Flooding Tolerance and Phosphorus Uptake of Southeastern Native Plants in Bioretention Gardens

by

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Abstract

Bioretention gardens restore hydrologic function of urban landscapes and capture stormwater runoff pollutants, such as phosphorus, a main pollutant in urban cities and residential neighborhoods. Monoculture plantings are common in bioretention gardens; however, polyculture plantings can improve biodiversity and ecosystem resilience. Thus, objectives of this study were to evaluate phosphorus removal from simulated stormwater in two bioretention substrates, and evaluate four landscape plant species, alone and in monoculture and polyculture plantings, for phosphorus uptake and tolerance of bioretention garden conditions. Part 1: Liners of *Ilex vomitoria* Ait. 'Schillings dwarf', *Andropogon tenarius* Michx., *Echinacea purpurea* L. Moench. 'Magnus Superior', and *Coreopsis verticillata* L.'Zagreb' were planted in containers in either 85:15 sand:organic matter or 50:50 sand:organic matter substrates. Plants were irrigated or flooded with 0.0, 0.4, 0.8, or 1.6 $mg{\cdot}L^{-1}$ P solutions of phosphorus. Part 2: Four planting combinations, *C. verticillata* 'Zagreb' monoculture, *A. ternarius* monoculture, *I. vomitoria* 'Schilling's Dwarf' monoculture, and a polyculture of *C. verticillata* 'Zagreb', *A. ternarius,* and *I. vomitoria* 'Schilling's Dwarf' were planted into 94 L nursery containers in a substrate of a 50:50 sand : organic matter. Containers were irrigated or flooded with 1.6 $mg{\cdot}L^{-1}$ P solution. Overall, plant growth across species was lower in flood treatments, and higher in organic than sand substrate. Leachate and substrate P was higher in flood treatments and substrate P was higher in sand, however, plant tissue P in all treatments was higher than either leachate or substrate P. Polyculture plantings had the lowest leachate P, suggesting a polyculture planting may be more effective in preventing excess P from entering waterways from bioretention gardens. In the simulated gardens, two species had higher growth in the fall and another in the spring, suggesting that with a monoculture, there may be vegetation gaps across seasons. Thus, while all species tested (excluding *E. purpurea*) were tolerant of bioretention conditions, growth was higher in organic substrates and non-flood conditions, and P was highest in plant tissue.

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Chapter I: Literature Review

Introduction

Urbanization plays an important role in transforming ecological systems. Over half of the world's population lives in urban environments, and more than 80% of people in the United States live within cities (Pickett et al., 2001). Cities have more precipitation, more impervious surfaces and contain less vegetation than their rural counterparts (Pickett et al., 2001). The extent of impervious surfaces and vegetation plays a crucial role for a functional hydrologic system. Compared to rural areas, cities have up to 30% more surface runoff and up to 50% decrease in groundwater recharge (Schueler, 1994). Urban streams begin to degrade at a threshold of 10% imperviousness with warmer stream temperatures, increased pollutant loads, stream channel instability and loss of biodiversity (Schueler, 1994).

Reduction of infiltration areas leads to increased stormwater runoff, which carries pollutants, such as pathogens, sediment, phosphorus, and heavy metals directly to adjacent waterways (Arnold and Gibbons, 2006; Paul and Meyer, 2001; Steuer et al., 1997; Waschbusch et al., 1997). When excess nutrients, such as phosphorus, enter surface waters, the aquatic water chemistry is altered, which can lead to eutrophication and impairments, such as algae blooms, decreased available oxygen and large fish kills (Mueller and Helsel, 1996; Smith et al.,1999; United States Environmental Protection Agency, 2007). Although total restoration of the natural hydrology is unlikely in urban ecosystems, reducing stormwater runoff and nutrient inputs can be accomplished by increasing stormwater retention and infiltration prior to reaching riparian shorelines (Booth and Jackson, 1997; Smith et al., 1999).

Low Impact Development and Bioretention Gardens

Low Impact Development (LID) describes a land use planning and development method of mitigating the negative effects of urbanization by emphasizing conservation of natural hydrology and stormwater infiltration, and limiting disturbance of soils, vegetation, and native terrestrial and aquatic ecosystems (Dietz, 2007; Prince George's County, Maryland, 2006). While previous methods of stormwater management and urban development sought solely to manage peak in-stream flow rates, LID techniques seek to reduce peak in-stream flow rates, maintain pre-development runoff volumes and maintain ecological integrity of the landscape by recognizing watersheds, topographical land areas draining to a common basin, in development plans (Arnold and Gibbons, 1996; Dietz, 2007; Schueler, 1997). Practices of LID focus include installation of green roofs, pervious pavements, vegetated swales, infiltration trenches, and bioretention gardens (Dietz, 2007; Price George's County, Maryland 2006; Schueler, 1997).

Bioretention gardens are an LID practice popular in commercial, industrial, and residential areas, and are designed to capture stormwater runoff and pollutants with a depressed ponding area, native vegetation, and help restore the hydrologic function of landscapes (Dietz, 2007; Prince George's County, Maryland, 2006; Virginia Department of Forestry, 2011). Bioretention gardens in the landscape are designed with engineered soils (in contrast to rain gardens, which typically utilize native soils) capture and infiltrate stormwater runoff, treat runoff pollutants, recharge groundwater, while creating insect and wildlife habitat (Dietz, 2007; Prince George's County, Maryland, 2006; Virginia Department of Forestry, 2011). As one of the most versatile LID methods, local governments and municipalities have developed design and installation guidelines for bioretention gardens, geared toward maximum runoff reduction and pollutant removal (Davis et al., 2009; Dietz, 2007; Prince Georges County, 2006; and Virginia Department of Forestry, 2011). Though research on the capture and infiltration of stormwater runoff is promising, scientific exploration is still needed to determine the ability of bioretention gardens to treat certain stormwater pollutants and identify plants that can tolerate flooded conditions (Davis et al., 2009; and Dietz, 2005).

Pollutant Removal in Bioretention Gardens

Bioretention gardens are efficient in the removal of suspended solids, nitrogen, heavy metals, and oils (Davis et al., 2009; Schueler, 1997). However, research on removal of phosphorus is highly variable between laboratory experiments showing a decrease of up to 85% total phosphorus (Davis et al., 2006; Davis et al., 2009) to increases of phosphorus in bioretention garden outlets in field experiments (Dietz, 2005). Phosphorus is a nationwide aquatic pollutant of concern, as defined by the Clean Water Act (USEPA, 2009). Phosphorus, a main pollutant in urban cities, enters waterways with surface water runoff, and degrades urban waterways through the over production of algae and aquatic plant growth (USEPA, 2009; Mueller and Helsel, 1996; Carpenter et al., 1998).

The main source of urban phosphorus is residential lawns and streets, potentially creating an opportunity for bioretention gardens to capture phosphorus, prior to degrading local streams (Steuer et al., 1997; Waschbusch, 1999). As Hunt and Lord (2006) suggest, soils within the bioretention garden affect nutrient removal capabilities, where the initial phosphorus soil content affects the ability of a bioretention area to capture additional phosphorus.

Bioretention Substrates

Bioretention gardens designs are currently geared toward optimal pollutant storage and removal from waterways, and many design manuals currently recommend ‘appropriate’ soil mixes. Early bioretention designs specified the use of native soils; however, substrates with high clay content can lead to system failure due to low infiltration capacity (Davis et al., 2009). Depending on the level of phosphorus input and soil type, native and organic soils with higher initial phosphorus content can also lead to decreased phosphorus pollutant retention and potential downstream phosphorus release during rain events (Burge et al., 2007; Dietz, 2005; Hunt and Lord, 2006).

Some design manuals suggest substrates with higher contents of sand with lower proportions organic matter to increase infiltration ability or vice versa to increase water holding capacity (Burge et al., 2007; Davis et al., 2006, 2009; Prince George’s County, Maryland, 2006). Substrates with high sand content encourage infiltration and have lower initial phosphorus levels, and can lead to increased phosphorus storage, and removal from stormwater (Burge et al. 2007; Davis et al., 2006, 2009). In contrast, substrates with high organic matter content encourage water holding capacity, but tend to have higher initial phosphorus levels (Burge et al, 2007; Hunt and Lord, 2006). While Hunt and Lord (2006) suggest using 85-88% sand, 8-12% fines, and 3-5% organic material. Virginia Department of Forestry recommended 20% leaf mulch, 50% sandy soil, and 30% topsoil (2011). Thus, there are currently different recommendations over which substrates are the best for plant survivability, pollutant removal, and stormwater infiltration. Selecting the appropriate substrate for a bioretention system is complex, and more research is needed to evaluate substrates for improved stormwater infiltration, plant pollutant removal ability and plant survivability.

Soil Flooding

Bioretention gardens typically experience periodic soil flooding for up to two days (Prince George's County, Maryland; 2006; Kraus and Spafford, 2009). Waterlogged conditions in soil can affect plant nutrient availability and uptake, photosynthesis rates, food and nutrient storage, root and shoot growth, and overall survivability (Chen et al., 2005; Rubio et al., 1997). During flooding events, nutrients are consumed by plants or move through the soil profile. Rubio et al. (1997) found that waterlogged soils increased the ability of plants to uptake phosphorus and could increase the soil phosphorus availability. However, repeated flooding can result in a phosphorus release from soils, which introduces additional phosphorus to soils and waterways (Jernigan, 2010; Olila et al., 2007). The release of phosphorus from flooding events is highly dependent on the soil characteristics, as sediment can act as a nutrient source or sink (Olila et al., 1997). Though flooded soils can increase phosphorus availability for plant uptake, plants face reductions in growth, biomass, photosynthetic activity (Chen et al., 2005).

Soil flooding is a complex issue and scientific research on focused plant physiology surrounds wetland plants (Chen et al., 2005; Olila et al., 1997; Tanner, 1996). Thus, plants that are physiologically tolerant of local environmental conditions are recommended in bioretention gardens (Krauss and Spafford, 2009). Additional research is needed to investigate landscape plant health and ability to phosphorus uptake, and potential phosphorus release in different bioretention soils under flooded conditions.

Flooding Response in Plants

Flooding of a growth substrate, such as soil, depletes oxygen and alters the metabolism, nutrient uptake, and overall survivability of plants (Kolzlowski, 1984b). When a substrate is flooded, the limited oxygen supply present in the soil is depleted by roots, microorganisms, and reduction reactions, and flooding during the growing season is much more harmful than flooding during the non-growing season (Kozlowski, 1982; Pezeshki, 2001). While flooding response will vary with the age of the plant, flood tolerance, and the duration of the flood, flooding can cause injury, inhibition of seed production and germination, inhibition of vegetative and reproductive growth, changes in plant anatomy, and mortality (Dylewski et al., 2011, 2012; Kozlowski, 1997).

Stress tolerant species, such as those accustomed to flood and disturbances, will generally acquire more minerals in their plant tissue than flood intolerant species (McJannet et al., 1995; Chen et al., 2005). Flood tolerant plants often have morphological adaptations to flooding, such as the formation of aerenchyma tissue, the creation of lenticels to allow to gas exchange, and regeneration of new root tissue (Kozlowski, 1984b). However, many landscape plants are currently being recommended for planting in bioretention gardens, which are subject to periodic flooding. Thus, it is important to identify landscape plants more tolerant to flood conditions, to improve plant survivability in bioretention gardens.

Native Plants

Native plants, or those that have co-evolved with other native ecosystem species, are better equipped to support wildlife and insects, which are often dependent on plants for food and habitat (Kendle and Rose, 2000; Tallamy, 2007). While both native and exotic plants contribute to species richness and biodiversity, native plants are preferred for use in bioretention gardens, due to their tolerance of local ecosystem conditions (Deutschewitz et al, 2003; Kraus and Spafford, 2009). The influx of exotic species and threat of invasive plants in urban and wet areas have led to native plant recommendations for urban, and therefore, bioretention gardens (Kuhn and Klotz, 2006; Smith et al., 2006). Though urban areas are more vulnerable to invasion by exotic species, urban areas are not specifically attractive for exotic plants alone. (Mack et al, 2000). Urban centers have high nutrient inputs, warmer climate, high light availability, disturbances in soil structure and a variety of habitats such as parks, gardens, cemeteries, or remnants of natural vegetation or construction sites with bare soil (Deustchewitz et al, 2003). With this diversity of growing conditions, and levels of light and nutrient availability, urban areas may provide better living and reintroduction conditions for great variety of native and exotic plant species alike (Deustchewitz et al, 2003**).**

Although exotic, hardy landscape species are often used for rain gardens, planting native plant species in an urban garden can improve biodiversity of indigenous plants (Smith et al., 2006). To promote the preservation of native plant species in urbanizing ecosystems requires an educational and practical approach, which is offered by bioretention gardens and LID projects, often located in public use areas (Dietz, 2005; Hatt et al., 2007). Plant survivability, biodiversity and perceived aesthetics are also essential for the success of urban LID landscapes (Larson et. al., 2009). The use of native plants in urban gardens avoids the introduction of potential invasive species, reduces the risk of biological homogenization, and improves insect biomass for wildlife food sources (Alpert et al., 2000; McKinney, 2006; Smith et. al. 2006; Tallamy, 2004, 2010).

Though diverse and extensive lists of native plants for bioretention gardens exist, most scientific research on native plants for pollutant removal and flooding tolerance is related to wetland ecosystems (Del Bubba et al., 2003; Kraus and Spafford, 2009; Olila et al., 1997; Ranney et al., 1994; Tanner, 1996). Scientific identification of native plants capable of pollutant removal and tolerant of short term repeated flooding for use in bioretention gardens will promote the preservation of native species in urbanizing habitats.

Planting Composition and Functional Plant Groups

Functional plant diversity is emerging as a crucial aspect important for ecosystem health and resilience. Functional plant groups can be defined by differences in life history traits (ruderal, stress tolerant, competitive), above ground morphology, or physiology. Members of different functional groups will occupy more complementary ecosystem niche spaces than members of the same functional group, and with more niche spaces occupied and resources allocated, comes a higher degree of ecosystem productivity and functionality (Diaz and Cabido, 2001). Evaluating bioretention plant species for pollutant tolerance, potential resource use, pollutant removal, and flood water uptake of different functional groups can provide important information on bioretention effectiveness.

A greater number of functional groups in an ecosystem has also been associated with a higher level of competition against invasive species and weeds, a common concern in LID projects and bioretention gardens (Byun et al., 2012, Pokorny et al., 2005). Competition for light can lead to increased biomass in above ground parts, and increased leaf area per leaf biomass, which can also reduce the influx of invasive and weedy species. Additionally, communities with only one or two functional types (grasses and perennial forbs, for example) can lead to vegetation gaps during the growing or dormant seasons (Spehn et al., 2001). In monocultures and low diversity plantings, it is less likely that one other species could compensate for a mortality or poor growth of another single species, thus, functional group 'richness' can improve establishment and mean vegetation cover (Spehn, et al., 2001) and thus effectiveness of bioretention.

Often, monoculture plantings are common in large-scale bioretention gardens and LID projects due to lower costs and perceived ease of maintenance (Dewar, 2007; Bracken, 2008; Kuhn and Klotz, 2006). Monoculture plantings are less resistant to biotic and abiotic stressors and decrease biotic heterogeneity (Sphen et al., 2001). Diversifying planting composition between monocots and dicots, evergreen and deciduous, and shallow and deep rooted species will increase competition for nutrients, and encourage higher biomass productivity and stress tolerance (Dewar, 2007; Bracken, 2008; Kuhn and Klotz, 2006). In addition, plants that are genetically unrelated allocate more resources to competition – increased root growth and water and nutrient uptake ability (Dudley and File, 2007). Polyculture planting could increase competition in water uptake, thus flooding tolerance and increase competition in nutrient uptake and removal (Dudley and File, 2007; Bracken, 2008). Thus, it is essential to explore the effect of polyculture planting and diverse functional plant groups on bioretention garden resilience and functionality.

Conclusion

The urbanization of landscapes has led to an increase in stormwater runoff from impervious surfaces and a decrease in native plant biodiversity. Bioretention gardens can improve stormwater infiltration, while increasing plant biodiversity and adding aesthetic beauty to the environment. As bioretention gardens can remain flooded with stormwater runoff for two days, plants should be tolerant of periodic flooding and capable of removing key stormwater pollutants. Additionally, bioretention garden substrates should encourage infiltration, capture pollutants, and reduce the release of phosphorus into waterways.

The objectives of this study are 1) evaluate the removal of phosphorus from stormwater runoff in two currently recommended bioretention substrates, 2) evaluate four landscape plants from different functional groups for phosphorus uptake and tolerance to bioretention garden conditions, and 3) evaluate monoculture and polyculture plantings for phosphorus uptake and tolerance of bioretention garden conditions.

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Chapter II

The Effect of Flooding Cycles on Phosphorus Uptake in Four Native Plant Species in Two Bioretention Substrates

Index Words: rain gardens, pollution removal, low impact development

Abstract

Bioretention gardens are designed to restore the hydrologic function of urban landscapes and capture stormwater runoff pollutants, such as phosphorus. Phosphorus is a pollutant of concern in urban cities and residential neighborhoods. The objectives of this study were to evaluate phosphorus removal from simulated stormwater in two bioretention substrates and evaluate four landscape plant species for tolerance to bioretention garden conditions and phosphorus uptake. Liners of *Ilex vomitoria* authority 'Schillings dwarf', *Andropogon tenarius* Michx., *Echinacea purpurea* L. Moench. 'Magnus Superior', and *Coreopsis verticillata* L.'Zagreb' were planted in containers in either 85:15 sand:organic matter or 50:50 sand:organic matter substrates. Plants were irrigated or flooded with 0.0, 0.4, 0.8, or 1.6 $mg \cdot L^{-1}$ P solutions of phosphorus. At experiment termination, size index and root and shoot dry weight were recorded, and leachate, substrate, and plant tissue were analyzed for P concentrations. Plant growth was lower in flood conditions than non-flood conditions and lower in sand than organic substrate, however, the species tested survived and appeared to be tolerant of repeated flooding (with the exception of *E. purpurea*). Substrate P was highest in sand for all

species and highest in flood conditions with *C. verticillata* and *A. ternarius*. Leachate P was highest in flood over non-flood treatments, and highest in organic flood with *C. verticillata* and *A. ternarius*. Shoot tissue P was highest in non-flood treatments for *I. vomitoria* and *A. ternarius*, and root tissue P was highest in organic treatments for *I. vomitoria* and *A. ternarius, and in flood treatments for C. verticillata*. Overall, plant tissue P was highest in organic and non-flood treatments, and though, substrate P was high in sand and leachate P was high in flood treatments, maximum P concentration was found in plant tissues.

Introduction

Phosphorus, a main pollutant in urban cities, enters waterways with surface water runoff, and degrades urban waterways through the over production of algae and aquatic plant growth (USEPA, 2009; Mueller and Helsel, 1996; Carpenter et al., 1998). The main source of urban phosphorus is residential lawns and streets, potentially creating an opportunity for bioretention garden to capture phosphorus, prior to degrading local streams (Steuer et al., 1997; Waschbusch, 1999). Research on the capture and infiltration of stormwater runoff is promising, but scientific exploration is still needed to determine the ability of bioretention gardens to treat certain stormwater pollutants and identify plants that can tolerate flood conditions (Davis et al., 2009; and Dietz, 2005).

Bioretention gardens are designed to help restore the hydrologic function of urban landscapes by capturing stormwater runoff and pollutants with a depressed ponding area, engineered soils, and native vegetation. (Dietz, 2007; Prince George's County, Maryland, 2006; Virginia Department of Forestry, 2011). While bioretention gardens are effective for the removal of suspended solids, nitrogen, heavy metals, and oils, research on removal of phosphorus is highly variable (Davis et al., 2006; Davis et al., 2009; Dietz, 2005; Schueler, 1997). Substrate used in bioretention gardens affect nutrient removal capabilities; likewise, the initial phosphorus content of the substrate affects the ability of a bioretention area to capture additional phosphorus (Hunt and Lord, 2006). Substrates with high sand content encourage infiltration and have lower initial phosphorus levels and can lead to increased phosphorus storage and removal from stormwater (Burge et al. 2007; Davis et al., 2006, 2009). In contrast, substrates with high organic matter content have higher water holding capacity but tend to have higher initial phosphorus levels

(Burge et al, 2007; Hunt and Lord, 2006). Thus, there is currently a conflict over which substrates are the best for plant survivability, pollutant removal, and stormwater infiltration.

Bioretention gardens typically experience periodic flooding for up to two days at a time (Prince George's County, Maryland; 2006; Kraus and Spafford, 2009). Rubio et al. (1997) found that waterlogged soils increased the ability of plants to uptake phosphorus and could increase the soil phosphorus availability. However, repeated flooding can result in a phosphorus release from soils, which introduces additional phosphorus to soils and waterways (Jernigan, 2010; Olila et al., 2007). The release of phosphorus from flooding events is highly dependent on the soil characteristics, as sediment can act as a nutrient source or sink (Olila et al., 1997). Though flooded soils can increase phosphorus availability for plant uptake, plants face reductions in growth, biomass, photosynthetic activity (Chen et al., 2005). Selecting the appropriate soil for a bioretention system is complex, and more research is needed to evaluate soils for improved stormwater infiltration and pollutant removal as well as to evaluate plants for flooding tolerance and pollutant removal.

Though diverse and extensive lists of native plants for bioretention gardens exist, most scientific research on native plants for pollutant removal and flooding tolerance is related to wetland ecosystems (Del Bubba et al., 2003; Kraus and Spafford, 2009; Olila et al., 1997; Ranney et al., 1994; Tanner, 1996). Currently, plants that are physiologically tolerant of local environmental conditions are recommended in bioretention gardens (Krauss and Spafford, 2009). However, since bioretention gardens are subject to periodic flooding, additional research is needed to identify landscape plant species tolerant of periodic flooding and to investigate plant phosphorus uptake under these conditions.

The objectives of this experiment are to evaluate the removal of phosphorus from simulated stormwater runoff in two currently recommended bioretention substrates and to evaluate four diverse landscape plants for phosphorus uptake and tolerance to bioretention garden conditions. Specifically, the goal of this research is to quantify the effect of phosphorus concentration in simulated runoff on plant growth and the removal of phosphorus by plants and substrates in simulated bioretention conditions.

Materials and Methods

Species

Species used in this experiment included one woody shrub, one grass, and two herbaceous perennials native to the southeastern United States and currently recommended in bioretention garden designs. These included, respectively, *Ilex vomitoria* ‘Schillings dwarf’ Ait. (Yaupon Holly), (Liner Source, Inc. Nursery, Eustis, FL), *Andropogon tenarius* Michx. (Split Beard Broomsedge) (Hoffman Nursery, Inc., Rougemount, NC), *Echinacea purpurea* ‘Magnus Superior’ L. Moench. (Purple Coneflower) and *Coreopsis verticillata* ‘Zagreb’ L. Glab. (Whorled Coreopsis) (Emerald Coast Growers, Pensacola, FL).

Substrates and Containers

Liners (3 cm) were planted into 3.8 L containers. Half of the containers were filled with 85% sand, 5% pine bark, 5% peat moss, 5% calcined clay (Profile Greens Grade, Profile Products, Buffalo Grove, IL) (herein referred to as ‘sand’), and half of the containers were filled with 50% sand, 22.5% pine bark, 22.5% peat moss (herein referred to as ‘organic’). Both substrates were pre-plant amended with 0.04 $kg{\cdot}m^{-3}$ dolomitic limestone and 0.45 kg·m^{-3} phosphorus free fertilizer with micronutrients (Tru-prill,19N-0P_2O_5-17K_2O, Plant Science, Inc., Ontario, Canada). Each container received an additional top-dressed fertilizer application of 6.7g (half the original rate) at the fourth flood cycle (described below) after experiment initiation with the exception of the first run of *I. vomitoria* in which each container received an additional fertilizer treatment of 6.7 g (half the original rate) at six weeks after experiment initiation.

Flooding Treatments

Half of the plants were exposed to flood treatments using a pot-in-pot method. To flood a container, the container with a drainage hole containing substrate and plant was placed inside another container without drainage holes. Each container was flooded with 1200 mL of tap water added by hand. No additional water was added during each flood cycle. Plants were flooded for 48 hours followed by draining for 7 day (flood-drain cycle) with no water added during the draining period. Plants were exposed to eight flood-drain cycles with the exception of *I. vomitoria* in the first run which received 10 flood-drain cycles. The other half of the plants were 'non-flood' and irrigated with 400 mL tap water three times weekly (with the exception of *I. vomitoria* in the first run which was irrigated as needed with 300 mL of tap water).

Phosphorus Concentrations

Plants were irrigated or flooded with one of four solutions of phosphorus. The phosphorus source was 85% ortho-phosphoric acid (Fisher Scientific, Pittsburgh, PA). Solutions contained 0.0, 0.4, 0.8, or 1.6 mg·L^{-1} P with the exception of the first run of *I. vomitoria,* which used 0.0, 0.2, 0.4, 0.8, 1.0 mg·L^{-1} P solutions.

Data Collection and Analysis

Size index [(shoot height + shoot widest width + shoot width (perpendicular to widest)/ 3)] was measured for each plant at run initiation, midway, and termination. At termination of each run (described below), plant shoots 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 to determine shoot dry weight and root dry weight.

Four 50 g substrate samples were collected (for each substrate) prior to planting before and after the addition of lime and fertilizer. Upon termination, four 50-g substrate samples were collected for each substrate x phosphorus x flooding treatment combination for each species. Tissue samples were collected by grinding down the entire shoot tissue of each plant to 5mm particle size. A 0.5 g sample of the tissue from each of three plants per substrate x phosphorus x flooding treatment combination was collected. Leachate samples (200mL) were collected from three containers in each substrate x flooding x phosphorus treatment for each species. Leachate was collected using the Virginia Tech Pour Through Method (Wright, 1986). Leachate samples were analyzed for pH and electrical conductivity with a hand held Beckman Coulter pHI© 460 meter, epoxy body 3-in-1 gel-filled pH electrode and conductivity probe, and filtered through 42 Whatman filter paper. Substrate samples were processed using Mehlich 1 double acid extraction method and tissue samples were processed using the dry ash and double acid extraction method, both processes adopted by the Auburn University Plant and Soil Analysis Lab (Hue and Evans, 1986). Leachate, substrate and tissue samples were analyzed for P concentrations for the second run (excluding *E. purpurea*) at Auburn University Plant and Soil Analysis Lab using Inductively Coupled Argon Plasma (ICAP) machine.

Substrate Volumetric Moisture

Substrate volumetric moisture percentage was measured and logged using ECH$_2$O Soil Moisture Sensors, model EC-5, and Em5b dataloggers (Decagon Devices, Inc., Pullman, WA). Soil moisture was measured in 60-minute intervals in three containers per substrate x flood x phosphorus treatment combination. Sensors were calibrated for each substrate prior to use, and the calibration was applied to the data output. Substrate volumetric moisture percentage was measured and logged in run 2 for each species (excluding *E. purpurea*).

Experimental Design and Statistical Analysis

Plants were placed on raised benches at the Paterson Horticulture Greenhouse Complex, Auburn University, AL, and were grown in an un-shaded 8 mm twin-wall, polycarbonate covered greenhouse under natural photo-periods with a heating set point of 18.3°C and ventilation beginning at 25.6°C. Dates for each run are shown in Table 1. The experimental design was a completely randomized design with a complete factorial treatment design of substrate, flood, and phosphorus concentration, and five single-container replications of each treatment. Each species was treated as a separate experiment. Analysis of variance was conducted using PROC GLIMMIX in SAS version 9.2 (SAS Institute, Cary, NC). The two experimental runs were analyzed separately where trends among significant treatment means were different between runs; otherwise, the two runs were combined into one analysis and designating run as a random variable. Where residual plots and a significant COVTEST statement using the HOMOGENEITY option indicated heterogeneous variance, a RANDOM statement with the GROUP option was used to correct heterogeneity. Differences among treatment least squares means were determined using Tukey's test with the STEPDOWN option and a family-wise error rate of 0.05 in LSMEANS statements. Linear and quadratic trends over phosphorus concentrations were tested using orthogonal polynomials in CONTRAST statements. All significances were at $\alpha = 0.05$. Results for main effects and interactions are presented when significant and not presented if not significant. If interactions were significant, then the simple effects of each factor are presented. The results for *I. vomitoria* are presented separately by run, since the P concentrations were different for each run; 0.0, 0.2, 0.4, 0.8, or 1.0 mg/L P in run 1 and 0.0, 0.4, 0.8 and 1.6 mg/L P in run 2. In all other species the results are pooled over run, unless noted otherwise. Additionally, for simplicity of presentation, some of the data for this species is presented within the text and not within tables. SI for *I. vomitoria* P irrigation rate 0.8 mg/L P was removed from data set due to contamination in the irrigation solution for that rate.

Results

The initial substrate phosphorus (P) concentration was 2.33 mg/kg for organic and 0.11 mg/kg for sand. Organic had higher minimum volumetric moisture content (VWC) than sand in both non-flood and flood treatments. The minimum VWC for organic was 0.22 m^3/m^3 for non-flood and 0.25 m^3/m^3 for flood. The minimum VWC for sand substrate was 0.16 m^3/m^3 VWC for non-flood and 0.16 m^3/m^3 for flood . The maximum VWC for organic was 0.38 m^3/m^3 VWC for non-flood and 0.48 m^3/m^3 VWC for flood. The maximum VWC for sand was 0.28 m^3/m^3 VWC for non flood and 0.36 m^3/m^3 VWC for flood.

Ilex vomitoria ‘Schilling’s Dwarf’

In run 1 root dry weight (RDW) was higher in organic than in sand and higher in non-flood than in flood (Table 2). In run 2, RDW was higher in organic (1.1g) than in sand (0.7g). In run 1 shoot dry weight (SDW) was higher in organic (2.1g) than in sand (1.6g). In run 2, SDW was higher in non-flood than in flood and higher in organic than sand (Table 2). Additionally, in run 2, midway and final size index (SI) was higher in organic than in sand (Table 2).

Final substrate P was higher in sand than in organic (Table 4). Substrate P increased linearly with increasing P irrigation rate (Table 4). Leachate P was higher in flood than non-flood and increased linearly with increasing P irrigation rate (Table 4). Sand had 3 times lower leachate P than organic (Table 4). . For both root and shoot tissue P, there was a two way interaction between substrate and flood treatments (Table 4). In flood, root and shoot tissue P was similar between organic and sand (Table 4). In non-flood, root and shoot tissue P was higher for organic than sand (Table 4). In organic substrate, root and shoot tissue P was higher in non-flood than flood, while in sand there was no difference in root and shoot tissue P between flood and non-flood (Table 4). Root and shoot tissue P was highest at the highest P irrigation rate (Table 4). Overall, total P per plant, shoot and root tissue combined, was 1.63 mg P.

Andropogon ternarius

RDW and SDW were higher in organic than sand substrate and higher in non-flood than flood treatments (Table 2). Midway size index was higher in organic than sand (Table 3). Final size index was higher in organic than sand, and higher in non-flood than flood treatments (Table 3).

For leachate P there was a three-way interaction among treatments. When different, leachate P was higher in flood substrate than non-flood substrate. In sand, substrate P was higher in flood than non-flood (Table 5). At the highest P irrigation rate leachate P of flood substrates was higher in organic than sand. In organic flood substrate, leachate P increased linearly with increasing P irrigation rate (Table 5). Root tissue P was higher in organic than sand substrate and increased quadratically with increasing P irrigation rate, where root tissue P was highest at P irrigation rate of 0.8 mg/L P (Table 5). Shoot tissue P was higher in non-flood treatments than flood treatments (Table 5) and also increased quadratically over P treatments (Table 5). Overall, total P per plant, shoot and root tissue combined, was 14.28 mg P.

Echinacea purpurea

In both runs of *E. purpurea*, survivability was lower in flood (14%) than non-flood (32%) treatments. In run 1, RDW was smaller in organic (0.2g) than sand (0.6g). In run 1 and run 2, SDW was smaller in flood (0.2g) than non-flood (0.9g). No additional data was collected in this species due to overall lower survivability across treatments.

Coreopsis verticillata 'Zagreb'

RDW and SDW were higher in organic than sand substrate and higher in non-flood than flood treatments (Table 2). RDW and SDW for non-flood was twice that in flood. Size index was higher in non-flood over flood at both midway and final measurements (Table 3).

Substrate P was higher in flood than non-flood, and also higher in sand than organic. Substrate P increased linearly with increasing P irrigation rate (Table 6). For leachate P, three 2-way interactions were significant (substrate x flood, substrate x P rate, and flood x P rate) (Table 6). In sand and organic, and non-flood and flood, leachate P increased linearly with increasing P irrigation rates (Table 6). Within each P irrigation rate, leachate P was higher in organic than sand and higher in flood than non-flood (Table

6). Root tissue P was higher in flood than in non-flood. There were no significant differences in shoot tissue P (Table 6). Overall, total P per plant, shoot and root tissue combined, was 24.51 mg P.

Discussion

It is expected that substrates with higher organic matter content would have higher water holding capacity, and the VWC measured in the substrates in this research agree with this (Burge et al., 2007; Davis et al., 2006, 2009). Substrates with lower organic matter content likely provide drier substrate conditions between irrigation periods (flood or non-flood), and as a result, it is possible plants in sand substrates experienced more water stress than plants in organic substrate (Burge et al., 2007; Davis et al., 2006, 2009). Across all species, RDW and SDW were higher in organic than sand (Table 2). The addition of organic matter to bioretention substrate tends to raise the initial P content, in addition to other nutrients, and increase cation exchange capacity which can improve plant health (Hunt and Lord, 2006). It may also improve microbial activity, which can increase the efficacy of bioretention gardens in terms for pollutant removal (Pasucal et al., 1997; Bettez and Groffman, 2012; Nogaro et al., 2007).

RDWs and SDWs of all species was higher in non-flood than flood substrates (Table 2). Final SI of *A. ternarius* and *C. verticillata* was also higher in non-flood substrates, while final SI of *I. vomitoria* was highest in organic substrates (Table 3). Despite lower growth in flood substrates, all species, except *E. purpurea*, tolerated flood conditions, nonetheless. This result is similar to Dylewski et al. (2011), who found that among three native landscape shrubs (*Ilex glabra, Itea virginica, Viburnum nudum*), RDW and SDW was lower in flood conditions than non-flood conditions, but overall, the shrubs tested were tolerant despite differences in growth (2011). In another experiment, SDW and RDW of *Muhlenberia capillaris* (Lam.) Trin., a landscape native grass, were higher in non-flood than flood plants yet still survived and grew in flood conditions (Christian et al., 2012).

The differences in plant growth among flood and non-flood treatments reinforce the understanding that water logged soil conditions create a stressful growing environment for some plants, including the ones in this research. This was particularly true for *E. purpurea*, which experienced the lowest survivability of all species tested here. In addition to decreased root and shoot growth, flood stress can also cause decreased photosynthetic rates, decreased chlorophyll content in leaves, and inhibit nutrient transfer between root and shoots, further limiting potential plant establishment, survivability, and nutrient uptake in flooded conditions (Chen et. al. 2005; Vartapetian and Jackson 1997). While the species tested here are classified by the USDA (2013) to have no tolerance to anaerobic conditions, all (with the exception of *E. purpurea*) had decreased growth but high survivability under flood conditions.

Root and shoot tissue P in *I. vomitoria* was highest in organic non-flood substrates (Table 4). Root tissue P of *C. verticillata* was highest in flood treatments, across substrate treatments, and for *A. ternarius* it was highest in organic substrates, across flood treatments (Tables 5 and 6). Additionally, root and shoot tissue P for *A. ternarius* increased quadratically with P irrigation rate, where it had the highest tissue concentration at 0.8mg/L P (Table 5). Shoot tissue P in *A. ternarius* was also highest in non-flood treatments (Table 5). Additionally, shoot tissue P for *I. vomitoria* increased linearly with increasing P irrigation rate (Table 4). Overall, there was a higher root and shoot P in organic substrates and non-flood treatments, and there was an increase in P concentration *I. vomitoria* with P irrigation rate (Tables 4, 5, 6).

A. ternarius, C. verticillata, and *I. vomitoria* had higher growth in organic substrates (Tables 2 and 3). When additional nutrients are available, such as those provided by organic matter and from stormwater effluent, native plants may store nutrients as an 'insurance' against future loss and as a support for growth when conditions are favorable (Chapin et al., 1990). In contrast, some studies have found that if a native plant species is tolerant to low nutrient conditions, they may not absorb much nutrition, and thus nutrients could be stored in the soil or enter surrounding waterways (Chabot and Hicks, 1982).

C. verticillata had higher root tissue P in flood treatments (Table 6), which is similar to Rubio et al. (1997) who showed that waterlogged soils irrigated with phosphorus can improve uptake of phosphorus per unit of biomass. Additionally, that work also found that *Paspalum dilatatum* Poir., a flood tolerant grass, showed higher physiological capacity to absorb P under flood conditions. Rubio et al. (1997) concluded that some plants, like *C. verticillata,* may be more stress tolerant and have developed morphological adaptations, such as adventitious root and shoot growth, to favor nutrient uptake. Root tissue P was higher for *A. ternarius* and *I. vomitoria* in non-flooded soils (Tables 4 and 5), which is similar to Lorenzen et al. (2001), who found that flooded soils decrease a plant's ability for growth and nutrient uptake (Lorenzen et. al. 2001).

In *I. vomitoria* there was an increase in root and shoot tissue P with increasing P irrigation rate (Tables 4 and 5). Similar to the increased P concentrations in tissues of *I. vomitoria*, Denman et al. (2007) found that trees irrigated with stormwater pollutants showed increased growth, presumably due to nutrient input. Species with long life expectancies, such as *I. vomitoria*, or from a high nutrient ecosystem, such as *C. verticillata*, often store nutrients over time as 'insurance' for future stress (Chapin et al., 1990). Lorenzen et al. (2001) found that, *Typha domingensis* Pers., a species from a high nutrient ecosystem, had higher growth, higher P accumulation, and higher biomass partitioning in response to P application. This type of nutrient-rich ecosystem could be expected in the forest understory of *I. vomitoria* where leaf litter would provide nutrients and organic matter for growth, or in the prairie ecosystem of *C. verticillata*.

In contrast, *A. ternarius*, a plant from a typically low nutrient sand plains ecosystem, had a peak in root and shoot tissue P at P irrigation rate of 0.8mg/L, and then decreased tissue concentrations at the higher P irrigation rate. Plants tolerant to low levels of soil nutrients, such as *A. ternarius*, can be negatively affected by phosphorus addition (Bowen, 1981). In a study with another grass species, *Cladium jamaicense* (Crantz) Kuk., also from a nutrient poor habitat (limestone soils on fresh-water ecosystem edges), there was no clear relationship between increased P availability and growth or nutrient uptake. *C. jamaicense*, had slow growth, low P uptake, and inflexible biomass partitioning in response to P inputs (Lorenzen et al., 2001). In a study with *Andropogon scoparious* Michx., which typically grows in P deficient soils, *A. scoparious* had less P accumulation in response to increasing P supply, than *Poa pratensis* L., which absorbed more P resulting in a lower maximum concentration of P in shoot tissue for *A. scoparious* than *P. pratensis* (Wuenscher and Gerloff, 1971). Traits such as such as slow growth and low capacity for nutrient uptake, common among low resource ecosystem plants, often mean that even when presented with supplemental nutrients and resources plants native to infertile soils are particularly unable to absorb high quantities of those nutrients (Bloom, 1985; Chapin, 1980; Grime, 1977; Parsons, 1968). Thus, to improve P removal from bioretention through accumulation in plant tissue, it may be more beneficial to select species native to nutrient rich soils, such as those with higher organic matter, than species native to nutrient poor soils, such as sand, since those species native to more fertile soils appear to have a higher capacity for nutrient uptake and accumulation in plant tissue (Lorenzen et al., 2001). When comparing the total P per plant, regardless of treatment, *C. verticillata* had the highest concentration, nearly double the total P of *A. ternarius*, which further supports these claims.

While plants from nutrient rich soils, such as those with more organic matter, can uptake more P in root and shoot tissue, in this research, sand substrates retained more P than organic substrates regardless of flooding treatment. This is similar to Hunt et al. (2006), who found that in bioretention cells, the lower the initial substrate P, such as sand, the more likely there is to be a P adsorption in the soil. While sandy soils generally retain less P than other soil types, Harris et al. (1996) found that sandy soils that were coated with clay particles actually retained more P than non-coated sandy soils, which improves the CEC capacity of the sandy soil (Hunt et al., 2006).

Higher substrate P in sand could explain the lower growth of species in sand substrates, as species from low-nutrient habitats, such as many native plants, can be negatively affected by phosphorus addition (Bowen, 1981; Lorenzen et al., 2001). Additionally, since many plants have phosphorus sensitivities, Burge et al. (2007) recommend bioretention substrates with low phosphorus levels, to avoid injuring plants. There was a linear increase in substrate P with increasing P irrigation rate in *I. vomitoria* and *A. ternarius*, which suggests that P is being held in the substrate with more P addition.

Despite the negative effect of flooding on plant growth, both sand and organic flood substrates retained more phosphorus than their non-flood counterparts (Table 4-6). This is similar to Olila et al. (2007) who noted that ions have a longer time to interact with the soil matrix in flood conditions. While flood substrates retained more phosphorus than non-flood substrates, flood leachates contained more P than non-flood leachates, suggesting a complex nutrient dynamic in flood conditions. Despite increased substrate P in flood conditions, flooding perhaps decreased the plants' ability to absorb P, and thus more was found in leachate. Additionally, organic flood contained the highest leachate P (Tables 4-6). This is similar to Hunt et al. (2006), who found that in bioretention cells, soils with higher initial P had more P in outflow and floodwater leachates. However, Hunt et al. (2006) also found that typically total P outflow concentrations exceeded inflow concentrations.

Nonetheless, in this study, it was found that overall P concentrations in the leachate were lower than P concentrations in the substrate, which suggests there was P storage in the substrate and uptake in the plant, which was similar to findings by Christian et al. (2012). It was also found that the higher the P irrigation rate, the higher the P concentration in leachates, which would be expected. Also similar to Christian et al. (2012), was that P concentration was higher in flood leachates than non-flood, and there was an increasing P in leachate with P irrigation rate. This is in agreement with Olila et al., (1997) who observed additional P release in completely drained and re-flooded soils, where rewetting of completely dried soils resulted in a flush of available P into leachates.

Conclusion

Overall, plants in non-flood treatments had higher tissue P concentrations than plants in flood treatments, with the exception of *C. verticillata.* Plants in organic substrates also had higher plant tissue P, RDW, and SDW, than those in sand substrates. This suggests that substrates with more organic matter are better for P uptake in plant tissue and for creating a healthier environment for plant establishment and growth in bioretention gardens.

While organic substrates did have higher leachate P and less substrate P than sand substrates, root and shoot tissue P was often 10 fold the concentration of substrate P (Tables 5 and 6). This suggests that plant tissue has a greater capacity to remove P from stormwater effluent than substrate.

As suggested by previous research, the removal of phosphorus by bioretention substrates was effective, but varied, and P accumulation may be better controlled by managing, growing, and harvesting healthy plant tissue (Davis et al., 2006). Additionally, bioretention substrates by themselves were less effective in the removal of P than when compared to storage in plant tissue (Read et al., 2008). While some plants have a higher pollutant removal capacity than others, it appears that this ability is improved when the species is tolerant to bioretention conditions, and has the capacity to survive and thrive in intermittent wet/dry cycles (Read et al., 2008). With the exception of *E. purpurea*, all species tested were tolerant to bioretention conditions, and healthiest in non-flood substrates with more organic matter.

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Table 1. Run dates for each species.

Species	Run	Potting Date	Treatment Initiation	Experiment Termination
I. vomitoria	1	17 Sept. 2011	26 Sept. 2011	27 Dec. 2011
	2	19 Feb. 2012	5 Mar. 2012	9 May 2012
A. ternarius	1	19 Feb. 2012	5 Mar. 2012	9 May 2012
	2	20 July 2012	27 July 2012	9 Oct. 2012
C. verticillata	1	20 July 2012	27 July 2012	9 Oct. 2012
	2	15 Mar. 2013	22 Mar. 2013	1 June 2013
E. purpurea	1	19 Feb. 2012	5 Mar. 2012	9 May 2012
	2	20 July 2012	27 July 2012	9 Oct. 2012

Table 2. Effect of substrate (n=48) and flood treatments (n=48) on root dry weight (RDW) and shoot dry weight (SDW) of three southeastern United States native plants grown in a greenhouse in Auburn, AL.

	RDW (g)				SDW (g)			
	Flood [z]		Substrate [y]		Flood		Substrate	
Species	Non-Flood	Flood	Organic	Sand	Non-Flood	Flood	Organic	Sand
Ilex vomitoria [x]	1.2a [w]	0.9b	1.2a	0.9b	2.2a	1.5b	2.5a	1.3b
Andropogon ternarius	15.8a	11.4 b	16.64a	10.6b	19.9a	14.1b	18.8a	15.2b
Coreopsis verticillata	13.7a	6.8 b	11.3a	9.2b	8.6a	4.9b	7.7a	5.8b

[z] Flood treatments were non-flood (irrigated three times weekly) or flood (submerged for 48 hours and drained for 7 days, repeated eight times).
[y] Substrate treatments were 'organic' 50:50 organic matter : sand or 'sand' 85:15 sand : organic matter.
[x] RDW for *I. vomitoria* is presented for run 1, SDW is presented for run 2 . Data for other species are pooled over runs.
[w] Lowercase letters denote least squares mean separation between flood or between substrate treatments within a species within a row using ANOVA F-test in the Glimmix procedure ($p<0.05$)

Table 3. Effect of substrate (n=48) and flood treatments (n-48) on midway and final size indices (SI) of three southeastern United States native plants grown in a greenhouse in Auburn, AL, where the midway size index was measured after the fourth flood cycle and the final size index was measured at termination.

Species	Midway SI (cm) [z]				Final SI (cm)			
	Flood [y]		Substrate [x]		Flood		Substrate	
	Non- Flood	Flood	Organic	Sand	Non-Flood	Flood	Organic	Sand
Ilex vomitoria [w]	- [w]	-	12.12a [v]	10.21b	-	-	11.80a	7.50b
Andropogon ternarius [u]	-	-	55.26a	51.07b	42.74a	34.04b	39.87a	36.91b
Coreopsis verticillata	29.05a	23.74b	-	-	32.87a	28.38b	-	-

[z] SI = [(shoot height + shoot widest width + shoot width (perpendicular to widest)/ 3)].

[y] Flood (treatments) were non flooded (irrigated three times weekly with 400 mL treatment solution) and flooded (submerged in 1200mL treatment solution for 48 hours and drained for 7 days, with eight cycles).

[x] Substrate treatments were 'organic' = 50:50 organic matter : sand or 'sand' = 85:15 sand : organic matter.

[w] "–" = main effect was not significant

[v] Lowercase letters denote least squares mean separation between flood or between substrate treatments within a species within a row using ANOVA F-test in the Glimmix procedure ($p<0.05$)

[u] Midway SI for *A. ternarius* was only collected for second run, final SI includes both runs.

Table 4. Effect of substrate, flood, and phosphorus (P) irrigation rate treatments on final P concentrations (mg/L) of substrate, leachate, and root and shoot tissue of *Ilex vomitoria* 'Schilling's Dwarf' when grown in a greenhouse in Auburn, AL. Data are not presented for P irrigation rate 0.8 mg/L P.

Fraction	P-concentration								
Substrate (mg/kg)	Substrate [z]				P rate (mg/L) [y]				Trend
	Organic	Sand			0	0.4	0.8	1.6	
	2.55b[x]	8.94a			4.87	5.15	-[w]	7.22	L***
Leachate (mg/L)	Flood Treatment[v]				P rate (mg/L)				
	Non-flood	Flood		Substrate	0	0.4	0.8	1.6	Trend
	0.91b	1.14a		Organic	0.88A[u]	1.26A	-	2.30A	L***
				Sand	0.50B	0.48B	-	0.74B	L***
Root tissue (mg/kg)		Flood Treatment			P rate (mg/L)				
	Substrate	Non-flood	Flood		0	0.4	0.8	1.6	
	Organic	785.98Aa	628.10b		585.02b	-	-	719.42a	
	Sand	583.75B	671.04						
Shoot tissue (mg/kg)		Flood Treatment			P rate (mg/L)				Trend
	Substrate	Non-flood	Flood		0	0.4	0.8	1.6	
	Organic	736.72Aa	569.28b		585.73	638.92	-	696.35	L**
	Sand	604.04B	628.31						

[z] Substrate treatments were 'organic' 50:50 organic matter : sand, and 'sand' 85:15 sand : organic matter.

[y]Least square means comparisons at each phosphorus rate using Tukey's test where significant liner (L) trends using orthogonal polynomials at a=0.001 (**) or a<0.0001 (***).

[x]Uppercase letters denote least squares mean separation within a treatment, within a species within a column using ANOVA F-test in the Glimmix procedure (p<0.05).

[w]"–" = data not present for this P irrigation rate due to sample loss.

[v] Flood (treatments) were non flood (irrigated three times weekly with 400 mL treatment solution) and flood (submerged in 1200mL treatment solution for 48 hours and drained for 7 days, with eight cycles).

[u]Lowercase letters denote least squares mean separation within a treatment, within a species within a row using ANOVA F-test in the Glimmix procedure ($p<0.05$).

Table 5. Effect of substrate, flood, and phosphorus (P) rate treatments on final P concentrations (mg/L) of substrate, leachate, and root and shoot tissue of *Andropogon ternarius* when grown in a greenhouse in Auburn, AL.

				P-concentration				
Fraction								
Substrate		Flood Treatment^{z}						
(mg/kg)	Substrate^{y}	Non-flood	Flood					
	Organic	2.41B^{xw}	3.08B					
	Sand	6.41Ab	8.54Aa					
				Substrate				
Leachate				Organic		Sand		
(mg/L)^{uv}				Flood Treatment				
		P rate (mg/L)		Non-Flood	Flood	Non-Flood	Flood	
		0		0.27b	0.86a	0.25	0.71	
		0.4		0.41	1.72	0.38b	0.73a	
		0.8		0.58	1.04	0.24b	0.72a	
		1.6		0.41b	2.64aA	0.35b	0.98aB	
					L***			
Root tissue	Substrate			P rate (mg/L)				Trend
(mg/kg)	Organic	Sand		0	0.4	0.8	1.6	
	568.77a	469.18b		473.49	494.05	631.53	476.83	Q*
Shoot tissue	Flood Treatment			P rate (mg/L)				
(mg/kg)	Non-flood	Flood		0	0.4	0.8	1.6	
	941.44a	739.36b		686.34	814.84	1010.85	849.59	Q**

^{z}Flood treatments were non flood (irrigated three times weekly with 400 mL treatment solution) and flood (submerged in 1200mL treatment solution for 48 hours and drained for 7 days, with eight cycles).

^{y} Substrate treatments were 'organic' 50:50 organic matter : sand, and 'sand' 85:15 sand : organic matter.

^{x}Lowercase letters denote least squares mean separation within a row using ANOVA F-test in the Glimmix procedure ($p<0.05$).

[w]Uppercase letters denote least squares mean separation within a column using ANOVA F-test in the Glimmix procedure ($p<0.05$).
[u]Uppercase letters compares differences between substrate within a flood treatment for each phosphorus rate and lowercase letters compares differences in flood treatments within a substrate for each phosphorus rate.
[v]Least square means comparisons at each phosphorus rate using Tukey's test where significant liner (L) and quadratic (Q) trends using orthogonal polynomials at a = 0.05 (*), a=0.001 (**), and a<0.0001 (***).

Table 6. Effect of substrate, flood, and phosphorus (P) rate treatments on final P concentrations (mg/L) of substrate, leachate, and root and shoot tissue of *Coreopsis verticillata* 'Zagreb' when grown in a greenhouse in Auburn, AL.

Fraction	P-concentration									
Substrate	Substrate [z]		Flood treatment [y]			P rate (mg/L) [x]				Trend
(mg/kg)	Organic	Sand	Non-flood	Flood		0	0.4	0.8	1.6	
	3.20b[w]	6.34a	3.74b	5.79a		3.80	4.55	5.27	5.46	L***
Leachate		Flood Treatment				P rate (mg/L)				
(mg/L)	Substrate	Non-flood	Flood		Substrate	0	0.4	0.8	1.6	
	Organic	0.48Ab[v]	1.47Aa		Organic	0.42	0.95A	1.08A	1.46A	L***
	Sand	0.19Bb	0.57Ba		Sand	0.22	0.27B	0.50B	0.54B	L*
					Flood Treatment	0	0.4	0.8	1.6	
					Non-flood	0.17B	0.28B	0.41B	0.49B	L*
					Flood	0.47A	0.94A	1.17A	1.50A	L***
Root tissue	Flood Treatment									
(mg/kg)	Non-flood	Flood								
	628.11b	1012.96a								

[z] Substrate treatments were 'organic' 50:50 organic matter : sand, and 'sand' 85:15 sand : organic matter.

[y]Flood treatments were non flood (irrigated three times weekly with 400 mL treatment solution) and flood (submerged in 1200mL treatment solution for 48 hours and drained for 7 days, with eight cycles).

[x]Least square means comparisons at each phosphorus rate using Tukey's test where significant liner (L) and quadratic (Q) trends using orthogonal polynomials at a = 0.05 (*), a=0.001 (**), and a<0.0001 (***).

[w]Uppercase letters denote least squares mean separation within a treatment, within a column using ANOVA F-test in the Glimmix procedure (p<0.05).

[v]Lowercase letters denote least squares mean separation within a treatment, within a row using ANOVA F-test in the Glimmix procedure (p<0.05).

Chapter III:
The Effect of Planting Composition and Flooding Cycles on Growth and Phosphorous Uptake of Three Native Plant Species in Simulated Bioretention Gardens

Index Words: low impact development, pollution removal, biodiversity, green infrastructure

Abstract

Bioretention gardens restore hydrologic function of urban landscapes and capture stormwater runoff pollutants, such as phosphorus, a main pollutant in urban cities and residential neighborhoods. Monoculture plantings are common in bioretention gardens; however, polyculture plantings can improve biodiversity and ecosystem resilience. Thus, the objective of this study was to evaluate landscape plant species in monoculture and polyculture plantings for phosphorus uptake and tolerance of bioretention garden conditions. Four planting combinations, *C. verticillata* 'Zagreb' monoculture, *A. ternarius* monoculture, *I. vomitoria* 'Schilling's Dwarf' monoculture, and a polyculture of *C. verticillata* 'Zagreb', *A. ternarius,* and *I. vomitoria* 'Schilling's Dwarf' were planted into 94 L nursery containers in a substrate of a 50 sand : 50 organic matter. Containers were irrigated or flooded with 1.6 $mg{\cdot}L^{-1}$ P solution, At experiment termination, size index and root and shoot dry weight were recorded, and leachate, substrate, and plant tissue were analyzed for P concentrations. SDW of *I. vomitoria* was higher in fall, while SDW of *C. verticillata* was higher in spring, and overall, SDW was

smallest in flood conditions. RDW was highest for *A. ternarius* monoculture, and smallest with *C. verticillata*. Root and shoot tissue P was highest among species in *C. verticillata.* Substrate and leachate P was highest in flood treatments, and leachate P was lowest in the polyculture planting. Overall, two species had higher growth in the fall and another in the spring, suggesting that with a monoculture, there may be vegetation gaps across seasons. Additionally, polyculture combination had the lowest leachate P, suggesting a polyculture planting may be more effective in preventing excess P from entering waterways from bioretention gardens.. Thus, in bioretention gardens, polyculture plantings could regulate seasonal vegetation caps, and reduce leachate P, preventing P release into waterways.

Introduction

Bioretention gardens are a LID practice popular in commercial, industrial, and residential areas and are designed to capture and infiltrate stormwater runoff, treat runoff pollutants, recharge groundwater, while creating insect and wildlife habitat (Dietz, 2007; Prince George's County, Maryland, 2006; Virginia Department of Forestry, 2011). Bioretention gardens are also efficient in the removal of suspended solids, nitrogen, heavy metals, and oils (Davis et al., 2009; Schueler, 1997). Phosphorus, a main pollutant in urban cities, enters waterways with surface water runoff and degrades urban waterways through the over production of algae and aquatic plant growth (Davis et al., 2006; Dietz, 2005; Davis et al., 2009; USEPA, 2009; Mueller and Helsel, 1996; Carpenter et al., 1998). Since the main source of urban phosphorus is residential lawns and streets, bioretention gardens could be used to capture phosphorus prior to degrading local streams (Steuer et al., 1997; Waschbusch, 1999).

Bioretention gardens are designed to experience periodic flood conditions for up to two days (Price George's County, Maryland; 2006; Kraus and Spafford, 2009). Rubio et al. (1997) found that waterlogged soils increased the ability of plants to uptake phosphorus and could increase the soil phosphorus availability. However, repeated flooding can result in a phosphorus release from soils, which introduces additional phosphorus to soils and waterways (Jernigan, 2010; Olila et al., 1997). The release of phosphorus from flooding events is highly dependent on the soil characteristics, as sediment can act as a nutrient source or sink (Olila et al., 1997). Though flooded soils can increase phosphorus availability for plant uptake, plants face reductions in growth, biomass, photosynthetic activity (Chen et al., 2005).

Stress tolerant plant species, such as those accustomed to flood and disturbances, will generally acquire more minerals in their plant tissue than flood intolerant species (McJannet et al., 1995; Chen et al., 2005). Plants that are physiologically tolerant of local environmental conditions are recommended in bioretention gardens (Krauss and Spafford, 2009). However, additional research is needed to investigate landscape plant performance and ability to absorb phosphorus under flood conditions.

Monoculture plantings are common in large-scale bioretention gardens and LID projects due to lower costs and perceived ease of maintenance (Dewar, 2007; Bracken, 2008; Kuhn and Klotz, 2006). Diversifying planting composition between functional groups - monocots and dicots, evergreen and deciduous, and shallow and deep rooted species - can increase competition for nutrients, biomass productivity, and stress tolerance (Dewar, 2007; Bracken, 2008; Kuhn and Klotz, 2006). Increasing the number of functional groups in an ecosystem has also been associated with a higher level of competition against invasive species and weeds, a common concern in low impact development projects and bioretention gardens (Byun et al., 2012, Pokorny et al., 2005). Polyculture planting could also increase competition in water uptake and thereby increase flooding tolerance and nutrient uptake and removal (Dudley and File, 2007; Bracken, 2008). The objectives of this experiment are to evaluate three diverse landscape plants and monoculture and polyculture planting combinations, for phosphorus uptake and tolerance of bioretention garden conditions.

Materials and Methods

On 20 July 2012, 3 cm liners of *Coreopsis verticillata* 'Zagreb' L. Glab. (Whorled Coreopsis) (Emerald Coast Growers, Pensacola, FL), 3 cm liners of *Ilex* *vomitoria* 'Schilling's Dwarf' Ait. (Yaupon Holly) (Liner Farm, Eustis, FL), and 9 cm liners of *Andropogen ternarius* Michx. (Splitbeard Bluestem) (Hoffman Nursery, Rougemont, NC), were planted into 3.8 L containers containing a substrate of 5:3:1 pine bark: peat moss : pearlite amended with 0.045 $kg \cdot m^{-3}$ of $17N\text{-}5P_2O_5\text{-}11K_2O$ (with micronutrients) fertilizer (Plant Science, Inc., Ontario, Canada), and 0.02 $kg \cdot m^{-3}$ dolomitic limestone. The plants were placed on raised benches in an un-shaded 8 mm twin-wall, polycarbonate covered greenhouse under natural photo-periods with a heating set point of 18.3°C and ventilation beginning at 25.6°C at the Paterson Greenhouse Complex, Auburn University, AL

On 19 Sept. 2012, plants were planted into 94 L (0.12 yd^3) nursery containers (Nursery Supplies, Inc., Mobile, AL), representing simulated bioretention gardens. There were three plants per container, and planting combinations included a monoculture of *C. verticillata* 'Zagreb', *A. ternarius,* or *I. vomitoria* 'Schilling's Dwarf' or a polyculture of *C. verticillata* 'Zagreb', *A. ternarius,* and *I. vomitoria* 'Schilling's Dwarf', with 12 containers per planting combination. Containers were filled with a substrate of a 50% sand, 25 % pine bark, 25% peat moss, amended with 0.04 $kg \cdot m^{-3}$ dolomitic limestone and 0.45 $kg \cdot m^{-3}$ micro-nutrient phosphorus free fertilizer (Tru-prill, $19N\text{-}0P_2O_5\text{-}17K_2O$, Plant Science, Inc, Ontario, Canada). This substrate was chosen to simulate bioretention soils currently suggested in low impact development projects (Hunt and Lord, 2006; Virginia Department of Forestry, 2011).

Containers that were to be in flood treatments had no holes on the bottom and were modified by installing a spigot, approximately 7.6 cm from the base of the container, to open or close to enable drainage and flooding. A 0.6 cm hole was drilled into the container,   and a 3.8 cm x 3.2 cm PVC DWV Spigot x Slip Joint Street Trap Adapter was hand-threaded through this hole. To prevent substrate loss during container drainage, a 10 cm^2 piece of fiberglass window screen was attached over the opening of the trap adapter with a zip tie. Once the slip joint was in place, a 3.8 cm PVC spigot was externally attached. The system was sealed with waterproof and weatherproof silicone sealant and patched as necessary. On the non-flood containers, there was no drainage modification, and water drained through holes stamped by the manufacturer bottom of each container.

Containers were placed under an outdoor shade structure at the Paterson Horticulture Greenhouse Complex, Auburn University, AL. The top of the structure was pitched with 3.4 m tall sloping to 1.8 m tall along the short side and covered with a double layer of 6 mil clear polyethylene plastic to prevent rainfall from entering and a 60% woven shade cloth. Containers were randomly arranged.

After one week of acclimation outdoors, on 27 Sept. 2012 (herein referred to as 'fall'), flood and non-flood treatments were initiated. Six containers from each planting combination were flooded with 26.5 L of tap water, and no additional water was added during each flood event of 48 hours. Following each flood event the container was drained for seven days. No additional water was added to the containers during the draining period of seven days that followed each flood event (flood – drain cycle). There were 12 flood - drain cycles. The non-flood containers, six from each planting combination, were irrigated three times weekly with 20 L of tap water. Plants were irrigated or flooded with a solution containing 1.6 $mg{\cdot}L^{-1}$ phosphorus (85% H_3PO_4, Fisher Scientific, Pittsburgh, PA) which is four times the median phosphorus concentration of 0.4 mg·L^{-1} common in urban stormwater runoff (Steuer et al, 1997, Waschbusch et al, 1999).

Size index [(shoot height + shoot widest width + shoot width (perpendicular to widest)/ 3)] was measured for each plant at run initiation, midway, and termination. At termination of each run (described below), plant shoots 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 to determine shoot dry weight and root dry weight. Four 50 g substrate samples were collected (for each substrate) prior to planting before and after the addition of lime and fertilizer and upon termination, samples were collected for each planting combination x flood treatment. Upon termination, tissue samples were collected by grinding down the entire shoot or root tissue of each plant to 5mm particle size. A 0.5 g sample of the root and shoot tissue from each of three plants per planting combination x flood treatment combination was collected. Leachate samples (200mL) were collected from three containers in each planting combination x flooding treatment. Leachate was collected using the Virginia Tech Pour Through Method (Wright, 1986). Leachate samples were analyzed for pH and electrical conductivity with a hand held Beckman Coulter pHI© 460 meter, epoxy body 3-in-1 gel-filled pH electrode and conductivity probe, and filtered through 42 Whatman filter paper.

Substrate samples were processed using Mehlich 1 double acid extraction method, and tissue samples were processed using dry ash and double acid extraction method, both adopted by the Auburn University Plant and Soil Analysis Lab (Hue and Evans, 1986). Leachate, substrate and tissue samples were analyzed for P concentrations for run 1 at Auburn University Plant and Soil Analysis Lab using Inductively Coupled Argon Plasma

(ICAP) machine. The experiment was repeated (run 2) on 4 Apr. 2013 to 7 June 2013 (herein referred to as 'spring') with no differences in methodology except that chemical analysis of root and shoot tissue, substrate, and leachate was omitted.

Analysis of variance was performed using PROC GLIMMIX in SAS version 9.2 (SAS Institute, Cary, NC). The experimental design for shoot and root dry weights and root and shoot phosphorus concentrations was a split plot with planting combination and flood completely randomized in the main plot and species as the subplot. The model included main effects of combination, flood, and species and interactions between combination x flood and flood x species. The experimental design for soil and water P concentrations was a completely randomized design with a complete factorial treatment design of combination and flood. The two runs were analyzed separately when treatment means were different between runs; otherwise, the two runs were combined into one analysis by including runs in the analysis as a random variable. When residual plots and a significant COVTEST statement using the HOMOGENEITY option indicated heterogeneous variance, a RANDOM statement with the GROUP option was used to correct heterogeneity. Differences among treatment least squares means were determined using Tukey's test with the STEPDOWN option and a family-wise error rate of 0.05 in LSMEANS statements. All significances were at $\alpha = 0.05$. Results for main effects and interactions are presented when significant and not presented if not significant. If interactions were significant, then the simple effects of each factor are presented.

Results

Shoot dry weight (SDW) was different among species in fall, where *A. ternarius* was highest and *C. verticillata* was lowest (Table 1). In spring, there was an interaction between species and flood. In flood and non-flood treatments, SDW of *C. verticillata* was highest (Table 2). SDW of *C. verticillata* was higher in flood than non-flood, while SDW of *I. vomitoria* and *A. ternarius* was similar in flood and non-flood (Table 1).

SDW of *I. vomitoria* was higher in fall than in spring, while SDW of *C. verticillata* was higher in spring than in fall (data not presented). In spring, for all planting combinations, SDW was smaller in flood than non-flood treatments (Table 1). In non-flood, SDW of *C. verticillata* monoculture was lower than that in other planting combinations (Table 1).

In fall, RDW was higher for *A. ternarius* than the other species (Table 1). In spring, RDW was highest for *A. ternarius* and lowest for *I. vomitoria* (Table 1). In spring, RDW of *A. ternarius* monoculture was highest, while RDW of *C. verticillata* monoculture and polyculture were lowest (Table 1). In fall, size index (SI) of non-flood and flood plants was highest for *A. ternarius* (Table 1). Although flood did not affect SI of *A. ternarius* and *I. vomitoria*, SI of *C. verticillata* was higher in non-flood than flood (fall, Table 1). In spring, SI of *A. ternarius* and *C. verticillata* were higher than that of *I. vomitoria* (Table 1).

Initial substrate phosphorus (P) was 2.33 mg/kg. Final substrate P was higher in flood than non-flood (Table 2). Similarly, leachate P was higher in flood than non-flood (Table 2). Leachate P in flood was lowest in the polyculture, however in non-flood there were no differences in leachate P concentrations (Table 2). Within planting combination, P leachate was higher in flood than non-flood for the monocultures, but was not different for the polyculture (Table 2).

Root and shoot tissue P was highest among species in *C. verticillata* (Table 2). Among planting combinations, root tissue P was highest for *C. verticillata* monocultures and lowest for *A. ternarius* and *I. vomitoria* monocultures (Table 2). There was no difference in shoot tissue P among planting combinations (data not shown). However, for total P in plant tissue, combined root and shoot tissue, *C. verticillata* had 10.47 mg P, *A. ternarius* had 101.71 mg P, and *I. vomitoria* 22.78 mg P.

Discussion

In the fall experimental run, beginning on 27 Sept.2012, SDW and RDW was smaller in *C. verticillata* and larger for *A. ternarius* and *I. vomitoria* (Table 1). In spring, *C. verticillata* had the highest SDW (Table 1). Most growth for *C. verticillata* typically occurs during spring and summer while growth for the other two species can extend into fall (USDA 2013). Size index was also highest in *A. ternarius* in both runs regardless of flood treatment (Table 1). This result reflects a change in seasonality and growth, inevitable within a bioretention garden.

Additionally, in spring, plant growth was affected by flooding treatments, where SDW was smaller in flooded treatments. Similarly, Dylewski et al. found that among three native landscape shrubs SDW and final size index was decreased in flood soils, compared to non-flood (2012). In experiment with *Muhlenberia capillaris* (Lam.) Trin., a native landscape grass, it was also found that SDW and RDW was higher in non-flooded than flooded treatments (Christian et al., 2012). Additionally, when a substrate is flooded during the growing season it is much more harmful to the plant than flooding during the non-growing season, such as in fall (Kozlowski, 1982; Pezeshki, 2001). Despite flooding stress, all species appeared to tolerate flood conditions, where *C. verticillata* was most affected.

In each planting combination in spring, SDW was lower in flood treatments than non-flood treatments, and among flood, SDW lowest for *C. verticillata* monocultures, and similar among all planting combinations (Table 1). In non-flood treatments, SDW was lowest in the *C. verticillata* monoculture planting combination. Additionally, RDW was highest in spring for *A. ternarius* monocultures, and similar for all other combinations. Species differ considerably in their response and susceptibility to flooding stress (Vartapetian and Jackson, 1997).

Both RDW and SDW values for polyculture combination were neither the highest in weight nor the lowest, and the 'middle' growth rates could be due to the inclusion of a faster growing species such as *A. ternarius* (Whitney, 1985). In study of functional group diversity on green roofs, it was found that functional group diversity decreased the potential benefit of a few species, because the container was shared with a poorer performing species, thus, results for water conversation and pollutant uptake were weaker for diverse pots, than monoculture pots, due to the a 'dilution effect' (Lundholm et al., 2010). In bioretention gardens, faster growing species, such as *A. ternarius*, could eventually out-compete slower growing species, potentially reducing biodiversity, and canopy and vegetation gaps. Urban colonizers and exploiters such as grasses and can tolerate high levels of disturbance (common in bioretention gardens) and colonize an area quickly (Whitney, 1985). Although functional annuals (i.e. perennial plants with high seeding and growth rates) can grow quickly and can provide immediate ground cover in a new bioretention area, grasses allocate relatively few resources for nutrient storage; therefore, tend to have reduced pollutant removal capacity (Chapin et al., 1990; Lorenzen et al., 2001).

Polyculture planting in this study included three different functional plant groups – herbaceous perennial, shrub, and grass. When competition exists between different functional groups, compared to within the same functional group, there is a higher biomass yield than when a functional group is grown alone, as the plants are occupying different resource niche spaces (Bracken, 2008). Additionally, mixtures of shallow and deep rooted species (shallow to deep rooted in this experiment*: C. verticillata, A. ternarius, I. vomitoria*) can improve the uptake of limiting nutrients, such as P, leading to higher productivity over time, and potentially, more nutrient removal from stormwater (Bracken, 2008).

Since *A. ternarius* and *I. vomitoria* were high growth species in fall, and *C. verticillata* was a high growth species in spring, and the polyculture treatments represented intermediate RDW and SDWs, having a variety of plants in a simulated garden can reduce vegetation gaps caused by seasonal growth variations among species (Table 1). In a study of diversity and canopy structure on grassland ecosystems, it was found that species and functional group diversity increased mean vegetation cover from 64% in monocultures to 100% in a 32-species mixture (Spehn et al., 2000). The most species rich community produced 143% more biomass than monoculture plantings over several seasons Additionally, Wardle et al. (2000), found that plant functional group richness exerted positive effects on plant biomass and productivity, which indicates that ecosystem stability and resistance may be improved by above ground community diversity.

Among species, root and shoot tissue P was highest in *C. verticillata*, and similar in *I. vomitoria* and *A. ternarius*. Among planting combinations, root tissue P was also highest in *C. verticillata* monoculture pots, and the next highest root tissue P was in polyculture pots, and *A. ternarius* and *I. vomitoria* monoculture pots were similar. Perennial plants from higher nutrient ecosystems, such as *C. verticillata*, may have higher nutrient uptake than plants than faster growing plants, such as grasses (Read et al., 2008, Vance et al. 2003). Nutrient storage can occur in perennial plants as an 'insurance' against future loss and as a support for growth when conditions are favorable, thus *C. verticillata* may have had the highest root and shoot tissue P due to its natural ability to uptake more nutrients (Chapin et al., 1990). However, when total P for each plant was measured, *C. verticillata* had 10.47 mg P, *A. ternarius* had 101.71 mg P, and *I. vomitoria* 22.78 mg P. While these measurements were conducted in the fall, greenhouse experiments in Chapter II, conducted in these spring seasons, show that *C. verticillata* had 24.51 mg P, *A. ternarius* had 14.28 mg P, and *I. vomitoria* 1.63 mg P, which further emphasizes the seasonal variations among plant functional groups.

Since *A. ternarius* is a fast growing functional annual, rather than storing additional P in tissue, it may immediately allocate additional P to growth, therefore, may have a perceived reduction in P uptake capacity, when really it is allocating nutrients to biomass rather than storage (Chapin et al., 1990). Additionally, slower growing plants from low-resource ecosystems, such shade understory, have low capacity for nutrient uptake, which may explain why *I. vomitoria*, an understory shrub had low shoot and root P concentrations. (Chapin, 1980; Grime, 1977; Parsons, 1968). Overtime, *I. vomitoria* may have higher P storage, however, in the short run, moderate growth perennials native to high resource ecosystems, such as *C. verticillata*, may have higher root and shoot tissue P.

Resource allocation varies widely among plant functional types. Since polyculture plantings had the second highest level of root tissue P, next to *C. verticillata* monocultures, it is possible that with a higher diversity of functional groups there is a higher uptake of nutrients, since a variety of niche spaces are occupied (Table 2) (Milla et al., 2009; Read et al., 2008; Spehn et al., 2009). Thus, for increased P uptake over seasons and across species, it could be beneficial to have a variety of plant functional groups. While *C. verticiallata* monoculture had highest tissue P of all planting combinations, *C. verticillata* also had low SDW and RDW in fall, representing a vegetation planting gap (Table 1). With a planting gap, another species, such as an invasive plant or weed, could move in more readily in a monoculture, since polyculture plantings are better able to resist invasion and are more resilient to mortality of another single species (Byun et al, 2012; Pokorny et al. 2005). While root tissue P was highest in *C. verticillata*, RDW was lowest for this species. In contrast, root tissue P was lowest for *A. ternarius* and *I. vomitoria*, and RDW was highest for *A. ternarius*. Thus it's possible that while some species, such as *C. verticillata*, are storing excess P in plant tissue, species from low nutrient ecosystems or those with long life history traits, such as *A. ternarius* and *I. vomitoria*, maybe be allocating additional P to biomass production. In another study testing functional plant groups and pollutant removal, it was found that plant species with high root mass accounted for 20-37% in the variation for effluent removal, thus root architecture, plant functional group, and life history traits may play a role the reason for high P storage in the root tissue of *C. verticillata* (Read et al., 2008).

Leachate P was higher in flood treatments than in non-flood treatments. In non-flood treatments, leachate P was similar regardless of planting combination (Table)

When flooded, leachate P was lowest in polyculture plantings, which suggests there is more nutrient competition for P in polyculture plantings than monoculture plantings (Milla et al., 2009; Read et al., 2008; Spehn et al., 2009).

Christian et al. (2012), also found that phosphorus concentration in leachate was higher in flood plants than non-flood Olila et al., (1997) observed P release in completely drained and re-flooded soils, where rewetting of completely dried soils resulted in a flush of available P. Such P outflows would likely not meet water quality requirements in a phosphorus sensitive area (Baldwin and Mitchell, 2000). Partial drying of wet soil can result in a sediment affinity for available P, thus, perhaps, limiting complete soil desiccation can result in less P release from soils upon repeat flood events (Baldwin and Mitchell, 2000).

Conclusion

All planting combinations had higher root and shoot tissue P than combined substrate and leachate P suggesting that the removal of P in bioretention systems could be improved by managing, growing, and harvesting plant tissue (Davis et al., 2006: Read et al. 2006: Denman et al. 2007). Plants can make direct use of P for growth and storage in tissue, and while some plants have a higher pollutant uptake and removal capacity than others, it appears the varying plant tissue P concentrations could be managed over seasons by incorporating more diverse plant functional groups.

Additionally, two species had the higher SDW in the fall, and another species had a higher SDW in spring, suggesting that in a monoculture planting or a planting of the same functional group (all grasses or all shrubs, for example), there may be vegetation gaps within seasons. The polyculture planting combination had moderate SDW and RDW across seasons, suggesting that a polyculture planting could reduce the problem of vegetation gaps, with more successful species eventually filling in the gaps of unsuccessful species.

While leachate P is a concern for phosphorus sensitive waterways, it was found that flood treatments, a common condition in bioretention gardens, had the highest leachate P. However, the polyculture planting combination had the lowest leachate P, which suggests that to reduce additional P entering the waterways, a polyculture planting may be more effective than a monoculture planting. Thus, in simulated bioretention gardens, polyculture plantings regulate P removal across seasons, reduce potential seasonal vegetation caps, and reduce leachate P from entering nearby waterways. Therefore, when considering planting combinations for bioretention gardens, a more diverse species mix is most effective for aesthetic value, resistance to invasion of weed species, and for continued P uptake in roots and shoots.

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Tables and Figures

Table 1. Effect of flood (n=24), species (n=48), and planting combination (n=12) on root dry weight (RDW), shoot dry weight (SDW), and size index (SI) of *Coreopsis verticillata* 'Zagreb', *Ilex vomitoria* 'Schilling's Dwarf', and *Andropogon ternarius* when grown outdoors in fall 2012 (run 1) and spring 2013 (run 2) in simulated gardens at the Paterson Greenhouse Complex in Auburn, AL.

Run	Flood Treatment[z]	Species			Combination[y]			
		I. vomitoria	*C. verticillata*	*A. ternarius*	*I. vomitoria*	*C. verticillata*	*A. ternarius*	Poly
		SDW						
1		13.45b [x]	0.67c	50.29a				
2	Non-Flood	0.75b	25.84Ab[w]	6.20b	11.28Aa	4.03Ab	13.45Aa	11.00Aa
	Flood	0.68b	12.01Ba	4.12b	5.91Ba	0.42Bb	5.61Ba	6.25Ba
		RDW						
1		3.09b	1.80b	13.14a				
2		0.76c	2.90b	3.96a	2.46ab	1.75b	3.51a	2.45b
		SI[v]						
1	Non-Flood	26.21b	19.66Ab	37.60a				
	Flood	28.46b	11.46Bc	41.57a				
2		9.94b	46.39a	43.70a				

[z]Flood treatments were non flood (irrigated three times weekly) and flood (submerged in treatment solution for 48 hours and drained for 7 days, with eight cycles).
[y]Combination represents a monoculture of the species listed or a polyculture including of all three species.
[x]Lowercase letters denote least squares mean separation between flood treatment within a row using ANOVA F-test in the Glimmix procedure ($p<0.05$).
[w]Uppercase letters denote least squares mean separation among species, within a column using ANOVA F-test in the Glimmix procedure ($p<0.05$).
[v]SI – Size index measured for each plant is [height + long width + perpendicular width)/3]

Table 2. Effect of flood (n=24), species (n=48), and planting combination (n=12) of final P concentrations (mg/L) of substrate, leachate, and root and shoot tissue of *Coreopsis verticillata* 'Zagreb', *Ilex vomitoria* 'Schilling's Dwarf', *Andropogon ternarius* when grown outdoors in simulated gardens at the Paterson Greenhouse Complex in Auburn, AL.

Fraction		P-concentration			
Substrate (mg/kg)	Flood Treatment[z]				
	Non-flood	5.78B[y]			
	Flood	7.46A			
Leachate (mg/L)		Combination[x]			
		A. ternarius	*C. verticillata*	*I. vomitoria*	Poly
	Non-flood	6.10B[w]	5.80B	6.68B	8.28
	Flood	15.08Aa	17.80Aa	13.45Aa	11.95b
Root tissue (mg/kg)		Species			
		A. ternarius	*C. verticillata*	*I. vomitoria*	
		1944.64B	4089.10A	1606.97B	
		Combination			
		A. ternarius	*C. verticillata*	*I. vomitoria*	Poly
		1728.41c	4876.44a	722.55c	2860.22b
Shoot tissue (mg/kg)		Species			
		A. ternarius	*C. verticillata*	*I. vomitoria*	
		2443.70b	3088.22a	2636.41b	

[z]Flood treatments were non flood (irrigated three times weekly) and flood (submerged in treatment solution for 48 hours and drained for 7 days, with eight cycles).
[y]Uppercase letters denote least squares mean separation among species or combination within a colum using ANOVA F-test in the Glimmix procedure ($p<0.05$).
[x] Combination = the monoculture of each species, where single species are named are a monoculture of that species, and 'poly' is a combination of all three species.
[w]Lowercase letters denote least squares mean separation between flood treatment within a row using ANOVA F-test in the Glimmix procedure ($p<0.05$).