

Nutrient Uptake and Plant Selection in Southeastern Rain Gardens

by

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Abstract

A bioretention rain garden is an innovative stormwater management practice that integrates stormwater infiltration and storage to improve the quality of runoff. *Part 1.* Two bioretention rain garden designs constructed at the Donald E. Davis Arboretum on the Auburn University campus were compared, one using conventional (CONV) treatment through filtration and adsorption, and the other incorporating an internal water storage (IWS) layer. Contaminant removal from stormwater was observed in warm and cool seasons. The CONV rain garden (RG) provided improved removal of nutrients when compared to a rain garden with an IWS layer. Even when rain gardens included evergreen and perennial plants, a total net loss (release) of nutrients occurred during perennial die back during the cool season. Warm season plant uptake of nutrients was greater than cool season plant uptake in both rain gardens, indicating that evergreen species used in place of perennial plants may provide increased winter uptake. *Part 2.* Because rain garden plants must be able to tolerate repeated short intervals of flooding, research was conducted to screen five native landscape shrub taxa for tolerance to repeated flooding events. *Fothergilla ×intermedia* L. ‘Mt. Airy’ (dwarf witchalder), *Ilex verticillata* (L.) A. Gray ‘Winter Red’ (winterberry), *Clethra alnifolia* L. ‘Ruby Spice’ (summersweet), *Callicarpa dichotoma* (Lour.) K. Koch ‘Early Amethyst’ (purple beautyberry), and *Viburnum nudum* L. Brandywine™ (possumhaw) were flooded in 11.3 L (3 gal) containers in a greenhouse. Root zones of plants were flooded to the substrate surface for 0 (non-flooded), 3, or 6 days followed by 6 days of draining with no

additional irrigation. *Run 1.* Flooding duration did not affect growth index (GI), shoot dry weight (SDW), or root dry weight (RDW) of *I. verticillata* ‘Winter Red’ and *V. nudum* Brandywine™. Growth index was lower in plants flooded for 6 days for *C. alnifolia* ‘Ruby Spice’, but flooding duration did not affect SDW or RDW. Growth index and RDW of *C. dichotoma* ‘Early Amethyst’ were not affected by flooding treatments, however SDW decreased. Growth index of *F. ×intermedia* ‘Mt. Airy’ decreased with increasing flood duration, and SDW and RDW were lower in plants flooded for 6 days. Generally, the lowest values for photosynthesis (P_n) and stomatal conductance (SC) occurred in plants flooding or draining for 6 days. Photosynthesis and SC were lower at 5th flood cycle of the experiment. Stem water potential (SWP) was higher in plants at 5th flood cycle of the experiment. *Run 2.* Growth index and RDW of *F. ×intermedia* ‘Mt. Airy’ were lowest in plants flooded for 6 days. The SDW of *I. verticillata* ‘Winter Red’ was highest in plants flooded for 6 days and in *V. nudum* Brandywine™ the GI was highest in plants flooded for 6 days. Overall, in both runs, all taxa, with the exception of *F. ×intermedia* ‘Mt. Airy’, maintained good visual quality, did not have any reduction in RDW, and exhibited minimal effects of flooding on shoot growth. *F. ×intermedia* ‘Mt. Airy’ exhibited poor visual quality, with growth adversely affected by flooding. Conversely, all other taxa appeared tolerant of flooding and would be appropriate native shrub selections for rain gardens. *Part 3.* Rain gardens are recommended as a way to remove pollutants from runoff. A pollutant of particular concern for waterways is phosphorus. Research was conducted to evaluate phosphorus (P) uptake by and growth of the native grass *Muhlenbergia capillaris* in flooded and non-flooded conditions. *Muhlenbergia capillaris* (Lam.) Trin. (gulf muhly grass) was placed into 3.8 L (1 gal) containers and root zones were flooded for 0 or 3 days and drained for 6 days. *Run 1.*

Height (HT), SDW, and RDW were higher in non-flooded plants than flooded plants. Shoot dry weight increased linearly with an increasing P irrigation rate, while RDW changed cubically with increasing P irrigation rates. Phosphorus concentration in leachate increased linearly with an increasing P irrigation rate in non-flooded plants at the 1st flood cycle of the experiment. At the 5th flood cycle of the experiment, phosphorus concentration in the leachate of non-flooded plants increased linearly with increasing P irrigation rate and in flooded plants the phosphorus concentration in leachate changed quadratically with increasing P irrigation rates. *Run 2.* Plant HT, SDW, and RDW were higher in non-flooded plants than flooded plants, and plants with phosphorus fertilizer added to substrate had higher HT, SDW, and RDW than plants without phosphorus fertilizer added to substrate. Phosphorus concentration in leachate was highest in non-flooded or flooded plants with phosphorus fertilizer added to the substrate at the 1st, 3rd, or 5th flood cycles of the experiment. All plants exhibited growth during flooding and appeared tolerant of flooding and would be appropriate native shrub selections for rain gardens.

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Chapter I

Literature Review

Introduction

Water quality is a major focus in many urban areas, particularly stormwater runoff and the pollutants it contains. Stormwater runoff is the primary factor in the degradation of many streams and other water bodies (Hunt et al., 2006; U.S. Environ. Protection Agency, 2000). Common pollutants found in stormwater runoff include bacteria, nutrients, metals, oils, grease, and sediments (LeBleu et al., 2007). Stormwater research has focused primarily on nitrogen (N), phosphorus (P), and heavy metals, including, but not limited to, copper (Cu), lead (Pb), and zinc (Zn) (Davis et al., 2001; Dietz, 2007; Hunt et al., 2006; Pitt et al., 1995; Wu et al., 1998). Rapid urbanization has resulted in an increase of impervious surface areas, thereby increasing the amount of surface runoff. In highly urbanized watersheds, stormwater management infrastructure redirects runoff captured by artificial channels (e.g. road culverts, curb and gutter systems) into stormwater retention and detention facilities or directly to streams (Hogan and Walbridge, 2007). Artificial channels carry runoff to streams, nearby water bodies, or other retention facilities, with minimal time allowed for pollutants to settle out or for water to infiltrate back into the ground. The amount of land area covered by impermeable surfaces can dramatically affect the quality of a state's waters, adjacent wetlands and forests, and other natural areas (Hunt and White, 2001).

Low Impact Development

Low impact development (LID) aims to design, construct, and maintain a development site to protect or restore the natural hydrology (the properties, distribution, and effects of water on the earth's surface) of a site so that the overall integrity of the watershed is protected (Jones, 2007). The LID approach is referred to as creating a “hydrologically” functional landscape, and has been recommended as an alternative to traditional stormwater design (Dietz, 2007). Common LID practices include bioretention areas such as rain gardens, green roofs, grassed swales and pervious pavements (Dietz, 2007). Low impact development is the driving force behind the use of bioretention in many parts of the country (Davis et al., 2009).

Bioretention

Bioretention is a form of LID and a best management practice (BMP) that has the potential to improve stormwater quality in developed areas (Davis et al., 2003). Best management practices are techniques that aim to minimize flooding, erosion, and the amount of metals, nutrients, and bacteria that enter state’s waters (Hunt and White, 2001). Bioretention as a stormwater treatment practice has gained popularity due to its aesthetics, potential to reduce flooding, and early documented improvements to stormwater quality (Hunt et al., 2008). Bioretention typically treats storm water that has run over impervious surfaces at commercial, residential, and industrial areas (U.S. Environ. Protection Agency, 1999). Bioretention was originally designed to minimize surface water runoff volume by slowing runoff velocity and allowing it to infiltrate into the ground, but increasingly it is being used to improve ground water and surface water quality (Morzaria-Luna et al., 2004). Bioretention is an integral part of LID (Hager,

2003), as the practice has the potential to reduce stormwater runoff volumes, minimize peak flows, recharge ground water, increase evapotranspiration, and reduce the mass of pollutants entering surface and ground waters (Hunt et al., 2008). Clogging of the bioretention area may be a problem, especially if it is near an area of that receives runoff with high sediment loads (U.S. Environ. Protection Agency, 1999). Other problems to consider are sloping lands, mature tree removal before or after installation, freezing soils, and unstable soils (U.S. Environ. Protection Agency, 1999).

The use of grass as the sole vegetation type in bioretention has become a controversial issue among many developers (Davis et al., 2009). Concerns include long term permeability, soil compaction and the potential reduction in pollutant removal abilities, even though a grassed bed would be considerably less expensive to install and maintain than other conventional bioretention materials that use native vegetation (Davis et al., 2009). Passeport et al. (2009) studied two *Cynodon Rich. spp.* (Bermuda sod) grassed bioretention cells and found the lowest nutrient event mean concentrations (EMC) (a method for characterizing pollutant concentrations in a receiving water from a runoff event) and highest load reductions occurred during the warm and humid seasons. When considering effluent concentrations in addition to removal rates, the grassed cells showed promising results for fecal coliform (FC) and nutrient pollution abatement when compared to conventionally vegetated bioretention areas (Passeport et al., 2009). Still, only limited research has been done to suggest that bioretention can effectively manage pollutants such as pathogenic bacteria (Davis et al., 2009).

Rain Gardens

A rain garden is a natural or dug shallow depression designed to capture and absorb stormwater runoff from a roof or other impervious surface such as a driveway, walkway, and even compacted lawn area (Univ. of RI, 2009). Rain gardens are an appealing landscape feature designed to a certain standard, with a specific soil profile, vegetation receiving area, and topography (Dussaillant, 2002). Rain gardens are generally used in residential or commercial settings and are typically planted with shrubs, perennials, or trees. They are often covered with a layer of shredded mulch (Dietz, 2007). Rain gardens can be included in new designs or retrofitted inexpensively into existing areas (Prince George's County, MD, 2002). Rain gardens combine environmentally sensitive site design with pollution prevention to form a comprehensive approach to water quality problems (Coffman, 1995). Rain gardens manage stormwater through bioretention, combining physical, biological, and chemical processes to maximize pollutant removal (Coffman, 1995; Hunt 2001). The settling of sediments in shallow areas, the natural processes of plants and microbes, and chemical reactions occurring in the soil allow rain gardens to absorb and purify stormwater runoff (Coffman, 1995). Pollutant treatment in rain gardens has been attributed to adsorption, decomposition, ion exchange, and volatilization (Dietz and Clausen, 2006; Prince George County, MD, 1993). The long-term efficiency of rain gardens is difficult to determine since most rain gardens are still evolving and maturing in relation to their hydrologic, biologic, and chemical settings (Tornes, 2005).

Fill-soil for rain gardens is a crucial component of design because the fill substrate must provide adequate drainage, reduce pollutant levels, and support plant

growth (Hunt and Lord, 2006). According to Hunt and Lord (2006) bioretention substrates should be comprised of roughly 85% sand, 10% fines (both clay and silt) and 5% organic matter. Other rain garden installations have modified Hunt's fill-soil recommendation. Auburn University used the Virginia Department of Forestry's (2007) recommendation for blending organic matter (20%) into a sandy soil (50%) with about 30% of native top soil (LeBleu et al., 2007). The physical and chemical substrate characteristics play an important role in the removal of pollutants, particularly phosphorus (P). Phosphorus content in the original soil of the bioretention area appears to be critical to P removal performance; the variation is dependent upon initial levels of soil P (Davis et al., 2009). The substrate used in bioretention studies in North Carolina have demonstrated ranges of performance for P, from up to 65% removal to the addition of 240% P at various field sites (Davis et al., 2009; Hunt et al., 2006). The large addition of P comes from using a soil with a high P content. The assumption in rain garden use is that sediment, nutrient, and other chemical removal occurs as the runoff comes in contact with the soil, bacteria, and roots of shrubs or other vegetation within the rain garden (Tornes, 2005).

The use of plants to remediate contaminated soils and wastewater has been practiced internationally for some time, but new research is being conducted to determine how effective plants are at removing contamination from waters polluted by both stormwater and wastewater discharges (Ruby and Appleton, 2009; Wang et al., 2002). Phytoremediation is a relatively new technology that uses plants to degrade, extract, contain, or immobilize contaminants such as metals, pesticides, explosives, oil, excess nutrients, and pathogens from soil and water (U.S. Environ. Protection Agency, 2000).

Phytoremediation has been identified as a more cost effective, noninvasive, natural, and publicly acceptable method of removing environmental contaminants than most chemical and physical methods (Arthur et al., 2005). Plant uptake of nutrients and chemicals from soil is a form of immobilization of a pollutant and the uptake process is a vital part of the transpiration process of plants (Novotny, 2003). Vegetation type likely affects the infiltration capacity, nutrient uptake, and evapotranspiration of a rain garden and probably the resulting water quality (Tornes, 2005). It is best to use native, non-invasive plant species that are resistant to stress from both brief periods of flooding during rainfall as well as dry periods in between events (Univ. of RI, 2009). Native plants are those that are indigenous to a region and possess traits that make them uniquely adapted to local conditions, such as climate, moisture, soils, other plants, animals, and insects (DuBois et al., 2009). Native plants can be attractive aesthetically and tend to be hardier and better able to resist drought, insects, and disease when used in locations that approximate their native environments when compared to some exotic species. Native plants are in addition frequently well-suited for the current trend of "low-maintenance" landscaping (DuBois et al., 2009), however, insufficient research has been done to characterize native plants in terms of tolerance to the alternating flooding and drought conditions that rain gardens present. However, many municipalities have compiled lists that contain suitable native plant species for rain gardens (Bannerman and Considine, 2003; U.S. Environ. Protection Agency, 1999). Research is still needed, including information on proper plant selection, choosing an assortment of plants for particular pollutants of concern, understanding mechanisms for nutrient and heavy metal removal, and ideal environments for maximum nutrient uptake and removal (Ruby and Appleton, 2009).

Phosphorus is now one of the most ubiquitous forms of water quality impairments in the developed world and thus has become a concern and important area of research and emphasis (Sharpley et al., 2001). Water contaminated with excess N and P can impact the quality of surface water and groundwater. Phosphorus enrichment of the soil surface layer can result in increased P losses from soils to surface waters when soil particles move downwards to storm drains or are lost as a result of surface erosion (Richards et al., 1998; Ryden et al., 1973). Recent research has shown that a loss of P from soils to surface runoff and subsurface flow originates primarily from small areas within watersheds during a few storms. These areas occur where high soil P or P application in mineral fertilizer or manure coincide with sites of high runoff or erosion potential (Sharpley et al., 2001). Repeated intermittent flooding can result in significant phosphorus release from soils (Olila et al., 1997). Movement of small amounts of P through the soil profile can have adverse affects on water quality and cause excessive enrichment of surface soils (Richards et al., 1998).

Phosphorus Accumulation and Release

Soil sediment characteristics are important since sediments act as either sources or sinks of nutrients to the water column (Olila et al., 1997). Nutrients are either taken up by plants or move through the soil to surface waters or subsurface waters. The effect of flooded soil on P uptake by plants is complicated and is strongly dependent on soil type. In some alkaline soils that are relatively low in P, flooding may cause an increase in P availability, leading to a temporary increase in P uptake concentration in plants. On the other hand, prolonged flooding reduces P concentration and uptake in plants due to root dysfunction, damage, and death (Chen et al., 2005; Kozlowski, 1984b). Chen et al.

(2005) found that after flooding, the concentrations of N, P, potassium (K), and Zn in leaves of flooded plants were lower than in control plants, however, N, P, K and Zn concentrations in roots of flooded plants were slightly higher than in non-flooded plants. Rubio et al. (1997) found that waterlogging reduced root: shoot ratios but did not reduce total P concentration in the plant. Rubio et al. (1997) also found P fertilization of waterlogged plants increased shoot P concentration. Soil P availability was higher during waterlogging periods, and the roots showed a morphology more favorable to nutrient uptake with a higher intrinsic capacity to absorb P (Rubio et al., 1997).

Sources of Phosphorus

Phosphorus is a critical nutrient for all forms of life, but like N, P that enters the environment from anthropogenic sources may exceed the needs and capacity of the terrestrial ecosystem (U.S. Environ. Protection Agency, 2009b). Nonpoint P and N pollution is caused primarily by agricultural (fertilizer and manure) and urban activities (Novotny and Olem, 1994; Sharpley et al., 1994). A significant amount of N and P enters surface waters from urban nonpoint sources such as car washes, construction sites, runoff of lawn fertilizers, pet wastes, and inputs from unsewered developments. Urban point sources include sewage and industrial discharges, which are heavily monitored (Carpenter et al., 1998). The most common sources of P in rivers are fertilizer and wastewater, including stormwater and treated wastewater discharged directly into the river (U.S. Environ. Protection Agency, 2009b).

Problems Associated with Phosphorus Contamination

Phosphates are nutrients that cause rapid growth of algae in bodies of water. As the algae decay, the process depletes the supply of oxygen and results in eutrophication

or premature "aging" of a lake or pond (U.S. Environ. Protection Agency, 2009a). Eutrophication caused by excessive inputs of N and P is the most common impairment of surface waters in the United States (U.S. Environ. Protection Agency, 1990). Impairment is measured as the area of surface water not suitable for designated uses such as drinking, irrigation, industry, recreation, or fishing (Carpenter et al., 1998). Excess P may contribute to unsightly algal blooms, which cause taste and odor problems and deplete oxygen required by fish and other aquatic species (U.S. Environ. Protection Agency, 2009b). Although P management is an integral part of profitable agrisystems, continued inputs of P in fertilizer and manure in excess of crop requirements have led to a build-up of soil P levels in some areas, which are of environmental rather than agronomic concern, particularly in areas of intensive crop and livestock production (Sharpley et al., 1994).

Phosphorus Adsorption to Soils

Phosphorus can be removed in constructed wetlands by plant uptake, assimilation by microorganisms, and physico-chemical processes involving the wetland soil. Among the physico-chemical processes, sorption by soil and precipitation reactions plays an important role (Del Bubba et al., 2003; Vymazal et al., 1998). Substrates can both adsorb phosphate ions and/or promote precipitation by supplying the solution with metals, which can react with phosphorus to produce sparingly soluble phosphates. In addition, calcium (Ca) present in the wastewater itself can promote phosphorus precipitation (Del Bubba et al., 2003; Maurer et al., 1999). However, the adsorption process can be studied only in the absence of phosphate precipitation (Del Bubba et al., 2003). Variations of pH, ionic strength, and concentration of competitive ions are known to strongly influence the adsorption process (Del Bubba et al., 2003; Polyzoopoulos et al., 1985). Del Bubba et al.

(2003), using a sorption-isotherm study found that the removal of P varied greatly among the types of sands studied, but it was particularly high in the sands with the greatest concentrations of Ca. Among the physico-chemical properties of the sands, Ca and magnesium (Mg) content, grain size, porosity, bulk density, and hydraulic conductivity were also significantly related. Zhou et al. (2005) also reported P removal varied considerably according to the type of sediment, but was found to be particularly high for the sediments which contained the highest concentrations of Fe, Al, and Ca. Furumai and Ohgaki (1989) found an increase in pH accelerated dissolution of the phosphorus compounds in sediments and the amount of released phosphorus ranged from 11.4% to 14.5% of total phosphorus in sediments for pH ranging from 4.7 to 7.4, respectively.

Studies on the Saugahatchee watershed surrounding Auburn, Alabama have reported P levels in the soil and water to be $85 \mu\text{g}\cdot\text{L}^{-1}$ for urban areas and $25 \mu\text{g}\cdot\text{L}^{-1}$ for rural areas (Bayne et al., 2004). Rubio et al. (1997) used fertilization rates of 0 and 73 mg P per pot (18 cm wide, 18 cm high, 4.6 L, $15.9 \text{ mg}\cdot\text{L}^{-1}$) as superphosphate. *Paspalum dilatatum* Poir. (dallisgrass), a waterlogging-tolerant grass from the Flooding Pampa, Argentina was flooded to a water depth of 20 mm above the soil surface and the experiment used native topsoil (Rubio, 1997). Ristvey et al. (2007) used 0, 5 and 25 mg/week in a greenhouse study to evaluate N and P uptake efficiency using pine bark substrate and young azaleas in 7.6 L (2 gal) pots with fertilizer applied to each container in 250 mL aliquots once per week. Elcan and Pezeshki (2002) used *Taxodium distichum* (L.) Rich. (bald cypress) seedlings in pots filled with field soil (Falaya silt loam) in a greenhouse that were fertilized weekly with 20-20-20 % N:P:K, respectively; no

concentration was given. Flooded soils were maintained at a water level 5 cm above the soil surface.

Flooding Responses in Plants

Flooding of soil or substrate depletes soil oxygen and alters plant metabolism (Kozlowski, 1984b). Upon flooding, the limited supply of oxygen is depleted rapidly by roots, microorganisms, and soil reductants (Pezeshki, 2001; Ponnampereuma, 1972). In many wetland plants, an extensive oxygen transport system (aerenchyma tissue) may exist in roots, stems, and leaves (Pezeshki, 2001; Armstrong et al., 1994). Aerenchyma allows a plant to transport to the roots above ground oxygen needed for maintaining aerobic respiration and oxidizing reducing compounds in the rhizosphere (Pezeshki, 2001). Responses to flooding therefore will vary with the type of plant, the duration and timing of flooding, and the condition of the flood water. Flooding during the growing season is much more harmful than flooding during the dormant season (Kozlowski, 1982). Moving flood water is less harmful than standing water, as even flood tolerant plants are injured by flooding in standing water (Kozlowski, 1982). In *Nyssa sylvatica biflora* (Walt.) Sarg. (swamp tupelo) and *N. aquatica* L. (water tupelo), stagnant water restricts or inhibits adjustment of seedlings to flooded conditions, whereas in moving flood water, seedlings were able to recover in height and growth characteristics (Hook et al., 1969). The water in the stagnant treatments was brownish, had an oily appearance on the surface, and gas bubbles escaped from the soil. The moving water treatments expressed no negative physiological effects (Hook et al., 1969).

Upon flooding, a flood-tolerant species will generally acquire more minerals than flood-intolerant species (Chen et al., 2005; Pezeshki et al., 1999). Plant responses to

flooding during the growing season include injury, inhibition of seed germination and vegetative and reproductive growth, changes in plant anatomy and promotion of early senescence and mortality (Kozlowski, 1997). Morphological adaptations to flooding include formation of aerenchyma tissue, hypertrophy of lenticels (openings in stems and roots that permit gas exchange between internal tissues and the atmosphere), and regeneration of new root structures (Kozlowski, 1984a).

Types of Flooding

The influence of different flooding regimes on plant growth has been investigated in greenhouse experiments. Arbona et al. (2009) used continuous flooding or flooding and recovery methods to test photosynthetic performance on *Citrus* L. spp. as it relates to flooding under greenhouse conditions. Control plants were watered regularly, while others were continuously flooded by submerging plants in 4 L containers filled with tap water so the water level was 2 cm above the soil surface for 33 days. Arbona et al. (2009) also used the flooding and recovery method, where plants experienced continuous soil flooding for 7 days. At the end of this period, the excess water was drained and plants were allowed to recover for 7 days. Elcan and Pezeshki (2002) also used continuous flooding (62 days) in addition to flooding followed by drought (flooded plants followed by drought for 5 days) and control followed by drought (well watered then 5 days of drought). Mielke and Schaffer (2009) used continuous flooding for 35 days to study the photosynthetic and growth responses of *Eugenia uniflora* L. seedlings to soil flooding and light intensity. Liu and Dickman (1992) used cyclic flooding and draining to look at abscisic acid accumulation in leaves of flooded *Populus* L. species with water withheld from plants for eight to ten days so that soil matric potential reached about -50

kPa. Permanent wilting point is reached at -1500 kPa. At -50 kPa, the soil was re-watered to field capacity (-33 kPa) and allowed to dry down again to -50 kPa. The flooding treatment was accomplished by immersing pots in plastic bags filled with tap water. Arbona et al. (2009) studied seedlings of citrus, *Citrus paradisi* (L.) Macf. × *Poncirus trifoliata* (L.) Raf. (Citrumelo CPB 4475) (Cit), *Citrus sinensis* (L.) Osb. × *P. trifoliata* (L.) Raf. (Carrizo citrange) (CC) and *Citrus reshni* (Hort.) ex Tan. (Cleopatra mandarin) (CM) for different flood tolerances. Visible leaf damage, net CO₂ assimilation rate (A) and stomatal conductance (g_s) were measured. Under continuous soil flooding, leaf damage was seen at 5% for CC leaves, 25.7% in Cit and 100% in Cm. Stomatal conductance was significantly decreased by flooding in all studied genotypes but to different extents and at different times. In general, leaves of CM and Cit had higher A and g_s than leaves of CC. Flooding decreased A in Cit and CM plants but it did not significantly affect A in CC. Under flooding and recovery, CM showed significantly decreased A values whereas Cit and CC exhibited slightly but significantly increased values. However, after continuous soil waterlogging and subsequent drainage, only CC fully recovered and had A values similar to those found in well-watered plants. On the contrary, in flooded Cit and CM seedlings A remained at low levels even after 7 days of recovery. Stomatal conductance was not affected by soil flooding in CC whereas in Cit, it significantly increased and in Cleopatra it decreased. After recovery, g_s in previously flooded CC seedlings did not show any significant variation whereas in Cit, g_s remained lower than controls and in CM, it recovered up to control levels. Most trees have growth reductions, reduced P_n from a period of weeks to months, and leaf fall increases during the high water period, but leaf flush, flowering and fruiting also occur during the

waterlogging period (Parolin, 2001). Overall, the extent of the damage from flooding appears to vary from plant to plant but many of the same symptoms are observed. Each species responds differently and a large percentage are able to recover from the flooded state.

Effects of Drought on Plants

With a growing population, water will become an even scarcer commodity in the future. Therefore, a better understanding of the effects of drought on plants is vital for improved management practices and for predicting the long-term fate of natural or planted vegetation (Chaves et al., 2003). Water stress can decrease growth directly through its effect on turgor, especially since turgor is the main driving force for cell elongation (Elcan and Pezeshki, 2002). Drought stress is not evident in plants growing in a drying soil until a critical soil water potential is reached (Aronson et al., 1987; Penman, 1948; Holmes, 1961; Richards and Marsh, 1961). Drought stress will affect visual quality, growth rate, evapotranspiration (ET) rate, and recuperative ability following drought-induced dormancy (Aronson et al., 1987; Beard, 1973). In nature, plants can either be subjected to slowly developing water shortages (days to months) or face short term water deficits (hours to days) (Chaves et al., 2003). Fast or slow desiccation can have completely different results in terms of physiological response or adaptation (Chaves et al., 2003; McDonald and Davies, 1996). Whether it is a short term or long term deficit, generally, plants respond to drought in some of the same ways as flooding; stomatal closure (Elcan and Pezeshki, 2002; Kozłowski and Pallardy, 1997; Pezeshki and Chambers, 1985; Smith and Ager, 1988) and decreases in net photosynthetic rate (P_n) (Elcan and Pezeshki, 2002). Elcan and Pezeshki (2002) reported that flooding did not

have any significant effects on net P_n . However, after the onset of drought, P_n was significantly reduced in control-drought plants and flood-drought plants and reduced root mass was also noted.

Stomatal Closure and Abscisic Acid

Physiological responses to waterlogging are often considered to be similar to those induced by drought and many plants. Even plants not adapted for wetland conditions can respond to anaerobic root conditions by closing stomata to restrict water loss (Bradford and Hsiao, 1982). Stomatal closures together with leaf growth inhibition are among the earliest responses to drought, protecting the plants from extensive water loss. Stomata closure is likely to be mediated by chemical signals traveling from the dehydrating roots to the shoots (Chaves et al., 2003). Bradford and Hsiao (1982) found that stomatal conductance and transpiration are reduced by 30% to 40% after approximately 24 hours of flooding. Stomatal closure during water stress is believed to be mediated by abscisic acid (ABA) (Radin, 1984; Raschke, 1975; Hsiao, 1973). Abscisic acid is synthesized in the shoot and root in response to various stresses including drought, low temperature, and hypoxia (Chaves et al., 2003). Radin (1984) found a link between nutrient concentrations and stomatal sensitivity to water stress and ABA concentrations. Low P concentrations not only sensitized stomata to water stress, it also increased the accumulation of ABA during drying. Leaves of P-deficient plants accumulated more ABA in response to water stress, but the difference was evident only at low water potentials, after initiation of stomatal closure (Radin, 1984). In contrast, Else et al. (1996) found that there may not be a connection between stomatal conductance

and ABA. Increased levels of ABA could be due to interferences of other substances found in plants, which have not yet been identified.

Root Respiration

Upon flooding, the most important change in the plant environment is the depletion of soil oxygen, which inhibits the normal respiration of plants and other aerobic organisms (Rubio et al., 1997). The initial effects of flooding in plants are found in the root system (Blom, 1999). The depletion of oxygen results in a shift from aerobic to anaerobic microbial processes, which leads to reduction of oxidized compounds and the production of phytotoxic compounds in flooded soils (Blom, 1999; Ernst, 1990; Laanbroek, 1990; Smolders et al., 1997). The most important response in roots to extended periods of flooding is the formation of aerenchyma (aerated tissue) either by cell collapse (lysigeny) or by the enlargement of intercellular spaces resulting from cell separation without collapse (schizogeny) (Armstrong et al., 1994; Blom and Voesenek, 1996). Roots will also lose oxygen to the surrounding soil or sediment by means of radial oxygen loss (ROL). The rhizosphere and root tissues are in competition for the cortically transported oxygen. In fact, parts of the roots may be denied oxygen because of competition from the rhizosphere (Blom, 1999). Root conductance to water uptake decreased under waterlogged or anaerobic conditions (Bradford and Yang, 1981). Bradford and Hsiao (1982) reported that within 8 hours of flooding there was a 50% reduction in sap flow, indicating a halving of root conductance.

Conclusion

A major environmental concern due to dispersal of industrial and urban wastes generated by human activities is the contamination of soil (Ghosh and Singh, 2005) and

water. Through stormwater runoff, urban areas further contribute nonpoint source pollutants such as sediment, nitrogen, phosphorus and heavy metals, impairing downstream habitat and water quality (Dietz and Clausen, 2005; Novotny and Olem, 1994). While erosion of particulate P from agricultural soils remains a dominant concern, the transport of dissolved P or soluble P in surface runoff and subsurface flow is also a critical environmental issue (Sharpley et al., 2001). There is a lack of research and replicated study regarding the use of landscape plants for phytoremediation of P and other pollutants, which emphasizes the need for this research. This research seeks to quantify some of the effects of stormwater flooding on select plants in a bioretention environment and to identify novel solutions to concerns with nonpoint source pollutants, especially P, that incorporate plant and soil processes, thereby improving water quality.

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Chapter II

Evaluating Nutrient Uptake in a Bioretention Rain Garden

Index Words: Rain garden, flood tolerance, landscape

Abstract: A bioretention rain garden is an innovative stormwater management practice that integrates stormwater infiltration and storage to improve the quality of runoff. Two bioretention rain garden designs constructed at the Donald E. Davis Arboretum on the Auburn University campus were compared, one using conventional (CONV) treatment through filtration and adsorption, and the other incorporating an internal water storage (IWS) layer. Contaminant removal from stormwater was observed in warm and cool seasons. Even when rain gardens included 30% evergreen plants and 40% perennial plants, a total net loss (release) of nutrients occurred during perennial die back during the cool season. The CONV rain garden (RG) provided improved removal of most nutrients when compared to a rain garden with an IWS layer. Warm season plant uptake of nutrients was greater than cool season plant uptake in both rain gardens, indicating that evergreen species used in place of perennial plants may provide increased winter uptake.

Introduction

Water quality, particularly of stormwater runoff and the pollutants it contains, is a major focus for researchers, municipalities, and homeowners in many urban areas. Stormwater runoff is the primary factor in the degradation of many streams and other water bodies (Hunt et al., 2006; U.S. Environ. Protection Agency, 2000). Common

pollutants found in stormwater include bacteria, nutrients, metals, oils, grease, and sediments (LeBleu et al., 2008). Rapid urbanization has resulted in an increase of impervious surface areas, thereby increasing the amount of surface runoff (Hogan and Walbridge, 2007).

Low impact development (LID) is a best management practice (BMP) that aims to design, construct, and maintain a developed site to protect or restore the natural hydrology so that the overall integrity of the site and the watershed is protected (Jones, 2007). Best management practices and LID began as a way to mitigate the negative effects of increasing urbanization and impervious surfaces (Dietz, 2007). Bioretention is an integral part of LID (Hager, 2003). Bioretention has gained popularity due to its aesthetic appeal, potential to reduce flooding, and early documented improvements to stormwater quality by reducing stormwater runoff volumes, minimizing peak flows, recharging ground water, increasing evapotranspiration, and reducing the mass of pollutants entering surface and ground water (Hunt et al., 2008). Bioretention was originally designed to minimize surface water runoff volume by slowing runoff velocity and allowing the water to infiltrate into the ground, but increasingly bioretention has been used to improve ground water and surface water quality (Morzaria-Luna et al., 2004).

A rain garden, one form of bioretention, is a natural or dug shallow depression designed to capture and absorb stormwater runoff from a roof or other impervious surface such as a driveway, walkway, and even compacted lawn area (Univ. of RI, 2009). Rain gardens are designed to capture the first 0.5 to 1 in of stormwater, allowing runoff to soak into the soil where treatment is facilitated by microbial activity. The first flush or first inch of stormwater is generally considered to contain the highest concentration of

pollutants. Pollutant treatment in rain gardens has been attributed to adsorption, decomposition, ion exchange, and volatilization (Dietz and Clausen, 2006; Prince George's County, MD, 1993). Rain gardens are an appealing landscape feature designed to a certain standard, with a specific soil profile, vegetation receiving area, and topography (Dussailant, 2002). Rain gardens are generally used in residential or commercial settings and are typically planted with shrubs, perennials, or trees then covered with a layer of shredded mulch (Dietz, 2007). According to the Virginia Department of Forestry (2007), when constructing rain gardens, native top soil can be blended with organic matter and sand to achieve approximately 50% sand fraction. Rain gardens can be included in new designs or retrofitted inexpensively into existing areas (Prince George's County, MD, 1993).

Two examples rain garden construction strategies are conventional (CONV) rain gardens and rain gardens that make use of an internal water storage (IWS) system. Conventional rain gardens are designed to prevent saturated layer formation, which also inhibits denitrification and allows pollutants to be treated by means of interception, storage, filtration, and adsorption (Fig. 1) (U.S. Environ. Protection Agency, 1999). Rain gardens with an IWS layer allow a saturated, reduced environment, with the intention of removing some of the nitrate (NO_3) by denitrifying it into nitrogen (N_2) gas (Hunt et al., 2006). Currently, very little information exists regarding the difference in these two systems in terms of nutrient retention (soil adsorption) and plant removal potential, yet there may be differences in nutrient removal by different plant species that can be used in rain gardens. In order to more fully understand nutrient retention and removal in rain gardens, it would be helpful to understand how nutrient removal varies at different times

of year, under different rain garden systems, and with different plant species. Therefore, the objectives of this research were to 1.) monitor and compare nutrient and metal concentrations in stormwater entering and exiting two rain garden systems (CONV and IWS), and 2.) monitor and compare concentrations of nutrients and metals in each rain garden in plant tissue and soil.

Materials and Methods

Two rain gardens were constructed at the Donald E. Davis Arboretum at Auburn University (AU) in Auburn, AL (Figure 2). Construction of the two rain gardens was completed in the summer of 2006. Each rain garden was approximately 1.2 m (3.9 ft) deep with a surface area of 21 m² (226 ft²). Rain gardens had a trapezoidal shape approximately 5 m (16.4 ft) long on the top and 9 m (29.5 ft) along the base by 3 m (10 ft) wide. A 23 cm (9 in) high continuous 0.3 m (1 ft) wide earthen berm was created around the perimeter of each rain garden to prevent surface runoff from entering the rain garden and allow surface ponding. The natural drainage area of each rain garden was delineated and the rain gardens were designed to hold the amount of water a 2.5 cm (1 in) storm would produce. The rain gardens were sized to accommodate a total runoff volume from the drainage area to a ponded depth of 15.2 cm (6 inches). After excavation, native soil (Marvyn sandy loam) was mixed with 50% sand and 20% organic matter fill soil and shredded pine bark mulch (Wilson's Woodyard, Opelika, AL) to improve both the drainage and organic matter content of the rain garden substrate (Table 1). This substrate was used to fill in the rain garden and was spread over approximately 7.5 m (25 ft) of 10 cm (4 in) slotted drain tile (Ewing Irrigation and Landscape Products, Montgomery, AL) installed inside a 15 cm (6 in) layer of gravel laid over a 1.4 mm (45

mil) impermeable rubber pond liner (Pond Wise, Inc., Huntsville, AL). The liner also extended up the sides of the rain garden in order to contain all water received.

The CONV RG treated pollutants by having an aerobic (oxygen-rich), unsaturated zone. A 10 cm (4 in) drain pipe was connected to the slotted drain tile in bottom 15 cm (6 in) of the rain garden and extended out from the rain garden drainage outlet through the soil to allow water to exit the rain garden and be collected at the collection site. The IWS RG resulted in partially saturated soil conditions by attaching an elbow and pipe to the outlet at the collection site, which raised the water level in the rain garden 0.46 m (1.5 ft) above the top of the gravel drain layer. The purpose of the 0.46 m IWS layer was to reduce nitrogen within the saturated, anaerobic (oxygen-deprived) zone through denitrification (Hunt et al., 2006). The impermeable layer and raised drainage outlet were sealed with a rubber gasket attached to the outlet pipe, which permitted capture of all applied runoff water. Water collected from the drainage outlet is hereafter referred to as outflow.

In May 2008, each rain garden was planted with *Dryopteris erythrosora* Adans. (autumn fern), *Carex lurida* Wahlenb. (shallow sedge), and *C. comosa* Boott (perennial sedge). *Iris* L. sp. (iris) had been planted previously in each rain garden during the original construction in 2006. The CONV RG was planted in 2006 with plants suited for shade, *Itea virginica* L. (sweetspire) and *Hibiscus coccineus* L. (swamp hibiscus). The IWS RG was planted in 2006 with three sun-loving species, *Echinacea purpurea* (L.) Moench (purple cone flower), *Rudbeckia fulgida* Aiton (orange coneflower), and *Muhlenbergia capillaris* (Lam.) Trin. (muhly grass). The substrate surface in each rain

garden was covered with a shredded pine bark mulch layer 3 to 4 inches deep to conserve water and reduce weed growth.

Stormwater Application

A flooding event was simulated in a rain garden using a 0.37 kW (½ hp) submersible pump to transfer water from the existing stormwater (SW) pond to each of the two bioretention rain gardens. The SW pond is approximately one-quarter of an acre, is located in the southern portion of the arboretum, and receives runoff from adjacent roads, buildings, and green areas. Seven 7.6 L·m⁻¹ (2 gpm) and one 3.8 L·m⁻¹ (1 gpm) landscape bubblers (Rainbird[®] 1400 series, Ewing Irrigation and Landscape Products, Montgomery, AL) were fixed onto 15.2 cm (6-inch) pop-up spray heads installed within each rain garden. Bubblers applied 3407 L (900 gallons) of water to each rain garden at each event at an operating rate of 56.8 L·m⁻¹ (15 gpm) to simulate stormwater runoff onto the rain garden surface. The time was recorded when water began to flow from the bubblers into the rain garden. Bubblers were operated for 60 minutes to apply a consistent volume of water to each rain garden and at each runoff event. A water meter located in the 5 cm (2 in) PVC supply line verified a consistent flow rate into each rain garden. If flow rate was lower than expected due to partial clogging of bubblers, the run time was lengthened so that the total 3407 L (900 gallons) was applied to each rain garden at each event. The time between runoff application and steady outflow from the drain were recorded. A V-notch weir (Thel-Mar, NC) was attached to each 10 cm (4 in) rain garden drain pipe outlet with a small air line connected to the bubbler flowmeter to log flow rate.

Water Sample Collection

Water samples (1.5 L) were collected once per week by hand from a single location in the stormwater runoff pond before each simulated runoff event. The outflow samples from rain garden drain outlets and flow rates were automatically collected and recorded at the drain outlet via an automatic sampler (Model 3700, ISCO, CA) and bubbler flowmeter (Model 4230, ISCO, CA). Discrete 250 mL samples were collected automatically at 15 minute intervals after water began to drain from the rain garden. Four samples per hour were combined to make up one 1 L sample. After 24 hours of outflow collection, a flow-weighted composite sample was made for analysis of event mean nutrient and metal concentrations. The flow-weighted samples (constant time - volume proportional to flow rate) were taken at equal increments of time and were composited proportional to the flow rate at the time each sample was taken (U.S. Environ. Protection Agency, 1997b). Equal volume samples at equal time intervals were collected into sample containers and composited after all samples were collected. A 1 L sample was made by taking a volume from each sample bottle that was proportional to the average flow rate during sample collection. If the highest flow rate occurred when samples were collected in bottle 4, the largest sample was taken from bottle 4, and if the lowest average flow rate occurred when the samples collected were in bottle 24, the smallest sample was taken from bottle 24; so as the flow from the rain garden increased or decreased, the amount of water used per sample bottle was adjusted accordingly to make up the flow-weighted sample, proportional to the volume collected.

Water samples were collected nine times during the warm season (July to October 2008) and four times during the cool season (November 2008 to February 2009) in the

IWS rain garden and eight times during the warm season (month to month year) and 11 times (month to month year) during the cool season in the CONV rain garden (Table 2). Fewer samples were collected from the IWS RG during the cool season sampling as a result of a tree that fell into the rain garden that temporarily damaged the sprinkler application system. Water samples were collected on a weekly basis, alternating between rain gardens.

Infiltration Rate Determination

Soil infiltration testing was performed on each rain garden (ASTM Standard D3385 - 03). The double-ring infiltrometer method consisted of driving two open cylinders, one inside the other, into the ground to a depth of six inches for the outer ring and four inches for the inner ring. The inner ring was used to measure the rate of infiltration. The inner ring was filled with water up to a designated height above the substrate surface then the outer ring was filled to the same height. Using a hook gage, the initial water level height was measured then the water level was allowed to drop and the water level was measured again after a designated amount of time. The rings were filled back up to the initial height after each measurement. This procedure was repeated every 15 minutes during the first hour, every 30 minutes during the second hour, and every 60 minutes for at least the next 6 hours or until after a relatively constant infiltration rate was obtained.

Plant and Soil Sample Collection

Plant and soil samples were taken on the same dates as outflow was sampled (Table 2). Planting design in each rain garden utilized equal areas of perennial (warm season) and evergreen (cool season) plants (Figure 3). Only plants that were located in

both rain gardens were sampled (Table 3). Samples of leaves and stems were severed at the substrate level from five plants within a species were combined to make one plant sample per species per sampling date. Samples were collected from *Iris* sp., *D. erythrosora*, and *C. lurida*, and *C. comosa*. Soil samples were taken using a soil probe at a depth of 25 cm (10in) from 5 locations within the rain garden. The five soil samples were combined into one composite soil sample per rain garden per sampling date. In November 2009, after the study was completed, an additional soil sample was collected from each rain garden in an effort to quantify the entire nutrient load found in the rain garden. Samples were collected at four locations in the rain garden at a depth of 1 m (3 ft) and combined into one composite sample per rain garden. The sample was taken as a cross sectional deep core soil sample of the entire rain garden soil profile.

Water, Plant, and Soil Sample Analyses

Water samples from the stormwater runoff pond were analyzed for concentrations of total Kjeldahl nitrogen (TKN), nitrate-nitrite ($\text{NO}_3\text{-NO}_2$), total phosphorus (TP), aluminum (Al), boron (B), copper (Cu), iron (Fe), manganese (Mn), sodium (Na), and zinc (Zn). Rain garden outflow samples were analyzed for concentrations of TKN, $\text{NO}_2\text{-NO}_3$, and TP. Outflow samples from three dates (July, October, and February) were analyzed for concentrations of Al, B, Cu, Fe, Mn, Na, and Zn. All water analyses were completed by Environmental Resource Analysts (ERA), Inc. (Auburn, AL). Metal concentrations were determined using inductively coupled argon plasma spectroscopy (ICAP) (EPA Method 200.7). Total Kjeldahl nitrogen, $\text{NO}_3\text{-NO}_2$, and TP were detected colorimetrically using EPA Methods 351.2, 353.2, and 365.4, respectively. Laboratory analysis of water samples indicated concentrations of some constituents below the

minimum detection limit (MDL). In these cases, a proxy value of one-half the MDL was assigned. The MDLs used are as follows: TKN (0.380), NO₃-NO₂ (0.774), and TP (0.0130).

Plant samples were analyzed for total percent nitrogen (%N, dry basis), total percent phosphorus (%P, dry basis), and concentrations of Al, B, Cu, Fe, Mn, Na, and Zn (ppm). Plant samples were analyzed for phosphorus and metals by dry ashing and using ICAP via a Varian Vista-MPX Axial Spectrometer (Isaac and Johnson, 1985; Odom and Kone, 1997). Nitrogen was detected using combustion analysis (Elementar Vario Macro CNS Analyzer, 1997) (Columbo and Giazzi, 1982). Soil samples were analyzed for concentrations of nitrate-nitrogen (NO₃-N), ammonium-nitrogen (NH₄-N), P, Al, B, Cu, Fe, Mn, Na, and Zn. For soil samples, plant available N was estimated as NO₃-N plus NH₄-N. Using a potassium chloride (KCl) extraction, NO₃-N and NH₄-N were determined colorimetrically (Kirsten, 1979; Murphy and Riley, 1962; Sims et al., 1995) and by a microplate reader (uQuant, Biotek Instruments, Winooski, VT). Plant available concentrations in soil of Al, B, Cu, Fe, Mn, Na, P, and Zn were determined using ICAP (SpectroCiros CCD, Kleve, Germany), after extraction with a dilute double acid (Mehlich extraction 1, 0.025 N sulfuric acid and 0.05N HCl acid) (Bohn et al., 1985; Mehlich, 1953). Soil sample analysis does not represent total constituent mass; rather it provides an estimate of plant available concentrations. Plant and soil analyses were conducted at the Auburn University (AU) Soils Testing Laboratory.

Results and Discussion

Physical characteristics and infiltration rates of the rain garden substrate in each rain garden were generally similar (Table 1). Both rain gardens have a sandy loam soil,

with an infiltration rate of $19 \text{ cm}\cdot\text{hr}^{-1}$. Clay, silt, sand, and organic matter average 10%, 27%, 60%, and 3%, respectively between both rain gardens.

Stormwater Runoff Pond

Stormwater runoff into the pond came from several campus parking lots and road surfaces, dormitories, classroom and research buildings, and greenspaces and therefore provided ample surfaces for contaminated stormwater runoff. In the Summer and Fall of 2008, much of the southeastern United States experienced mild drought conditions. Precipitation amounts did not appear to affect nutrient and metal concentrations in the stormwater runoff pond in the summer months, however fluctuations in nutrient and metal concentrations occurred following large rain events (4 cm, 1.5 in) throughout the study (Figs. 4-7). Over the course of the study, total nitrogen concentrations in the stormwater runoff pond averaged $0.81 \text{ mg}\cdot\text{L}^{-1}$, with fluctuations from 0.2 to $3.4 \text{ mg}\cdot\text{L}^{-1}$ observed. Total phosphorus concentrations in the stormwater pond were constant at an average of $0.11 \text{ mg}\cdot\text{L}^{-1}$ throughout the study, with the exception of one event ($2.3 \text{ mg}\cdot\text{L}^{-1}$) in early September 2008 (Fig. 4), which coincided with a major rainfall of 9.14 cm (3.6 in). The source of increased P was not identified.

Concentrations of Al, Na, and Fe in the stormwater runoff pond remained relatively stable throughout the study period, as shown in Fig. 5. Boron and Zn varied more than did Cu (Fig. 6). Increased concentrations of B and Zn are likely due to several months of low rainfall followed by a relatively large storm event in mid-November of 4.6 cm (1.8 in). Manganese concentrations (Fig. 7) exhibited a mid-November increase similar to B and Zn. No obvious explanation was found for this increase.

Rain Garden Outflow

Total N concentration in rain garden outflow was higher than in stormwater inflow, indicating a likely release of N from the rain gardens (Table 4). Total P concentrations in the rain garden with an average outflow concentration of $0.21 \text{ mg}\cdot\text{L}^{-1}$ for IWS RG and $0.05 \text{ mg}\cdot\text{L}^{-1}$ for CONV RG, indicated a reduction of P concentration when compared to an average stormwater inflow of $0.23 \text{ mg}\cdot\text{L}^{-1}$. These results are the opposite of previous studies (Hunt et al., 2006) who found a reduction of N load and an increase in P load in outflow samples. The P load increase in outflow was due to the high P concentration in the fill substrate (Hunt et al, 2006). The saturated subsoils of IWS RG resulted in a tremendous release of Fe ($25,247 \text{ mg}\cdot\text{L}^{-1}$) in the outflow as compared to $3,696 \text{ mg}\cdot\text{L}^{-1}$ in CONV RG and $1,623 \text{ mg}\cdot\text{L}^{-1}$ in the stormwater inflow. Aluminum concentrations in the stormwater runoff pond exceeded the maximum contaminant level (MCL) allowed in drinking water (Table 3). MCLs are set as close to the maximum contaminant level goal (MCLG) as feasible using the best available treatment technology for water treatment plants. Concentrations of Fe, Mn, and Zn in the runoff pond exceeded the MCLG for drinking water, while concentrations of Cu exceeded the action level, the concentration of a contaminant which, if exceeded, triggers a treatment or other requirement which a water system must follow.

Soil Analysis

Soil analysis indicated relatively low concentrations of P, intermediate concentrations of potassium (K), and high concentrations of calcium (Ca) and magnesium (Mg). Soil pH was 6.5 and 6.4 for IWS RG and CONV RG, respectively. Concentrations of nutrients and metals in soil samples were similar to values reported in

literature (Mengel and Kirkby, 1987; Tisdale et al., 1993) (Table 5). Boron concentration in the IWS RG soils decreased from July to October (0.4 ppm to 0.05 ppm) then increased from October to February (0.05 ppm to 2.3 ppm). Nitrogen concentrations in the soil increased in both rain gardens from October to February. Concentrations of Fe (56 ppm) and Na (44 ppm) in both IWS RG and CONV RG soils were high, as compared to literature (Mengel and Kirkby, 1987; Tisdale et al., 1993).

Plant Analysis

Plants typically contain between 1.5 to 6% nitrogen and about 0.15 to 1% phosphorus (Mengel and Kirkby, 1987). Nitrogen concentrations in plant tissue in this study were within the range of the expected average values, but are on the low end of the normal range (1.54% N on average). Phosphorus concentrations in plant tissue were within expected ranges for both rain gardens (0.21%) but were also on the low end of the normal range. Tissue concentrations of metals in ferns (Table 6) indicated possible potential for high uptake of metals, especially Al (up to 2355 ppm) and Fe (up to 1968 ppm), and Na (up to 857 ppm). The ferns also had a high uptake of Al in February in the CONV RG (Table 6).

Summary

Rain events over 4 cm resulted in increased concentration of nutrients in the stormwater runoff pond. The IWS layer did result in reduction of TP and B concentrations in the rain garden, while all other nutrient and metal concentrations were higher in outflow samples than in inflow samples. It is recognized that increased concentrations do not necessarily lead to increased loads. In the absence of accurate outflow volume estimates, loads and removal efficiencies were unable to be determined.

Copper and Na concentrations were only slightly higher in the outflow of the IWS rain garden than in the inflow. The release of Fe in the IWS RG is characterized by the reduction of Fe³⁺ to Fe²⁺ under anaerobic conditions when soils are waterlogged (Mengel and Kirkby, 1987). In the CONV RG, TP, B, Cu, and Zn concentrations were reduced in water outflow, while concentrations of other nutrients and metals were higher in the outflow than in the stormwater inflow. In the IWS RG, only TP and B concentrations were reduced, however, Cu and Na concentrations were only slightly higher in the outflow than in the inflow. Nutrient concentrations in soil were relatively consistent throughout the study, without any major differences found between the two rain gardens. With few exceptions, plant nutrient concentration was higher during the warm season, with less consistent plant nutrient concentrations during the cool season for both plant materials (perennial and evergreen). Nitrogen and P mass loads, in grams, by plant uptake are available for reference, along with the mass loading of N g and P g in stormwater into the rain gardens (Appendix I). All plant taxa in the rain gardens appeared healthy, with no visible signs of stress and were able to endure the bi-weekly flooding events. Removal of desiccated perennial plant material is recommended in an effort to keep desiccated material from decaying and releasing nutrients or metals back into the rain garden.

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Table 1. Physical properties and infiltration rate of soil in two types of rain gardens.

	Clay (%)	Silt (%)	Sand (%)	Textural Class	OM (%)	Infiltration Rate (cm/h)
IWS RG ^z	10.0 ^y	27.2	62.9	Sandy Loam	2.8	19.6
CONV RG ^z	13.8	28.1	58.2	Sandy Loam	2.8	18.5

^zThe rain garden with an internal water storage layer (IWS RG) utilizes a saturated water zone in the rain garden that permits water to collect in the bottom 1 m (3.5 ft) of the rain garden. The saturated layer allows nitrogen to be removed via denitrification. The conventional rain garden (CONV RG) has an aerobic zone which allows water to be treated via interception, storage, filtration, and adsorption.

^y Analysis completed by Auburn University (AU) Soils Testing Laboratory, Oct. 2007.

Table 2. Sampling schedule, showing the total number of sampling events for two types of rain gardens ^z combined during July 2008 to February 2009.

Samples	Warm Season Samples, 2008				Cool Season Samples, 2008 to 2009			
	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Water – Input Pond (grab ^y)	4	4	4	5	4	3	4	4
Water – Output Nutrients (composite ^x)	4	4	4	5	4	3	4	4
Water – Output Metals (composite)	2	x ^w	x	2	x	x	x	2
Soil – (core 6” avg. depth) ^v	2	x	x	2	x	x	x	2
Plant – (above ground material)	2	x	x	2	x	x	x	2

^z The rain garden with an internal water storage layer (IWS RG) utilizes a saturated water zone in the rain garden that permits water to collect in the bottom 1 m (3.5 ft) of the rain garden. The saturated layer allows nitrogen to be removed via denitrification. The conventional rain garden (CONV RG) has an aerobic zone which allows water to be treated via interception, storage, filtration, and adsorption.

^y Grab sample – A discrete, individual sample taken within a short period of time. A manual grab is collected by inserting a container under or downcurrent of a discharge with the container opening facing upstream (U.S. Environ. Protection Agency, 1997a).

^x Composite sample – A mixed or combined sample that is formed by combining a series of individual and discrete samples of specific volumes at specified intervals (U.S. Environ. Protection Agency, 1997b).

^w An X denotes a time when no samples were taken due to budget constraints.

^v Note – Additional deep core soil sample was taken (Nov. 2009) and not included in schedule. Sample was taken at a depth of 1 m (3 ft).

Table 3. Individual plant species, counts, estimated dry weights, and area extent used in a study with two types of rain gardens.

Species	Count ^z	July/Oct. 2008 ^y	Feb. 2009 ^y	Coverage (%) ^x
<i>Carex</i> spp.	150	4.9	2.3	30
<i>Iris</i> sp.	3	92.25	20.1	10
<i>Dryopteris erythrosora</i>	15	40.59	19.5	30
Other ^w				30

^z Number of plant species planted in each rain garden.

^y Estimated dry weight, in grams, according to plant size. Plants were harvested, dried and weighed in July and October 2008, and February 2009.

^x Area of the rain garden covered by each plant species.

^w Other plants in rain garden (perennials and shrubs) not accounted for in study; *Itea virginiana*, *Echinacea purpurea*, *Muhlenbergia capillaris*, *Rudbeckia hirta*, and *Vaccinium angustifolium*.

Table 4. Concentrations of nutrients and metals found in stormwater inflow and rain garden outflow, along with drinking water standards, mg·L⁻¹ for the city of Auburn, AL.

	TN (mg·L ⁻¹)	TP (mg·L ⁻¹)	Al (mg·L ⁻¹)	B (mg·L ⁻¹)	Cu (mg·L ⁻¹)	Fe (mg·L ⁻¹)	Mn (mg·L ⁻¹)	Na (mg·L ⁻¹)	Zn (mg·L ⁻¹)
SW	0.80	0.23	328	22.73	11.21	1,623	334	2,897	17.93
Inflow	(n ^z =32)	(n=32)	(n=32)	(n=32)	(n=32)	(n=32)	(n=32)	(n=32)	(n=32)
IWS	3.15	0.21	868	20.67	14.33	25,247	3,168	3,061	43.83
Outflow	(n=13)	(n=13)	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)
CONV	1.81	0.05	2,677	21.27	4.47	3,696	530	2,945	17.50
Outflow	(n=19)	(n=19)	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)
MCL ^y	- ^x	-	0.2	-	1.3 ^w	-	-	-	-
MCLG ^v	-	-	-	-	1.3	0.3	0.5	-	5

^z n denotes number of samples taken

^y MCL – Maximum Contaminant Level – The highest level of a contaminant that is allowed in drinking water. MCLs are set as close to the MCLG as feasible using the best available treatment technology (City of Auburn, 2008).

^x Missing data does not have an MCL or MCGL associated with corresponding nutrient, denoted by a ‘-’.

^w Action Level – The concentration of a contaminant which, if exceeded, triggers a treatment or other requirement which a water system must follow (City of Auburn, 2008).

^v MCLG – Maximum Contaminant Level Goal – The level of a contaminant in drinking water which there is no known or expected risk to human health. MCGLs allow for a margin of safety (City of Auburn, 2008).

Table 5. Plant available nutrient concentrations in top 15.2 cm (6 in) of soil, in two types of rain gardens^z, with comparative typical values, ppm. Values are shown on a dry basis.

	Date	N ^y ppm	P ^y ppm	Al ppm	B ppm	Cu ppm	Fe ppm	Mn ppm	Na ppm	Zn ppm
IWS RG ^z	July 2008	7.6	4.6	179	0.4	2.4	69	76	27	3.6
	Oct. 2008	15	6.5	158	0.05	2.4	70	53	46	5.0
	Feb. 2009	14.6	5.3	185	2.3	1.9	74	57	48	3.5
CONV RG ^z	July 2008	8.8	3.2	118	0.5	1.8	53	57	44	6.8
	Oct. 2008	8.0	2.6	136	0.05	1.5	35	35	36	4.5
	Feb. 2009	10.6	2.3	116	0.8	1.2	35	19	63	3.3
Typical Values ^x		300 -	200 -					20 -		
Tisdale, et al., 1993		4000	1000	200	7 - 80	9	- ^w	3000	0.5-5	10 - 300
Mengel and Kirkby, 1987					20 -			20 -		
		-	500 - 800	-	200	5 - 50	20	3000	-	17 - 160

^z The rain garden with an internal water storage layer (IWS RG) utilizes a saturated water zone in the rain garden that permits water to collect in the bottom 1 m (3.5 ft) of the rain garden. The saturated layer allows nitrogen to be removed via denitrification. The conventional rain garden (CONV RG) has an aerobic zone which allows water to be treated via interception, storage, filtration, and adsorption.

^y Values are reported as an estimate of plant-available N (NO₃-N + NH₄-N) and P, found in top 15.2 cm (6 in) of soil.

^x Represents total values found in mineral soils (Tisdale, et al., 1993; Mengel and Kirkby, 1987).

^w No typical values were cited in literature for corresponding nutrients denoted by a '-'.

Table 6. Concentrations of nutrients and metals in plant tissue for *Carex*, *Dryopteris erythrosora*, and *Iris* in two rain garden types, conventional (CONV) and internal water storage (IWS).

IWS RG ^z Nutrient and Metal Concentrations										
Date	Plant Type	N ^y %	TP %	Al ppm	B ppm	Cu ppm	Fe ppm	Mn ppm	Na ppm	Zn ppm
July 2008	<i>Carex</i>	1.58	0.24	107	5	35	145	265	306	27
	<i>D. erythrosora</i>	2.02	0.23	381	14	9	302	79	283	30
Oct. 2008	<i>Carex</i>	1.74	0.13	82	6	34	13	59	173	20
	<i>D. erythrosora</i>	1.52	0.24	257	20	27	129	169	267	42
	<i>Iris</i> sp.	1.99	0.15	73	15	17	11	70	198	30
Feb. 2009	<i>Carex</i>	2.08	0.22	398	22	37	314	308	342	22
	<i>D. erythrosora</i>	2.06	0.17	273	39	28	211	122	439	30
	<i>Iris</i> sp.	1.92	0.27	120	48	27	111	283	280	35
CONV RG ^z Nutrient and Metal Concentrations										
Date	Plant Type	N %	TP %	Al ppm	B ppm	Cu ppm	Fe ppm	Mn ppm	Na ppm	Zn ppm
July 2008	<i>Carex</i>	2.12	0.18	148	20	20	261	67	522	34
	<i>D. erythrosora</i>	1.46	0.22	2355	40	15	1968	389	356	50
Oct. 2008	<i>Carex</i>	1.36	0.36	131	7	12	59	124	296	17
	<i>D. erythrosora</i>	1.52	0.36	128	6	22	117	128	199	16
	<i>Iris</i> sp.	1.44	0.23	1109	29	19	882	241	356	50
Feb. 2009	<i>Carex</i>	1.17	0.09	192	21	25	181	119	239	14
	<i>D. erythrosora</i>	1.5	0.17	1149	39	19	878	254	857	43
	<i>Iris</i> sp.	0.72	0.05	432	33	15	332	112	167	30

^z The rain garden with an internal water storage layer (IWS RG) utilizes a saturated water zone in the rain garden that permits water to collect in the bottom 1 m (3.5 ft) of the rain garden. The saturated layer allows nitrogen to be removed via denitrification. The conventional rain garden (CONV RG) has an aerobic zone which allows water to be treated via interception, storage, filtration, and adsorption.

^y Analysis was completed by Auburn University Soil Testing Laboratory, Auburn, AL.

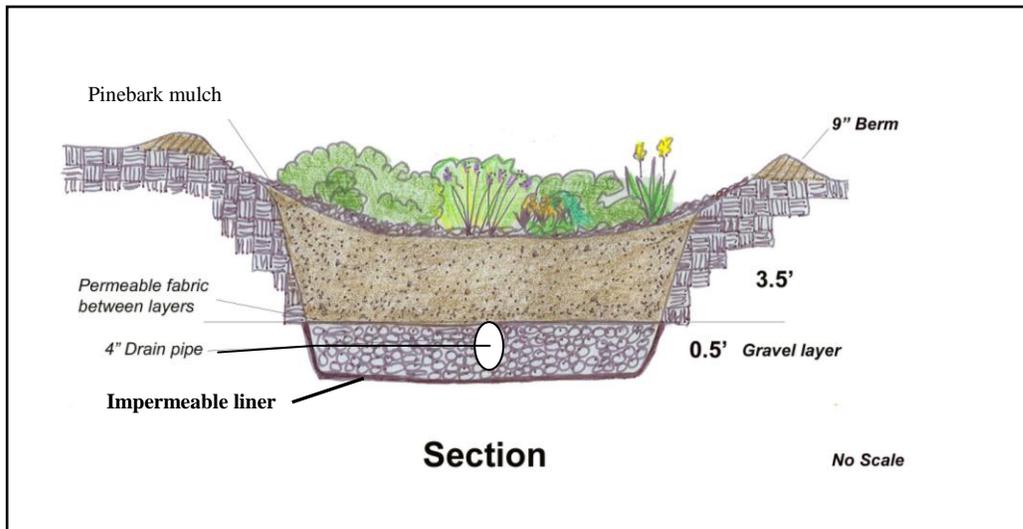


Fig. 1. A conventional rain garden showing aerated soil layer and gravel collection drain. Rain gardens with an internal water storage layer (IWS) incorporate a 0.46 m (1.5 ft) depth zone of saturation above the impermeable liner.

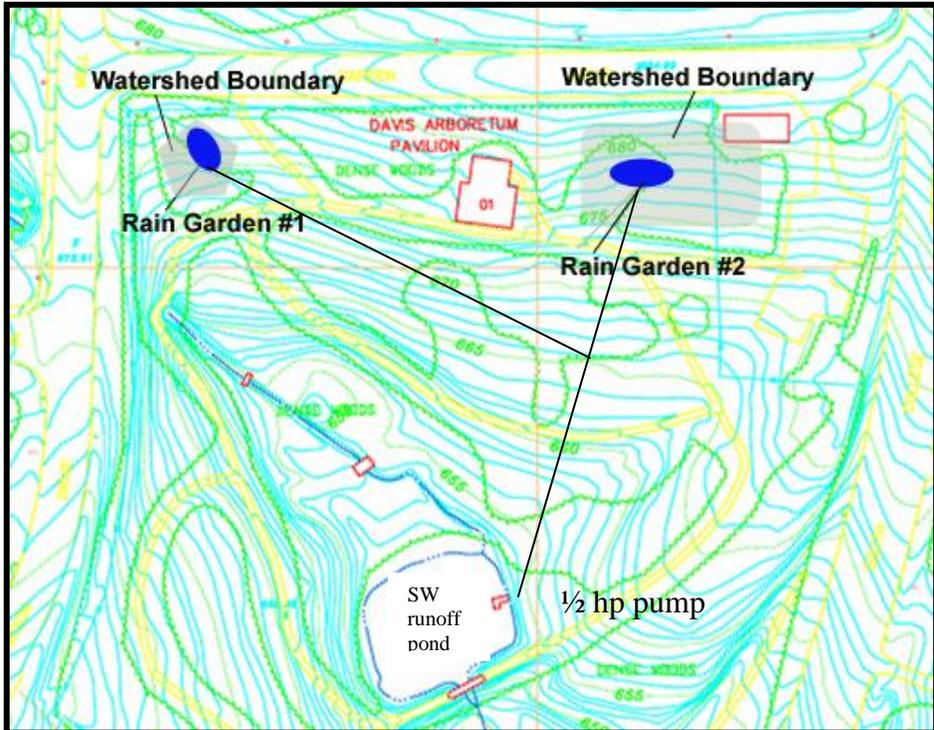


Fig. 2. Donald E. Davis Arboretum on Auburn University Campus in Auburn, AL map showing rain gardens, supply pipe, pumping facilities, and stormwater runoff source.

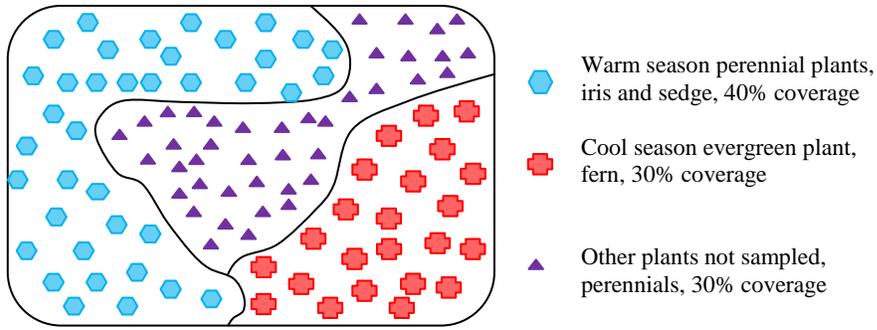


Fig. 3. Planting layout of rain gardens and showing percent coverage of each plant type.

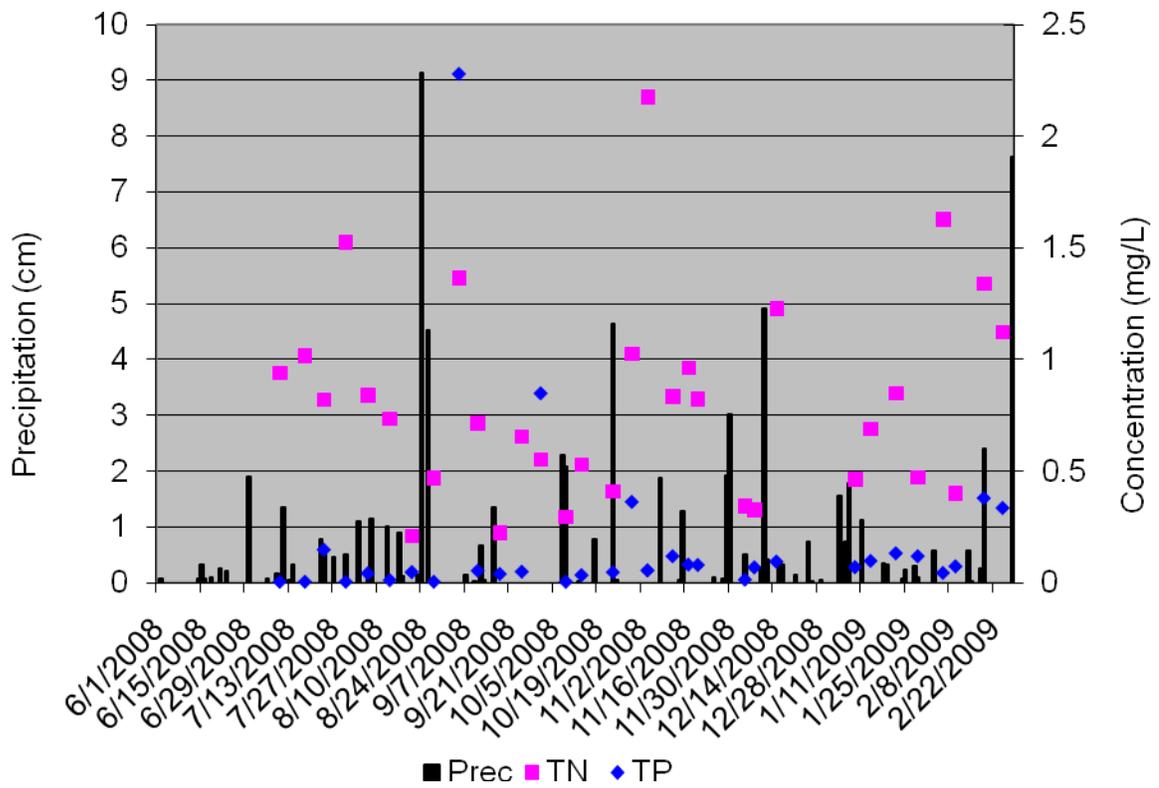


Fig. 4. Rainfall volume and frequency and total nitrogen and total phosphorus (TN and TP) concentrations in stormwater during July 2008 to Feb. 2009 located in Donald E. Davis Arboretum on Auburn University Campus in Auburn, AL.

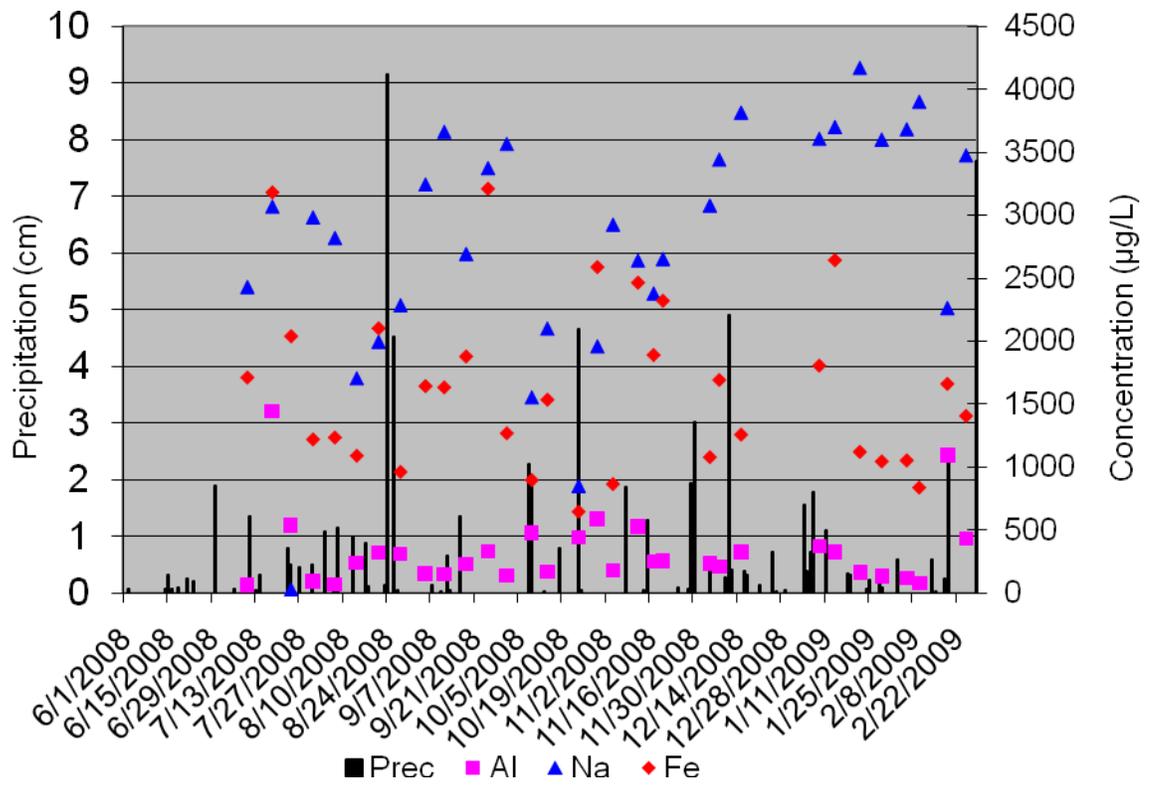


Fig. 5. Rainfall volume and frequency and aluminum (Al), sodium (Na), and iron (Fe) concentrations in stormwater during July 2008 to Feb. 2009 located in Donald E. Davis Arboretum on Auburn University Campus in Auburn, AL.

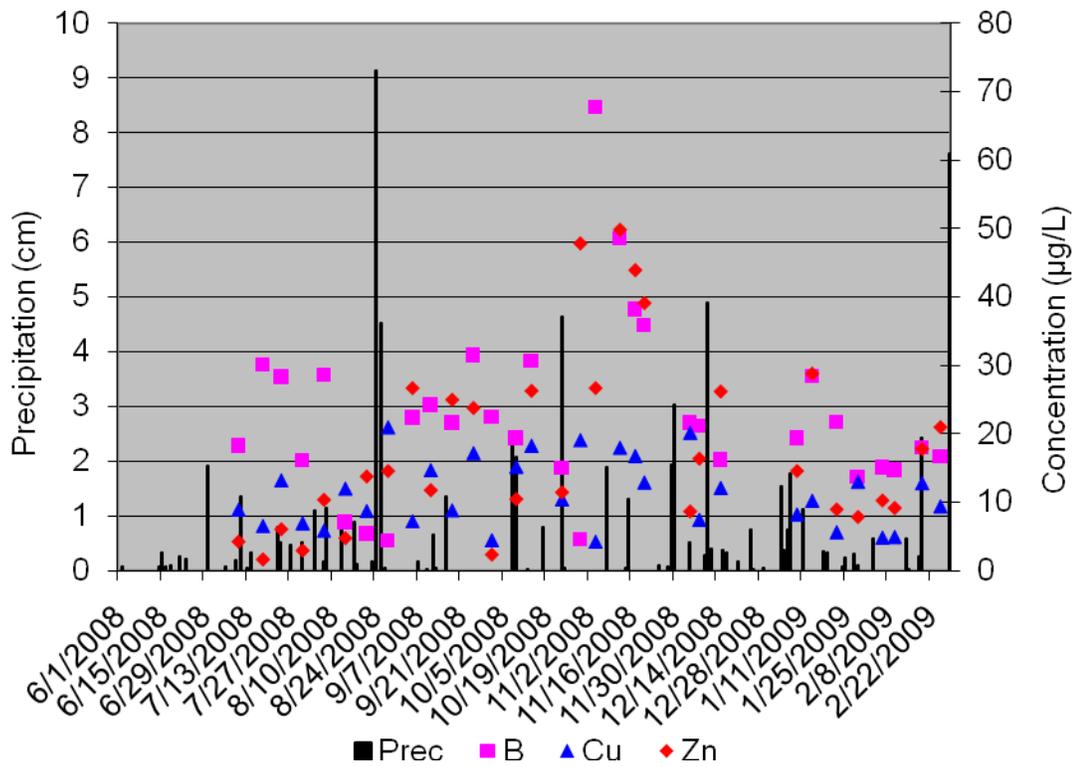


Fig. 6. Rainfall volume and frequency and boron (B), copper (Cu), and Zinc (Zn) concentrations in stormwater during July 2008 to Feb. 2009 located in Donald E. Davis Arboretum on Auburn University Campus in Auburn, AL.

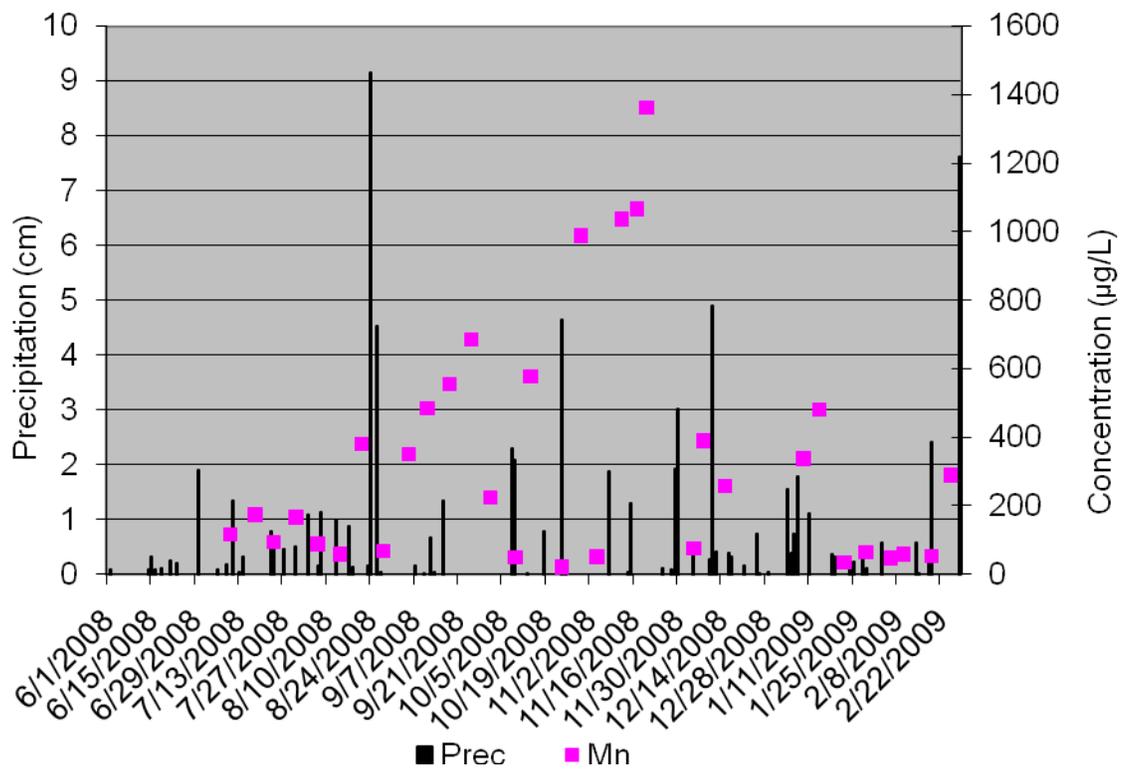


Fig. 7. Rainfall volume and frequency and manganese (Mn) concentrations in stormwater during July 2008 to Feb. 2009 located in Donald E. Davis Arboretum on Auburn University Campus in Auburn, AL.

Chapter III

Effect of Repeated Short Interval Flooding Events on Growth, Stem Water Potential, and Gas Exchange of Five Native Shrub Taxa

Index Words: Water, Rain garden, *Fothergilla ×intermedia* L. ‘Mount Airy’, *Ilex verticillata* ‘Winter Red’, *Clethra alnifolia* ‘Ruby Spice’, *Callicarpa dichotoma* ‘Early Amethyst’, *Viburnum nudum* Brandywine™

Abstract: Plants in rain gardens must be able to tolerate repeated short intervals of flooding. Research was conducted to screen five native landscape shrub taxa for tolerance to repeated flooding events. On 13 Aug. 2009, thirty 3.8 L (1 gal) plants each of *Fothergilla ×intermedia* L. ‘Mt. Airy’ (dwarf witchalder), *Ilex verticillata* (L.) A. Gray ‘Winter Red’ (winterberry), *Clethra alnifolia* L. ‘Ruby Spice’ (summersweet), *Callicarpa dichotoma* (Lour.) K. Koch ‘Early Amethyst’ (purple beautyberry), and *Viburnum nudum* L. Brandywine™ (possumhaw) were removed from their containers and potted into 11.3 L (3 gal) containers in 5:3:1 pine bark: peat: perlite substrate and placed in a greenhouse in Auburn, AL. Beginning on 28 Aug. 2009, root zones of plants were flooded to the substrate surface for 0 (non-flooded), 3, or 6 days (flood duration). Following the flooding period, plants were allowed to drain for 6 days. During the draining period, no water was added to containers. The flood-drain process was repeated for 6 weeks. Non-flooded plants were hand watered as needed. The experiment was terminated on 16 Oct. 2009 (run 1) and repeated (run 2) beginning 26 Oct. 2009 and ending 12 Dec 2009. *Run*

1. Flooding duration did not affect growth index (GI), shoot dry weight (SDW), or root dry weight (RDW) of *I. verticillata* ‘Winter Red’ and *V. nudum* Brandywine™. Growth index was higher in plants flooded for 0 and 3 days than in plants flooded for 6 days for *C. alnifolia* ‘Ruby Spice’, but flooding duration did not affect SDW and RDW. Growth index of *C. dichotoma* ‘Early Amethyst’ was not affected by flooding treatments, however SDW decreased with increasing flood duration. Root dry weight of *C. dichotoma* ‘Early Amethyst’ was not affected by flooding duration. Growth index of *F. ×intermedia* ‘Mt. Airy’ decreased with increasing flood duration, and SDW and RDW were higher in plants flooded for 0 or 3 days than in plants flooded for 6 days. Generally, the lowest values for P_n and SC occurred in plants flooding or draining for 6 days. Photosynthesis (P_n) and stomatal conductance (SC) were lower at the 5th flood cycle of the experiment while stem water potential (SWP) was higher in plants when measured at the 5th flood cycle of the experiment. Run 2. Growth index and RDW of *F. ×intermedia* ‘Mt. Airy’ were lowest in plants flooded for 6 days. The SDW of *I. verticillata* ‘Winter Red’ was highest in plants flooded for 6 days and in *V. nudum* Brandywine™ the GI was highest in plants flooded for 6 days. Overall, in both runs, all taxa, with the exception of *F. ×intermedia* ‘Mt. Airy’, maintained good visual quality, did not have any reduction in RDW, and exhibited minimal effects of flooding on shoot growth. *F. ×intermedia* ‘Mt. Airy’ exhibited poor visual quality, with growth adversely affected by flooding, suggesting this taxon would not be a good choice for rain garden use. Conversely, all other taxa appeared tolerant of flooding and would be appropriate native shrub selections for rain gardens.

Introduction

Rain gardens, an emerging stormwater management tool, are natural or dug shallow depressions designed to capture and absorb stormwater runoff from a roof or other impervious surface such as a driveway, walkway, and even compacted lawn area (Davis et al., 2009; Univ. of RI, 2009). Benefits of rain gardens and other bioretention areas include decreased surface runoff, increased groundwater recharge, and reduction of pollutants (Dietz, 2007; Prince George's County, MD, 1993). Bioretention areas are typically designed to minimize the impacts of stormwater runoff from impervious surfaces in residential, commercial, and industrial areas, with each component (soil, plants, and mulch) designed to perform a specific function in stormwater treatment (U.S. Environ. Protection Agency, 1999). Rain gardens, as a stormwater treatment practice, have gained popularity due to aesthetics, potential to reduce flooding, and early documented improvements to stormwater quality (Hunt et al., 2008). In rain gardens, as the runoff passes over mulch and vegetation, water velocity is slowed and then distributed evenly over the ponding area. The water then forms a shallow pond and gradually infiltrates into the soil (U.S. Environ. Protection Agency, 1999).

The nature of hydrology in rain gardens requires that plants selected for use in rain gardens be able to tolerate alternating periods of flooding and drying. Initial effects of flooding in plants are often found in the root system (Blom, 1999). For terrestrial plant systems, the depletion of oxygen results in a shift from aerobic to anaerobic microbial processes, which leads to reduction of oxidized compounds and the production of phytotoxic compounds in flooded soils (Blom, 1999; Ernst, 1990; Laanbroek, 1990; Smolders et al., 1997). Flooding affects soils by altering soil structure, depleting oxygen,

accumulating carbon dioxide, inducing anaerobic decomposition of organic matter, and thereby altering plant metabolism and inhibiting growth (Kozlowski, 1984; 1997). Soil inundation inhibits root respiration which leads to decay of the root system and inhibits photosynthesis (P_n), and the transport of carbohydrates (Kozlowski, 1997). Uptake of water decreases under waterlogged or anaerobic conditions (Bradford and Yang, 1981). Root weight declines in flooded plants due to deterioration and loss of most of the smaller roots (Bradford and Hsiao, 1982). Flooding of soil induces stomatal closure, with stomata beginning to reopen after a period of flooding if the plant is able to acclimate to the flooded soil. After termination of flooding, stomata begin to reopen to near pre-flood level, while some stomata exhibit permanent damage and are unable to recover from flood stress (Kozlowski and Pallardy, 1979). Stomatal conductance and transpiration are reduced after approximately 24 hours of flooding as a way to restrict water loss due to stress (Bradford and Hsiao, 1982). Stomatal closure and increased ethylene production are associated with the decrease in P_n (Chen et al., 2005).

Flood tolerance varies greatly among plant species, genotypes, and rootstock, and is influenced by plant age, time and duration of flooding, condition of the floodwater, and site characteristics (Kozlowski, 1997). Although native plants are often recommended for use in rain gardens, experiments are needed to quantify tolerance to short-term repeated flooding. Previous studies completed at Auburn University by Werneth et al. (2010) suggested a need for continued research to identify native plants with tolerance to repeated flooding for use in rain gardens. Therefore, the objective of this research was to determine the effect of repeated, short-term flooding events on growth, rate of

photosynthesis (P_n), stomatal conductance (SC), and stem water potential (SWP) of five native landscape shrubs taxa.

Materials and Methods

Run 1

On 13 Aug. 2009, 30 each 3.8 L (1 gal) *Fothergilla ×intermedia* ‘Mount Airy’ (dwarf witchalder), *Ilex verticillata* ‘Winter Red’ (winterberry), *Clethra alnifolia* ‘Ruby Spice’ (summersweet), *Callicarpa dichotoma* ‘Early Amethyst’ (purple beautyberry), and *Viburnum nudum* L. Brandywine™ (possumhaw) (original liners from Spring Meadow Nursery, Inc., Grand Haven, MI), were removed from their containers and placed into 11.3 L (3 gal) containers filled with 5: 3: 1 (by volume) of a pine bark (PB): peat moss: perlite blend. Substrate was amended with $1.2 \text{ kg}\cdot\text{m}^{-3}$ ($2 \text{ lbs}\cdot\text{yd}^{-3}$) dolomitic limestone, $0.9 \text{ kg}\cdot\text{m}^{-3}$ ($1.5 \text{ lbs}\cdot\text{yd}^{-3}$) micronutrient fertilizer (Micromax® Scott’s Company, Marysville, OH), and $8.2 \text{ kg}\cdot\text{m}^{-3}$ ($13.8 \text{ lbs}\cdot\text{yd}^{-3}$) controlled release fertilizer (CRF) (Polyon® 18N-2.6P-9.96K, Agrium Advanced Technologies, Sylacauga, AL). Pine bark was from Pineywoods Mulch Company (Alexander City, AL), peat moss was from Fafard (Agawam, MA), and perlite was from Sunshine (Sungro, Bellevue, WA). Plants were overwintered on a full sun nursery pad the previous winter. During the overwintering period, plants received daily overhead irrigation for 10 min at 10:30 AM and 1:30 PM. At temperatures of -4° C (25° F) or lower, plants were covered with white polyethylene plastic with ventilation holes.

Containers were placed on raised greenhouse benches at the Paterson Horticulture Greenhouse Complex, Auburn University, AL. Plants were grown in an unshaded 8 mm

(0.3 in) twin-wall, polycarbonate covered greenhouse under natural photoperiods with a heating set point of 18.3°C (65°F) and with ventilation beginning at 25.6°C (78°F).

Flooding cycle treatments were initiated on 28 Aug. 2009 (15 days after planting (DAP)). Plants were flooded using a pot-in-pot method. The pot containing the plant had holes in the bottom to allow for drainage and was placed inside an outer pot without holes. Tap water was added by hand to each container until the water level was even with the substrate level; additional water was added to the container as needed to keep the water level even with the substrate surface. The volume of water applied was recorded daily; approximately 4 L (1.1 gal) was added initially in order to flood plants, and approximately 400 mL (0.1 gal) was added daily to maintain the flood level. Plants were flooded for 0 days (non-flooded), 3, or 6 days. Following flooding, outer containers were removed and plants were allowed to drain for 6 days. No additional water was added to the containers during the 6 days of draining. Substrate moisture percent was measured using ECH₂O soil moisture sensors (model EC-5) every 60 minutes and recorded using Em5b data loggers (Decagon Devices, Inc., Pullman, WA). Plants were treated for broad mites as needed throughout the experiment using abamectin (Avid[®] 0.15 EC, Syngenta, Greensboro, NC).

Initial and final growth indices (GI) [(height + widest width + width perpendicular to widest width) / 3] were taken on 17 Aug and 15 Oct 2009, respectively. Net photosynthesis (P_n) and stomatal conductance (SC) of plants were measured using the LI-COR 6400 (Model 1000, LI-COR Biosciences, Inc., Lincoln, NE.). All measurements were taken between 10:00 AM and 1:30 PM. Photosynthetically active radiation (PAR) was set at 1800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, reference carbon dioxide was set at 400

mg·L⁻¹, stomatal ratio was set to 0, flow rate was set to 500 mol·s⁻¹, and temperature and humidity were set to ambient. Photosynthesis and SC were measured on the newest matured leaves on the plants. One leaf from three plants per treatment for each taxon was measured for each reading. Stem water potential (SWP) was measured using a pressure chamber (PMS Instruments, Corvallis, OR) on the same days and with the same plants used for P_n and SC measurements. For SWP, one 10 cm (4 in) terminal stem cutting was removed from each plant, placed in a plastic bag, put on ice in a cooler, and immediately returned to the lab. Stem sections were then re-cut to 7.6 cm (3 in), foliage was removed from the basal 2.5 cm (1 in) of the stem, and SWP was measured using the pressure chamber. For plants flooded for 3 days, P_n, SC, and SWP were measured on the final flood day and final drain day during the third and fifth flood-drain cycles (hereafter referred to as the middle of the experiment and the end of the experiment, respectively). For plants flooded for 6 days, P_n, SC, and SWP were measured on the final flood day and final drain day during the second and fourth flooding cycles (hereafter referred to as the middle of the experiment and the end of the experiment, respectively). Photosynthesis, SC, and SWP were measured for three non-flooded plants on the same days as the flooded treatments. Plants flooded for 3 days were harvested on 14 Oct. 2009 (62 DAP, 5 cycles), non-flooded plants and plants flooded for 6 days were harvested on 16 Oct. 2009 (64 DAP, 4 cycles). Shoots were severed at the substrate level, and roots were washed to remove substrate. Shoots and roots were dried separately in an oven for 48 hours at 66°C (150°F) for determination of shoot dry weight (SDW) and root dry weight (RDW).

Run 2

The above experiment was repeated with all five taxa planted as described above on 07 Oct. 2009 and placed on greenhouse benches under 400 watt high pressure sodium lamps set on a 14 hr photoperiod (6:00 AM to 8:00 PM CST). The lamps were used throughout the entirety of the experiment. Average PAR of the lamps was measured using a LI-250 lightmeter (LI-COR Biosciences, Inc., Lincoln, NE) and ranged from 103 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to 131 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Flooding cycle treatments were initiated on 26 Oct. 2009 (19 DAP). Treatments were terminated on 9 Dec. 2009 (64 DAP) for plants flooded for 3 days and on 12 Dec. 2009 (67 DAP) for non-flooded plants and plants flooded for 6 days. Since plant species used were deciduous, P_n , SC, and SWP were not measured. Plants were harvested on 14 Dec. 2009 (69 DAP) for all treatments. Initial and final GI were recorded on 22 Oct. 2009 and at harvest, respectively. Shoots were severed at the substrate level, with roots washed to remove substrate. Shoots and roots were dried separately in an oven for 48 hours at 66°C (150°F) for determination of SDW and RDW.

In both runs, plants were arranged in a completely randomized design with each taxon representing a separate experiment. Ten replications of each treatment were used for each taxon. Data were analyzed using glimmix and mixed models procedures with least square means ($P<0.05$) (SAS Institute, Cary, NC). Data were analyzed to determine the effect of flooding duration on GI, RDW, SDW, P_n , SC, and SWP. Values for P_n , SC, and SWP were analyzed to detect differences between flooding and draining periods within a treatment. Photosynthesis, SC, and SWP data collected during the 3rd flood cycle of experiment were compared to those data collected at the 5th flood cycle of

experiment. Data were collected from control plants at each measurement date, regardless of treatment being measured. Data are presented only where significant.

Results

***Fothergilla ×intermedia* ‘Mount Airy’**

In run 1, GI decreased with increasing flood duration, and SDW and RDW were higher in plants flooded for 0 or 3 days than in plants flooded for 6 days (Table 1). In run 2, GI of plants flooded for 0 days was higher than that of plants flooded for 6 days (Table 2). Flooding duration did not affect SDW. Root dry weight was lowest in plants flooded for 6 days.

During the 3rd flood cycle of the experiment and at the 5th flood cycle of the experiment, P_n was lowest during flooding in plants flooded for 6 days (Tables 3-4). When physiological measurements taken during the 3rd flood cycle of the experiment were compared to those taken at the 5th flood cycle of the experiment, there was no difference in P_n . During the 3rd flood cycle of the experiment and at the 5th flood cycle of the experiment, SC was lowest during flooding in plants flooded for 6 days (Tables 3-4). Stomatal conductance was similar when physiological measurements taken during the 3rd flood cycle of the experiment were compared to those taken at the 5th flood cycle of the experiment with the exception of when SC was measured during the draining period in plants that were flooded for 6 days (Table 5). During the 3rd flood cycle of the experiment, flooding did not affect SWP, however at the 5th flood cycle of the experiment SWP was lowest during flooding in plants flooded for 6 days (Table 5). When measured during the draining period, SWP was higher for plants at the 5th flood cycle of the experiment than during the 3rd flood cycle of the experiment (Table 5). In contrast, SWP

was higher in the 3rd flood cycle of the experiment than at the 5th flood cycle of the experiment when measured during the flooding period (Table 5). There was no difference in SWP between the 3rd flood cycle and the 5th flood cycle of the experiment in plants flooded for 3 days when measured during the flooding period (Table 5).

***Ilex verticillata* ‘Winter Red’**

Flooding duration did not affect GI, SDW, or RDW in run 1. In run 2 flooding duration did not affect GI. Shoot dry weight was lower in plants flooded 0 or 3 days than in plants flooded for 6 days (Table 2). Flooding duration did not affect RDW in run 2.

There were no differences among flooding treatments in P_n during the 3rd flood cycle of the experiment. At the 5th flood cycle of the experiment, P_n was lowest during the flooding period in plants flooded for 6 days (Table 4), and was lower at the 5th flood cycle of the experiment than in the 3rd flood cycle of the experiment only in plants flooded for 6 days (Table 5). In the 3rd flood cycle of the experiment, SC was highest during the flooding period in plants flooded for 3 days (Table 3). At the 5th flood cycle of the experiment, SC was lowest during the flooding period in plants flooded for 6 days (Table 4). Stomatal conductance measured during the 3rd flood cycle of the experiment compared to SC measured at the 5th flood cycle of the experiment did not differ when measured during the draining periods, but SC was lower at the 5th flood cycle of the experiment when measured during the flooding period (Table 5). There were no differences among flooding treatments in SWP during the 3rd flood cycle of the experiment. At the 5th flood cycle of the experiment, SWP was lowest when measured during the flooding period (Table 4), but SWP was not different between the 3rd flood cycle and the 5th flood cycle of the experiment.

***Clethra alnifolia* ‘Ruby Spice’**

In run 1, GI was higher in plants flooded for 0 or 3 days than in plants flooded for 6 days, however, flooding duration did not affect SDW or RDW (Table 1). In run 2, flooding duration did not affect GI, SDW, or RDW.

During the 3rd flood cycle of the experiment, P_n was not affected by flooding duration. At the 5th flood cycle of the experiment P_n was highest during flooding in plants flooded for 3 days (Table 4). Photosynthesis was consistently higher in the 3rd flood cycle of the experiment than at the 5th flood cycle of the experiment when measurements taken during the 3rd flood cycle of the experiment were compared to those taken at the 5th flood cycle of the experiment (Table 5). During the 3rd flood cycle of the experiment, SC was highest during the flooding period in plants flooded for 3 days (Table 3), but at the 5th flood cycle of the experiment, SC was not affected by flooding. Stomatal conductance was consistently higher in the 3rd flood cycle of the experiment than at the 5th flood cycle of the experiment (Table 5). During the 3rd flood cycle of the experiment, SWP was not affected by flooding duration. At the 5th flood cycle of the experiment, SWP was lower when measured during the flooding period (Table 4). Stem water potential was higher at the 5th flood cycle of the experiment than in the 3rd flood cycle of the experiment when measured during the draining period (Table 5).

***Callicarpa dichotoma* ‘Early Amethyst’**

In run1, GI was not affected by flooding duration, however, SDW of plants decreased with increasing flooding duration (Table 1). Root dry weight was not affected by flooding duration. Flooding duration did not affect GI, SDW, or RDW in run 2.

During the 3rd flood cycle of the experiment, P_n was lowest during the draining period in plants flooded for 6 days (Table 3). At the 5th flood cycle of the experiment, P_n was not affected by flooding duration. When measurements taken during the 3rd flood cycle of the experiment were compared to those taken at the 5th flood cycle of the experiment, P_n was higher in the 3rd flood cycle of the experiment in plants flooded for 6 days and plants draining for 3 days (Table 5). During the 3rd flood cycle of the experiment and at the 5th flood cycle of the experiment, SC was not affected by flooding duration. When measurements taken during the 3rd flood cycle of the experiment were compared to those taken at the 5th flood cycle of the experiment, SC was higher in the 3rd flood cycle of the experiment than at the 5th flood cycle of the experiment in all plants except during the flooding period in plants flooded for 3 days, which was not different (Table 5). During the 3rd flood cycle of the experiment and at the 5th flood cycle of the experiment, SWP was not affected by flooding duration. Stem water potential was higher at the 5th flood cycle of the experiment than in the 3rd flood cycle of the experiment only when measured during draining in plants flooded for 3 days (Table 5).

***Viburnum nudum* Brandywine™**

Flooding duration did not affect GI, RDW or SDW in run 1. In run 2, GI of plants increased with increasing flood duration; plants flooded for 6 days had a higher GI than plants flooded for 0 days (Table 2). Flooding duration did not affect SDW or RDW in run 2.

During the 3rd flood cycle of the experiment and at the 5th flood cycle of the experiment, flooding duration did not affect P_n or SC. When physiological measurements taken during the 3rd flood cycle of the experiment were compared to those taken at the 5th

flood cycle of the experiment, there were no differences found in P_n or SC. In the 3rd flood cycle of the experiment, SWP was highest when measured during flooding (Table 3). At the 5th flood cycle of the experiment, SWP was lowest when plants were flooded (Table 4). Stem water potential was higher at the 5th flood cycle of the experiment than during the 3rd flood cycle of the experiment when measured during the draining periods (Table 5).

Discussion

Fothergilla ×intermedia ‘Mt. Airy’ had poor visual quality, with growth adversely affected by flooding in both runs. Thus, this taxon would not appear to be a good choice for use in rain gardens. However, even under flooded conditions, *F. ×intermedia* ‘Mt. Airy’ was still able to produce flowers on some plants, and no plants were lost due to flooding. *Fothergilla gardenii*, a parent of *F. ×intermedia* ‘Mt. Airy’, is facultative wetland (FACW) plant (usually occurs in wetlands; estimated probability 67 to 99 %), but occasionally found in non-wetlands (U.S. Dept. of Agr., 2010), however, no information is specifically given for *F. ×intermedia*, which is not suited for rain garden conditions. All other taxa maintained good visual quality, had no reduction in root dry weight, and exhibited minimal effects on shoot growth regardless of flooding treatment. As a result, these taxa appeared to be flood tolerant and seem to be appropriate selections for rain garden use. It should be noted that in run 2, the growth index of *V. nudum* Brandywine™ actually increased with flooding, suggesting that this taxa may even thrive under flooded conditions.

Visual appearance of *I. verticillata* ‘Winter Red’ was fair. The plants did not produce growth that was as full or dense as in other taxa, but this could be contributed to

the slow growth rate and the overall growth habit of this cultivar. Although not compared statistically, *Ilex verticillata* ‘Winter Red’ seemed to have one of the slower growth rates of the taxa in the experiment. In spite of this, the plants produced new shoots, and all plants were relatively the same size, regardless of treatment, suggesting that flooding did not affect the growth rate. *Ilex verticillata* is an obligate wetland (OBL) plant, almost always found (estimated probability 99 %) under natural conditions in wetlands and FACW in the southeast (U.S. Dept. of Agr., 2010). Plants appeared generally healthy and over time, possibly with additional acclimation, might be able to develop into a larger, denser plant. Plants were susceptible to broad mites (*Polyhagotarsonemus latus* (Banks)) and aphids (species not identified), but after pesticide application no other problems occurred.

Clethra alnifolia ‘Ruby Spice’ seemed to thrive under flooded conditions in this experiment. The plants had dense canopies and prominent upright growth habit and had started to colonize in the container by sending up new suckers. This result is different from results by Werneth et al. (2010) who found that *C. alnifolia* ‘Ruby Spice’ did not tolerate flooding. The difference may be that in the current work, plants were grown in 11.4 L (3 gal) containers, while in the previous work, plants were grown in 2.5 L (trade gal) containers. It’s possible that bigger larger root system on older, more established plants made the larger plants more tolerant of flooding. *Clethra alnifolia* is FACW and facultative (FAC) plant (equally likely to occur in wetlands or non-wetlands; estimated probability 34 to 66 %) in the southeast (U.S. Dept. of Agr., 2010). No flowers were seen on the plants during flooding. Plants were susceptible to broad mites and aphids, but after pesticide application no other problems occurred.

Callicarpa dichotoma ‘Early Amethyst’ had a relatively high growth rate and maintained vigor under flooded conditions. *Callicarpa dichotoma* ‘Early Amethyst’ is an OBL plant in the southeast (U.S. Dept. of Agr., 2010). Plants in this taxa were able to produce flowers and fruit during flooding, regardless of the number of days flooded. No pest problems were observed.

Viburnum nudum Brandywine™ produced plants with good visual quality and dark green foliage. Plants consistently produce new shoots throughout the runs, and growth did not appear stunted by flooding. *Viburnum nudum* is a FACW plant in the southeast (U.S. Dept. of Agr., 2010). Plants did not have any pest problems throughout the entirety of experiment.

In general throughout run 1, the lowest P_n and SC values occurred most often in plants flooded for 6 days, and P_n and SC tended to be lower at the 5th flood cycle of the experiment. Photosynthesis did not differ over time for *V. nudum* Brandywine™ which had the best performance nor for *F. × intermedia* ‘Mt. Airy’ which had the worst performance. It is possible that *F. × intermedia* ‘Mt. Airy’ had a slow rate of growth and thus did not exhibit fluctuations in carbon assimilation. In contrast, *V. nudum* Brandywine™ was one of the faster growing taxa in this experiment and perhaps remained vigorous enough for P_n to remain unaffected by flooding.

For *V. nudum* Brandywine™, SWP was the only physiological parameter affected by flooding treatment. In the 3rd flood cycle of the experiment, stem water potential was not generally affected by flooding. One exception to this was for *V. nudum* Brandywine™ which had higher SWP during the flooding period in plants flooded for 3 and 6 days. At the 5th flood cycle of the experiment, however, SWP of *V. nudum*

Brandywine™ tended to be higher during the draining periods than during the flooding periods. If SWP differed between the 3rd flood cycle and the 5th flood cycle of the experiment, it tended to be higher at the 5th flood cycle, which could possibly be due plants being more stressed towards the end of the experiment and therefore having their stomata closed. Substrate percent moisture generally ranged from 53% at the end of a flooding period to 15% at the end of a draining period indicating intermediate levels of drought for plants in between flooding events.

Although flooded conditions in this experiment were of longer duration than may typically be expected for a rain garden, all plants survived, and in fact, many plants thrived in these conditions. Rain gardens typically remain inundated for no longer than 48 h but can hold water for up to 72 to 96 h (Davis et al., 2009). Although flooded plants did not receive any irrigation during their draining period to allow the substrate to dry, typically substrate percent moisture (by volume) did not drop below 12 to 15 %. Although this may be considered moderately dry, examining the extent of drought tolerance of these taxa may provide additional information regarding their suitability for use in rain gardens.

It appears as if plants that tend to have faster rates of root and shoot growth may be best equipped to tolerate the fluctuating hydrology of a rain garden and in particular short periods of inundation. This work, as well as past research (Werneth, 2010), has demonstrated that several of deciduous shrubs native to the southeastern United States provide flood tolerant, attractive options for use in rain gardens in the Southeast. As rain gardens continue to be incorporated into landscapes to remediate stormwater runoff, continued evaluation of the flood tolerance of other native shrub taxa will be valuable.

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Table 1. Main effect of flooding duration (days) on growth index (GI), shoot dry weight (SDW), and root dry weight (RDW) of *Fothergilla ×intermedia* ‘Mt. Airy’, *Clethra alnifolia* ‘Ruby Spice’, and *Callicarpa dichotoma* ‘Early Amethyst’ grown in a greenhouse, Aug. to Oct. 2009 (run 1).

Flooding Duration (days) ^z	<i>F. ×intermedia</i>			<i>C. alnifolia</i>	<i>C. dichotoma</i>
	GI (cm)	RDW (g)	SDW (g)	GI (cm)	SDW (g)
0	50.3a ^y	19.3ab	10.4a	65.2a	47.0a
3	44.6ab	22.3a	11.1a	58.2a	36.0b
6	38.5b	10.3b	2.8b	45.2b	22.3c

^z Plants were flooded for 0, 3, or 6 days followed by 6 days of draining. The flood-drain cycle occurred five times on plants flooded for 3 days and four times for plants flooded for 6 days.

^y Lowercase letters denote mean separation within a column using Proc Glimmix at $P < 0.05$ (SAS Institute, Cary, NC).

Table 2. Effect of flooding duration (days) on growth index (GI), shoot dry weight (SDW) and root dry weight (RDW) on *Fothergilla ×intermedia* ‘Mt. Airy’, *Ilex verticillata* ‘Winter Red’, and *Viburnum nudum* Brandywine™ grown in a greenhouse from Oct. to Dec. 2009 (run 2).

Flooding Duration (days) ^z	<i>F. ×intermedia</i>		<i>I. verticillata</i>	<i>V. nudum</i>
	GI (cm)	RDW (g)	SDW (g)	GI (cm)
0	34.6a ^y	61.0a	29.1b	37.9b
3	32.6ab	59.0a	28.6b	39.5ab
6	30.4b	48.0b	32.6a	41.8a

^z Plants were flooded for 0, 3, or 6 days followed by 6 days of draining. The flood-drain cycle occurred five times on plants flooded for 3 days and four times for plants flooded for 6 days.

^y Lowercase letters denote mean separation within a column using Proc Glimmix at $P < 0.05$ (SAS Institute, Cary, NC).

Table 3. Effect of flooding duration (days) on photosynthesis (P_n), stomatal conductance (SC), and stem water potential (SWP), during the 2nd flood and drain cycle (6 day flooding duration treatment) or 3rd flood and drain cycle (3 day flooding duration treatment) on *Fothergilla ×intermedia* ‘Mt. Airy’, *Ilex verticillata* ‘Winter Red’, *Clethra alnifolia* ‘Ruby Spice’, *Callicarpa dichotoma* ‘Early Amethyst’, and *Viburnum nudum* Brandywine™ grown in a greenhouse, Aug. to Oct. 2009 (run 1).

Flooding Duration (days) ^z	Stage ^y	<i>F. ×intermedia</i>		<i>I. verticillata</i>	<i>C. alnifolia</i>	<i>C. dichotoma</i>	<i>V. nudum</i>
		P_n ^x	SC ^w	SC	SC	P_n	SWP ^v
0	na	18.3a ^u	0.23ab	0.23b	0.23b	21.3a	-1.04b
3	F	17.3a	0.26a	0.48a	0.45a	16.9b	-0.72a
3	D	17.6ab	0.25a	0.25b	0.22b	21.5a	-1.17b
6	F	7.7c	0.09c	0.19b	0.24b	22.9a	-0.82a
6	D	15.6b	0.17b	0.26b	0.28b	15.7c	-0.97b

^z Plants were flooded for 0, 3, or 6 days followed by 6 days of draining. The flood-drain cycle occurred five times on plants flooded for 3 days and four times for plants flooded for 6 days.

^y Stage denotes whether measurements were taken during flooding (F) or draining (D).

^x $P_n = \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$

^w $\text{SC} = \text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$

^v $\text{SWP} = \text{MPa}$

^u Lowercase letters denote mean separation using Proc Mixed at $P < 0.05$ (SAS Institute, Cary, NC).

Table 4. Effect of flooding duration (days) on photosynthesis (P_n), stomatal conductance (SC), and stem water potential (SWP), during the 4th flood and drain cycle (6 day flooding) and 5th flood and drain cycle (3 day flooding) on *Fothergilla × intermedia* ‘Mt. Airy’, *Ilex verticillata* ‘Winter Red’, *Clethra alnifolia* ‘Ruby Spice’, and *Viburnum nudum* Brandywine™ grown in a greenhouse, Aug. to Oct. 2009 (run 1).

Flooding Duration (days) ^z	Stage ^y	<i>F. × intermedia</i>			<i>I. verticillata</i>			<i>C. alnifolia</i>		<i>V. nudum</i>
		P_n^x	SC ^w	SWP ^v	P_n	SC	SWP	P_n	SWP	SWP
0	na	18.2a ^u	0.24a	-0.74b	20.3a	0.24a	-0.55a	4.86b	-0.46a	-0.62b
3	F	18.5a	0.23a	-0.88b	20.2a	0.27a	-0.10b	11.7a	-1.0b	-0.8c
3	D	19.5a	0.25a	-0.23a	18.0ab	0.25ab	-0.48a	3.99b	-0.48a	-0.52ab
6	F	8.83c	0.06c	-1.47c	5.06c	0.04c	-1.22b	3.04b	-1.2b	-1.0d
6	D	12.3b	0.09b	-0.37b	12.4b	0.15b	-0.6a	3.69b	-0.6a	-0.43a

^z Plants were flooded for 0, 3, or 6 days followed by 6 days of draining. The flood-drain cycle occurred five times on plants flooded for 3 days and four times for plants flooded for 6 days.

^y Stage denotes whether measurements were taken, during flooding (F) or draining (D).

^x $P_n = \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$

^w $\text{SC} = \text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$

^v $\text{SWP} = \text{MPa}$

^u Lowercase letters denote mean separation using Proc Mixed at $P < 0.05$ (SAS Institute, Cary, NC).

Table 5. Effect of flooding duration (days) on photosynthesis (P_n), stomatal conductance (SC), and stem water potential (SWP), for the 2nd versus the 4th flood and drain cycle (6 day flooding treatment) and 3rd versus the 5th flood and drain cycle (3 day flooding treatment) on *Fothergilla × intermedia* ‘Mt. Airy’, *Ilex verticillata* ‘Winter Red’, *Clethra alnifolia* ‘Ruby Spice’, *Callicarpa dichotoma* ‘Early Amethyst’, and *Viburnum nudum* Brandywine™ grown in a greenhouse, Aug. to Oct. 2009 (run 1).

Time ^z	Stage ^y	Flooding Duration (days) ^x	<i>F. × intermedia</i>		<i>I. verticillata</i>		<i>C. alnifolia</i>			<i>C. dichotoma</i>			<i>V. nudum</i>
			SC ^w	SWP ^v	P_n ^u	SC	P_n	SC	SWP	P_n	SC	SWP	SWP
Mid	F	3	0.26 ^t	-1.17	18.9	0.49a ^v	16.5a	0.45a	-0.75	19.9	0.21	-0.9	-0.72
End	F	3	0.23	-0.83	20.2	0.27b	11.6b	0.11b	-0.47	14.4	0.15	-0.8	-0.8
Mid	F	6	0.09	-0.9a	15.4a	0.19a	14.8a	0.24a	-0.63	22.9a	0.28a	-0.98	-0.82
End	F	6	0.06	-1.47b	5.1b	0.04b	3.0b	0.03b	-0.67	10.7b	0.10b	-0.63	-1.0
Mid	D	3	0.25	-0.98b	18.9	0.25	17.0a	0.22a	-0.78b	21.5a	0.27a	-1.0b	-1.17b
End	D	3	0.25	-0.23a	18.0	0.25	3.9b	0.04b	-0.42a	8.1b	0.06b	-0.55a	-0.52a
Mid	D	6	0.17a	-0.82b	19.2a	0.26	16.8a	0.28a	-0.98b	15.7	0.21a	-0.77	-0.97b
End	D	6	0.09b	-0.37a	12.4b	0.15	3.7b	0.03b	-0.65a	12.0	0.08b	-0.38	-0.43a

^z Mid and End represent the time of the experiment in which the measurements were taken; the middle or end of the experiment. Mid represents the 2nd/3rd flooding or draining cycle and End represents the 4th/5th flooding or draining cycle. The 2nd/4th cycles correspond to the 6 day flooding duration and the 3rd/5th cycles correspond to the 3 day flooding duration.

^y Stage denotes whether measurements were taken during flooding (F) or draining (D).

^x Days denotes the number of days in the flooding and draining cycle.

^w SC = mmol·m⁻²·s⁻¹

^v SWP = MPa

^u P_n = μmol·m⁻²·s⁻¹

^t Lowercase letters denote mean separation using Proc Mixed at $P < 0.05$ (SAS Institute, Cary, NC); if no differences then letters are omitted.

Chapter IV

Phosphorus Uptake by *Muhlenbergia capillaris*, a Rain Garden Plant, Under Flooded and Non-Flooded Conditions

Index Words: Water, Phosphorus, Uptake, *Muhlenbergia capillaris*

Abstract: Rain gardens are recommended as a way to remove pollutants from runoff. A pollutant of particular concern for waterways is phosphorus. Research was conducted to evaluate phosphorus (P) uptake by and growth of the native grass *Muhlenbergia capillaris* in flooded and non-flooded conditions. On 17 Nov. 2009, 140 plants [0.25 L (2.25 in) liners] of *Muhlenbergia capillaris* (Lam.) Trin. (gulf muhly grass) were placed into 3.8 L (1 gal) containers. Each container was filled with approximately 2.5 L (0.7 gal) substrate of 85% sand and 15% peat moss and placed on raised benches in a greenhouse on 30 Mar. 2010. Beginning on 13 Apr. 2010, roots zones of plants were flooded to the substrate surface for 0 (non-flooded) or 3 days (flooded). Following the flooding period, plants were allowed to drain for 6 days. During the draining period, no water was added to containers. The flood-drain process was repeated for 6 weeks. Non-flooded plants were hand watered as needed. The experiment was terminated on 1 June 2010 (run 1) and repeated (run 2) beginning 7 June 2010 and ending 20 July 2010. *Run 1.* Height (HT), shoot dry weight (SDW), root dry weight (RDW) were higher in non-flooded plants than flooded plants. Shoot dry weight increased linearly with an increasing P irrigation rate, while RDW changed cubically with increasing P irrigation

rates, with the maximum and minimum dry weights at 5.5 g and 3.7 g. Phosphorus concentration in leachate increased linearly with an increasing P irrigation rate in non-flooded plants at the 1st flood cycle of the experiment. At the 5th flood cycle of the experiment, phosphorus concentration in the leachate of non-flooded plants increased linearly with increasing P irrigation rate and in flooded plants the phosphorus concentration in leachate changed quadratically with increasing P irrigation rates, with maximum and minimum concentrations occurring at 0.17 mg·L⁻¹ P and 0.07 mg·L⁻¹P.

Run 2. Height, SDW, and RDW of plants were higher in non-flooded plants than flooded plants, and plants with phosphorus fertilizer added to substrate had higher HT, SDW, and RDW than plants without any phosphorus fertilizer added to substrate. Phosphorus concentration in leachate was highest when in non-flooded or flooded plants with phosphorus fertilizer added to the substrate at the 1st, 3rd, and 5th flood cycles of the experiment. All plants exhibited growth during flooding and appeared tolerant of flooding and would be appropriate native shrub selections for rain gardens.

Introduction

Phosphorus is now one of the most ubiquitous forms of water quality impairment in the developed world and thus is receiving increased attention and is becoming an important area of research (Sharpley et al., 2001). Water contaminated with excess nitrogen and phosphorus can impact the quality of surface water and groundwater. Phosphorus enrichment of the soil surface layer can result in increased phosphorus losses from soil to surface waters when soil particles move downwards to storm drains or are lost as a result of surface erosion (Richards et al., 1998; Ryden et al., 1973). Recent research has shown that the primary loss of phosphorus from soil into surface runoff and

subsurface flow typically originates from only a small part of the landscape during a relatively large rain event. These areas generally occur where high soil phosphorus or phosphorus application via mineral fertilizer or manure coincide on sites with high runoff or erosion potential (Sharpley et al., 2001). Repeated cycles of soil saturation followed by infiltration and reflooding can also result in significant P release from soils (Olila et al., 1997). Soil sediment characteristics are important since sediments act as either sources of or sinks for nutrients within the water column (Olila et al., 1997). Nutrients are either taken up by plants or move through the soil to surface waters or subsurface waters. Movement of small amounts of phosphorus through the soil profile can have adverse effects on water quality and cause excessive enrichment of surface soils (Richards et al., 1998). While erosion of particulate P from soils remains a dominant concern, the transport of dissolved or soluble phosphorus in surface runoff and subsurface flow is also a critical environmental issue (Sharpley et al., 2001).

Phosphates are forms of phosphorus that cause rapid growth of algae in bodies of water. As the algae decay, the process depletes the supply of oxygen and results in eutrophication or premature "aging" of a lake or pond (U.S. Environ. Protection Agency, 2009a). Eutrophication caused by excessive inputs of N and P is the most common impairment of surface waters in the United States (U.S. Environ. Protection Agency, 1990). Excess P may contribute to unsightly algal blooms, which cause taste and odor problems and deplete oxygen required by fish and other aquatic species (U.S. Environ. Protection Agency, 2009b).

Phosphorus can be removed in constructed wetlands by plant uptake, assimilation by microorganisms, and physico-chemical processes involving the wetland soil. Among

the physico-chemical processes, sorption by soil and precipitation reactions plays an important role (Del Bubba et al., 2003; Vymazal et al., 1998). The effect of soil flooding or inundation on phosphorus uptake by plants is complicated and strongly dependent upon soil type. In some alkaline soils that are relatively low in phosphorus, flooding may cause an increase in P availability, leading to a temporary increase in P uptake by plants. On the other hand, prolonged flooding can reduce P uptake by plants due to root dysfunction, damage, and death (Chen et al., 2005; Kozlowski, 1984). Substrates can absorb phosphate ions and/or promote precipitation by supplying the solution with metals, which can react with phosphorus to produce sparingly soluble phosphates. Variations of pH, ionic strength, and concentration of competitive ions are known to strongly influence the adsorption process (Del Bubba et al., 2003; Polyzopoulos et al., 1985).

Flooding of soil or substrate depletes soil oxygen and alters plant metabolism (Kozlowski, 1984b). Upon flooding, the limited supply of oxygen is depleted rapidly by roots, microorganisms, and soil reductants (Pezeshki, 2001; Ponnampereuma, 1972). Responses to flooding will vary with the type of plant, the duration and timing of flooding, and the condition of the flood water. Flooding during the growing season is much more harmful than flooding during the dormant season (Kozlowski, 1982). Moving flood water is less harmful than standing water, as even flood tolerant plants are injured by flooding in standing water (Kozlowski, 1982). Stagnant water restricts or inhibits adjustment of seedlings to flooded conditions, whereas in moving flood water, seedlings are able to recover in height and growth characteristics (Hook et al., 1969). The water in the stagnant treatments was brownish, had an oily appearance on the

surface, and gas bubbles escaped from the soil (Hook et al., 1969). Upon flooding, a flood-tolerant species will generally acquire more minerals than flood-intolerant species (Chen et al., 2005; Pezeshki et al., 1999). Plant responses to flooding during the growing season include injury, inhibition of seed germination and vegetative and reproductive growth, changes in plant anatomy and promotion of early senescence and mortality (Kozlowski, 1997). Morphological adaptations to flooding include formation of aerenchyma tissue, hypertrophy of lenticels (openings in stems and roots that permit gas exchange between internal tissues and the atmosphere), and regeneration of new root structures (Kozlowski, 1984a).

As rain gardens receive increased attention and acceptance for use not only in commercial situations but also in residential plots, research is needed to identify appropriate plant species. Plant species used in rain gardens must be able to withstand repeated, short-term flooding events separated by periods of drying. In addition, it would be helpful to characterize a species' ability to absorb phosphorus as a step in remediating stormwater runoff. In previous work, phosphorus concentrations in a pond that captured stormwater runoff typically ranged from $0.01 \text{ mg}\cdot\text{L}^{-1}$ to $0.8 \text{ mg}\cdot\text{L}^{-1}$ P. When this stormwater was added to rain gardens, the concentration of phosphorus in the outflow from the rain garden was reduced. It is necessary to evaluate individual plant species for their ability to absorb phosphorus under flooded conditions. Therefore, the objective of this research is to evaluate phosphorus uptake by and growth of the native grass *Muhlenbergia capillaris* in flooded and non-flooded conditions.

Materials and Methods

Run 1

On 17 Nov. 2009, 140 [0.25 L (2.25 in) liners] *Muhlenbergia capillaris* (Lam.) Trin. (gulf muhly grass) from Magnolia Gardens Nursery, Magnolia, TX were placed into 3.8 L (1 gal) containers. Each container was filled with approximately 2.5 L (0.7 gal) substrate of 85% sand and 15% peat moss amended with $1.2 \text{ kg}\cdot\text{m}^{-3}$ ($2 \text{ lbs}\cdot\text{yd}^{-3}$) dolomitic limestone and $0.9 \text{ kg}\cdot\text{m}^{-3}$ ($1.5 \text{ lbs}\cdot\text{yd}^{-3}$) micronutrient fertilizer (Scott's Company, Marysville, Ohio). The plants were placed on a full sun nursery pad in Auburn, AL at the Paterson Greenhouse Complex. Plants were top dressed on 1 Dec. 2009 with 5.1 g (0.18 oz) 43N-0P-0K (Harrell's Professional Fertilizer Solutions, Sylacauga, AL) per container and 3.7 g (0.13 oz) 0N-0P-52.3K (Piedmont Fertilizer, Opelika, AL) per container. No phosphorus (P) was added to the substrate. During the overwintering period, plants received daily overhead irrigation for 10 min starting at 10:30AM, and 1:30PM. At temperatures of -4°C (25°F) or lower, plants were covered with white polyethylene plastic with ventilation holes.

On 30 Mar. 2010 (133 DAP) plants were moved into a greenhouse and placed on raised benches at the Paterson Greenhouse Complex, Auburn University, AL. Plants were grown in an unshaded 8 mm (0.3 in) twin-wall, polycarbonate covered greenhouse under natural photoperiods with a heating set point of 18.3°C (65°F) and ventilation beginning at 25.6°C (78°F). Initial and final shoot heights (HT) were measured on 12 Apr. 2010 and 1 June 2010, respectively. Height was measured by gathering all the leaves up towards the center of the pot and holding them upright and measuring to the

highest point. On 1 June 2010, shoot width at the level of the top of the pot was also measured.

Flooding treatments were initiated on 13 Apr. 2010. Plants were flooded using a pot-in-pot method. The container with the plant (with holes in the bottom to allow for drainage) was placed inside another container without drainage holes. To flood a container, tap water was added by hand to the container until the water level was even with the substrate level; additional water was added to the container daily as needed to keep the water level even with the substrate surface. The volume of water applied was recorded daily; approximately 600 mL (0.2 gal) was added initially in order to flood plants, and approximately 100 mL (0.03 gal) was added daily to maintain the flood level. Plants were flooded for 0 days (non-flooded) or 3 days. Following flooding, plants were allowed to drain for 6 days. No additional water was added to the containers during the 6 days of draining. The flood – drain cycle was repeated for four additional cycles. Non-flooded plants were irrigated by hand as needed. Plants were irrigated (non-flooded) or flooded with one of seven solutions, each with a different phosphorus concentration (source of phosphorus was 85% ortho-phosphoric acid, Fisher Scientific, Pittsburg, PA). Solutions contained 0, 0.1, 0.2, 0.3, 0.4, 0.5, or 0.8 mg·L⁻¹ P (hereafter referred to as P irrigation rate). Substrate moisture percent was measured using ECH₂O soil moisture sensors (model EC-5) every 60 minutes in 5 containers per flooding treatment and recorded using Em5b dataloggers (Decagon Devices, Inc., Pullman, WA). At the end of the 1st, 3rd, and 5th flood cycles, leachate and plant tissue samples were collected from five flooded and five non-flooded plants in each P irrigation rate. Leachate was collected from non-flooded plants using the Virginia Tech Pour-Through Method (Wright, 1986).

Each pour-through of non-flooded plants was conducted by applying 500 mL of the same solution used for irrigation to the container. For flooded plants, leachate was collected from water that drained from the container when the exterior pot was removed. Leachate samples were placed in 125 mL amber bottles (Fisher Scientific, Pittsburg, PA) and stored in a cooler at 5°C (41°F) until analysis. Plant tissue samples consisted of leaves severed at the substrate level. Tissue samples were placed in paper bags and dried in an oven for 48 hours at 66°C (150°F).

Leachate water samples were analyzed by inductively coupled plasma (ICP) (SpectroCiros CCD, Kleve, Germany) for calcium (Ca), magnesium (Mg), K, P, and sulfur (S). Nitrate (NO₂) and nitrite (NO₃) were analyzed by ion chromatography (IC) (Dionex IC-3000, Sunnyvale, CA), and ammonium (NH₄) was determined colorimetrically (Sims et al., 1995; Murphy and Riley, 1962) using a microplate spectrophotometer (μQuant, BioTek Instruments Inc., Winooski, VT). Tissue samples were ground using a plant tissue mill (Foss Tecator Cyclotec Sample Mill, Fisher Scientific, Pittsburg, PA) and analyzed by microwave digestion (MarsXpress, CEM Corp., Matthews, NC) and ICP for the same elements. All analyses were completed by the Department of Agronomy and Soils Soil Chemistry Laboratory at Auburn University.

The experiment was terminated on 1 June 2010 (195 DAP). Leaves were severed at the substrate level, and roots were rinsed to remove substrate. Shoots and roots were dried separately in an oven for 48 hours at 66°C (150°F) for determination of shoot dry weight (SDW) and root dry weight (RDW).

Run 2

On 24 May 2010, 96 [0.25 L (2.25 in) liners] *M. capillaris* from Stepping Stone Nursery, Homestead, FL were potted into 3.8 L (1 gal) containers as described above (substrate and amendments the same). All plants were top dressed with 5.1 g (0.18 oz) of 43N-0P-0K (Harrell's Professional Fertilizer Solutions, Sylacauga, AL) per container and 2.9 g (0.1 oz) 0N-0P-52.3K (Piedmont Fertilizer, Opelika, AL) per container. One half of the plants were top dressed with 2.0 g (0.07 oz) 0N-20.1P-0K (Piedmont Fertilizer, Opelika AL) per container.

On 24 May 2010 containers were placed in a greenhouse as described above. Flooding treatments were initiated on 7 June 2010 (14 DAP) and were applied as described above. Solutions used for irrigation or flooding included 0, 0.2, 0.4, or 0.8 mg·L⁻¹ P. Leachate samples were collected and analyzed as described above. The experiment was terminated and plants were harvested on 20 July 2010 (58 DAP) as described above. Shoot dry weight and RDW were determined as described above. Initial (7 June 2010) and final (20 July 2010) HT were measured as described above.

Phosphorus Isotherm

In order to characterize the phosphorus adsorption capacity of the substrate used, a phosphorus sorption isotherm was developed for the sand-peat substrate used in the experiment either with 1) no nutrients added, 2) with nitrogen and potassium added (5.1 g N and 3.7 g K), or 3) with nitrogen, phosphorus, and potassium added (5.1 g 43N- 0P- 0K, 3.7 g 0N-20.1P-0K, and 3.7 g 0N-0P- 52.3K). Isotherms were developed using solutions containing 0, 0.01, 0.05, 0.1, 0.2, 0.5, 1, 5, 10, 20, or 50 mg·L⁻¹ P, based on techniques of Nair et al. (1984) and Liator et al. (2005). Isotherms were determined

using a substrate: solution ratio of 2 g substrate: 25 mL P solution. The suspensions were equilibrated for 24 h in polyethylene tubes on a reciprocal shaker, then centrifuged at 4500 rpm for 30 min. Samples were then filtered through 0.45 μm filter paper and analyzed for P using ICP.

In both experiments, plants were arranged in a completely randomized design. Data were analyzed using Proc Glimmix model procedure with least square means ($P < 0.05$) (SAS Institute, Cary, NC). In run 1, nine repetitions were used in each treatment. In run 2, six repetitions were used in each factorial combination of treatment x flooding duration x substrate fertilizer addition. Data were analyzed to determine the effect of flooding duration on HT, SDW, and RDW. Data were also analyzed to determine differences in the P irrigation rate applied to plants. In run 2, data were also compared to determine differences in the addition of phosphorus fertilizer application in substrates. Data was compared by the time the measurements were taken; in the beginning, middle, or end of the experiment. Data was collected from non-flooded control plants at each measurement date, regardless of treatment being measured. Data is presented only where significant.

Results

Phosphorus sorption isotherms indicated that when nutrients (N, P, and K) were added to the sand-peat substrate, 100% of phosphorus adsorbed to the substrate at P irrigation rates up to $1 \text{ mg}\cdot\text{L}^{-1}$ (equivalent to $12.5 \text{ mg P}\cdot\text{kg}^{-1}$ substrate) (0.01, 0.05, 0.1, 0.2, 0.5, and $1 \text{ mg}\cdot\text{L}^{-1}$). The P irrigation rates used in both runs were within this range and thus the phosphorus in the irrigation water had the potential to be completely adsorbed to the substrate. For P irrigation rates over $1 \text{ mg}\cdot\text{L}^{-1}$ (5, 10, 20, and $50 \text{ mg}\cdot\text{L}^{-1}$),

percent P adsorption potential declined rapidly as P irrigation rate increased. When no nutrients were added to the substrate, 100% P adsorption occurred for P irrigation rates less than $0.5 \text{ mg}\cdot\text{L}^{-1}$ P. For rates of $0.5 \text{ mg}\cdot\text{L}^{-1}$ and higher (0.5, 1, 5, 10, 20, and $50 \text{ mg}\cdot\text{L}^{-1}$), adsorption ability declined rapidly as P irrigation rate increased.

In run 1, HT, SDW, RDW were higher in non-flooded plants (0 day flood duration) than flooded plants (3 day flood duration) (Table 1). Shoot dry weight increased linearly with an increasing P irrigation rate, while RDW changed cubically with increasing P irrigation rates, with the maximum and minimum RDW of 5.5 g and 3.7 g (Table 1). In run 2, HT of non-flooded plants was higher than the HT of flooded plants (Table 2). Shoot dry weight of plants was highest in non-flooded plants and lowest in flooded plants (Table 2). Plants with phosphorus fertilizer added to the substrate had higher SDW than plants without any phosphorus fertilizer added to substrate (Table 2). Root dry weight in run 2 was highest in non-flooded plants without phosphorus added to the substrate (Table 2). Plants flooded for 0 or 3 days with phosphorus fertilizer added to the substrate had a similar RDW, and plants flooded for 3 days without phosphorus fertilizer added to the substrate had the lowest RDW.

At the 1st flood cycle of run 1 (Apr. 2010), phosphorus concentration in leachate increased linearly with an increasing P irrigation rate in non-flooded plants (Table 3). In flooded plants, there was no trend for P concentration in leachate (Table 3). Phosphorus concentration in leachate at a P irrigation rate of $0.1 \text{ mg}\cdot\text{L}^{-1}$ was higher in flooded plants than non-flooded plants (Table 3). At a P irrigation rate of $0.8 \text{ mg}\cdot\text{L}^{-1}$, phosphorus concentration in leachate was higher in non-flooded plants than in flooded plants (Table 3). In the 3rd flood cycle of run1 (May 2010), phosphorus concentration in leachate was

higher in flooded plants ($0.09 \text{ mg}\cdot\text{L}^{-1}$) than non-flooded plants ($0.05 \text{ mg}\cdot\text{L}^{-1}$). At the 5th flood cycle of run 1 (June 2010), phosphorus concentration in the leachate of non-flooded plants increased linearly with increasing P irrigation rate. In flooded plants, the phosphorus concentration in leachate changed quadratically with increasing P irrigation rates, with maximum and minimum concentrations occurring at $0.17 \text{ mg}\cdot\text{L}^{-1}$ P and $0.07 \text{ mg}\cdot\text{L}^{-1}$ P. At a P irrigation rate of $0.3 \text{ mg}\cdot\text{L}^{-1}$, phosphorus concentrations in leachates were higher in plants flooded for 3 days, but in P irrigation rate of 0.5 and $0.8 \text{ mg}\cdot\text{L}^{-1}$, phosphorus in leachate was higher in plants flooded for 0 days than in plants flooded for 3 days (Table 3).

In run 2, phosphorus concentration in leachate was highest when phosphorus fertilizer was added to the substrate of flooded plants at the 1st, 3rd, and 5th flood cycles of the experiment (10 June, 28 June, and 16 July, 2010, respectively) (Table 4). Phosphorus concentration in leachate was lowest when no phosphorus was added to the substrate of flooded plants at all three sampling dates, regardless of flooded or non-flooded plants (Table 4).

Discussion

Shoot dry weight and RDW were similar between run 1 and run 2 when no P fertilizer was added to substrate. Shoot dry weight and RDW were higher when P fertilizer was added to the substrate, but that resulted in increased phosphorus concentration in leachate in run 2. The concentrations of phosphorus in the irrigation rates were sufficient to support plant growth and development. The concentrations in P irrigation rates were based off of results found in the rain garden experiment in the Davis Arboretum, Auburn, AL, and results of Bayne et al. (2004) from local watersheds

surrounding Auburn, AL. The effect of P irrigation rates on P concentration in leachate was more consistent and predictable in non-flooded plants. In all cases, the P concentration in leachate was lower than the P irrigation rate applied to plants, indicating the plant is taking up the phosphorus, the phosphorus is adsorbing to the substrate, or is being leached out.

In run 2, in non-flooded plants without the addition of P fertilizer to substrate, the concentration of P in leachate decreased with each flooding cycle of the run. The phosphorus applied by the P irrigation rate was taken up by the plant for growth, adsorbed to the substrate, or leached out. At the 1st flood cycle of the experiment when plants had an addition of P fertilizer to substrate, there was a large release of P into the leachate concentrate of flooded (70.2 mg·L⁻¹) and non-flooded (43.9 mg·L⁻¹) plants. This is probably due to the initial release of the fertilizer applied to the substrate. Over time (the 3rd and 5th flood cycles of the experiment), the concentration of P in leachate declined, indicating the P was taken up by the plant and roots, adsorbing to the soil, or leaching out. Throughout the run, the P concentration in leachate was higher in flooded plants than in non-flooded plants, which agrees with Olila et al. (1997) that repeated flooding can cause a release of P from soils.

All plants in both runs appeared healthy and exhibited no problems with pests. Even under flooded conditions, the plants were able to spread and put out new leaves. *Muhlenbergia capillaris* has a moderate growth rate, with an average mature height of 1 m (3 ft) for optimal growing conditions (U.S. Dept. of Agr., 2010). *Muhlenbergia capillaris* is a facultative upland (FACU) plant in the southeast. It usually occurs in non-wetlands (estimated probability 67 to 99 %), but occasionally found on wetlands

(estimated probability 1 to 33 %) (U.S. Dept. of Agr., 2010). Plants in both flooded and non-flooded conditions were close to reaching the mature height in less than one growing season. At the end of the draining period, plants did seem to exhibit some signs of slight wilting and drought stress. Flooded plants did not receive any irrigation during their draining period to allow the substrate to dry, substrate percent moisture (by volume routinely averaged 5 to 6 %. This may be considered extremely dry, and examining the extent of drought tolerance of this taxon may provide additional information regarding its suitability for use in rain gardens. After re-flooding, plants regained full turgor pressure and recovered. No flowers were seen in either run.

Muhlenbergia capillaris appears to be able to tolerate the fluctuating hydrology of a rain garden and the short periods of inundation that accompany it. This work further justifies past research (Werneth, 2010), and demonstrates the need for more investigation that native deciduous shrubs make flood tolerant, attractive options for use in rain gardens in the Southeast.

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Table 1. Effect of flooding and phosphorus irrigation rates (P-Rate) on height (HT), shoot dry weight (SDW), and root dry weight (RDW) of *Muhlenbergia capillaris* grown in a greenhouse, Apr. to June 2010 (run 1).

Flooding Duration (days) ^z	HT (cm)	SDW (g)	RDW (g)
0	72.6a ^y	6.2a	5.1a
3	59.0b	4.2b	4.4b
P rate (mg·L ⁻¹)		SDW (g)	RDW (g)
0.0		4.8	5.3
0.1		5.0	3.7
0.2		4.7	4.1
0.3		4.4	4.8
0.4		6.0	4.9
0.5		5.1	5.5
0.8		6.2	4.8
Significance ^x		L**	C***
Equation		y= 1.79x + 4.58	y= -43.8x ³ + 52.1x ² -14x+5.1
R ² value		0.507	0.808

^z Plants were flooded for 0 (non-flooded) or 3 days followed by 6 days of draining. The flood-drain cycle occurred five times.

^y Lowercase letters denote mean separation within a column using Proc Glimmix at $P < 0.05$ (SAS Institute, Cary NC).

^x Significance of linear (L) or cubic (C) to determine trend with increasing phosphorus rate at $\alpha = 0.01$ (**) and 0.001(***) determined using contrast statements.

Table 2. Effect of flooding duration (days) and addition of phosphorus fertilizer to substrate on height (HT), shoot dry weight (SDW), and root dry weight (RDW) of *Muhlenbergia capillaris* grown in a greenhouse, May to July 2010 (run 2).

Flooding Duration (days) ^z	Substrate ^y	HT (g)	SDW (g)	RDW (g)
0	NP	80.0a ^x	6.4c	5.10a
0	P	79.75a	9.9a	3.77b
3	NP	64.33b	4.9c	1.96c
3	P	66.29b	7.4b	3.37b

^z Plants were flooded for 0 (non-flooded) or 3 days followed by 6 days of draining. The flood-drain cycle occurred five times on plants.

^y Substrate: P denotes the addition of 0N- 20.1P- 0K to substrate, NP denotes no phosphorus added to substrate.

^x Lowercase letters denote mean separation within a column using Proc Glimmix at $P < 0.05$ (SAS Institute, Cary NC).

Table 3. Effect of flooding duration (days) and phosphorus (P) irrigation rates (P-Rate) on concentration of phosphorus in leachate (P-Conc) ($\text{mg}\cdot\text{L}^{-1}$) of *Muhlenbergia capillaris* grown in a greenhouse, Apr. to June 2010 (run 1).

P-Rate ^z ($\text{mg}\cdot\text{L}^{-1}$)	Leachate P-Conc ($\text{mg}\cdot\text{L}^{-1}$)	
	Flooding duration (days) ^y	
	0	3
April 2010 ^x		
0	0.06	0.08
0.1	0.04b ^w	0.12a
0.2	0.09	0.12
0.3	0.1	0.11
0.4	0.15	0.13
0.5	0.15	0.12
0.8	0.21a	0.11b
Significance ^v	L***	NS
Equation	$y = 0.212x + 0.045$	
R ²	0.93	NS
June 2010 ^u		
0	0.03	0.07
0.1	0.03	0.07
0.2	0.11	0.07
0.3	0.08b	0.17a
0.4	0.15	0.16
0.5	0.22a	0.14b
0.8	0.36a	0.12b
Significance	L***	Q**
Equation	$y = 0.426x + 3E-5$	$y = -0.372x^2 + 0.386x + 0.51$
R ²	0.94	0.65

^z Phosphorus irrigation rate of water used to irrigate or flood plants.

^y Plants were flooded for 0 (non-flooded) or 3 days followed by 6 days of draining. The flood-drain cycle occurred five times.

^x Leachate samples were collected during the first flood-drain cycle, or beginning of the experiment.

^w Lowercase letters denote mean separation within a column using Proc Glimmix at $P < 0.05$ (SAS Institute, Cary NC).

^v Linear (L) or quadratic (Q) contrast used to determine trend with increasing phosphorus rate at $\alpha = 0.01$ (**) and 0.001 (***). Not significant is denoted by NS.

^u Leachate samples were collected during the fifth flood-drain cycle, or end of the experiment.

Table 4. Effect of flooding duration (days) and addition of phosphorus fertilizer to substrate on concentration of phosphorus in leachate (P-Conc) ($\text{mg}\cdot\text{L}^{-1}$) of *Muhlenbergia capillaris* grown in a greenhouse, May to July 2010, (run 2).

Flooding duration (days) ^z	Substrate ^y	Leachate P-Conc ($\text{mg}\cdot\text{L}^{-1}$)
10 June 2010 ^x		
0	NP	0.50c ^w
0	P	43.86b
3	NP	0.34c
3	P	70.16a
28 June 2010 ^v		
0	NP	0.22c
0	P	9.31b
3	NP	2.06c
3	P	33.64a
July 2010 ^u		
0	NP	0.06c
0	P	3.87b
3	NP	0.58c
3	P	18.5a

^z Plants were flooded for 0 (non-flooded) or 3 days followed by 6 days of draining. The flood-drain cycle occurred five times on plants.

^y Substrate: P denotes the addition of phosphorus to soil, NP denotes that no phosphorus added to soil.

^x Leachate samples were collected during the first flood-drain cycle, or beginning of the experiment.

^w Lowercase letters denote mean separation within a column using Proc Glimmix at $P < 0.05$ (SAS Institute, Cary NC).

^v Leachate samples were collected during the third flood-drain cycle, or middle of the experiment.

^u Leachate samples were collected during the fifth flood-drain cycle, or end of the experiment.

Appendix I

Chapter II

Table 1. Mass load of nutrients in stormwater inflow (g) into two types of rain gardens, conventional (CONV) or internal water storage (IWS).

IWS RG ^z Mass Load in Water				
Date	TN Inflow (mg·L ⁻¹)	TN Load (g)	TP Inflow (mg·L ⁻¹)	TP Load (g)
10 July 2008	0.94 ^y	3.21 ^x	0.01	0.02
24 July 2008	0.82	2.8	0.15	0.51
7 Aug. 2008	0.84	2.87	0.04	0.15
21 Aug. 2008	0.21	0.72	0.05	0.17
5 Sept. 2008	1.36	4.65	2.28	7.77
18 Sept. 2008	0.22	0.76	0.04	0.14
1 Oct. 2008	0.55	1.88	0.85	2.9
14 Oct. 2008	0.53	1.81	0.04	0.12
30 Oct. 2008	1.03	3.5	0.36	1.24
12 Nov. 2008	1.73	5.89	0.12	0.41
20 Nov. 2008	0.82	2.8	0.08	0.28
8 Dec. 2008	0.33	1.12	0.07	0.24
25 Feb. 2009	0.92	3.13	0.34	1.15
CONV RG ^z Mass Load in Water				
Date	TN Inflow (mg·L ⁻¹)	TN Load (g)	TP Inflow (mg·L ⁻¹)	TP Load (g)
18 July 2008	1.02 ^y	3.46	0.01	0.02
31 July 2008	1.52	5.19	0.01	0.02
14 Aug. 2008	0.73	2.5	0.01	0.05
28 Aug. 2008	0.47	1.6	0.01	0.02
11 Sept. 2008	0.71	2.43	0.56	1.91
25 Sept. 2008	0.66	2.24	0.05	0.18
9 Oct. 2008	0.29	1	0.01	0.02
24 Oct. 2008	0.41	1.39	0.05	0.17
4 Nov. 2008	1.19	4.07	0.58	1.96
17 Nov. 2008	0.96	3.28	0.08	0.28
5 Dec. 2008	0.34	1.17	0.02	0.05
15 Dec. 2008	1.23	4.18	0.1	0.33
9 Jan. 2009	0.46	1.58	0.72	2.45
14 Jan. 2009	0.69	2.34	5.73	19.52
22 Jan. 2009	0.85	2.9	0.13	0.46
29 Jan 2009	0.47	1.61	0.12	0.41
6 Feb. 2009	1.63	5.55	0.05	0.16
10 Feb. 2009	0.4	1.36	0.08	0.26
19 Feb. 2009	1.34	4.56	0.38	1.3

^z The rain garden with an internal water storage layer (IWS RG) utilizes a saturated water zone in the rain garden that permits water to collect in the bottom 1 m (3.5 ft) of the rain garden. The saturated layer allows nitrogen to be removed via denitrification. The conventional rain garden (CONV RG) has an aerobic zone which allows water to be treated via interception, storage, filtration, and adsorption.

^y Total Kjeldahl nitrogen (TKN) + nitrate-nitrite (NO₂-NO₃) = TN

^x Load (g) = (volume of water into RG 3607 L * Inflow mg·L⁻¹) / 1000 mg·g⁻¹

Table 2. Estimated mass loads of nutrients found in plant tissue for *Carex*, *Dryopteris erythrosora*, and *Iris* sp. in two rain garden types, conventional (CONV) or internal water storage (IWS).

IWS RG ^z Nutrient Loads in Plant Tissue								
Date	Plant Type	Total Plants	Avg. Plant Weight (g)	Total Plant Mass ^y (g)	N ^x (%)	N ^w (g)	TP ^x (%)	TP ^w (g)
July 2008	<i>Carex</i>	150	2.8	420	1.58	5.68	0.24	0.86
	<i>D. erythrosora</i>	15	21.03	315.45	2.02	6.07	0.23	0.71
Oct. 2008	<i>Carex</i>	150	7.2	1080	1.74	18.79	0.13	1.40
	<i>D. erythrosora</i>	15	60.15	902.25	1.52	13.75	0.24	2.18
	<i>Iris</i> sp.	2	92.25	184.5	1.99	3.67	0.15	0.28
Feb. 2009	<i>Carex</i>	150	2.3	345	2.08	7.49	0.22	0.80
	<i>D. erythrosora</i>	15	18.95	284.25	2.06	6.20	0.17	0.51
	<i>Iris</i> sp.	2	36.9	73.8	1.92	1.42	0.27	0.20
CONV RG ^z Nutrient Loads in Plant Tissue								
Date	Plant Type	Total Plants	Avg. Plant Weight (g)	Total Plant Mass ^y (g)	N (%)	N (g)	TP (%)	TP (g)
July 2008	<i>Carex</i>	150	2.4	360	2.12	7.6	0.18	0.75
	<i>D. erythrosora</i>	15	20.05	300.75	1.46	4.60	0.22	0.68
Oct. 2008	<i>Carex</i>	150	7.2	1080	1.36	14.69	0.36	3.92
	<i>D. erythrosora</i>	15	60.15	902.25	1.52	13.71	0.36	3.28
	<i>Iris</i> sp.	2	92.25	184.5	1.44	2.66	0.23	0.42
Feb. 2009	<i>Carex</i>	150	2.4	360	1.17	4.04	0.09	0.33
	<i>D. erythrosora</i>	15	20.05	300.75	1.5	4.26	0.17	0.49
	<i>Iris</i> sp.	2	36.9	73.8	0.72	0.53	0.05	0.04

^zThe rain garden with an internal water storage layer (IWS RG) utilizes a saturated water zone in the rain garden that permits water to collect in the bottom 1 m (3.5 ft) of the rain garden. The saturated layer allows nitrogen to be removed via denitrification. The conventional rain garden (CONV RG) has an aerobic zone which allows water to be treated via interception, storage, filtration, and adsorption.

^y Total Plant Mass = Total Plants * Avg Plant Weight (g)

^x Concentration of nutrient in plant tissue, analysis completed by Auburn University Soil Testing Laboratory, Auburn, AL.

^w Plant Load (g) = Ttl Plant Mass (g) * Concentration (%)