of P in Drainage Water:** Dissolved P is leaving the restored Juniper Bay in surface drainage water exiting the Bay at the perimeter ditch outflow point. Concentrations of total P have exceeded 0.2 mg/L after large storm events, and management methods should be used to reduce these levels to <0.1 mg/L, which were observed prior to restoration, in order to avoid causing excessive weed and algal growth in downstream waters. The source of the P is residual fertilizer left in the soil after agriculture had ceased. Mechanisms causing the release occur

under anaerobic conditions which are common in wetland soils. Results suggest that it is not possible to prevent the dissolution of P in restored wetlands, but methods to retain the P onsite must be employed. At present, plant uptake appears to be the most viable method of P control.

6. **Controlling water movement through soils will not reduce P loss**: Varying water flow velocities through soils did not result in a significant decrease in the amount of P dissolved and leached out of the soil systems used in laboratory experiments. The concentrations detected at the outlet of experimental flow cells were consistently above the water quality limits and may cause eutrophication in adjacent surface water bodies. These findings matched field observations at the Bay. Much of the P leached out of the system was from 5 cm above the water table and below. The dominant mechanisms believed to contribute to P dissolution in the experimental system include: i) dissolution of surface Fe and Al with concomitant release of P, ii) competitive adsorption of dissolved organic carbon on particle surfaces and displacement of P to the water. These processes will occur in most wetland soils.

7. **Hydrologic Trespass:** Water table levels in the area outside the southwest section of the Juniper Bay perimeter ditch appear to be higher after restoration than before. The water table was observed in the 0 to 25 cm (0 to 10 in.) depth range for periods up to 103 days after restoration, compared to 26 to 38 days before the Bay's drainage ditches were plugged. Identification of the actual source of this water was beyond the scope of the study. However, previous work indicated that should water levels in the perimeter ditch rise within or above the estimated critical range (see chapter 8), then the water would move from the ditch into the surrounding land area. Water levels were observed to be within the critical range along three of the four ditch locations evaluated, but were below the critical range in the southwest sector where the high water tables were observed outside the Bay.

Chapter 10

RECOMMENDATIONS AND TECHNOLOGY TRANSER PLAN

RECOMMENDATIONS

1. The perimeter ditch must not be plugged or dammed to avoid Juniper Bay becoming too wet, and to avoid water seeping into surrounding areas and causing hydrologic trespass. Beaver dams were observed at a number of locations in the perimeter ditch, and we believe these are responsible for the water levels in some portion of the ditch being within the critical range where problems will develop. Such dams should be removed periodically to prevent ditch water from causing hydrologic trespass. A permanent beaver maintenance program should be supported.

2. Phosphorus is leaving the restoration site in drainage water, and will contribute to eutrophication of downstream waters. The problem should diminish in time, but action taken now could lessen the impact of the current losses. A porous dam structure composed of carbonate rocks could be installed at the outflow point to absorb P.

3. Dead trees should be replaced if at all possible, to help reduce the loss of P from the bay. Bald cypress is a recommended tree for the organic soils in the wettest areas of the Bay. Pond pine is also well adapted to the drier mineral soil areas. Atlantic white cedar and sweet bay are two trees that should not be planted.

4. Trees should be planted on smooth ground. If the original topsoil is present, then no additional soil treatment should be necessary. In areas where the topsoil has been removed to fill ditches, an application of fertilizer could increase the rate of tree growth.

5. To avoid the possibility of hydrologic trespass becoming a problem, ditch water levels should be monitored and maintained below the critical levels described in chapter 8. In addition, water table monitoring should be continued outside the Bay in the southwest section where hydrologic trespass is expected to begin if it occurs at all.

TECHNOLOGY TRANSFER PLAN

This information will be disseminated through research articles, presentations at professional meetings, and field trips. Information in chapters 2, 3, 4, 6, and 7 of this report each represent a scientific article that will be submitted for publication.