EFFECT OF DORMANT AND FOLIAGED PLANTS

ON NITRATE AND PHOSPHATE RETENTION

IN BIORETENTION CELLS

By

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Bachelor of Science in Biology

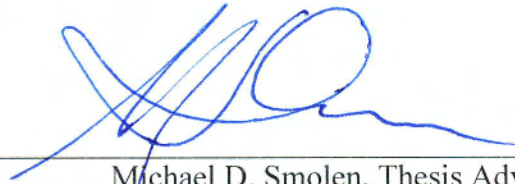Southwestern Oklahoma State University

Weatherford, Oklahoma

2005

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2010

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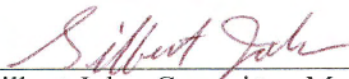IN BIORETENTION CELLS

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ACKNOWLEDGMENTS

I am very grateful to have received time and guidance from my thesis committee. This was an exciting study and I learned a great deal about water quality through bioretention, and in general what it takes to contribute to academia. My thesis advisor is a most pleasant and patient person, he also was a shining light during my time in Stillwater. One day, I would like to be able to share patience, enthusiasm, and objectivity as naturally as Michael Smolen. The same is true for Mike Schnelle and Gilbert John whom all exemplify what it means to be a mentor. This path has been walked and now we are at a new beginning. Thank you all for maintaining the enthusiasm and confidence during this study. Chukma'shki!

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CHAPTER I

INTRODUCTION

Water Quality

Our modern definition of water quality and approaches to maintaining this
limiting resource has undergone rapid changes in the last several decades. Increased
urbanization of our populations and unchecked growth following the Industrial
Revolution has placed the state of our water resources in peril. It has only been since the
1970s that Lake Erie was thought to be altogether "dead" and fires on the Cuyahoga
River caught national attention. These incidents and other historic events have shaped
the way we approach the conservation of our natural resources.

In the early 1970s Congress revised existing water laws in the spirit of a more
comprehensive means to address the needs of a growing population and increasing
industry. Several decades later these changes are still being implemented in the
protection of our waters and have continued to changed with the increasing pressures of
technology and a growing population.

Originally, Congress targeted municipal sewer systems and direct industrial
dumping or point sources (PS) of pollution into our natural waterways but since the
1980s non-point sources (NPS) and stormwater have received increasingly

more attention. As the nation and our economy have changed so too have our population dynamics and sources of water degradation. Non-point sources of pollution are now the leading contributors to impairment and PS are less of a problem following decades of targeted management (USEPA 2003). With continued improvements to the methods that address these two types of water pollution there are increasingly advanced technologies for protecting and restoring the nations "waters".

Bioretention and Stormwater Management

Urban sprawl and densely populated municipalities alter the natural hydrology and have been shown to contribute significant amounts of pollution to stormwater that enters natural waterways, contributing to reduced quality and altered flow rates. With conventional land development there is an increase in the amount of impervious surface in the respective watershed and a subsequent increase in the volume of stormwater and peak flow. These anthropogenic sources of stormwater can have many impacts on the environment contributing to erosion, silting, and habitat deterioration.

Stormwater leaving urban/suburban landscapes may carry oil and metals from automobiles, pesticides, herbicides, nutrients, and animal wastes. Some of these pollutants can lead to eutrophication of water bodies from erosion and nutrient loading, others simply altogether impair water quality if present in significant concentrations such as pesticides, heavy metals and hydrocarbons.

Use of bioretention as a Best Management Practice (BMP) has only been utilized in the recent decades. This novel approach to stormwater management was first applied in the use of Bioretention Cells. Bioretention Cells are landscaped areas with amended soil that is engineered to increase infiltration, and designed to mitigate runoff associated with a delineated area. These BMPs are used to reduce stormwater pollution and naturally manage quantities of runoff following precipitation events through an integrated onsite approach (PGDER 2002)(NCES 2005). Stormwater has been conventionally managed by detention ponds, which are centralized BMPs that store large qualities of water that is then slowly released off-site. Bioretention Cells, also known as rain gardens, can be integrated throughout a site to suite developers' needs or can be installed post development to address unanticipated storm-water issues.

This technology is continuously being improved. As such, there is inadequate knowledge of the effects of ecological processes occurring within these systems. Ecological processes in the context of this study refer to the cumulative effects of dormant and foliaged plants and vesicular arbuscular mycorrhizal fungi (VAM) on uptake and sequestration of elements in the root zone (*i.e.* their influence on rhizospheric nutrient cycling and other soil processes).

Plants & Mycorrhizal Fungi

The influence of plants and symbiotic VAM on soil properties has not been addressed in the Bioretention Cell literature. However, there is extensive documentation of the plant and VAM role in the uptake of elements and modification of the rhizosphere (Bolan 1991)(Varma and Hock 1998). For instance, vascular plants have been used for some time for their known phytoremediative attributes and ability to modify the soil environment (*i.e.* soil organic matter, CEC, soil stability)(EPA 2006).

The same is true of VAM, an endophytic symbiont that is increasingly being used for enhanced bioremediation, especially for heavy metals (Read and Perez-Moreno 2003)(Varma and Hock 1998). The lack of quantification of the intrinsic benefits of plants and their symbionts in Bioretention Cells may lead to a gross underestimation of the ameliorative potential of these BMPs which could otherwise further encourage their implementation.

CHAPTER II
REVIEW OF LITERATURE

Water Quality

In 1948 Congress passed the Federal Water Pollution Control Act, which in 1977 after further amendment became known as the Clean Water Act (CWA), a statute that provides both regulatory and non-regulatory tools to protect the surface waters of the United States. This Congressional Act is intended to restore and maintain the chemical, physical, and biological integrity of the nation's waters; specifically to support "the protection and propagation of fish, shellfish, and wildlife and recreation in and on the water (GPO 1972)."

The CWA was initially used to address industrial and municipal PS and the chemical aspects of "integrity" (USEPA 1999). Point source pollution, as defined by the CWA, includes all pollution originating from discrete conveyances (*i.e.* end-of-pipe). Since the 1980's, increasingly more attention is given to NPS such as agricultural and residential overland runoff following precipitation events and the biological aspects of "integrity" (USEPA 1999). Non-point sources, as interpreted by the CWA, include all pollution that is not classified as PS.

In recent years the Environmental Protection Agency (EPA) reported that NPS is linked to over 40% of the nations impaired waters while only 10% of impairments can be solely attributed to PS (USEPA 1999). More specifically, pollutants and erosion from agriculture and urbanized areas (UAs) are leading causes of water quality impairment in the nation.

Non-point sources are typically addressed by the CWA with measures other than regulatory forms. Concentrated Animal Feeding Operations (CAFOs) are the exception and are treated as PS. Similarly, some pollution from UA originates as NPS but as stormwater is directed through Municipal Separate Stormwater Systems (MS4s) it becomes PS and is regulated as such.

Section 319 of the CWA addresses NPS by providing monies to states, tribes, and local governments for the development and implementation of programs aimed at the reduction of NPS pollution. Stormwater from urban areas and densly populated municipalities can be indirectly addressed with 319 funds and programs, since the implemented practices target NPS to overall reduce stormwater pollutants (*i.e.* the resultant PS pollution from MS4s).

The CWA does not provide regulatory constraints for NPS, but stormwater BMPs are mandatory. Urban BMPs that target stormwater include detention ponds, grassed swales, and bioretention. Non-regulatory strategies utilize public educational materials and other forms of outreach as a major component of the BMP strategy.

In 1987 the scope of Section 402(p) under the CWA was expanded requiring permitting for some MS4s under the National Pollutant Discharge Elimination System (NPDES) regulatory program. This program requires most entities with MS4s to obtain a NPDES permit. Urbanized areas and densely populated municipalities fall under this regulatory framework. The EPA can delegate permitting for NPDES to states and tribal governments once they demonstrate at least equally stringent regulations as the EPA.

The implementation of this comprehensive regulatory plan for municipal PS discharge was to be achieved in two phases. Progress is monitored with a biannual Integrated Water Quality Assessment Report that is required of entities that issue permits. Phase I under section 402(p) was announced in 1990 requiring permits for stormwater discharge from MS4s serving populations of 100,000 or more. Phase II under section 402(p) began in 1999 requiring permits for stormwater discharge from small MS4s located within an UA. In the EPA Stormwater Phase II Final Rule document (USEPA 2005) "an UA is a land area comprising one or more places, central place(s), and the adjacent densely settled surrounding area, urban fringe, that together have a residential population of at least 50,000 and an overall population density of at least 1,000 people per square mile."

Oklahoma Water Quality

As of 2010, Oklahoma is in compliance with Phase I and Phase II requirements as determined by the EPA and Oklahoma Department of Environmental Quality (ODEQ) is authorized to administer most of the NPDES permitting. Oklahoma has two cities regulated under Phase I (Tulsa and Oklahoma City) and 45 Phase II entities (GCSA).

Oklahoma's 2008 Integrated Water Quality Assessment Report covered 622,176.5 lake acres of a total 1,041,884.0 lake acres and 12,415.7 river and stream miles of a total 78,778 river and stream miles. This report identified Total-phosphate as the main source of nutrient impairment in 24,317 lake acres and 160.2 river and stream miles. Nitrates and ammonium were causes of impairment in 117.7 and 115.5 river and stream miles, respectively. Urban-related runoff (stormwater) was listed as a probable source of contamination for 20,553.0 lakes, reservoirs, and pond acres and 6,573.8 river and stream miles (ODEQ 2008). The EPA reported in 2003 "The most recent National Water Quality Inventory identified runoff from urbanized areas is the leading source of water quality impairments to surveyed estuaries and the third-largest source of impairments to surveyed lakes" (EPA 2003).

Oklahoma water quality standards are variable and dependent upon locality and defined beneficial use, in terms of nutrient limitations in water bodies and are more often narrative than numerical. For instance, the only Oklahoma numeric water quality standard for phosphorus (0.037 mg/L) solely applies to waters designated as a "Scenic

River" (OWRB 2007). Oklahoma currently has only one designated "Scenic River"; the Illinois River but Flint Creek and Barren Fork Creek, tributaries of the Illinois River, also fall under this designation.

Oklahoma Water Quality Standard 785:45-5-9 describes applicable general narrative criteria and states "Nutrients from point source discharges or other sources shall not cause excessive growth of periphyton, phytoplankton, or aquatic macrophyte communities which impairs any existing or designated beneficial use (OWRB 2007)."

Municipal stormwater is regulated as PS under Section 402(p) of the CWA but is typically managed with BMPs. In Oklahoma and surrounding states the use of Stormwater Detention Facilities is a dominant urban BMP in the management of stormwater. These facilities are artificial detention basins (ponds) that trap and store excessive stormwater and slowly release it to minimize peak flow. This BMP primarily targets water quantity issues allowing some pollutants to settle out of solution and be retained in the pond sediment. Most importantly, these BMPs manage the peak flow and reduce down stream erosion that would otherwise result in sedimentation of streams, nutrient release, and lead to habitat disturbance or destruction.

Stormwater management in Oklahoma is generally addressed by conventional strategies but increasing populations and regulatory constraints will continue to demand a more natural and integrated approach. It has been these pressures that led to a more holistic strategy in both development and resource management across the nation. Conventional stormwater management has undergone many changes, some mere

modifications of a theme and some vast improvements or novel practices altogether, all of which are used in a Low Impact Development (LID) strategy.

Low Impact Development

Conventional stormwater management has undergone many changes since the inception of the CWA. Decades of efforts to manage stormwater, and the resultant natural water degradations, have led to innovative strategies and insight into ecological processes associated with stormwater. In the last 30 years different methods and policies have been evaluated, improved, and/or abandoned. Low Impact Development (LID), a more recent development, is an approach combining many of these successful strategies and policies with a decentralized methodology aimed at restoring the natural hydrologic regime to pre-development conditions (ACB 2002; PGDER 2002; PSAT 2003; PGDER 1999).

The increasing demands on conventional stormwater management infrastructure and increasing regulatory constraints led to the concerted efforts of resource managers across the United States. Among the pioneers of the LID approach are Prince George's County, Maryland Department of Environmental Resources (PGDER) and the Center for Watershed Protection (CWP). Since the inception of LID several entities, local and regional, have incorporated these practices into their development guidelines and many have contributed to the improvement and acceptance of LID strategies.

In *"Low Impact Works!"* Hood *et al* (2006) share results of a 10-year USEPA section 319 National Management Project that directly compares water quality and quantity impacts from a integrated LID site approach and a centralized conventional stormwater approach. They reported the site with LID more resembled pre-development conditions than the conventional site in all the variables measured: peak discharge, runoff volume, coefficient, and lag time.

Although LID has met enthusiastic acceptance within the water quality community, existing regulations and guidelines can be preventative for implementation. For example, a city ordinance requiring the use of a centralized drainage system, curb specifications, and required widths for roadways could limit or prevent many LID designs. Minimizing road widths, sharing driveways and the use of permeable pavement are all LID practices that may conflict or not fit within the scope of conventional standards being implemented.

An LID practitioner is not confined to the predominately engineered, structural, or end-of-pipe approach of the past. Low Impact Development is a more natural strategy that utilizes landscape elements and benefits from decades of experience with site-specific issues. Conventional stormwater management often leads to a quick evacuation of precipitation from the development site then detention and slow release to natural waterways. This method primarily treats quantity rather than quality of stormwater entering natural waterways and is not capable of maintaining pre-development conditions (ACB 2002; Davis 2005; PGDER 1999).

In Coffman's (2003) *Discussion of an Ecosystem Basis for Protecting Receiving Waters* he succinctly details the need for an ecosystem-based approach and the advantages of a more natural strategy to manage runoff quality and quantity. In application this is achieved with the "toolbox" of strategies when coupled with conventional stormwater practices to reduce pollutant loading and volume of stormwater entering natural waterways by 20 to 60% (CWP 1998).

> "In a natural setting, stormwater is controlled by a variety of mechanisms (interception by vegetation, small depression storage, channel storage, infiltration and evaporation) uniformly distributed throughout the landscape. LID mimics these mechanisms by uniformly distributing small infiltration, storage, and retention and detention measures throughout the developed landscape. What we soon began to see is that every development feature (green space, landscaping, grading, streetscapes, roads, and parking lots) can be designed to provide some type of beneficial hydrologic function (PGDER 1997)."

Low Impact Development approaches pre-development conditions utilizing existing vegetation and natural landscape elements with an ultimate goal of minimizing impact to natural habitats and entire ecosystems, both immediate and downstream, additionally achieving cost benefits by reducing the need for centralized stormwater infrastructure. An onsite evaluation is conducted to determine hydrologic soil groups,

ground water depth, seasonal precipitation, etc. Conservation of onsite natural resources such as floodplains, forested stream buffers, hydrologic soil groups A and B, and long-lived trees can reduce the cost and need for installation of conventional BMPs as well as enhance the overall aesthetics and value of the site.

Many of the elements of an LID practitioner's "toolbox" are site-specific, smaller scale, and more natural in structure and function working with the in-situ conditions. These and other LID strategies can also fulfill the "Green Building" and "Smart Growth" perspectives.

Bioretention Cells, water features, and grassed swells retain runoff, which through infiltration, evaporation, and transpiration reduces the resultant stormwater (PGDER 2002; Davis 2005; PGDER 1999). An integral difference between LID and conventional stormwater management is onsite reduction of stormwater, achieved by decentralizing runoff then utilizing infiltration and evapotranspiration. Bioretention cells use the onsite topography and hydrologic soil groups paired with selected plant species.

Bioretention Cells are fundamental examples of the LID approach; site-specific, non-structural, utilized throughout the site. These practices like many LID strategies can be designed in a multitude of ways depending on the needs and desires of the developer and the topography of the site. Many LID practices utilize some form of infiltration and/or bioretention thus knowledge of these systems can lead to a greater understanding of LID.

Bioretention Cells and other LID strategies are being used increasingly and studied more thoroughly since the release of the *Low-Impact Development Design Manual* (PGDER 1997). In application to all other benefits LID exhibits versatility, aesthetic appeal, and carries the shared cost and goals of onsite landscaping requirements. The versatility and effectiveness of the LID approach to mitigate stormwater will soon lead to LID as the conventional approach to stormwater management.

Most recently, Congress enacted the *Energy Independence and Security Act* of 2007 (EISA) that includes Section 438 establishing stormwater management requirements for federal development and redevelopment projects. The scope of this section reads as follows:

> **"**The sponsor of any development or redevelopment project involving a Federal facility with a footprint that exceeds 5,000 square feet shall use site planning, design, construction, and maintenance strategies for the property to maintain or restore, to the maximum extent technically feasible, the predevelopment hydrology of the property with regard to the temperature, rate, volume, and duration of flow."

To facilitate this new mandate the Obama administration issued Executive Order 13514 *Federal Leadership in Environmental, Energy, and Economic Performance* calling for the publication of EPA document 841-B-09-001 *Technical Guidance on Implementing the Stormwater Runoff Requirements for Federal Projects under Section*

438 of the Energy Independence and Security Act. This technical guidance illustrates the environmental, sociological, and economical benefits of the LID approach to stormwater management. This initiative and the increased intensity of related efforts across the nation can serve to stimulate the use of LID approaches in Oklahoma's stormwater management.

Bioretention

Bioretention is a process that is applied in the use of Bioretention Cells. It was this process that led to the paradigm shift towards the more holistic approach of LID.

"The bioretention technique has led to the creation of a new, holistic development philosophy known as Low Impact Development (PGDER 2002)."

Rain gardens as defined by *The Bioretention Manual* are preexisting and natural low topographic features that are landscaped with water tolerant plants, soil is not engineered as in a Bioretention Cell (PGDER 2002). However, term "Rain Garden" is applied to both LID strategies throughout the literature.

Bioretention Cells harbor a complex plant-soil-microbe-microfauna relationship; these units are engineered to function similarly to an upland riparian natural ecological community in terms of nutrient cycling, retention, and soil stability (Coffman 2002;

PGDER 2002). This is a terrestrial based approach that utilizes the relationships between vascular plants, soil microfauna, and soil microorganisms and how these communities influence soil and hydrologic processes and nutrient cycling. In simpler terms these systems are strategically placed volumes of engineered soil, with high infiltration rates, that are used to backfill a void in the ground that was prepared and situated accordingly to a size determined to treat a specified amount of stormwater, then landscaped with recommended native plant species.

Other LID strategies harbor similar relationships but differ fundamentally in design. A majority of these other systems remain saturated throughout the year such as constructed wetlands and ponds. The soil of Bioretention Cells must have a high infiltration rate that is usually engineered to achieve full drainage of all water remaining above the surface within a 48-hour period. This is in reference to the shallow basin associated with these units designed to hold a height of 15 to 30 centimeters of stormwater.

Bioretention Cells are part of the decentralized approach integrated throughout the site. Through evapotranspiration, detention, and infiltration these units balance the requirement of onsite management of pollutants and runoff volumes while mimicking the natural hydrological regime (PGDER 2002; Davis 2005). This approach can allow bioretention to treat more runoff yet require less space (Hood *et al.* 2006). Conventional detention ponds reduce the potential profitability of a site by requiring large areas of land compared. Bioretention Cells are integrated into the site as landscaping thus requiring

little to no land explicitly used for stormwater control (PGDER 2002; Hood *et al.* 2006).

However, in regions with high intensity annual precipitation some conventional detention

areas may be required to capture flash flood events.

Bioretention Cells are integrated management practices designed to suit

developers' needs or to retrofit post-development sites, for unanticipated storm-water

issues. These site-specific integrated management practices can be utilized in all

terrestrial-based applications (i.e. residential, commercial, and industrial) and have been

shown, in some cases, to be a suitable alternative to conventional stormwater detention

basins (PGDER 2002; Hunt 2005). Some intrinsic values of Bioretention Cells as listed

by PGDER include, establishing a unique sense of place by using native plants,

encouraging environmental stewardship and community pride, increasing real estate

values up to 20%, and a host of other benefits associated with mitigating the impacts of

urban development.

The Bioretention Manual highlights four fundamental performance categories of

Bioretention Cells that designers may modify to mitigate site-specific development

impacts (PGDER 2002). These categories differ mainly in the way they treat water

and/or interface with the subsoil: the basic design is the same for each. The first and

most basic design is an Infiltration/Recharge Facility. This design is utilized when the

groundwater is a recommended distance below the cell and the subsoil has the minimum

required infiltration rate. The second category, Filtration/Partial Recharge, only differs

from the first design in the inclusion of an under-drain discharge tube and partial pea-

gravel lined bottom covered in filter fabric. These cells are usually routed to the centralized stormwater drainage system or directly into a body of water, as is also the case with the next two categories. Category three, Infiltration/Filtration/Recharge, features a raised under-drain that is blanketed in pea gravel, as is the entire bottom of the cell. The final category is Filtration only. This category is the only facility that employee a liner/barrier to segregate the in-situ soils from the Bioretention Cell engineered soils.

In a laboratory and pilot scale Bioretention Cell study that utilized a Synthetic Stormwater (SSW), Davis *et al* (2001) observed reductions greater than 90% for copper, lead, and zinc but only moderate reductions in Total Kjeldahl nitrogen (TKN), ammonium, and phosphates with little to no nitrate reduction. Likewise, Clausen and Dietz (2005) found, in a field evaluation of a rain garden, heavy metal concentrations below detectable limits but poor retention rates for nitrate and negative retention rates for phosphates.

Hunt (2003) reported Bioretention Cells installed without the inclusion of an anaerobic zone may not properly attenuate nitrate, and there is a relationship between depth of the cell and nitrate retention capacity. Kim *et al* (2003) observed modified Bioretention Cells utilizing an anaerobic zone and microbial denitrification achieved nitrate plus nitrite mass removal rates of up to 80%. They also found the use of shredded newspaper, as a source of carbon for a solid-phase-electron-donor (compost), facilitates the denitrification process and worked better than many alternatives tested.

Studies consistently found greater amounts of phosphates in the resultant effluent than that placed in SSW or that present in inflow. Clausen and Dietz (2005) observed significant reductions of nitrates but negative reductions (increases) of Total-phosphate. Soil disturbance and settling was the speculated source of this phosphorus export. Recent modifications to bioretention guidelines have illustrated the importance of media (soil) sampling and the utilization of sandy-loams with preexisting low nutrient levels.

Implementation of Bioretention Cells requires a multidisciplinary approach and understanding that recommended guidelines and relationships vary regionally and locally. Bioretention is a complex approach that requires the appropriate use of environmentally dependant guidelines. For example, atmospheric nitrogen deposition varies regionally and locally and results in varied levels of nitrates in stormwater.

Bioretention Cells utilize the absorptive and adsorptive (sorption) properties of sandy-loams to retain phosphorus primarily in the rhizosphere and nitrogen in the last stage or anaerobic portion of the cell (PGDER 2002). The sorption properties of sandy-loams vary at the state and national level (NRCS 2010) and are reduced by large initial concentrations of *in situ* pollutants. Sandy-loams acquired in historically agricultural regions are likely to have elevated phosphorus levels (NRCS 2010). Regardless, all sandy-loams have a finite capacity for retention of nutrients, in the absence of biogeochemical processes, and once this capacity is exceeded they become a potential source of pollution.

Life expectancy and performance of a typical Bioretention Cell is determined via the aforementioned physical soil properties without consideration of biological process (Davis *et al* 2001; NCES 2005; PGDER 2002). Bioretention Cells studies in the present literature have explored many properties that influence performance, especially inclusion of an anaerobic zone and testing of alternative bioretention mediums but none have focused on the effect of plants on these systems. Also, the effect of seasonality has largely been overlooked.

Plants and Mycorrhizal Fungi

The role of plants and soil microorganisms and how their interactions influence soil structure, hydrologic processes, and nutrient cycling are not well understood in the Bioretention Cell environment. However, there is extensive documentation of the vascular plant and vesicular arbuscular mycorrhizal (VAM) role in the uptake of nutrients and modification of the rhizosphere (Bolan 1991; Varma and Hock 1998; Godbold *et al*. 2006). For instance, vascular plants have been used for some time for their known phytoremediative attributes and ability to modify the soil environment (*i.e*. soil organic matter, CEC, soil stability). The latter is also true of plants in a symbiotic association with VAM; an endophytic symbiont that is increasingly being used for enhanced bioremediation especially in heavy metal contaminated soils (Gerdemann 1968; Varma and Hock 1998; Khan 2006; Giasson *et al* 2006).

Knowledge of vascular plant uptake of nutrients and minerals, plant nutrition, is extensively studied and their ubiquitous symbiotic relationship with soil microbes has been well documented to facilitate this process (Coffman 2002). Mycorrhizal fungi are especially known for facilitating the uptake of poorly soluble minerals like zinc and phosphorus in oligotrophic (nutrient limited) environments. More specifically enhanced uptake of phosphorus as orthophosphate is the primary element that mycorrhizal plants benefit from (Bolan 1991) and is of particular interest in stormwater and land management in Oklahoma (Correll 1998; ODEQ 2006).

Two distinct types of MF dominate this ecological niche, vesicular-arbuscular mycorrhizal fungi (VAM) and ectomycorrhizal fungi (EM). Vesicular-arbuscular mycorrhizal fungi are characterized by the formation of external hyphal networks in the surrounding soil and intracellular growth on symbiont(s) root cortex. Ectomycorrhizal fungi form dense mycelial sheaths with intercellular invasion of the root cortex and are limited to mostly temperate forest trees. Of the two, VAM are most widely distributed and associated with the vast majority of species with the exception of a few families. Chenopodiaceae, Cruciferaceae, Cyperaceae, Juncaceae, and Proteaceae are typically ruderal species and rarely form MF symbiosis (Harley and Harley 1987).

Vesicular-arbuscular mycorrhizal fungi exhibit less host specificity; this typically results in a suite of VAM species on a single host in a natural setting (Bolan 1991). Although VAM are generally considered symbiotic in high nutrient environments they

can exhibit pathogenic properties, depleting photosynthate (carbohydrate sources) from their symbionts and overall inhibiting growth-rate of their symbiont

Vesicular-arbuscular mycorrhizal fungi collectively increase the effective rhizosphere, exude organic acids and phosphatase enzymes, and are involved in soil weathering processes (George 1995). These influences are accomplished by exploration of a larger soil volume both decreasing the physical distance phosphorus ions must travel and increasing the surface area for adsorption, solubilization of mineral phosphates, and increasing affinity for P ions (decreasing threshold concentration required for adsorption), respectively (Bolan 1991). Other attributes of VAM associations include competition against pathogenic fungi and bacteria, increased water use efficiency in host plants, and aeration of the soil above and below the root zone but are thought to be less significant than the latter attributes (Bolan 1991).

Selection of inoculum is important since there are over 5000 known species of naturally occurring VAM (Varma and Hock 1998). Primarily, members of the order *Glomales* are known for their beneficial attributes and are also the most abundantly found of all the VAM in soil communities. Inoculums are typically administered as a powder or solution but MF can also be introduced by naturally occurring communities (Varma and Hock 1998).

The significance of the intrinsic benefits of VAM have long been debated; scientific and horticultural communities alike have sought the applicability of VAM despite the lack of extensive understanding and commercial availability. It is well know

that more that 90% of all vascular plants form symbiotic VAM relationships in natural environments (Varma and Hock 1998). However, plant responses to VAM are highly variable and some plants may host a suite of MF, while others only form associations with fewer species of VAM.

Vesicular arbuscular mycorrhizal fungi although not entirely understood have been shown to enhance the uptake of certain nutrients and increase the growth rate of their symbionts (Bolan 1991). There are many biochemical process influenced by soil microorganisms including VAM and the lack of quantification of the intrinsic benefits of plants and these orgamisms in Bioretention Cells may lead to a gross underestimation of the ameliorative potential and duration of these BMPs which could otherwise further encourage their implementation.

Plants can significantly influence soil properties and are necessary for healthy communities of soil micro-organisms and development of complex soil horizons (Sparks 2003). There are many species of plants and the selection of plants can be very site specific. With a better understanding of how recommended plants and soil microorganisms influence the soil properties and the overall ameliorative potential of Bioretention Cells we could potentially improve these systems. Knowledge of soil microbe communities such as VAM and nitrifying bacteria could also lead to increased performance of these systems. The significant effect of nitrifying bacteria and the dynamic nature of the Nitrogen Cycle on bioretention performance (Siegel 2008) is a primary example of how knowledge of these microbial communities and plants that

largely influence soil ecology can increase our overall knowledge of the process of

bioretention.

Chapter III
METHODOLOGY

Overview and Scope

The desirable outcome of this study was to evaluate the significance of plant presence in laboratory-scale Bioretention Cells by quantifying reductions of nitrate, phosphate, and ammonium in effluent following volumetric additions of known concentration of a prepared synthetic stormwater (SSW). Although many complex biogeochemical and geophysical processes are responsible for these results the scope of this work is focused on determining percent reduction of nutrient concentration and load in effluent from the laboratory-scale Bioretention Cells and does not attempt to quantify soil processes nor changes in Bioretention cell medium. In general, this study is intended to prompt further investigation into the role of plants in the bioretention process by quantifying percent reduction of nutrient concentration and load as influenced by the presence of plants in a Bioretention cell.

From 2_April_2007 through 25_May_2008 four species of vascular plants were utilized in a laboratory scale bioretention study using a Complete Factorial Randomized Experimental Design: *Panicum virgatum* (Switchgrass), *Chilopsis linearis* (Desert willow), *Sambucus canadensis* (Elderberry), and *Itea virginica*

(Virginia sweetspire). These plants were treated with an inoculum containing three species of VAM *Glomus clarum, G. intraradices,* and *G. mosseae* and grown in commercially available topsoil for 16 weeks, then exposed to a SSW as shown in Table 1 (see Appendix-C for SSW preparation). An ANOVA (Analysis of variance) was used to analyze data with a PROC MIXED statement in SAS 9.2 (Statistical Analysis Software). This model included volume, nutrient, time, and seasonality data (the SAS model and output tables can be found in Appendix (b) and Appendix(c), respectively). Probability values (p-values < 0.05 as significant) were obtained from the SLICE option in the LSMEANS statement to include interactions in the level of significance.

Effluent was collected and weighed at three time intervals: 6 hrs, 24 hrs, and 168 hrs following SSW application (weight of effluent was converted to volume based on 1ml of water equals 1mg). The total mixed leachate was sampled to determine concentrations of nitrate, ammonium, Ortho-phosphate, and Total-phosphate concentrations in the SSW. Nitrates and ammonium levels were determined using automated cadmium reduction and phosphate levels were determined using elemental analysis by inductively coupled plasma (ICP).

Treatment one was Bioretention Cells with plants and treatment two was Bioretention Cells with plants in the presence of symbiotic VAM fungus. The following interactions where statistically analyzed: plant vs. control, plants + VAM vs. control, species vs. control, and species + VAM vs. control. Bioretention Cells with only topsoil

and either control inoculum or bulk inoculum were used for controls (VAM inoculum and the inoculum control was obtained from INVAM see Appendix-C).

Initially, each treatment-species combination had six replicates for a total of 48 Bioretention Cells. Elderberry and Virginia sweetspire died before data was collected (this was due to a heater failure before the plants were bare-rooted and potted, the plants were potted anticipating recovery during the acclimation period, neither species recovered). Switchgrass and Desert willow were not adversely affected by the heater failure. However, three Switchgrass plants died before data SSW application and one control was removed (due to complete failure to drain). There was no mortality for Desert willow. Synthetic Stormwater data was collected from 32 laboratory-scale Bioretention Cells.

Table 1: Synthetic stormwater (SSW) constituents

Components	Concentration	Solubility	MWT (g/M)
Mono-Sodium Nitrate (NNaNO$_3$)	2mg/L as N	soluble at 20°C	218.39
Ammonium Chloride (NH$_4$Cl)	2mg/L as N	soluble at 20°C	137.45
Dibasic-Sodium Phosphate (Na$_2$HPO$_4$)	2mg/L as P	10g/100ml at 20°C	95.23

Laboratory Scale Bioretention Cells

Each plant and control was placed in a 3-gallon drain-less container modified with a drainage tube (Figure 1). Bioretention Cells were modified with a 16mm diameter hole in the center of the container bottom. Each hole was fitted with a 16mm insert adapter and rubber grommet (NETAFIM™) attached to 31 centimeters of 16mm vinyl tubing. Tubes were piped to individual correspondingly numbered 1-liter effluent sample containers (Nalgene™). The 1-liter effluent sample containers were weighed and emptied after each sampling period. Weights were recorded and converted to volumes.

Figure 1: Nursery container (3gal black plastic drain-less) used for laboratory scale Bioretention Cells

Soil Preparation

Approximately 400 liters of topsoil (Minick Materials Oklahoma City, OK) was mixed in a 424-liter cement mixer for 1 hour at full throttle. This soil was used to prepare inocula and pots all specimens. A 127-liter cement mixer was used to prepare a 42-liter bulk inoculum and a 42-liter control inoculum. Each inoculum mixture was allowed to mix for 10 minutes at full throttle.

Experimental soil was prepared by mixing 42 liters of topsoil that was utilized in a 1:35 ratio of bulk inoculum to topsoil mixture by adding 1.2 liters of bulk inoculum containing 1:1:1 (v/v/v) of *Glomus clarum, G. intraradices,* and *G. mosseae*. The bulk inoculum/topsoil combination was allowed to mix for 10 minutes in a 127-liter cement mixer. This procedure was also used to prepare a 1:35 ratio sterile control inoculum known to be devoid of VAM.

Plant Preparation

One-gallon commercial nursery stock (Greenleaf Nursery Company, Tahlequah, OK) was used for all plant specimens. Desert willows were selectively pruned to obtain an initial visually uniform plant size for all specimens. Non-photosynthetic Switchgrass foliage above the joint of the first culm was removed. Photosynthetic foliage of both species was left.

In order to obtain a homogenous experimental soil, commercial nursery media was removed from the root ball of all specimens (*i.e.* all plants were bare-rooted). After removal of commercial soil medium, the roots were rinsed with tap water until all nursery media was removed. All bare-root balls were wrapped in a moistened paper towel then foliage was allowed to air-dry overnight. One-gram root samples were taken from both species to assess initial VAM percent colonization (see Appendix: Standard Operating Procedures). Once the soil was removed from the roots of all specimens the plants were pruned to remove non-photosynthetic foliage, then root samples were taken from all specimens. An initial weight was obtained and recorded for each specimen after root samples were taken (this was later used to obtain % change).

Inoculation

To facilitate a more rapid VAM colonization a 16 cm diameter aluminum duct pipe was used to selectively place two liters of the inoculum mixtures in the upper center portion of the container, in direct contact with bare-roots. This pipe was placed in the center of the Bioretention Cells atop a 8.5 centimeter depth of topsoil. More topsoil was added around the outside of the pipe until it reached the rim of the pipe (Figure 2).

Root-balls of each plant were centrally placed in the pipe and the inoculum mixture was used to fill the pipe. Two liters of the 1:35 bulk inoculum mixture was placed in the pipe under and around the root-ball of all treatment-II specimens and within

the pipe of the treatment-II controls. Treatment-I plants and controls were treated the same except a 1:35 control inoculum to topsoil mixture was used. As each container was potted a few grams of topsoil was added or removed as needed until each contained 9.65 kilograms of topsoil (including inoculum mixtures).

Figure 2: Selective placement of inoculum to increase symbiont contact with roots

Greenhouse and Tables

Two 92 x 305 centimeter greenhouse tables were located in the center of the Biosystems and Agricultural Engineering greenhouse (Oklahoma State University, OK) against the Southern edge. All Bioretention Cells were randomly arranged on these tables with 10 centimeters distance between each container (Figure 3).

PV3-15	CV6-30	CN2-32	PN6-24	CV4-28	~~IV1-1~~	C9-45-N	CV1-25
~~IN6-12~~	CN5-35	PN2-20	PV6-18	~~IV4-4~~	~~C10-46-N~~	CV2-26	PV2-14
CN6-36	CN1-31	C12-48-N	C11-47-N	C7-43-N	C6-42-V	C2-38-V	PN3-21

~~IN2-8~~	CN3-33	PV1-13	C3-39-V	~~IN4-10~~	~~IN3-9~~	C4-40-V	PN4-22
~~IV5-5~~	~~IN5-11~~	PN1-19	CV5-29	C1-37-V	C5-41-V	~~IV6-6~~	~~PN5-23~~
~~IN1-7~~	~~PV5-17~~	CV3-27	~~IV2-2~~	C8-44-N	~~IV3-3~~	CN4-34	~~PV4-16~~

NE *NW*

Figure 3: Plant position and greenhouse orientation for the duration of the experiment. (Crossed-out specimens died and were not included in this study)

During winter months two Dayton™ propane heaters were employed to attain the most uniform greenhouse temperature possible, heaters were set to 24°C. Once the overnight-low temperature was above 24°C the heaters were retired. A swamp-pump and greenhouse cooling cells were employed to moderate elevated summer temperatures and the greenhouse was covered with a 20% shade cloth during the summer.

Each Bioretention Cell was systematically assigned a pot number, with all specimens within a species in sequential order, controls numbered last. Bioretention Cells were also marked with the genus initials and an accession number from 1 to 6 for each treatment-I and treatment-II replicates of a species (Table 2).

The random number generator in Excel (Macintosh version 11.2) was used to generate all whole numbers from 1 to 99. Numbers were selected in the order generated (repeating numbers disregarded) and used for pot position on the table. The

corresponding pot number for the first randomly generated number was placed on the southeast corner of the most southern table. The following randomly generated numbers were then used to determine pot row position of correspondingly numbered Bioretention Cells from Southeast to Southwest for eight Bioretention Cells in a row. For each new row, the succeeding randomly generated number started from the remaining most southeastern position of the table and moved from east to west. This procedure was followed for each table. Each table held 3 rows of 8 Bioretention Cells for a total of 48.

Table 2: Pot number and treatment list (crossed out specimens were not used for this study)

Species	Pot #	Treatment
Panicum virgatum	PV1-13	inoculated
P. virgatum	PV2-14	inoculated
P. virgatum	PV3-15	inoculated
~~P. virgatum~~	~~PV4-16~~	~~inoculated~~
~~P. virgatum~~	~~PV5-17~~	~~inoculated~~
P. virgatum	PV6-18	inoculated
P. virgatum	PN1-19	control inoculum
P. virgatum	PN2-20	control inoculum
P. virgatum	PN3-21	control inoculum
P. virgatum	PN4-22	control inoculum
~~P. virgatum~~	~~PN5-23~~	~~control inoculum~~
P. virgatum	PN6-24	control inoculum
Chilopsis linearis	CV1-25	inoculated
C. linearis	CV2-26	inoculated
C. linearis	CV3-27	inoculated
C. linearis	CV4-28	inoculated
C. linearis	CV5-29	inoculated
C. linearis	CV6-30	inoculated
C. linearis	CN1-31	control inoculum
C. linearis	CN2-32	control inoculum
C. linearis	CN3-33	control inoculum
C. linearis	CN4-34	control inoculum
C. linearis	CN5-35	control inoculum
C. linearis	CN6-36	control inoculum
Controls	C1-37-V	inoculated
Controls	C2-38-V	inoculated
Controls	C3-39-V	inoculated
Controls	C4-40-V	inoculated
Controls	C5-41-V	inoculated
Controls	C6-42-V	inoculated
Controls	C7-43-N	control inoculum
Controls	C8-44-N	control inoculum
Controls	C9-45-N	control inoculum
~~Controls~~	~~C10-46-N~~	~~control inoculum~~
Controls	C11-47-N	control inoculum
Controls	C12-48-N	control inoculum

Treatment Randomization

Prior to potting plants in the Bioretention Cells, each bare-root specimen was numbered from 1 to 12 for each of the species. The random number generator in Excel (Macintosh version 11.2) was used to generate all whole numbers from one to twelve for each of the three species. Numbers where then selected in the order generated (repeating numbers disregarded) and used to determine potting order.

Plants were potted in Bioretention Cells in ascending order, starting with the VAM treatment, with respect to pot ascension number as their randomly generated numbers appeared in the excel column. For example, the first randomly generated number was twelve so this specimen was placed in container IV1-1. The next randomly generated number was five, so this specimen was placed in IV2-2. This procedure was used to randomize the order in which all specimens were selected for the treatment replicates.

Watering

On Monday 2_Apr_07 plants were placed in the BAE greenhouse. Each container received approximately 2 liters of tap water following placement on the greenhouse tables. The plants were misted daily for 2 weeks following placement in the greenhouse. Plants received 2-liter applications of tap water as needed for the first 6 weeks to initialize roots and promote VAM colonization. If one container appeared to

need water each container received a 2-liter application of tap water. This initialization period concluded Sunday 13_May_07.

Data Acquisition

Sampling Effluent

Following the initialization period, each container received a 2-liter volumetric application of deionized water (DI), applied weekly for 33 weeks, to leach nutrients below SSW concentrations. Effluent from five consecutive preliminary leaching were collections following the initialization period (August 4th through September 1st), and one was collected in January a week before the first SSW application. One specimen was selected to represent each treatment-species combination and one for each of the two controls. Representatives were selected for their median total effluent volume, plants were foliaged and had no visual signs of disease or stress.

Leaching data was taken from every Bioretention Cells for leaching 5 during week 15 (the last of the five preliminary leaching collections) and for leaching 6 during week 33, the last DI application the week prior to SSW application to dormant plants. During the period between SSW application to dormant plants and SSW application to foliaged plants two liters of DI was added weekly.

Effluent samples were collected in one-liter Nalgene ™ sample Bioretention Cells for the preliminary leaching experiments and SSW applications. The labeled Nalgene ™ sample containers were suspended below the correspondingly labeled Bioretention Cells and a 16mm vinyl tubing was placed into the sample containers. Each sample container was covered with foil, to prevent light entry, and paraffin wax covered the mouth to reduce evaporation. A small slit was placed in the center of the paraffin wax and the 16mm vinyl tube was placed through this slit. At the end of the collection period a total Effluent volume was measured and recorded for each container by weighing the sample containers and converting grams to milliliters, weight of sample containers were subtracted. After each sampling period the Nalgene™ sample containers were washed and dried (see Appendix-C).

Synthetic Stormwater Application to Dormant and Foliaged Plants

Saturday 12_Jan_08, week 34 of the experiment, 2-liter volumetric applications of DI were substituted with 2-liter volumetric applications of SSW for three weeks of the experiment. This SSW application was added while the plants were dormant. The SSW effluent was collected and a volume recorded for a 6, 24, and 168 hour collection period. Each effluent collection was held for the duration of the 168 hr sampling period and mixed with subsequent collections from the same experimental container during the sampling period to acquire a total volume. A 120 ml sub-sample of the total SSW

effluent was taken for each specimen. All 120 ml effluent samples were sent to the OSU

Soil, Water, and Forage Analytical lab for analysis of nitrate, ammonium, Ortho-

phosphate, and Total-phosphate for each of three SSW applications.

Saturday 19_April_08, week 48 of the experiment, the 2-liter volumetric

applications of DI were again substituted with 2-liter volumetric applications of SSW for

three weeks of the experiment. These SSW applications were added while the plants were

fully foliaged. The SSW effluent was collected and a volume recorded for a 6, 24, and

168-hour collection period. A 120 ml sub-sample of the total SSW effluent was taken for

each specimen. All samples were sent to the OSU Soil, Water, and Forage Analytical lab

for analysis of nitrate, ammonium, Ortho-phosphate, and Total-phosphate from each of

three SSW applications. Total volume and concentration data was used to determine

load. Volume, concentration, and load data can be found in Appendix-A (Table 1a

through Table 12a).

CHAPTER IV
RESULTS

Arbuscular Mycorrhizal Fungi

Preliminary one-gram root samples taken as plants were potted yielded no sign of VAM. Once the experiment concluded (total duration of 58 weeks) one-gram root samples were taken from each specimen and examined for VAM presence. VAM was not present and statistical analysis indicated no significant effect of VAM.

Preliminary Leaching

Volume and leachate concentration data (Table 3, Table 4, and Table 5) were collected from weeks 11 through 15, after plants had time to establish (*i.e.* show visual signs of health such as continued growth and no visible signs of disease or stress). This pre-experiment was to assess the state of the experimental containers by analyzing nitrate, ammonium, and Ortho-phosphate concentration in leachate following 2-liter applications of DI. The desirable outcome was two fold; determine if data was consistent weekly and determine if leachate concentrations were below SSW levels

(*i.e.* nitrate, ammonium, and Ortho-phosphate concentration below 2 mg/L). Leachate

was analyzed for week 33 to determine if concentrations were below SSW levels, so the

effect of SSW could be observed.

Containers with plants had much lower nutrient leachate concentrations than

containers without plants (controls), ammonium leachate concentration was very low in

all leachate samples. Overall leachate concentrations were still high during week 15 and

required seventeen more weeks of 2-liter applications of DI to reach leachate levels

below initial SSW nutrient concentration. Leachate data from 2-liter applications of DI

from weeks 11 through 15 represent individuals that were selected for their median

effluent volume; week 33 represents averages from all replicates.

Table 3: Nitrate leachate concentration from DI application to Bioretention Cells

	Week 11	Week 12	Week 13	Week 14	Week 15	Week 33
	------------------------------ mg/L ------------------------------					
Grass + VAM	0.36	0.18	0.07	0.20	0.37	0.41
Grass	0.41	0.2	0.05	0.17	0.30	0.16
Willow + VAM	0.11	0.05	0.06	0.07	0.33	0.40
Willow	12.8	0.19	0.06	0.29	2.58	0.41
Control + VAM	43.6	3.37	0.37	2.48	6.21	15.10
Control	75.8	13.2	1.56	1.63	2.61	13.50

Table 4: Ammonium leachate concentration from DI application to Bioretention Cells

	Week 11	Week 12	Week 13	Week 14	Week 15	Week 33
	---------------------------- mg/L ----------------------------					
Grass + VAM	0.14	0.03	0	0.13	0.52	5.31
Grass	0.1	0.03	0	0.14	0.51	0.29
Willow + VAM	0	0.01	0.01	0.13	0.35	0.18
Willow	0	0.01	0.01	0.12	0.33	0.15
Control + VAM	0	0	0.03	0.12	0.24	0.22
Control	0	0.01	0	0.14	0.40	0.16

Table 5: Ortho-phosphate leachate concentration from DI application to Bioretention Cells

	Week 11	Week 12	Week 13	Week 14	Week 15	Week 33
	---------------------------- mg/L ----------------------------					
Grass + VAM	1.13	1.11	1.41	1.19	1.08	0.45
Grass	0.91	0.94	1.15	0.95	0.83	0.62
Willow + VAM	1.3	1.43	1.77	1.67	1.42	1.46
Willow	1.41	1.59	1.85	1.81	2.2	1.63
Control + VAM	1.94	2.07	2.17	2.39	2.48	2.69
Control	1.96	2.23	1.94	2.28	2.45	2.67

Vascular Plants

Overall plants maintained observable healthy growth free of disease and pestilent insect species. There were no problems with water-use or greenhouse temperature.

However, three Switchgrass specimens died before SSW application and were not included in this study. There was no mortality for Desert willow. This species appears to be an outstanding specimen for bioretention; it never had signs of wilt or other stress indicators and bloomed throughout summer. Switchgrass also grew well and filled-in the containers nicely, developing culms throughout the experiment. Each species had considerable root growth as shown in Figure 4 through Figure 7. Neither species was root-bound at the conclusion of the experiment, but every specimen could have been transplanted to larger containers.

Figure 4: Photograph of *Chilopsis linearis* (Desert willow) entire rootball

Figure 5: Photograph of *Chilopsis linearis* (Desert willow) rootball vertically halved

Figure 6: Photograph of *Panicum virgatum* (Switchgrass) entire rootball

Figure 7: Photograph of *Panicum virgatum* (Switchgrass) rootball vertically halved

Specimen PV1-13, a Switchgrass specimen with VAM, grew relatively slow and almost died, thus performing more like controls than other plants with VAM. This specimen consistently responded like controls throughout the experiment. Without this specimen included in "Plants + VAM" the data would look more like the "Plants" data that is represented in the following figures and can be clearly seen when compared to "Grass + VAM" vs. "Grass".

After the third effluent samples were collected from Bioretention Cells with foliaged plants, plants were harvested to obtain a final plant weight (using the same method used for initial weight). Initial and final plant weights were used to calculate the percent change in total biomass found in Table 6.

Table 6: Total biomass and percent change (above and below ground).

Species	Initial	Final	Increase
	---------- g ----------		--- % ---
Panicum virgatum	147	347.5	136
P. virgatum	106.5	402	277
P. virgatum	126.5	426	237
P. virgatum	145	192.5	33
P. virgatum	99.5	439	341
P. virgatum	134	632.5	372
P. virgatum	124	366	195
P. virgatum	121.5	369	204
P. virgatum	92	292	217
Chilopsis linearis	43	132	207
C. linearis	54.5	159	192
C. linearis	37.5	95	153
C. linearis	53.5	155	190
C. linearis	34	108	218
C. linearis	48.5	126.5	161
C. linearis	57.5	183.5	219
C. linearis	48	130.5	172
C. linearis	70	126.5	81
C. linearis	48	146	204
C. linearis	35.5	102.5	189
C. linearis	40.5	114.5	183

Synthetic Stormwater Application to Bioretention Cells with Dormant Plants

On Saturday 12_Jan_08, week 34 of the experiment 2-liters of SSW was applied weekly for three weeks to all Bioretention Cells in place of DI. All plants were dormant or in a non-photosynthetic state as shown in Figure 8 (*i.e.* transpiration was not taking place in Bioretention Cells with plants). Sampling while plants were dormant allowed assessment of plant influence on the ameliorative soil properties, independent of transpiration and relative to controls.

Figure 8: Photograph of experimental setup in the greenhouse with dormant plants

Figure 9 shows Bioretention Cells with dormant plants released between 60 and 85% of total effluent volume during the 6 hr collection period whereas controls released about 20% of total effluent volume during the 6 hr period. As shown in Figure 10 a, species effect is not apparent.
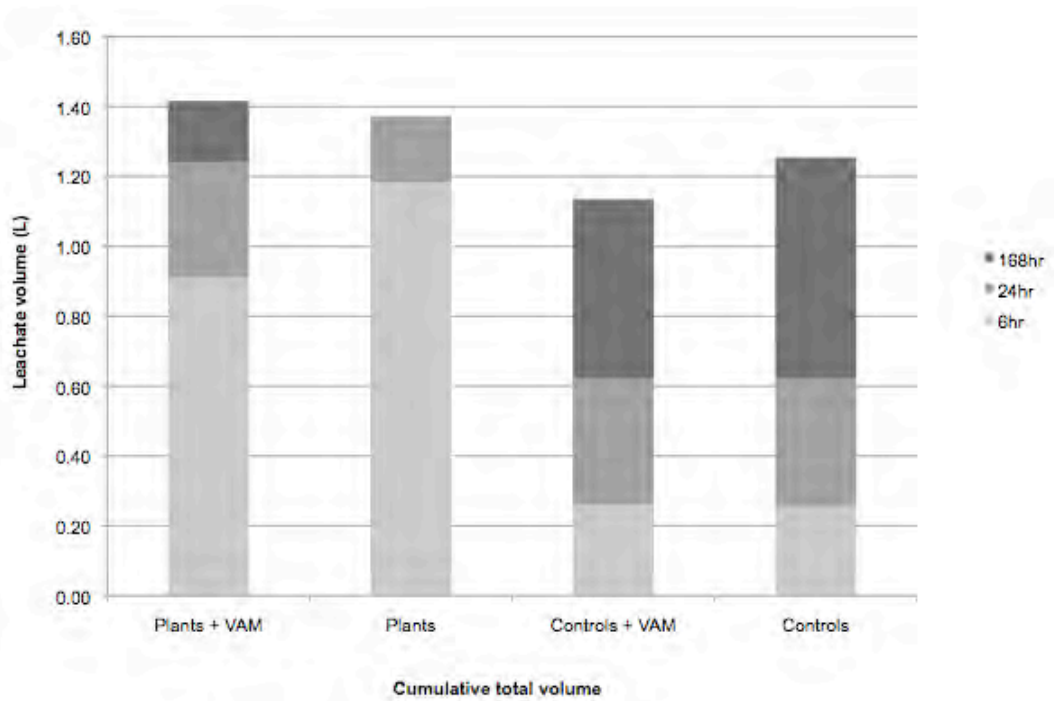
Figure 9: Cumulative effluent volumes from 2-liter SSW applications to Bioretention Cells with dormant plants
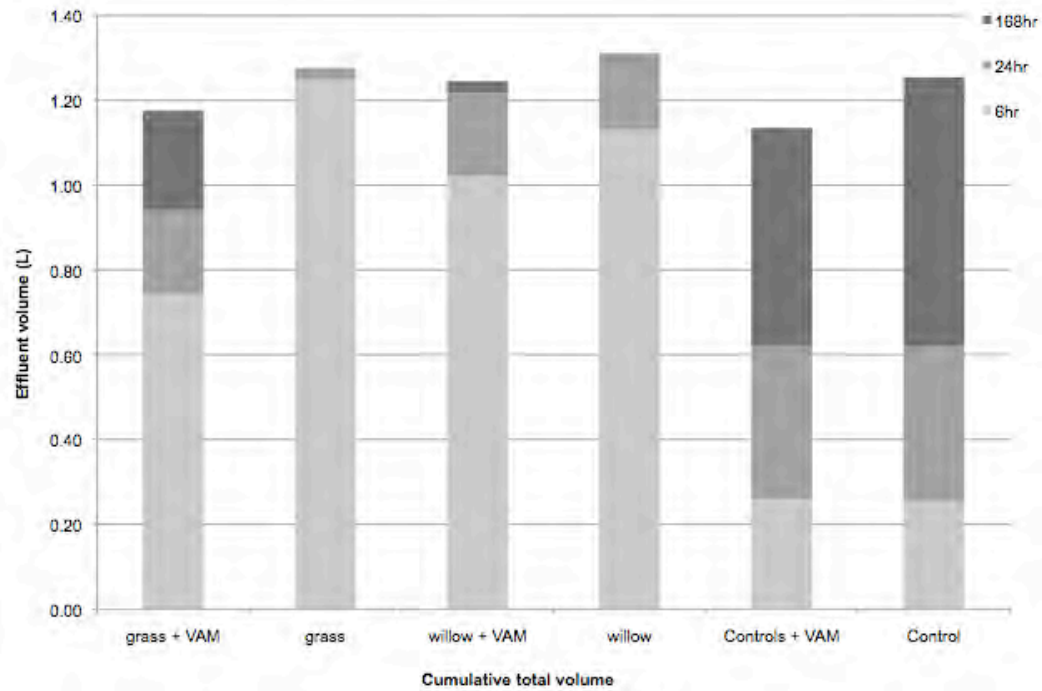
Figure 10: Cumulative effluent volumes from 2-liter SSW applications to Bioretention Cells with dormant plants (species effect)

When volume collected from each time period is observed independently from cumulative volumes, large differences are apparent between Bioretention Cells with dormant plants vs. controls in the 6 hr and 168 hr periods with only a small difference for the 24 hr collections.

Effluent Concentration from Bioretention Cells with Dormant Plants

In Table 7 Bioretention Cells with dormant plants reduced nitrate and phosphate concentrations below SSW levels (2mg/L), whereas Bioretention Cells without plants (controls) increased concentrations of both nutrients in the effluent above SSW levels. Effluent ammonium concentration was small from plants and controls.

Table 7: Effluent concentration from SSW application (2mg/L) to Bioretention Cells with dormant plants

Treatments	NO_3-N	NH_4-N	Ortho-P	Total-P
	------------------- mg/L ---------------------			
Plant + VAM	1.17	0.15	1.43	1.67
Plant	1.04	0.15	1.29	1.44
Control + VAM	16.47	0.15	3.44	3.70
Control	13.86	0.15	3.06	3.43

As shown in Table 8 there was over a 41% reduction of nitrate effluent concentration from Bioretention Cells with dormant plants, whereas Bioretention Cells without plants released about six times as much nitrate. Phosphate effluent concentration reduction from Bioretention Cells with dormant plants was between 28 and 35%. Bioretention Cells without plants released between 53 and 85% more phosphate.

Table 8: Percent reduction of concentration from SSW application to Bioretention Cells with dormant plants

Treatments	NO$_3$-N	NH$_4$-N	Ortho-P	Total-P
	---------------------- % ----------------------			
Plant + VAM	41.44	92.42	28.67	16.72
Plant	48.21	92.54	35.56	28.21
Control + VAM	-723.64	92.64	-72.06	-85.03
Control	-593.20	92.53	-53.10	-71.53

In Table 9 the reductions shown for Total-phosphate effluent concentration from Bioretention Cells with dormant Switchgrass were between 20 and 50%. Reductions of Ortho-phosphate effluent concentration from Bioretention Cells with dormant Switchgrass were between 30 and 55%. Dormant Desert willow percent reduction was below half that of dormant Switchgrass percent reduction for Total-phosphate and just about half that of Desert willow for Ortho-phosphate.

Table 9: Percent reduction of effluent concentration from SSW applications to Bioretention Cells with dormant plants (species effect)

Treatments	NO_3-N	NH_4-N	Ortho-P	Total-P
	---------------------- % ----------------------			
Grass + VAM	33.13	92.20	34.59	22.02
Grass	49.49	92.33	55.41	48.94
Willow + VAM	49.76	92.63	22.75	11.42
Willow	46.93	92.75	15.72	7.48
Control + VAM	-723.64	92.64	-72.06	-85.03
Control	-593.20	92.53	-53.10	-71.53

Effluent Load from Bioretention Cells with Dormant Plants

In Table 10 we can see the overall effect of plants on pollutant effluent loading. Bioretention Cells, with and without plants, reduced load below SSW levels (4 mg) for all nutrients and treatments except loading of nitrate from controls. Effluent load from Bioretention Cells with dormant plants was observably lower than effluent from controls for nitrates and about half of phosphate loading from controls. Ammonium effluent load was very small from all Bioretention Cells.

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Table 10: Effluent load from SSW application (4 mg) to Bioretention Cells with dormant plants

Treatments	NO_3-N	NH_4-N	Ortho-P	Total-P
	------------------------mg ------------------------			
Plant + VAM	1.49	0.19	1.84	2.13
Plant	1.59	0.22	1.92	2.12
Control + VAM	19.30	0.18	4.15	4.46
Control	17.29	0.20	4.51	4.98

In Table 11 over a 60% decrease of nitrate loading from Bioretention Cells with dormant plants is shown. Bioretention Cells without plants released over three times as much nitrate as SSW levels. Phosphate reduction from Bioretention Cells with dormant plants was between 40 and 50% whereas Bioretention Cells without plants increased phosphate effluent load between 3 and 24% above SSW levels.

Table 11: Percent reduction of effluent load from SSW application to dormant plants

Treatments	NO_3-N	NH_4-N	Ortho-P	Total-P
	----------------------- % ------------------------			
Plant + VAM	62.84	95.33	53.98	46.63
Plant	60.34	94.41	51.95	46.97
Control + VAM	-382.41	95.62	-3.75	-11.50
Control	-332.29	94.94	-12.74	-24.61

In Table 12 Bioretention Cells with both dormant plant species achieved at least a 41% reduction of effluent loading below SSW levels for all pollutants. Bioretention Cells without plants increased nitrate and phosphate load; releasing about four times the amount of nitrate and between 4 and 24% more phosphate.

Table 12: Percent reduction of effluent load from SSW application to dormant plants (species effect)

Treatments	NO_3-N	NH_4-N	Ortho-P	Total-P
	------------------- % -----------------------			
Grass + VAM	58.55	95.29	58.61	51.25
Grass	57.80	93.59	62.39	56.97
Willow + VAM	67.13	95.38	49.35	42.00
Willow	62.89	95.24	41.51	36.98
Control + VAM	-382.41	95.62	-3.75	-11.50
Control	-332.29	94.94	-12.74	-24.61

Synthetic Stormwater Application to Bioretention Cells with Foliaged Plants

On Saturday 19_April_08, week forty-eight, of the experiment 2-liters of SSW was applied for three weeks to all Bioretention Cells in place of DI. All plants were foliaged or in a photosynthetic state as shown in Figure 11 (*i.e.* transpiration was taking place in Bioretention Cells with plants).

Figure 11: Photo of experimental setup in the greenhouse with foliaged plants

Effluent Volume from Bioretention Cells with Foliaged Plants

Cumulative effluent volumes as shown in Figure 12 were observably different for 6 hr, 24 hr and 168 hr sampling periods. Bioretention Cells with foliaged plants released all effluent during the 6 hr period, controls released between 50 and 60% of total effluent during the 6 hr period. In Figure 13 we can see foliaged Desert willow released about half as much effluent volume as foliaged Switchgrass.
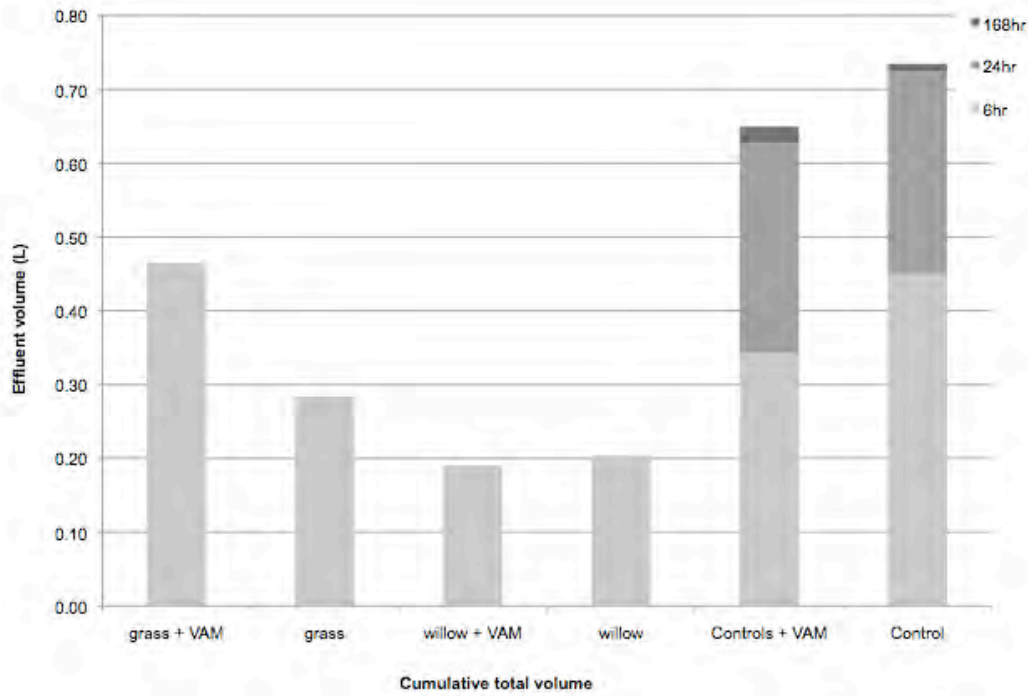
Figure 12: Cumulative effluent volumes from 2-liter SSW application to Bioretention Cells with foliaged plants
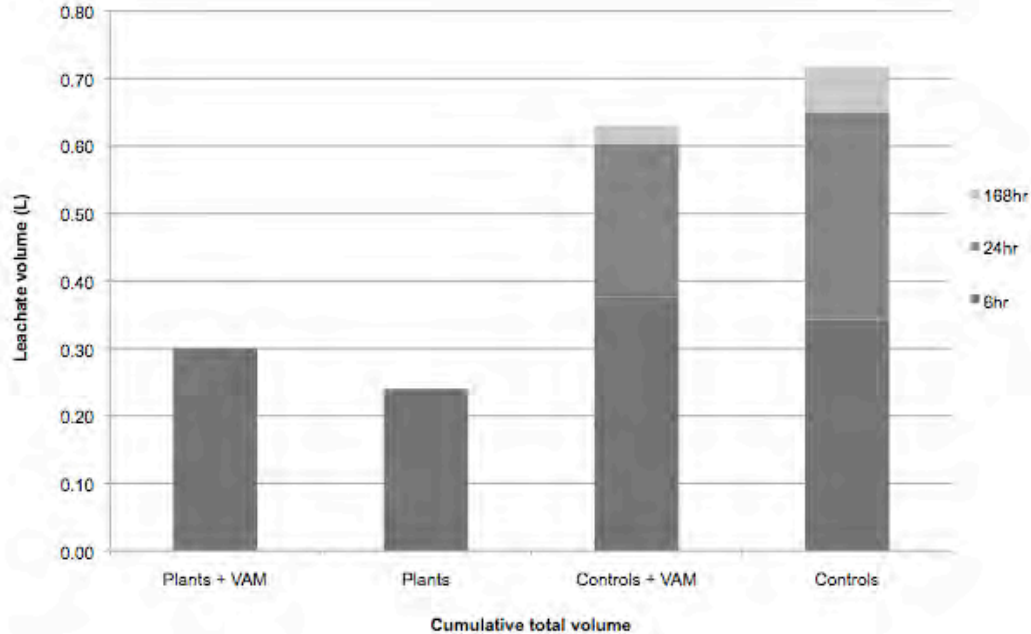
Figure 13: Cumulative effluent volumes from 2-liter SSW application to Bioretention Cells with foliaged plants (species effect)

When we look at the volume collected from each time period independently from cumulative volumes we see an observable difference between foliaged plants vs. controls in the 6 hr, 24 hr, and 168 hr periods.

Bioretention Cells with foliaged plants released all effluent volume during the 6 hr period whereas controls released about half of the total effluent volume. Volume of effluent released from foliaged plants and controls during the 6 hr period was not clearly different but total effluent volume from Desert willow was about half that of Switchgrass.

Total effluent was collected from Bioretention Cells with foliaged plants before the 24 hr period and the remaining majority of total effluent from controls was collected during this period.

Effluent Concentration from Bioretention Cells with Foliaged Plants

Table 13 shows Bioretention Cells with foliaged plants reduced effluent phosphate concentration below SSW levels, whereas controls increased phosphate effluent concentration. Plants and controls released higher nitrate effluent concentrations than that of SSW levels. There was very little ammonium effluent concentration collected from any Bioretention Cell.

Table 13: Effluent concentration from SSW application (2mg/L) to Bioretention Cells with foliaged plants

Treatments	NO_3-N	NH_4-N	Ortho-P	Total-P
	------------------ mg/L ---------------------			
Plant + VAM	3.78	0.20	1.29	1.30
Plant	3.48	0.27	1.13	1.09
Control + VAM	17.66	0.12	2.60	2.84
Control	20.08	0.09	2.44	2.68

As shown in Table 14 there was about a 90% increase of nitrate effluent concentration from Bioretention Cells with foliaged plants. Bioretention Cells without plants released as much as nine times the concentration of nitrate in the effluent. Bioretention Cells with foliaged plants reduced phosphate effluent concentration by over 35%. Bioretention Cells without plants released over 33% more Total-phosphate and over 21% more Ortho-phosphate than that of SSW levels.

Table 14: Percent concentration reduction from SSW application to Bioretention Cells with foliaged plants

Treatments	NO_3-N	NH_4-N	Ortho-P	Total-P
	---------------------- % ----------------------			
Plant + VAM	-89.22	90.01	35.53	35.13
Plant	-73.76	86.28	43.41	45.50
Control + VAM	-783.06	94.02	-29.81	-41.91
Control	-904.00	95.48	-21.88	-33.81

In Table 15 percent reduction of phosphate effluent concentration from Bioretention Cells with foliaged plants was between 32 and 54%. All Bioretention Cells had increased nitrate loading relative to SSW levels. Bioretention Cells with foliaged Switchgrass released over 68% more nitrate and Bioretention Cells with foliaged Desert willow released over 79% more nitrate.

Table 15: Percent reduction of concentration in effluent from SSW application to Bioretention Cells with foliaged plants (species effect)

Treatments	NO$_3$-N	NH$_4$-N	Ortho-P	Total-P
	------------------- % -------------------			
Grass + VAM	-70.46	94.59	36.97	32.86
Grass	-68.53	82.02	51.23	54.06
Willow + VAM	-107.97	85.42	34.08	37.40
Willow	-79.00	90.53	35.58	36.94
Control + VAM	-783.06	94.02	-29.81	-41.91
Control	-904.00	95.48	-21.88	-33.81

Effluent Load from Bioretention Cells with Foliaged Plants

As we can see in Table 16 nutrient loading in effluent from Bioretention Cells with foliaged plants was reduced well below SSW levels for nitrates and phosphates. Bioretention Cells without plants reduced phosphate loading in effluent and increased nitrate loading in effluent relative to SSW levels. There was very little ammonium effluent loading from any Bioretention Cells.

Table 16: Effluent load from SSW application (4 mg) to Bioretention Cells with foliaged plants

Treatments	NO$_3$-N	NH$_4$-N	Ortho-P	Total-P
	--------------------- mg ------------------------			
Plant + VAM	1.28	0.05	0.94	0.40
Plant	1.05	0.07	0.91	0.26
Control + VAM	11.34	0.07	2.06	1.39
Control	14.60	0.07	2.62	1.67

In Table 17 Bioretention Cells with foliaged plants reduced nitrate loading by about 70%. Bioretention Cells without plants released twice as much nitrate load. Bioretention Cells with foliaged plants reduced Ortho and Total-phosphate loading by over 70 and 90%, respectively. Controls reduced Ortho and Total-phosphate loading by over 30 and 60%, respectively.

Table 17: Percent reduction of load from SSW application to Bioretention Cells with foliaged plants

Treatments	NO$_3$-N	NH$_4$-N	Ortho-P	Total-P
	---------------------- % ------------------------			
Plant + VAM	67.93	98.85	76.52	90.11
Plant	73.86	98.20	77.14	93.61
Control + VAM	-183.48	98.13	48.52	65.33
Control	-264.90	98.35	34.52	58.33

Table 18 shows reduction of loading in the effluent following SSW application to Bioretention Cells with foliaged plants does not appear to be influenced by species. Percent reduction of phosphate loading from Bioretention Cells with Desert willow was just slightly better than Switchgrass.

Table 18: Percent reduction of load from SSW application to Bioretention Cells with foliaged plants (species effect)

Treatments	NO_3-N	NH_4-N	Ortho-P	Total-P
	------------------- % -----------------------			
Grass + VAM	57.04	98.71	73.53	86.29
Grass	67.88	97.24	78.25	93.13
Willow + VAM	78.81	98.98	79.50	93.93
Willow	79.84	99.16	76.02	94.09
Control + VAM	-183.48	98.13	48.52	65.33
Control	-264.90	98.35	34.52	58.33

Reduction of nitrate and phosphate by Bioretention Cells with both foliaged plant species reduced loading below SSW levels. Bioretention Cells without plants increased nitrate loading in the effluent; releasing more than twice as much nitrate than SSW levels. Controls reduced phosphate loading by 34 to 65%.

CHAPTER V
DISCUSSION

Plants had observable and quantifiable effects on Bioretention Cell effluent nutrient concentration, loading, volume, and infiltration. In addition dormant and foliaged plants responded differently (*i.e.* seasonality). We initially hypothesized that plant species could significantly influence retention of nutrients in a Bioretention Cell. We also expected an altered hydrologic regime from Bioretention Cells with plants vs. no plants (controls). Within one year we saw the latter assumptions bear true with significance and in most cases with great magnitude. We gained further insight into the effect of plants by application of SSW to dormant and foliaged plants.

At the beginning of this experiment each container had an equal mass and volume of bioretention soil medium with the same *in situ* nutrient levels. Containers with and without plants responded similarly to the weekly 2-liter additions of DI. However, as preliminary leaching results indicated: differences between plants and controls became observable relatively soon.

Vesicular Arbuscular Mycorrhizal Fungi

Vesicular arbuscular mycorrhizal fungi were not found to be present before or after the experiment and had no significant effect on Bioretention Cell performance. This result was not surprising since commercial nursery potting medium is a soil-less medium generally consisting of peat, bark and vermiculite. VAM propagules are predominantly found in soil, and are naturally associated with nutrient limited environments (Varma and Hock 1998). Many nursery specimens are propagated through cuttings, which also decreases the chance of vegetative material coming into contact with soil and VAM fungus. Nursery potting medium contains all the necessary nutrients in abundance thus also decreasing the likelihood of developing a symbiotic VAM relationship. There was a high level of phosphorus and nitrogen in the initial experimental soil medium (preliminary P-index 317). It is known that VAM preferentially develop symbiosis with vascular plants when found in phosphorus-limited environments and soils with high phosphate levels inhibit VAM inoculation (Gerdemann 1968).

Vascular Plants

The species selected for this study were chosen for their appropriate use in Bioretention Cells based on aesthetic value, rapid growth, root type, and their ability to survive in saturated and xeric conditions. Desert willow is a native species to the

Southwestern United States that can be found naturalized in the western counties of Oklahoma. This species was planted in windbreaks to prevent erosion and has a taproot. Switchgrass is a recommended species in several Bioretention manuals and is known to be a facilitative wetland species; this species has coarse roots that form a dense root ball. Both species are utilized in landscaping for their aesthetic appeal and horticultural cultivars are available for this species. As mentioned earlier, species effect was apparent sometimes but was not always clear and was not statistically analyzed.

Nitrogen and Ammonium

Significant nitrate retention in the Bioretention Cell literature without an anaerobic zone has been reported to be poor (Clausen and Dietz 2005, Hunt 2003), and in some cases likely unattainable (Kim *et al* 2003). Davis *et al* (2001) found moderate reductions of ammonium but nitrate reduction was low and in some cases nitrate was increased in the effluent. In this study dormant and foliaged plants reduced nitrate loading in effluent by more than 60% and 70%, respectively. Ammonium effluent concentration and load was very low for all containers (below 0.45 mg) with and without plants.

Nitrate effluent concentration from Bioretention cells with plants was increased relative to SSW concentration while plants were foliaged and decreased while plants were dormant. However, ammonium effluent concentration and correspondingly

ammonium load were reduced well below the initial 2 mg/L and 4 mg (containers with and without plants reduced concentration below 0.60 mg/L and load below 0.45 mg). The nitrification processes where nitrifying bacteria convert ammonium to nitrite then nitrate explain this result.

In the nitrogen cycle ammonium cations are oxidized by ubiquitous nitrifying bacteria living in soil, most commonly from the genus *Nitrosomonas*. This reduction yields nitrite, which is then further oxidized to nitrate, most commonly by bacteria of the genus *Nitrobacter*. Plants and other microbial organisms can interfere with the nitrifying process, albeit much slower than nitrification, and assimilate ammonium into organic compounds. The assimilation process is also called immobilization since the organic nitrogen complex must go through the process of mineralization and subsequent nitrification before leaching from the soil is possible (Sparks 2003).

The nitrogen cycle processes are dependant on pH, temperature, soil moisture and the presence of oxygen. Nitrification occurs at increased rates with increasing soil temperature (Seifert 1980). However, nitrifying bacteria are active over a wide temperature range (between 4ºC and 50ºC). Other environmental variables such as presence of oxygen and soil moisture can be assumed to have been favorable for nitrifying bacterial growth thus nitrification since all plants increased biomass throughout the experiment (Table 6). Although the nitrification process was not quantified under the scope of this study it is noteworthy and best explains conversion of ammonium to nitrate and increased concentration and loading of nitrate in Bioretention cell effluent.

Dormant vs. Foliaged (Seasonal Effect)

Effluent Volume

We can see in Figure 14 the lower total effluent volumes from Bioretention Cells with foliaged plants vs. dormant plants (*i.e.* seasonality) was highly significant (p < 0.001). However, total effluent volumes from Bioretention Cells with plants vs. Bioretention Cells without plants (controls) are only significantly different while plants are foliaged (p < 0.001). Bioretention cells with and without plants always had a highly significant (p < 0.001) effect on the effluent collection time periods but differences between time periods were variable so only the 6-hour and 24-hour periods are discussed.
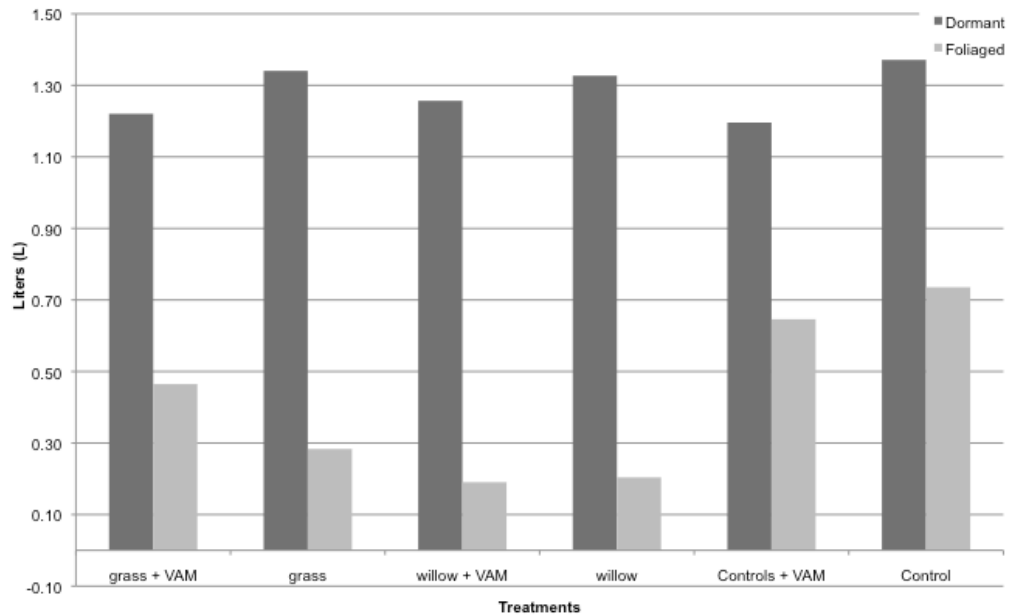
Figure 14: Total effluent volumes from SSW application to Bioretention Cells with dormant and foliaged plants

The data represented in Figure 15 provides a visual representation of increased infiltration for Bioretention Cells with dormant vs. foliaged plants. However, this result does not provide a good estimation of infiltration rate since many of the Bioretention Cells with plants (dormant and foliaged) released all effluent before the end of the 6 hr collection period.
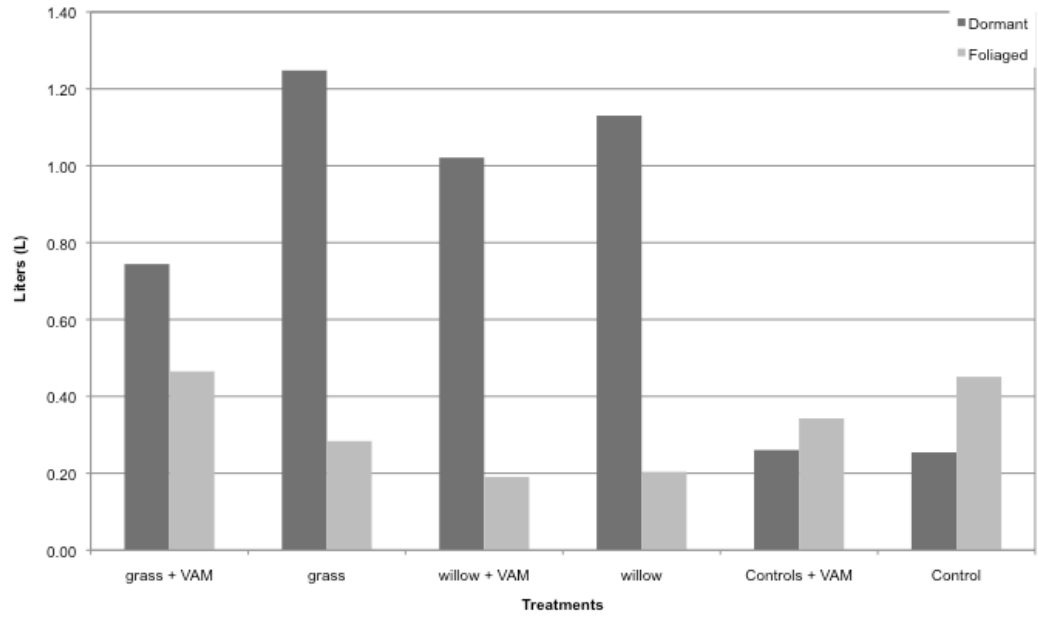
Figure 15: Six hr effluent volumes from SSW application to Bioretention Cells with dormant and foliaged plants
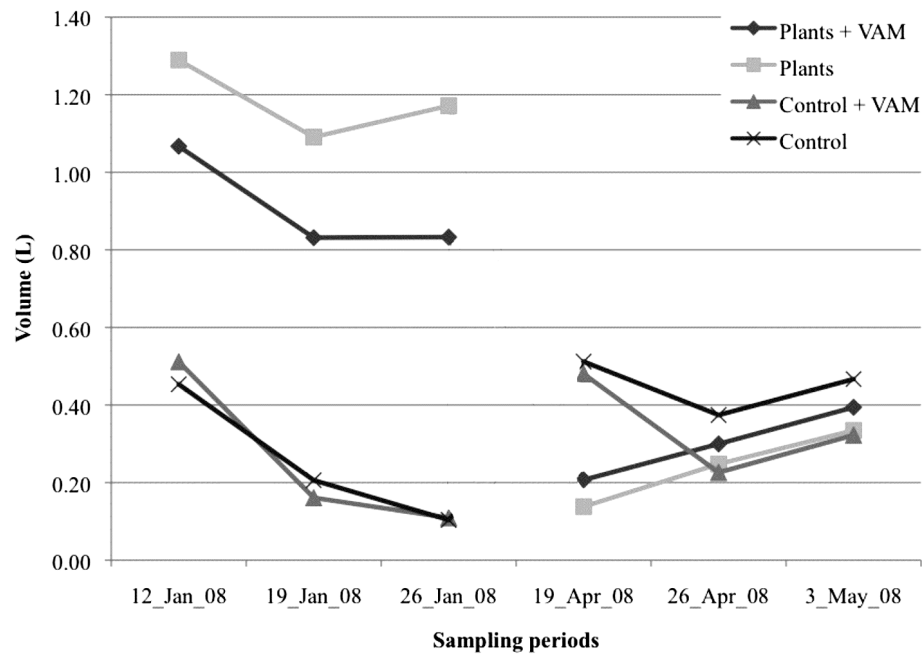
Figure 16: Effluent volume averages from 6 hr sampling periods for each SSW application to Bioretention Cells

An important distinction between Bioretention Cells with plants and controls as shown in Figure 16 is the effect of seasonality on stability (*i.e.* smaller differences between 6hr effluent volumes during dormant vs. foliaged events). Bioretention Cells with dormant plants released between 62 and 87% of total effluent during the 6 hr collection periods, controls released between 20 and 24%. Foliaged plants released 100% of total effluent during the 6 hr collection period, controls released between 48 and 60%. Effluent volume collected beyond the 6 hr sampling period, shown in Figure 17 and 18, was reduced to zero for foliaged plants.
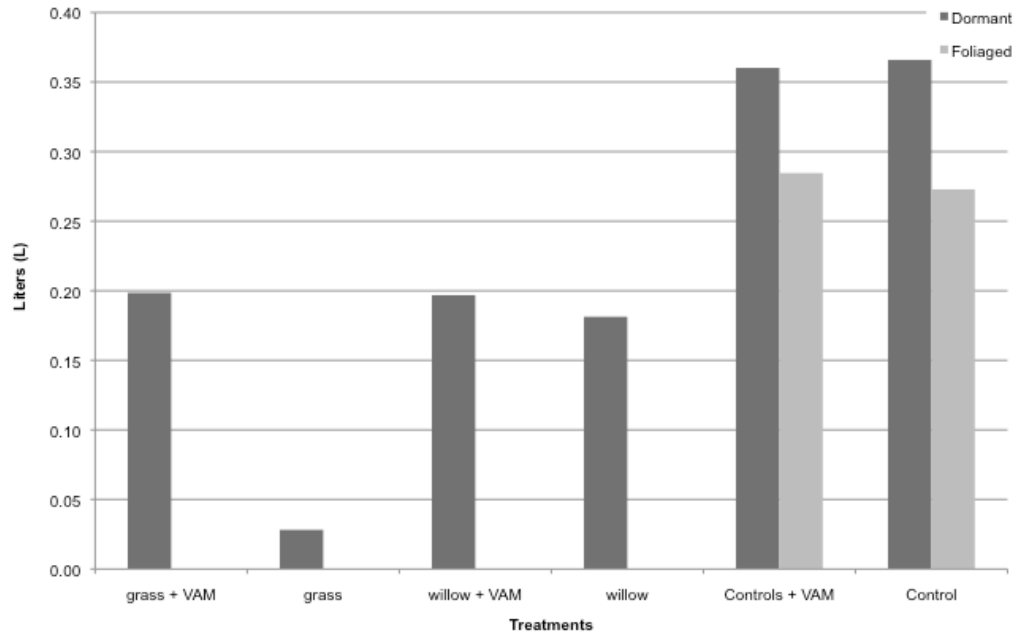
Figure 17: Effluent volume for 24 hr periods from SSW application to Bioretention Cells (dormant and foliaged plants)
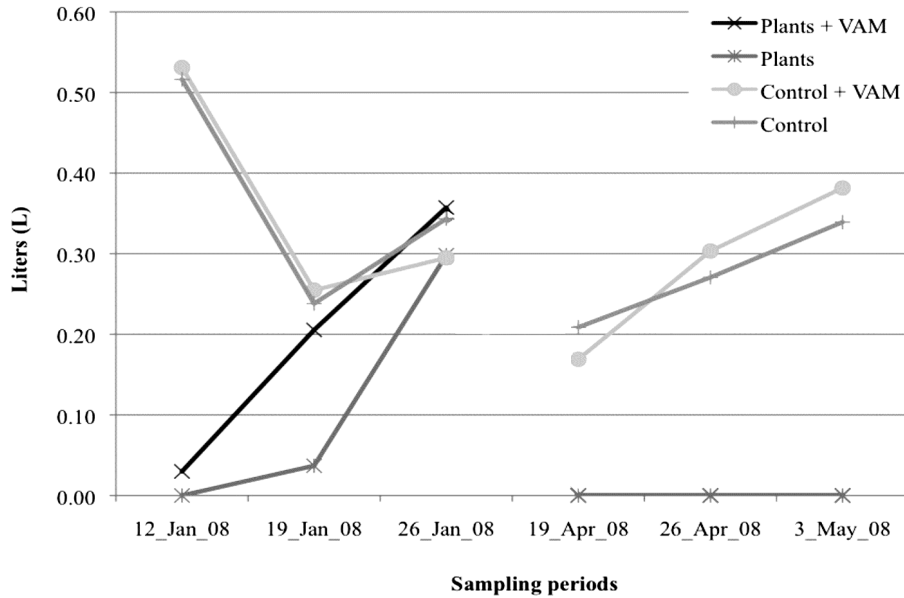
Figure 18: Effluent volume averages from 24 hr sampling periods for each SSW application to Bioretention Cells with foliaged and dormant plants

Effluent Concentration

The effect of plants on nitrate effluent concentration was highly significant from dormant and foliaged SSW events ($p < 0.001$), seasonality was also significant for controls ($p = 0.018$). When we compare percent reduction of nitrate concentration from SSW applications in dormant and foliaged plants we see less variability between events from Bioretention Cells with plants (Figure 19). Bioretention Cells with dormant plants reduced the concentration by over 40% and below SSW levels (2 mg/L) for each event, whereas foliaged plants increased nitrate effluent concentration by more than 70%.

Although foliaged plants released more nitrate effluent concentration than that found in

SSW it was still about ten times less than the controls contributed increases.
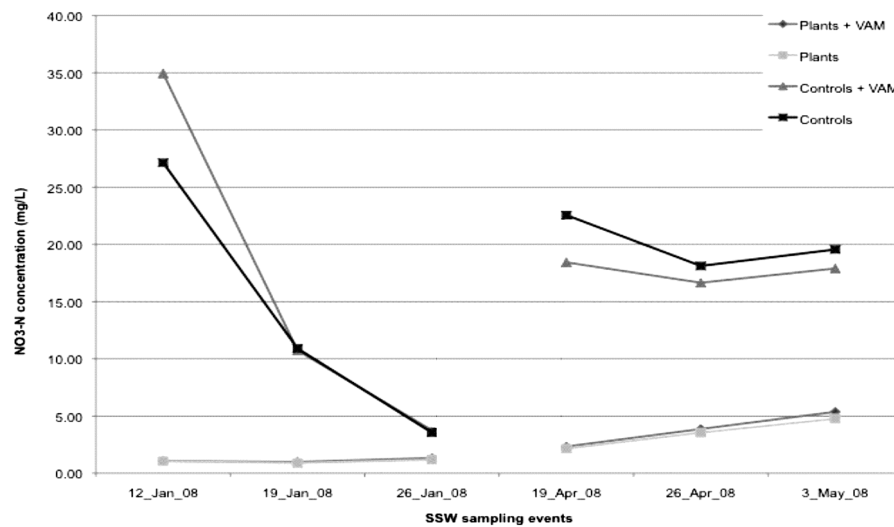
Figure 19: Nitrate effluent concentration from SSW application for each SSW
application to Bioretention Cells with foliaged and dormant plants

Bioretention Cells with dormant plants reduced nitrate effluent concentration by

41%, whereas Bioretention Cells with foliaged plants released over 73% more nitrate

than found in SSW. Bioretention Cells with dormant and foliaged plants greatly out-

performed Bioretention Cells without plants as shown more plainly in Figure 20. In

Figure 21 there appears to be no apparent species effect on nitrate effluent concentration

from application of SSW from dormant or foliaged plants.
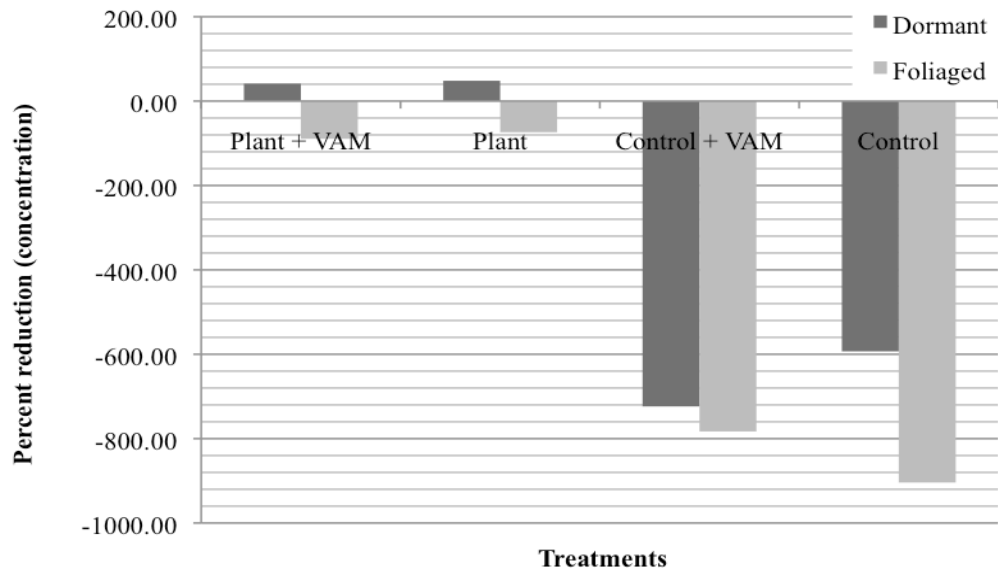
Figure 20: Percent reduction of nitrate effluent concentration from SSW application to dormant and foliaged plants
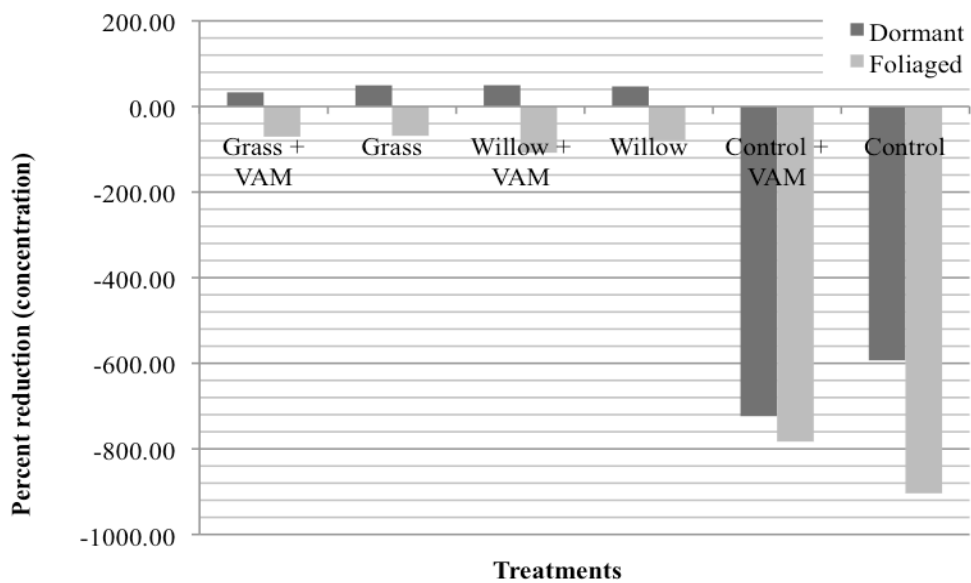
Figure 21: Percent reduction of nitrate effluent concentration from SSW application to dormant and foliaged plants (species effect)

Reduction of Ortho and Total-phosphate concentration in effluent by Bioretention Cells with dormant and foliaged plants was highly significant ($p < 0.001$). Seasonality had a significant influence on reduction of Ortho-phosphate effluent concentration, with plants ($p = 0.048$) and controls ($p < 0.001$). The seasonality effect on reduction of Total-phosphate effluent concentration was only significant for controls ($p = 0.025$). Differences between Bioretention Cells with plants vs. controls were highly significant ($p < 0.001$).

Figure 22 shows less variability in Total-phosphate effluent concentration from Bioretention Cells with plants relative to controls. Synthetic Stormwater application to Bioretention Cells with dormant and foliaged plants released an average stable concentration at or below 2.00 mg/L.
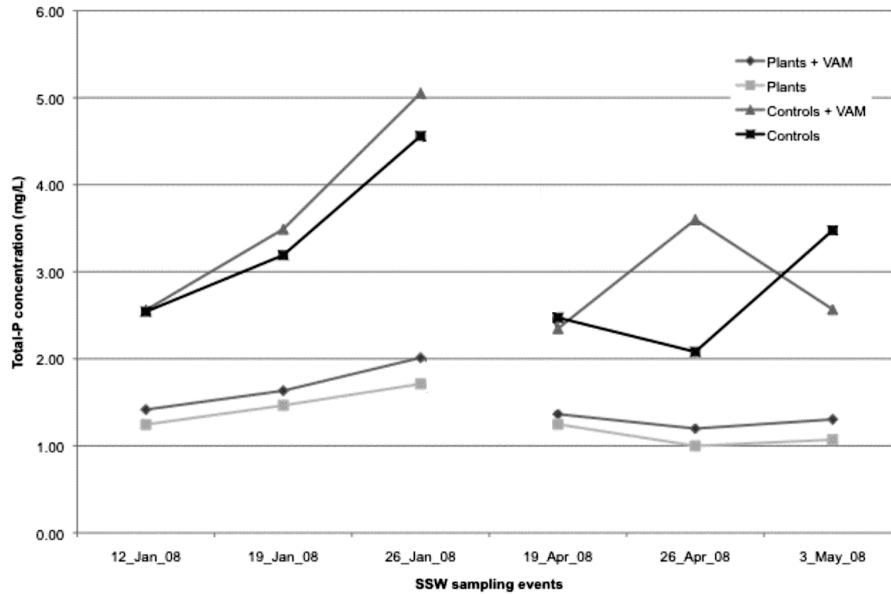
Figure 22: Total-phosphate concentration in effluent for each SSW application to Bioretention Cells with foliaged and dormant plants

Bioretention Cells with dormant plants reduced Total-phosphate effluent concentration by more than 16% whereas Bioretention Cells with foliaged plants reduced concentration by over 45%. In each case, Bioretention Cells with plants greatly out-performed Bioretention Cells without plants as shown in Figure 23. As we can see from Figure 24 species effect for percent reduction of Total-phosphate appears to be stronger for Switchgrass.
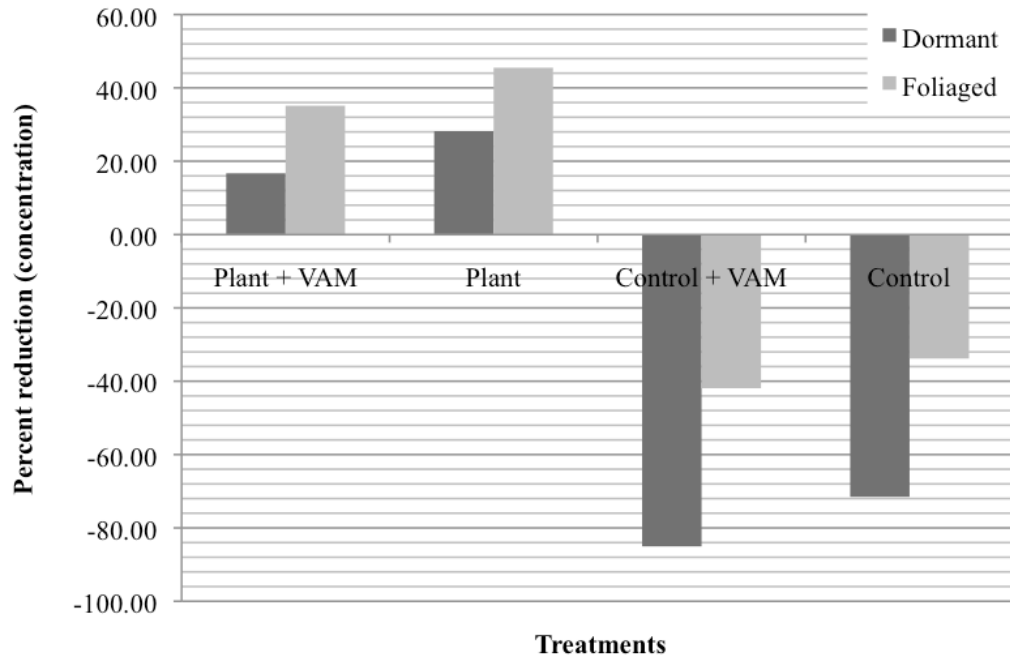
Figure 23: Percent reduction of Total-phosphate effluent concentration from SSW application to dormant and foliaged plants
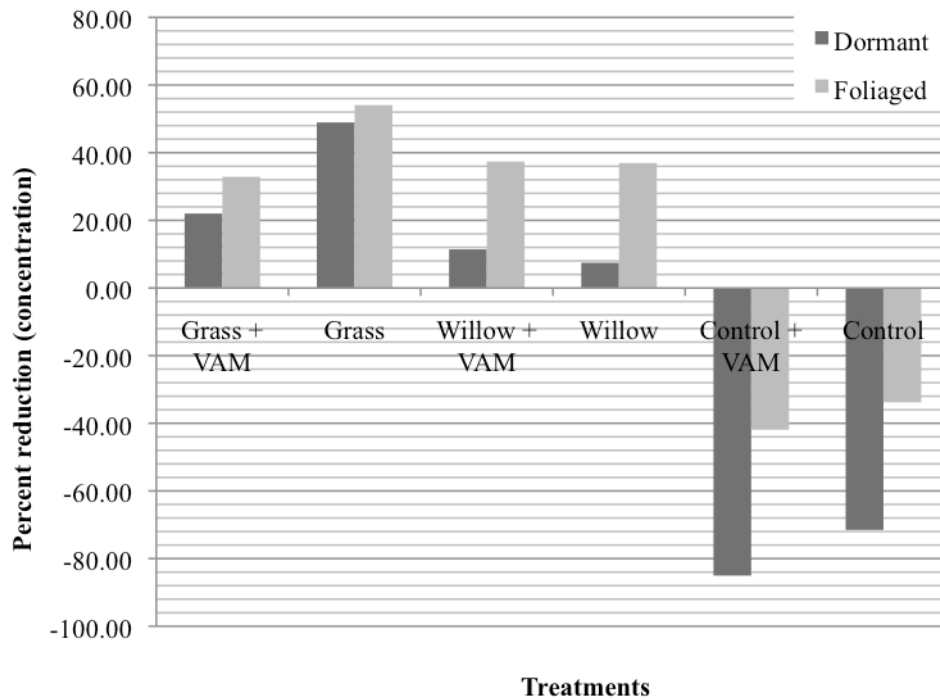
Figure 24: Percent reduction of Total-phosphate effluent concentration from SSW application to dormant and foliaged plants (species effect)

Reduction of ammonium concentration in the effluent was significant for Bioretention Cells with plants ($p = 0.031$), but when compared to controls, only foliaged plants were significantly different than controls ($p = 0.009$). Seasonality was significant for the effect of plants ($p = 0.027$). Differences between Bioretention Cells with foliaged plants vs. dormant plants were highly significant ($p < 0.001$).

Ammonium concentration from SSW applications to dormant vs. foliaged plants as shown in Figure 25 showed little to no variability between events. In all cases SSW application to Bioretention Cells with and without plants released much less than 2.00 mg/L ammonium, well below the ammonium concentration of the SSW.
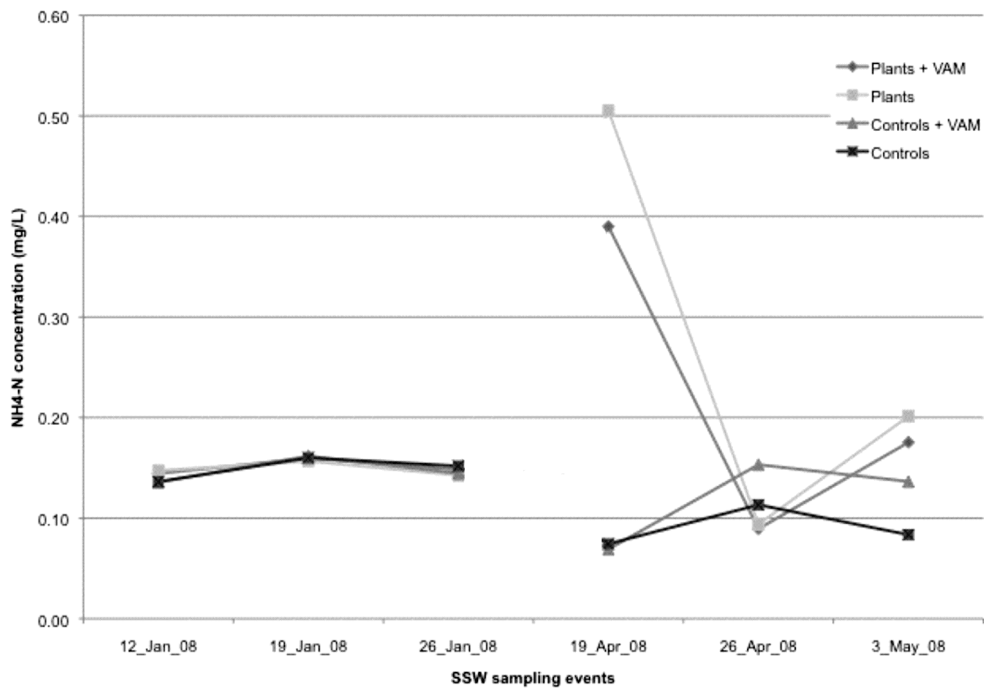
Figure 25: Ammonium concentration in effluent for each SSW application to Bioretention Cells with foliaged and dormant plants

Effluent Load

The effect of plants on reduction of nitrate load in the effluent was highly significant for Bioretention Cells with plants ($p < 0.001$). Seasonality was only significant for controls ($p = 0.003$). Differences between Bioretention Cells with plants vs. controls were highly significant ($p < 0.001$).

As shown in Figure 26 load responses of nitrate from SSW applications to dormant and foliaged plants had less variability between events from Bioretention Cells with plants relative to controls. Synthetic Storm Water application to Bioretention Cells with dormant and foliaged plants released a stable load below 4.00 mg, well below the nitrate load of SSW; this is also true for SSW application to foliaged plants. Foliaged plants released about 10% less nitrate than dormant plants.
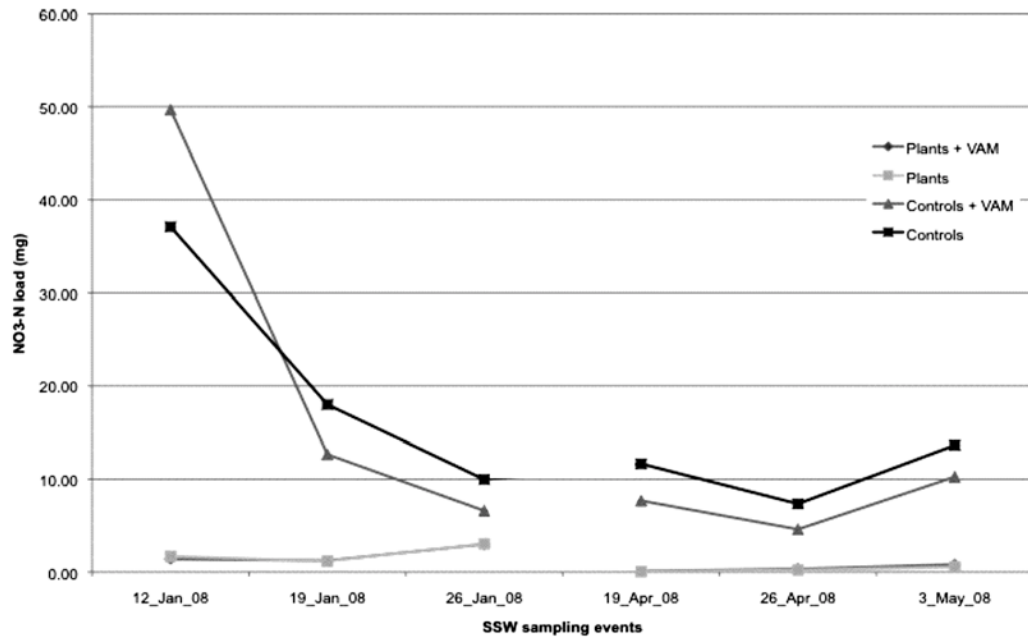
Figure 26: Nitrate load in effluent for each SSW application to Bioretention Cells with foliaged and dormant plants

Bioretention Cells with plants, dormant and foliaged, reduced nitrate loads by 60 and 70%, respectively whereas Bioretention Cells without plants increased nitrate loading by as much as 150 and 90%, respectively as shown in Figure 27. When we look at Figure 28 we see no apparent species effect on nitrate effluent loading from application of SSW to Bioretention Cells with Dormant vs. Foliaged plants however much less loading occurred while plants were dormant.
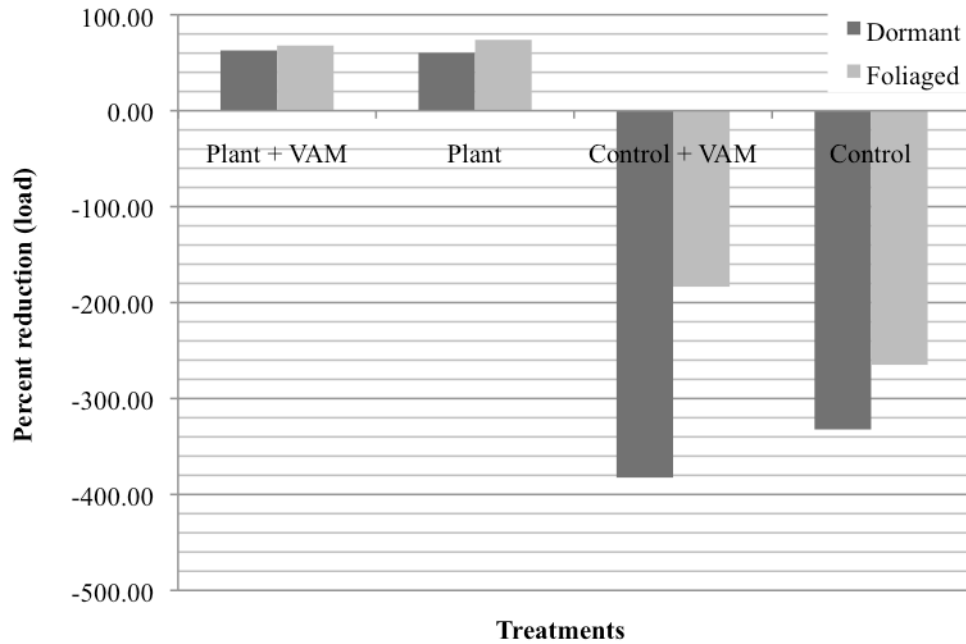
Figure 27: Percent reduction of nitrate effluent load from SSW application to Bioretention Cells with dormant and foliaged plants

The effect of plants on reduction of Ortho and Total-phosphate loading in the effluent was highly significant ($p < 0.001$). Seasonality was also highly significant ($p < 0.001$) for reduction of Ortho and Total-phosphate for plants and controls. Differences between Bioretention Cells with plants vs. controls were highly significant ($p < 0.001$).
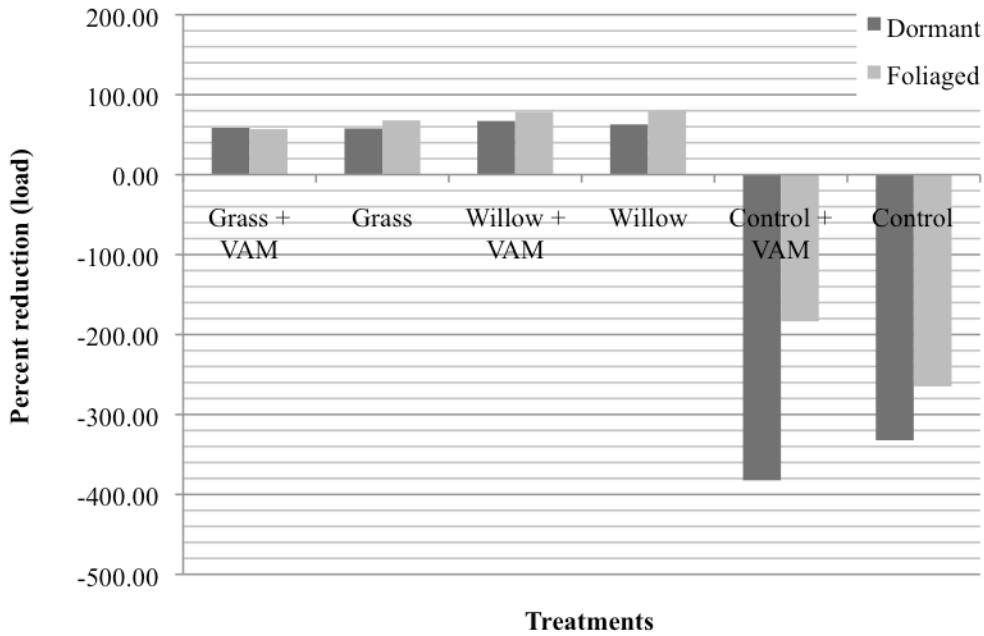
Figure 28: Percent reduction of nitrate effluent load from SSW application to Bioretention Cells with dormant and foliaged plants (species effect)

We can see in Figure 29 Total-phosphate loading from SSW applications to Bioretention Cells with dormant and foliaged plants shows less variability from containers with plants. In Figure 30 we can see SSW application to dormant and foliaged plants released an average stable loading below 4 mg, well below the Total-phosphate load of SSW. In Figure 31we see a species effect on phosphate load is not apparent.
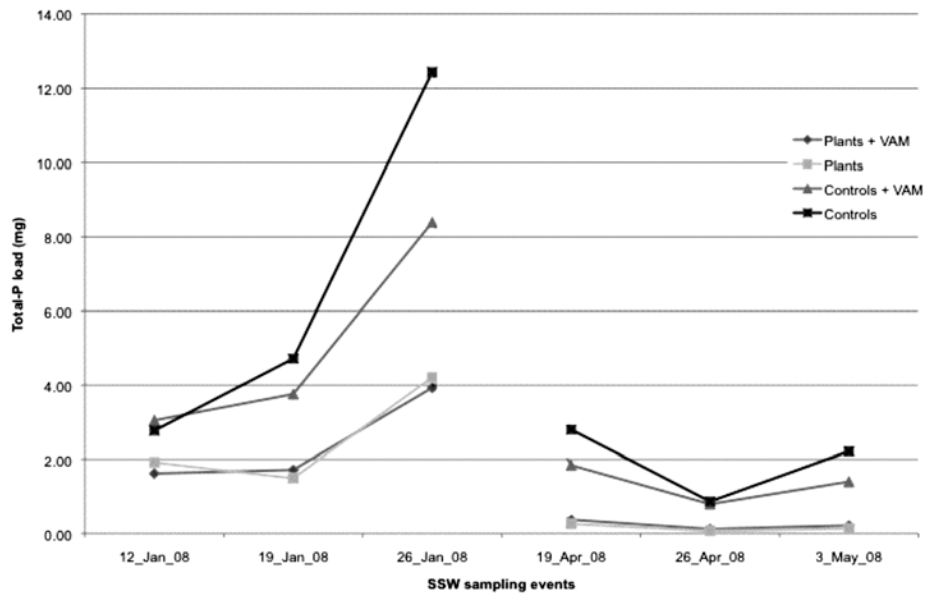
Figure 29: Total-phosphate load in effluent for each SSW application to Bioretention Cells with foliaged and dormant plants
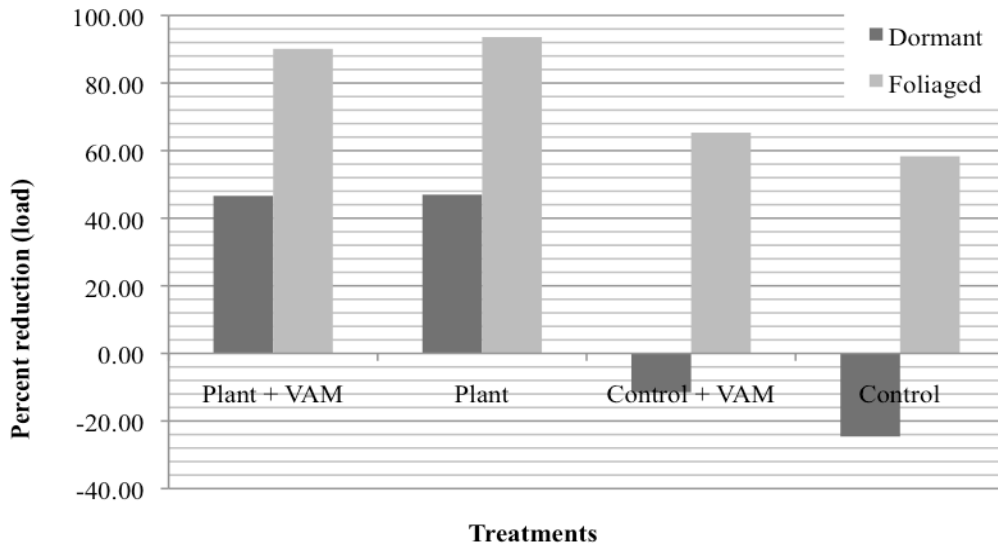
Figure 30: Percent reduction of Total-phosphorus effluent load from SSW application to Bioretention Cells with dormant and foliaged plants
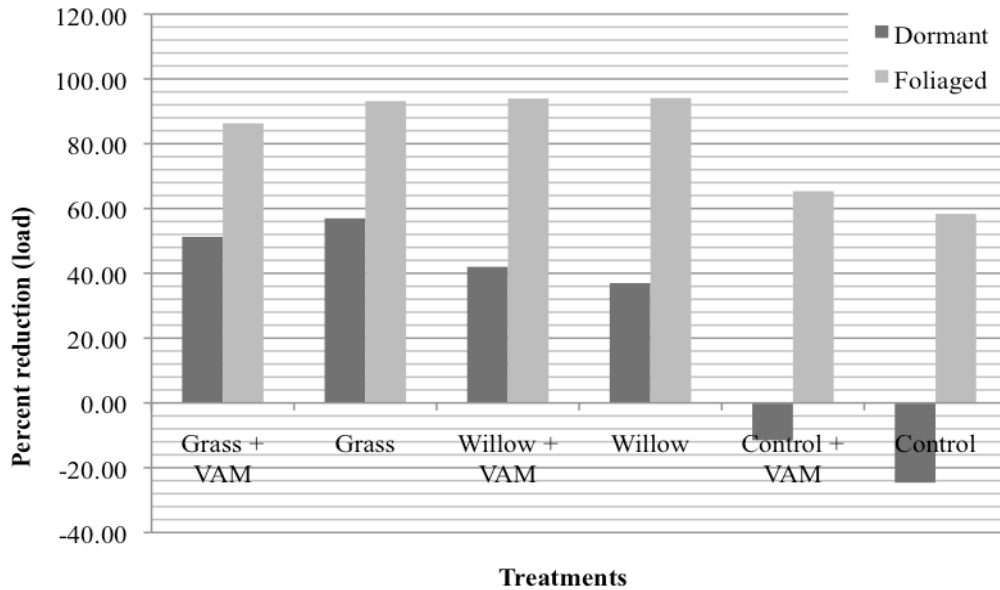
Figure 31: Percent reduction of Total-phosphorus effluent load from SSW application to Bioretention Cells with dormant and foliaged plants (species effect)

The effect of plants on reduction of ammonium effluent load and the difference between containers with plants and controls was not significant for dormant or foliaged SSW events (p = 0.446) but seasonality was highly significant for containers with plants and controls (p < 0.001). Ammonium loading from SSW applications shown in Figure 32 resulted in little to no variability between events from Bioretention Cells with plants relative to controls but differences between dormant and foliaged SSW applications are relatively large.
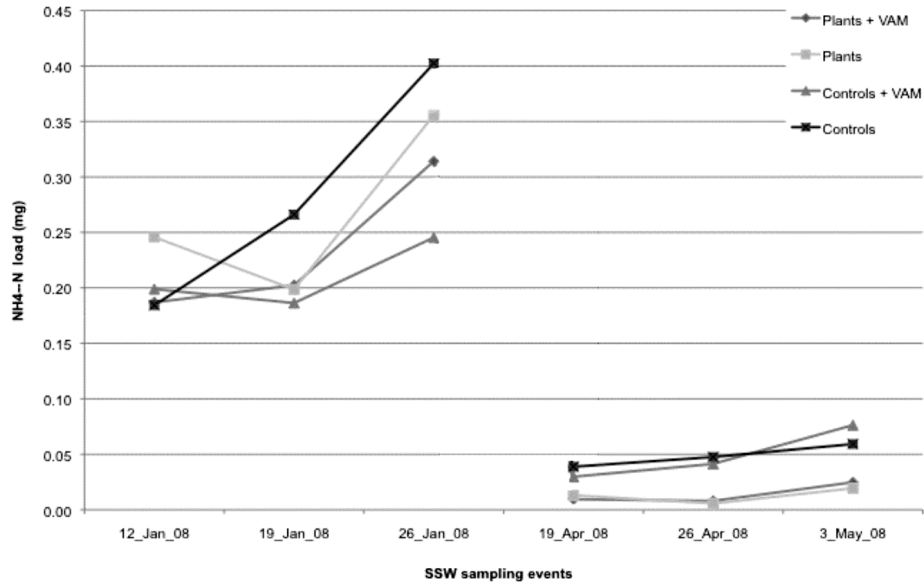
Figure 32: Ammonium effluent load from SSW application to Bioretention Cells with foliaged and dormant plants

CHAPTER VI

CONCLUSION

In this study plants had observable and quantifiable effects on Bioretention Cell effluent nutrient concentration, loading, volume, and infiltration rate. In addition dormant and foliaged plants responded differently. Vesicular arbuscular mycorrhizal fungi had no significant effect on effluent nutrient concentration, loading, volume, or infiltration rate nor was it related to a seasonal effect.

Foliaged plants increased infiltration and decreased total effluent volume relative to controls, whereas dormant plants only increased infiltration. Total-volume from dormant plants was not significantly different from controls. During the six-hour collection period containers with dormant and foliaged plants released all effluent whereas controls continued to drain throughout the 168hr period. In some cases there were higher concentrations from containers with foliaged plants but these containers still had lower loading than that of controls.

Dormant plants reduced nitrate concentration by more than 40% below SSW levels (2mg/L as N and P), whereas foliaged plants increased nitrate concentration by

more than 70%. Although foliaged plants increased nitrate concentration it was only 10% that of controls contributed increase.

The effect of seasonality on nitrate concentration in the effluent was only significant for controls (p = 0.018), but the difference between containers with plants and controls were highly significant (p < 0.001) for each season. Overall, nitrate effluent concentration from containers with plants was less variable than controls and significantly lower.

Dormant and foliaged plants reduced nitrate loading in effluent by more than 60% and 70%, respectively. Differences between containers with and without plants were highly significant (p < 0.001) whereas seasonality was only significant for controls (p = 0.003). Nitrate loading in effluent from containers with plants was less variable than controls and significantly lower.

Dormant and foliaged plants reduced phosphate effluent concentration by more than 16 and 45%, whereas controls released 85% more phosphorus while plants were dormant and 33% while plants were foliaged. Seasonality had a significant influence on reduction of Ortho-phosphate effluent concentration by Bioretention Cells with plants (p = 0.048) and controls (p < 0.001). Seasonality effect on reduction of Total-phosphate effluent concentration was only significant for controls (p = 0.025). Differences between Bioretention Cells with plants vs. controls were highly significant (p < 0.001). Total-phosphorus effluent concentration was less variable for containers with plants than that of

controls and was consistently lower than SSW levels. Reduction of phosphate effluent concentration was observably stronger for Switchgrass.

Dormant and foliaged plants reduced phosphate load by more than 46 and 90%, respectively. Controls during the dormant sampling increased loading of phosphate by more than 11%, whereas controls during the foliaged sampling period reduced phosphate loading by more than 58%. Seasonality had a highly significant ($p < 0.001$) influence on reduction of Ortho and Total- phosphate effluent loading by Bioretention Cells with plants and controls. Differences between Bioretention Cells with plants vs. controls were highly significant ($p = 0.009$, $p < 0.001$).

Ammonium effluent concentration and load was very low for containers (in all cases concentration was below 0.60 mg/L and loading below 0.45 mg) with and without plants. Seasonality significantly affected concentration in the effluent of plants ($p = 0.027$), whereas it was highly significant ($p < 0.001$) for loading from containers with and without plants. Differences between ammonium concentration and loading from Bioretention Cells with plants vs. controls were not significant.

Ameliorative effects of plants in the rhizosphere of Bioretention Cells could be attributed to many factors. Plants increase macro-pores and the permeability of Bioretention Cell medium. They increase organic matter thus increasing sorption (adsorption and absorption) properties of the soil. Transpiration decreases effluent volume possibly retaining more nutrients in the Bioretention Cells for further plant uptake but can lead to relative increases in nutrient concentrations. These plant attributes

are variable both influenced by species and rate of growth thus ultimately species selection could be significant in increasing Bioretention Cell performance.

Some considerations for species selection could include type of root structure, rate of growth, N:P ratio in foliage, wetland indicator status, relative depth of rhizosphere and the use of locally collected germplasm (provenance). Species selection should be site-specific: dependent on precipitation and average annual minimal temperatures. For example appropriate species for the Southeastern corner of Oklahoma would tolerate more inundation but may have increased sensitivity to drought such as *Taxodium distichum* (Bald cyprus) or *Itea virginica* (Virginia sweetspire) whereas potential species for Western Oklahoma such as *Salix exigua* (Coyote willow) or *Chilopsis linearis* (Desert willow) have excellent drought resistance yet prolonged inundation may lead to death. Oklahoma has vast differences in precipitation from east to west, from about 17 inches in the panhandle to 56 inches in the Southeast, and falls under 3 USDA "Plant Hardiness Zones".

Initial soil nitrate and phosphate levels were high in the sandy loam used for this study (P-index 317). It took over 30 weeks of weekly 2-liter additions of DI to leach nutrients below SSW levels (2mg/L as N and P). Initial soil nutrient levels appear to have a profound effect on nutrient effluent concentration and load but the establishment of plants significantly reduces this concern and could lead to increased life expectancy and further utilization of these LID practices.

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APPENDICES

Appendix (a): Experimental Data

Table 1a: Effluent volumes from SSW addition one

Pot #	6hr	24hr	168hr	Total
	--------------- mL --------------			
PV1-13	335.05	298.60	402.10	1035.75
PV2-14	1464.65			1464.65
PV3-15	1027.55			1027.55
PV6-18	1172.15			1172.15
PN1-19	1485.65			1485.65
PN2-20	1476.65			1476.65
PN3-21	1389.65			1389.65
PN4-22	1324.65			1324.65
PN6-24	1218.15			1218.15
CV1-25	1014.05			1014.05
CV2-26	1028.55			1028.55
CV3-27	1214.15			1214.15
CV4-28	1018.05			1018.05
CV5-29	1377.15			1377.15
CV6-30	1019.15			1019.15
CN1-31	1508.65			1508.65
CN2-32	1026.55			1026.55
CN3-33	1303.15			1303.15
CN4-34	1393.15			1393.15
CN5-35	1025.05			1025.05
CN6-36	1031.55			1031.55
C1-37V	244.05	315.10	351.10	910.25
C2-38V	1010.55	501.60	83.10	1595.25
C3-39V	292.05	425.60	349.10	1066.75
C4-40V	369.05	501.10	172.10	1042.25
C5-41V	514.05	711.10	55.60	1280.75
C6-42V	639.05	731.10	0.00	1370.15
C7-43N	232.55	337.10	297.60	867.25
C8-44N	322.05	440.10	414.10	1176.25
C9-45N	703.55	150.60	0.00	854.15
C11-47N	499.05	973.60	36.60	1509.25
C12-48N	510.05	680.10	212.60	1402.75

Table 2a: Effluent volumes from SSW addition two

Pot #	6hr	24hr	168hr	Total
		--------------- mL --------------		
PV1-13	197.10		895.10	1092.20
PV2-14	586.05	582.10		1168.15
PV3-15	1017.65			1017.65
PV6-18	559.55	193.60		753.15
PN1-19	1557.15			1557.15
PN2-20	1013.55			1013.55
PN3-21	896.65	45.60		942.25
PN4-22	1426.15			1426.15
PN6-24	1002.10			1002.10
CV1-25	996.55	26.10		1022.65
CV2-26	1020.55	24.10		1044.65
CV3-27	1016.05	568.60		1584.65
CV4-28	1009.55			1009.55
CV5-29	887.05	638.10		1525.15
CV6-30	1023.55	21.10		1044.65
CN1-31	1023.55	51.60		1075.15
CN2-32	1015.55			1015.55
CN3-33	1018.55	21.60		1040.15
CN4-34	1014.05	0.00		1014.05
CN5-35	1008.05	263.10		1271.15
CN6-36	1021.55	24.60		1046.15
C1-37V		390.10		390.10
C2-38V	171.05	79.10	1024.10	1274.25
C3-39V	200.05	195.60	859.10	1254.75
C4-40V	222.55	177.60	536.60	936.75
C5-41V		358.60	946.60	1305.20
C6-42V	372.05	327.60	566.10	1265.75
C7-43N	147.05	230.10	871.10	1248.25
C8-44N	95.05	139.10	1023.60	1257.75
C9-45N	395.05	371.10	427.60	1193.75
C11-47N	209.55	219.60	946.60	1375.75
C12-48N	183.55	230.60	965.60	1379.75

Table 3a: Effluent volumes from SSW addition three

Pot #	6hr	24hr	168hr	Total
	--------------- mL --------------			
PV1-13	160.65	369.60	979.60	1035.75
PV2-14	1029.65			1464.65
PV3-15	1107.15			1027.55
PV6-18	273.15	938.10	527.10	1172.15
PN1-19	1612.15			1485.65
PN2-20	1020.65			1476.65
PN3-21	1243.15			1389.65
PN4-22	1028.15			1324.65
PN6-24	1025.15	376.60		1218.15
CV1-25	1063.65	84.10		1014.05
CV2-26	1103.15	609.10		1028.55
CV3-27	512.05	1032.10		1214.15
CV4-28	1030.65	58.60		1018.05
CV5-29	637.05	36.10	487.60	1377.15
CV6-30	1411.15	445.10		1019.15
CN1-31	1455.15	479.10		1508.65
CN2-32	20.10	44.60		1026.55
CN3-33	1445.15	483.10		1303.15
CN4-34	1450.65	490.10		1393.15
CN5-35	1118.15	895.60		1025.05
CN6-36	1468.15	510.10		1031.55
C1-37V	43.15	137.10	612.10	910.25
C2-38V	39.65	110.60	1019.60	1595.25
C3-39V	162.65	373.10	839.10	1066.75
C4-40V	63.15	87.60	244.60	1042.25
C5-41V	56.15	178.60	1022.10	1280.75
C6-42V	290.15	882.10	578.60	1370.15
C7-43N	55.65	154.10	484.60	867.25
C8-44N	69.65	211.60	1014.20	1176.25
C9-45N	213.65	537.60	741.10	854.15
C11-47N	144.15	447.60	1018.10	1509.25
C12-48N	35.65	365.60	1062.60	1402.75

Table 4a: Effluent volumes from SSW addition four

Pot #	6hr	24hr	168hr	Total
	--------------- mL --------------			
PV1-13	296.10			296.10
PV2-14	0.00			0.00
PV3-15	417.55			417.55
PV6-18	561.05			561.05
PN1-19	116.60			116.60
PN2-20	170.10			170.10
PN3-21	232.60			232.60
PN4-22	69.60			69.60
PN6-24	270.05			270.05
CV1-25	81.60			81.60
CV2-26	74.55			74.55
CV3-27	120.10			120.10
CV4-28	28.05			28.05
CV5-29	145.60			145.60
CV6-30	345.05			345.05
CN1-31	96.60			96.60
CN2-32	128.55			128.55
CN3-33	62.10			62.10
CN4-34	72.40			72.40
CN5-35	200.60			200.60
CN6-36	97.60			97.60
C1-37V	301.60	197.10		498.70
C2-38V	406.55			406.55
C3-39V	430.10	368.10		798.20
C4-40V	520.10	46.60		566.70
C5-41V	440.60	338.10		778.70
C6-42V	783.05	64.10		847.15
C7-43N	373.55	370.10		743.65
C8-44N	555.60	135.10		690.70
C9-45N	620.55	160.60		781.15
C11-47N	718.60	25.60		744.20
C12-48N	291.55	352.10		643.65

Table 5a: Effluent volumes from SSW addition five

Pot #	6hr	24hr	168hr	Total
	--------------- mL --------------			
PV1-13	466.10			466.10
PV2-14	227.10			227.10
PV3-15	574.10			574.10
PV6-18	709.10			709.10
PN1-19	186.60			186.60
PN2-20	195.60			195.60
PN3-21	427.10			427.10
PN4-22	105.10			105.10
PN6-24	592.60			592.60
CV1-25	200.10			200.10
CV2-26	208.60			208.60
CV3-27	51.60			51.60
CV4-28	161.60			161.60
CV5-29	229.60			229.60
CV6-30	168.60			168.60
CN1-31	185.10			185.10
CN2-32	233.60			233.60
CN3-33	114.60			114.60
CN4-34	200.10			200.10
CN5-35	294.60			294.60
CN6-36	191.60			191.60
C1-37V	176.10	245.10		421.20
C2-38V	278.60	375.10		653.70
C3-39V	416.60	187.10		603.70
C4-40V	45.60	115.10		160.70
C5-41V	210.10	288.10		498.20
C6-42V	229.10	609.10		838.20
C7-43N	296.60	393.10		689.70
C8-44N	248.60	162.10		410.70
C9-45N	485.10	181.10		666.20
C11-47N	582.60	155.60		738.20
C12-48N	257.60	461.10		718.70

Table 6a: Effluent volumes from SSW addition six

Pot #	6hr	24hr	168hr	Total
	--------------- mL --------------			
PV1-13	539.10			539.10
PV2-14	319.60			319.60
PV3-15	662.10			662.10
PV6-18	808.10			808.10
PN1-19	275.60			275.60
PN2-20	239.60			239.60
PN3-21	539.10			539.10
PN4-22	195.60			195.60
PN6-24	638.60			638.60
CV1-25	297.10			297.10
CV2-26	320.10			320.10
CV3-27	144.60			144.60
CV4-28	268.60			268.60
CV5-29	319.60			319.60
CV6-30	262.10			262.10
CN1-31	270.10			270.10
CN2-32	326.60			326.60
CN3-33	209.10			209.10
CN4-34	295.10			295.10
CN5-35	397.60			397.60
CN6-36	294.60			294.60
C1-37V	265.10	323.60	53.60	642.30
C2-38V	338.60	445.10	54.10	837.80
C3-39V	509.10	240.10	75.60	824.80
C4-40V	154.60	220.10	114.60	489.30
C5-41V	321.10	338.10	35.10	694.30
C6-42V	345.10	722.10	66.60	1133.80
C7-43N	375.60	439.60	46.10	861.30
C8-44N	359.60	253.10		612.70
C9-45N	592.10	244.60		836.70
C11-47N	638.10	236.60	64.10	938.80
C12-48N	368.60	522.10	53.60	944.30

Table 7a: Effluent concentration of total volume from SSW addition one

Pot #	NO$_3$-N	NH$_4$-N	Orth-P	Total-P
	--------------- mg/L --------------			
PV1-13	1.56	0.15	1.11	1.21
PV2-14	0.77	0.17	0.83	0.80
PV3-15	1.30	0.17	1.02	1.06
PV6-18	1.62	0.14	1.69	2.08
PN1-19	0.71	0.16	0.89	0.83
PN2-20	0.36	0.15	0.94	0.84
PN3-21	0.98	0.17	0.90	0.86
PN4-22	1.71	0.17	0.89	0.81
PN6-24	2.00	0.17	0.93	0.91
CV1-25	1.01	0.15	1.21	1.37
CV2-26	0.63	0.13	1.27	1.41
CV3-27	0.66	0.14	1.52	1.79
CV4-28	0.82	0.14	1.18	1.24
CV5-29	1.34	0.13	1.38	1.65
CV6-30	1.10	0.13	1.35	1.55
CN1-31	0.79	0.13	1.44	1.68
CN2-32	1.19	0.14	1.29	1.46
CN3-33	1.42	0.14	1.36	1.49
CN4-34	0.44	0.13	1.30	1.46
CN5-35	1.55	0.13	1.55	1.86
CN6-36	0.39	0.14	1.36	1.51
C1-37V	20.60	0.14	1.90	2.33
C2-38V	24.20	0.14	2.34	3.04
C3-39V	26.20	0.14	1.60	1.88
C4-40V	76.70	0.14	2.24	2.86
C5-41V	44.60	0.14	2.42	3.16
C6-42V	17.30	0.14	1.82	2.09
C7-43N	5.90	0.14	1.50	1.73
C8-44N	55.10	0.14	1.99	2.66
C9-45N	32.30	0.13	1.78	2.31
C11-47N	25.20	0.14	2.50	3.18
C12-48N	17.20	0.14	2.13	2.82

Table 8a: Effluent concentration of total volume from SSW addition two

Pot #	NO$_3$-N	NH$_4$-N	Orth-P	Total-P
	-------------- mg/L --------------			
PV1-13	1.37	0.17	1.11	1.45
PV2-14	1.01	0.16	0.81	1.02
PV3-15	0.93	0.17	0.94	1.40
PV6-18	1.80	0.15	1.61	2.06
PN1-19	0.74	0.14	0.60	1.09
PN2-20	0.42	0.15	0.88	1.09
PN3-21	0.81	0.15	0.79	0.98
PN4-22	1.42	0.17	0.81	1.02
PN6-24	1.21	0.16	0.68	0.87
CV1-25	0.76	0.16	1.32	1.57
CV2-26	0.50	0.15	1.39	1.65
CV3-27	0.69	0.16	1.83	2.03
CV4-28	0.79	0.17	1.15	1.34
CV5-29	1.07	0.15	1.68	2.12
CV6-30	1.05	0.16	1.44	1.70
CN1-31	0.56	0.16	1.57	1.79
CN2-32	0.92	0.16	1.37	1.67
CN3-33	1.27	0.17	1.22	1.70
CN4-34	0.28	0.17	1.46	1.66
CN5-35	1.70	0.15	2.03	2.34
CN6-36	0.45	0.15	1.65	1.90
C1-37V	6.41	0.15	2.91	3.16
C2-38V	11.00	0.17	3.50	3.79
C3-39V	8.50	0.15	2.11	2.36
C4-40V	17.00	0.17	3.88	4.05
C5-41V	15.20	0.15	4.19	4.40
C6-42V	6.30	0.17	2.92	3.17
C7-43N	6.56	0.16	1.81	2.17
C8-44N	19.40	0.15	3.20	3.68
C9-45N	12.40	0.18	2.42	2.94
C11-47N	7.99	0.16	3.64	3.92
C12-48N	8.20	0.15	2.96	3.24

Table 9a: Effluent concentration of total volume from SSW addition three

Pot #	NO$_3$-N	NH$_4$-N	Orth-P	Total-P
	-------------- mg/L --------------			
PV1-13	0.86	0.14	1.59	1.87
PV2-14	0.88	0.14	0.82	1.04
PV3-15	0.94	0.17	1.39	1.67
PV6-18	3.02	0.15	2.79	3.05
PN1-19	0.90	0.15	1.08	1.32
PN2-20	0.56	0.14	1.20	1.39
PN3-21	0.89	0.15	0.97	1.17
PN4-22	1.01	0.14	0.93	1.10
PN6-24	1.44	0.14	0.89	1.05
CV1-25	1.06	0.17	1.52	1.80
CV2-26	1.25	0.13	1.77	1.97
CV3-27	0.93	0.14	2.22	2.47
CV4-28	1.17	0.15	1.39	1.57
CV5-29	1.81	0.15	2.41	2.71
CV6-30	1.46	0.16	1.78	1.97
CN1-31	1.31	0.13	1.79	2.03
CN2-32	1.10	0.15	1.63	1.81
CN3-33	1.83	0.14	1.73	1.94
CN4-34	0.66	0.14	2.06	2.02
CN5-35	2.27	0.14	3.17	2.76
CN6-36	0.97	0.15	2.36	2.24
C1-37V	2.83	0.17	4.77	4.83
C2-38V	3.39	0.15	5.04	5.12
C3-39V	3.72	0.14	3.27	3.45
C4-40V	3.64	0.13	6.27	6.22
C5-41V	5.49	0.14	6.37	6.16
C6-42V	3.43	0.14	4.39	4.53
C7-43N	2.72	0.20	2.98	3.27
C8-44N	4.27	0.15	5.89	5.97
C9-45N	3.06	0.14	3.61	3.85
C11-47N	3.94	0.15	5.20	5.24
C12-48N	3.72	0.13	4.32	4.47

Table 10a: Effluent concentration of total volume from SSW addition four

Pot #	NO_3-N	NH_4-N	Orth-P	Total-P
	-------------- mg/L --------------			
PV1-13	1.41	0.28	1.37	1.55
PV2-14	0.00	0.00	0.00	0.00
PV3-15	2.27	0.09	1.28	1.47
PV6-18	1.85	0.05	1.76	2.07
PN1-19	1.56	0.30	0.85	0.77
PN2-20	1.95	0.33	1.14	1.25
PN3-21	1.99	0.21	0.98	1.04
PN4-22	2.23	0.35	0.79	0.77
PN6-24	0.64	2.55	1.09	1.14
CV1-25	4.43	0.94	1.27	1.37
CV2-26	1.85	0.25	1.01	1.03
CV3-27	1.94	0.20	1.16	1.19
CV4-28	3.86	1.52	1.12	1.19
CV5-29	2.49	0.23	1.31	1.43
CV6-30	3.27	0.34	1.87	2.35
CN1-31	2.69	0.21	1.36	1.49
CN2-32	2.68	0.32	1.34	1.53
CN3-33	2.50	0.38	1.19	1.32
CN4-34	2.20	0.33	1.19	1.21
CN5-35	3.07	0.25	1.59	1.89
CN6-36	2.07	0.33	1.21	1.32
C1-37V	22.20	0.05	2.30	2.74
C2-38V	17.10	0.07	1.64	1.88
C3-39V	17.60	0.09	2.19	2.53
C4-40V	19.00	0.06	0.89	0.82
C5-41V	18.20	0.07	2.90	3.44
C6-42V	16.50	0.07	2.24	2.67
C7-43N	17.30	0.07	0.59	0.43
C8-44N	30.40	0.08	2.62	3.04
C9-45N	16.80	0.08	2.22	2.64
C11-47N	28.10	0.08	2.71	3.22
C12-48N	20.20	0.06	2.65	3.04

Table 11a: Effluent concentration of total volume from SSW addition five

Pot #	NO$_3$-N	NH$_4$-N	Orth-P	Total-P
	-------------- mg/L --------------			
PV1-13	3.69	0.09	1.28	1.35
PV2-14	3.74	0.09	0.98	0.88
PV3-15	2.77	0.08	1.21	1.22
PV6-18	3.43	0.09	1.60	1.70
PN1-19	3.63	0.11	0.94	0.71
PN2-20	4.04	0.09	1.02	0.98
PN3-21	3.79	0.09	0.87	0.78
PN4-22	4.07	0.09	0.84	0.69
PN6-24	2.45	0.08	1.01	0.95
CV1-25	4.65	0.09	1.26	1.04
CV2-26	3.22	0.09	1.25	0.97
CV3-27	3.64	0.09	1.42	1.22
CV4-28	4.89	0.10	1.10	0.91
CV5-29	4.61	0.09	1.54	1.45
CV6-30	4.07	0.08	1.48	1.25
CN1-31	3.18	0.09	1.23	1.13
CN2-32	3.63	0.10	1.10	1.00
CN3-33	3.82	0.09	1.25	1.12
CN4-34	3.42	0.10	1.15	1.04
CN5-35	3.43	0.09	1.41	1.40
CN6-36	3.54	0.11	1.30	1.19
C1-37V	18.40	0.34	2.60	2.85
C2-38V	18.80	0.10	2.37	2.45
C3-39V	15.50	0.21	1.84	2.00
C4-40V	16.40	0.09	7.19	7.82
C5-41V	16.90	0.10	3.22	3.41
C6-42V	13.90	0.09	2.95	3.08
C7-43N	15.10	0.21	0.42	0.30
C8-44N	23.80	0.09	1.46	1.36
C9-45N	14.70	0.09	2.48	2.43
C11-47N	20.30	0.09	2.89	3.26
C12-48N	16.70	0.09	2.82	3.05

Table 12a: Effluent concentration of total volume from SSW addition six

Pot #	NO_3-N	NH_4-N	Orth-P	Total-P
	-------------- mg/L --------------			
PV1-13	3.61	0.26	1.32	1.36
PV2-14	8.54	0.09	1.34	1.42
PV3-15	5.54	0.09	1.25	1.26
PV6-18	4.06	0.09	1.74	1.85
PN1-19	4.86	0.13	1.04	0.91
PN2-20	4.43	0.56	1.31	1.29
PN3-21	5.46	0.08	0.94	0.84
PN4-22	5.05	0.32	0.79	0.71
PN6-24	4.41	0.09	1.04	0.96
CV1-25	7.31	0.23	1.17	1.00
CV2-26	4.07	0.21	1.15	1.03
CV3-27	5.02	0.20	1.47	1.28
CV4-28	5.90	0.27	1.17	1.00
CV5-29	5.00	0.20	1.63	1.55
CV6-30	4.65	0.13	1.35	1.30
CN1-31	3.41	0.11	1.28	1.15
CN2-32	4.57	0.20	1.26	1.10
CN3-33	4.09	0.23	1.22	1.11
CN4-34	4.32	0.18	1.24	1.09
CN5-35	4.89	0.11	1.60	1.45
CN6-36	6.93	0.20	1.27	1.18
C1-37V	17.20	0.25	1.28	1.29
C2-38V	20.40	0.08	3.21	3.60
C3-39V	17.80	0.22	1.96	1.98
C4-40V	18.60	0.08	3.05	3.25
C5-41V	17.30	0.09	2.22	2.41
C6-42V	16.10	0.09	2.68	2.88
C7-43N	20.80	0.09	3.50	3.78
C8-44N	25.00	0.08	3.46	4.08
C9-45N	18.20	0.08	2.71	2.90
C11-47N	15.50	0.08	3.02	3.38
C12-48N	18.30	0.08	3.02	3.25

Appendix (b): Statistical Model (SAS 9.2)

```
DM 'LOG; CLEAR; OUTPUT; CLEAR;';
OPTIONS PAGENO=1 LS=85 NODATE PAGESIZE=75;
*TITLE 'Bond2.sas';

PROC IMPORT OUT=ONE
           DATAFILE= "H:\Statistics Department\Consulting
Clients\Ag\Bond\BOND1.xls"
           DBMS=EXCEL REPLACE;
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
      SHEET='Volume';

DATA TWO; SET ONE;

PROC PRINT;

PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN TIME;
MODEL VOL = PLANT|FUNGUS|TRANS|TIME/DDFM=KR;
RANDOM POT(PLANT*FUNGUS);
REPEATED /SUBJECT=TRANS*POT(PLANT*FUNGUS) TYPE=AR(1);
LSMEANS PLANT*FUNGUS*TRANS*TIME/SLICE=(PLANT*FUNGUS*TIME
PLANT*TRANS*TIME FUNGUS*TRANS*TIME PLANT*FUNGUS*TRANS);

RUN;
```

Figure 1(b) PROC MIXED statement for ANOVA model used to analyze volume data

```
DM 'LOG; CLEAR; OUTPUT; CLEAR;';
OPTIONS PAGENO=1 LS=85 NODATE PAGESIZE=75;
*TITLE 'Bond1.sas';

PROC IMPORT OUT=ONE
            DATAFILE= "H:\Statistics Department\Consulting
Clients\Ag\Bond\BOND1.xls"
            DBMS=EXCEL REPLACE;
     GETNAMES=YES;
     MIXED=NO;
     SCANTEXT=YES;
     USEDATE=YES;
     SCANTIME=YES;
       SHEET='Pollutants';

DATA TWO; SET ONE;

*PROC PRINT;

PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN;
MODEL TOTVOL = PLANT|FUNGUS|TRANS/DDFM=SATTERTH;
RANDOM POT(PLANT*FUNGUS);
LSMEANS PLANT*FUNGUS*TRANS/SLICE=(PLANT*FUNGUS PLANT*TRANS
FUNGUS*TRANS);

PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN;
MODEL C_NO3 = PLANT|FUNGUS|TRANS/DDFM=SATTERTH;
RANDOM POT(PLANT*FUNGUS);
LSMEANS PLANT*FUNGUS*TRANS/SLICE=(PLANT*FUNGUS PLANT*TRANS
FUNGUS*TRANS);

PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN;
MODEL C_NH4 = PLANT|FUNGUS|TRANS/DDFM=SATTERTH;
RANDOM POT(PLANT*FUNGUS);
LSMEANS PLANT*FUNGUS*TRANS/SLICE=(PLANT*FUNGUS PLANT*TRANS
FUNGUS*TRANS);
```

Figure 2(b): PROC MIXED statement for ANOVA model used to analyze nutrient data

```
PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN;
MODEL C_ORTHO = PLANT|FUNGUS|TRANS/DDFM=SATTERTH;
RANDOM POT(PLANT*FUNGUS);
LSMEANS PLANT*FUNGUS*TRANS/SLICE=(PLANT*FUNGUS PLANT*TRANS
FUNGUS*TRANS);

PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN;
MODEL C_ICP = PLANT|FUNGUS|TRANS/DDFM=SATTERTH;
RANDOM POT(PLANT*FUNGUS);
LSMEANS PLANT*FUNGUS*TRANS/SLICE=(PLANT*FUNGUS PLANT*TRANS
FUNGUS*TRANS);

PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN;
MODEL NO3 = PLANT|FUNGUS|TRANS/DDFM=SATTERTH;
RANDOM POT(PLANT*FUNGUS);
LSMEANS PLANT*FUNGUS*TRANS/SLICE=(PLANT*FUNGUS PLANT*TRANS
FUNGUS*TRANS);

PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN;
MODEL NH4 = PLANT|FUNGUS|TRANS/DDFM=SATTERTH;
RANDOM POT(PLANT*FUNGUS);
LSMEANS PLANT*FUNGUS*TRANS/SLICE=(PLANT*FUNGUS PLANT*TRANS
FUNGUS*TRANS);

PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN;
MODEL ORTHO = PLANT|FUNGUS|TRANS/DDFM=SATTERTH;
RANDOM POT(PLANT*FUNGUS);
LSMEANS PLANT*FUNGUS*TRANS/SLICE=(PLANT*FUNGUS PLANT*TRANS
FUNGUS*TRANS);

PROC MIXED;
CLASS POT FUNGUS PLANT TRANS RUN;
MODEL ICP = PLANT|FUNGUS|TRANS/DDFM=SATTERTH;
RANDOM POT(PLANT*FUNGUS);
LSMEANS PLANT*FUNGUS*TRANS/SLICE=(PLANT*FUNGUS PLANT*TRANS
FUNGUS*TRANS);

RUN;
```

Figure 2(b) (continued): PROC MIXED statement for ANOVA model used to analyze nutrient data

Appendix (c): Statistical Outputs

The Mixed Procedure: Dependant Effluent Collection Time

Table 1c: Least square means effluent collection time

Effect	Fungus	Plant	Trans	Time	Pr > |t|
Fung*Plan*Trans*Time	N	C	D	6	<0.001
Fung*Plan*Trans*Time	N	C	D	24	<0.001
Fung*Plan*Trans*Time	N	C	D	168	<0.001
Fung*Plan*Trans*Time	N	C	F	6	<0.001
Fung*Plan*Trans*Time	N	C	F	24	<0.001
Fung*Plan*Trans*Time	N	C	F	168	0.879
Fung*Plan*Trans*Time	N	P	D	6	<0.001
Fung*Plan*Trans*Time	N	P	D	24	0.002
Fung*Plan*Trans*Time	N	P	D	168	0.903
Fung*Plan*Trans*Time	N	P	F	6	<0.001
Fung*Plan*Trans*Time	N	P	F	24	0.987
Fung*Plan*Trans*Time	N	P	F	168	0.906
Fung*Plan*Trans*Time	V	C	D	6	<0.001
Fung*Plan*Trans*Time	V	C	D	24	<0.001
Fung*Plan*Trans*Time	V	C	D	168	<0.001
Fung*Plan*Trans*Time	V	C	F	6	<0.001
Fung*Plan*Trans*Time	V	C	F	24	<0.001
Fung*Plan*Trans*Time	V	C	F	168	0.744
Fung*Plan*Trans*Time	V	P	D	6	<0.001
Fung*Plan*Trans*Time	V	P	D	24	<0.001
Fung*Plan*Trans*Time	V	P	D	168	0.008
Fung*Plan*Trans*Time	V	P	F	6	<0.001
Fung*Plan*Trans*Time	V	P	F	24	0.989
Fung*Plan*Trans*Time	V	P	F	168	0.918

Table 2c: Test of effect slices effluent collection time

Effect	Fungus	Plant	Trans	Time	DF	DF	F-Value	Pr > F
Fung*Plan*Trans*Time	N	C		6	1	524	7.35	0.007
Fung*Plan*Trans*Time	N	C		24	1	522	1.56	0.212
Fung*Plan*Trans*Time	N	C		168	1	524	668	<0.001
Fung*Plan*Trans*Time	N	P		6	1	524	339.62	<0.001
Fung*Plan*Trans*Time	N	P		24	1	522	4.85	0.028
Fung*Plan*Trans*Time	N	P		168	1	524	0.03	0.863
Fung*Plan*Trans*Time	V	C		6	1	524	1.67	0.197
Fung*Plan*Trans*Time	V	C		24	1	522	1.24	0.267
Fung*Plan*Trans*Time	V	C		168	1	524	49.39	<0.001
Fung*Plan*Trans*Time	V	P		6	1	524	127.35	<0.001
Fung*Plan*Trans*Time	V	P		24	1	522	13.77	<0.001
Fung*Plan*Trans*Time	V	P		168	1	524	3.39	0.066
Fung*Plan*Trans*Time		C	D	6	1	438	0.01	0.924
Fung*Plan*Trans*Time		C	D	24	1	433	0.01	0.940
Fung*Plan*Trans*Time		C	D	168	1	438	2.75	0.098
Fung*Plan*Trans*Time		C	F	6	1	438	2.2	0.139
Fung*Plan*Trans*Time		C	F	24	1	433	0.03	0.867
Fung*Plan*Trans*Time		C	F	168	1	438	0.01	0.914
Fung*Plan*Trans*Time		P	D	6	1	438	27.26	<0.001
Fung*Plan*Trans*Time		P	D	24	1	433	2.65	0.104
Fung*Plan*Trans*Time		P	D	168	1	438	4.08	0.044
Fung*Plan*Trans*Time		P	F	6	1	438	1.28	0.259
Fung*Plan*Trans*Time		P	F	24	1	433	0.01	0.999
Fung*Plan*Trans*Time		P	F	168	1	438	0.01	0.994
Fung*Plan*Trans*Time	N		D	6	1	438	205.26	<0.001
Fung*Plan*Trans*Time	N		D	24	1	433	15.21	0.000
Fung*Plan*Trans*Time	N		D	168	1	438	92.26	<0.001
Fung*Plan*Trans*Time	N		F	6	1	438	10.2	0.002
Fung*Plan*Trans*Time	N		F	24	1	433	17.4	<0.001
Fung*Plan*Trans*Time	N		F	168	1	438	0.01	0.952
Fung*Plan*Trans*Time	V		D	6	1	438	108.86	<0.001
Fung*Plan*Trans*Time	V		D	24	1	433	6.8	0.010
Fung*Plan*Trans*Time	V		D	168	1	438	40.68	<0.001
Fung*Plan*Trans*Time	V		F	6	1	438	0.41	0.523
Fung*Plan*Trans*Time	V		F	24	1	433	20.72	<0.001
Fung*Plan*Trans*Time	V		F	168	1	438	0.04	0.845
Fung*Plan*Trans*Time	N	C	D		2	407	12.22	<0.001
Fung*Plan*Trans*Time	N	C	F		2	407	16	<0.001
Fung*Plan*Trans*Time	N	P	D		2	407	302.55	<0.001
Fung*Plan*Trans*Time	N	P	F		2	407	13.32	<0.001
Fung*Plan*Trans*Time	V	C	D		2	407	6.2	0.002
Fung*Plan*Trans*Time	V	C	F		2	407	11.63	<0.001
Fung*Plan*Trans*Time	V	P	D		2	407	123.58	<0.001
Fung*Plan*Trans*Time	V	P	F		2	407	18.99	<0.001

The Mixed Procedure: Dependant Total Volume

Table 3c: Test of fixed effects total volume

Effect	Num DF	Den DF	F Value	Pr > F
Plant	1	28.1	16.09	0.004
Fungus	1	28.1	1.93	0.176
Fung*Plan	1	28.1	1.44	0.241
Transpiration	1	155	486.02	<0.001
Plant*trans	1	155	33.15	<0.001
Fung*trans	1	155	2.9	0.091
Fung*plan*trans	1	155	0.28	0.600

Table 4c: Least square means total volume

Effect	Fungus	Plant	Trans	Estimate	Standard Error	DF	t Value	Pr > |t|
Fung*Plan*Trans	N	C	D	1.3713	0.07516	60.5	18.24	<0.001
Fung*Plan*Trans	N	C	F	0.7356	0.07516	60.5	9.79	<0.001
Fung*Plan*Trans	N	P	D	1.332	0.05067	60.5	36.31	<0.001
Fung*Plan*Trans	N	P	F	0.2402	0.05067	60.5	4.74	<0.001
Fung*Plan*Trans	V	C	D	1.1959	0.06861	60.5	17.43	<0.001
Fung*Plan*Trans	V	C	F	0.646	0.06861	60.5	9.41	<0.001
Fung*Plan*Trans	V	P	D	1.2423	0.05315	60.5	23.38	<0.001
Fung*Plan*Trans	V	P	F	0.3115	0.05381	60.5	5.79	<0.001

Table 5c: Test of effect slices total volume

Effect	Fungus	Plant	Trans	Num DF	Den DF	F-Value	Pr > F
Fung*Plan*Trans	N	C		1	155	53.34	<0.001
Fung*Plan*Trans	N	P		1	155	348.89	<0.001
Fung*Plan*Trans	V	C		1	155	47.9	<0.001
Fung*Plan*Trans	V	P		1	156	224.51	<0.001
Fung*Plan*Trans		C	D	1	60.5	2.97	0.090
Fung*Plan*Trans		C	F	1	60.5	0.78	0.382
Fung*Plan*Trans		P	D	1	60.5	1.53	0.221
Fung*Plan*Trans		P	F	1	61.7	0.93	0.338
Fung*Plan*Trans	N		D	1	60.5	0.18	0.676
Fung*Plan*Trans	N		F	1	60.5	29.87	<0.001
Fung*Plan*Trans	V		D	1	60.5	0.29	0.595
Fung*Plan*Trans	V		F	1	61.4	14.71	<0.001

The Mixed Procedure: Dependant Nutrient Concentration

Table 6c: Test of fixed effects nitrate concentration

Effect	Num DF	Den DF	F Value	Pr > F
Plant	1	28.1	177.83	<0.001
Fungus	1	28.1	0.03	0.857
Fung*Plan	1	28.1	0.01	0.925
Transpiration	1	155	8.52	0.004
Plant*trans	1	155	0.23	0.630
Fung*trans	1	155	1.13	0.290
Fung*plan*trans	1	155	1.56	0.214

Table 7c: Least square means nitrate concentration

Effect	Fungus	Plant	Trans	Estimate	Standard Error	DF	t Value	Pr > |t|
Fung*Plan*Trans	N	C	D	13.864	1.8464	93.8	7.51	<0.001
Fung*Plan*Trans	N	C	F	20.08	1.8464	93.8	10.88	<0.001
Fung*Plan*Trans	N	P	D	1.0381	1.2448	93.8	0.83	0.406
Fung*Plan*Trans	N	P	F	3.4848	1.2448	93.8	2.8	0.006
Fung*Plan*Trans	V	C	D	16.4728	1.6855	93.8	9.77	<0.001
Fung*Plan*Trans	V	C	F	17.6611	1.6855	93.8	10.48	<0.001
Fung*Plan*Trans	V	P	D	1.1379	1.3056	93.8	0.87	0.386
Fung*Plan*Trans	V	P	F	3.9927	1.3278	96.8	3.01	0.003

Table 8c: Test of effect slices nitrate concentration

Effect	Fungus	Plant	Trans	Num DF	Den DF	F Value	Pr > F
Fung*Plan*Trans	N	C		1	155	5.71	0.018
Fung*Plan*Trans	N	P		1	155	1.94	0.165
Fung*Plan*Trans	V	C		1	155	0.25	0.618
Fung*Plan*Trans	V	P		1	156	2.37	0.126
Fung*Plan*Trans		C	D	1	93.8	1.09	0.299
Fung*Plan*Trans		C	F	1	93.8	0.94	0.336
Fung*Plan*Trans		P	D	1	93.8	0	0.956
Fung*Plan*Trans		P	F	1	95.4	0.08	0.781
Fung*Plan*Trans	N		D	1	93.8	33.18	<0.001
Fung*Plan*Trans	N		F	1	93.8	55.54	<0.001
Fung*Plan*Trans	V		D	1	93.8	51.74	<0.001
Fung*Plan*Trans	V		F	1	94.9	40.58	<0.001

Table 9c: Test of fixed effects ammonium concentration

Effect	Num DF	Den DF	F Value	Pr > F
Plant	1	183	4.72	0.031
Fungus	1	183	0.01	0.927
Fung*Plan	1	183	0.25	0.615
Transpiration	1	183	0.65	0.420
Plant*trans	1	183	4.53	0.035
Fung*trans	1	183	0.01	0.929
Fung*plan*trans	1	183	0.32	0.573

Table 10c: Least square means ammonium concentration

Effect	Fungus	Plant	Trans	Estimate	Standard Error	DF	t Value	Pr > |t|
Fung*Plan*Trans	N	C	D	0.1493	0.05556	183	2.69	0.008
Fung*Plan*Trans	N	C	F	0.09038	0.05556	183	1.63	0.106
Fung*Plan*Trans	N	P	D	0.1488	0.03746	183	3.97	<0.001
Fung*Plan*Trans	N	P	F	0.2668	0.03746	183	7.12	<0.001
Fung*Plan*Trans	V	C	D	0.1473	0.05071	183	2.9	0.004
Fung*Plan*Trans	V	C	F	0.1195	0.05071	183	2.36	0.020
Fung*Plan*Trans	V	P	D	0.1508	0.03928	183	3.84	0.000
Fung*Plan*Trans	V	P	F	0.2257	0.03996	183	5.56	<0.001

Table 11c: Test of effect slices ammonium concentration

Effect	Fungus	Plant	Trans	Num DF	Den DF	F Value	Pr > F
Fung*Plan*Trans	N	C		1	183	0/56	0.454
Fung*Plan*Trans	N	P		1	183	4.95	0.027
Fung*Plan*Trans	V	C		1	183	0.15	0.699
Fung*Plan*Trans	V	P		1	183	1.79	0.183
Fung*Plan*Trans		C	D	1	183	0	0.978
Fung*Plan*Trans		C	F	1	183	0.15	0.699
Fung*Plan*Trans		P	D	1	183	0	0.972
Fung*Plan*Trans		P	F	1	183	0.56	0.455
Fung*Plan*Trans	N		D	1	183	0	0.994
Fung*Plan*Trans	N		F	1	183	6.93	0.009
Fung*Plan*Trans	V		D	1	183	0	0.957
Fung*Plan*Trans	V		F	1	183	2.71	0.102

Table 12c: Test of fixed effects Ortho-phosphate concentration

Effect	Num DF	Den DF	F Value	Pr > F
Plant	1	27.9	92.33	<0.001
Fungus	1	27.9	1.66	0.208
Fung*Plan	1	27.9	0.01	0.911
Transpiration	1	155	42.65	<0.001
Plant*trans	1	155	10.66	0.001
Fung*trans	1	155	0.2	0.658
Fung*plan*trans	1	155	0.09	0.763

Table 13c: Test least square means Ortho-phosphate concentration

Effect	Fungus	Plant	Trans	Estimate	Standard Error	DF	t Value	Pr > |t|
Fung*Plan*Trans	N	C	D	3.3857	0.2376	52	14.25	<0.001
Fung*Plan*Trans	N	C	F	2.4376	0.2376	52	10.26	<0.001
Fung*Plan*Trans	N	P	D	1.4735	0.1602	52	9.2	<0.001
Fung*Plan*Trans	N	P	F	1.1461	0.1602	52	7.15	<0.001
Fung*Plan*Trans	V	C	D	3.7007	0.2169	52	17.06	<0.001
Fung*Plan*Trans	V	C	F	2.5962	0.2169	52	11.97	<0.001
Fung*Plan*Trans	V	P	D	1.6868	0.168	52	10.04	<0.001
Fung*Plan*Trans	V	P	F	1.3299	0.1698	53.8	7.83	<0.001

Table 14c: Test effect slices Ortho-phosphate concentration

Effect	Fungus	Plant	Trans	Num DF	Den DF	F Value	Pr > F
Fung*Plan*Trans	N	C		1	155	14.32	<0.001
Fung*Plan*Trans	N	P		1	155	3.76	0.055
Fung*Plan*Trans	V	C		1	155	23.32	<0.001
Fung*Plan*Trans	V	P		1	155	3.98	0.048
Fung*Plan*Trans		C	D	1	52	0.96	0.332
Fung*Plan*Trans		C	F	1	52	0.24	0.624
Fung*Plan*Trans		P	D	1	52	0.84	0.362
Fung*Plan*Trans		P	F	1	52	0.62	0.435
Fung*Plan*Trans	N		D	1	52	44.52	<0.001
Fung*Plan*Trans	N		F	1	52	20.31	<0.001
Fung*Plan*Trans	V		D	1	52	53.86	<0.001
Fung*Plan*Trans	V		F	1	52.7	21.13	<0.001

Table 15c: Test fixed effects Total-phosphate concentration

Effect	Num DF	Den DF	F Value	Pr > F
Plant	1	27.9	97.78	<0.001
Fungus	1	27.9	1.66	0.209
Fung*Plan	1	27.9	0.08	0.779
Transpiration	1	155	7.47	0.007
Plant*trans	1	155	1.75	0.188
Fung*trans	1	155	0.06	0.802
Fung*plan*trans	1	155	0.41	0.523

Table 16c: Least square means Total-phosphate concentration

Effect	Fungus	Plant	Trans	Estimate	Standard Error	DF	t Value	Pr > |t|
Fung*Plan*Trans	N	C	D	3.062	0.2524	60	12.13	<0.001
Fung*Plan*Trans	N	C	F	2.6763	0.2524	60	10.6	<0.001
Fung*Plan*Trans	N	P	D	1.3248	0.1702	60	7.79	<0.001
Fung*Plan*Trans	N	P	F	1.1056	0.1702	60	6.5	<0.001
Fung*Plan*Trans	V	C	D	3.4411	0.2304	60	14.93	<0.001
Fung*Plan*Trans	V	C	F	2.8382	0.2304	60	12.32	<0.001
Fung*Plan*Trans	V	P	D	1.4503	0.1785	60	8.13	<0.001
Fung*Plan*Trans	V	P	F	1.3257	0.18707	62.2	7.34	<0.001

Table 17c: Effect slices Total-phosphate concentration

Effect	Fungus	Plant	Trans	Num DF	Den DF	F Value	Pr > F
Fung*Plan*Trans	N	C		1	155	1.75	0.188
Fung*Plan*Trans	N	P		1	155	1.24	0.266
Fung*Plan*Trans	V	C		1	155	5.14	0.025
Fung*Plan*Trans	V	P		1	155	0.36	0.550
Fung*Plan*Trans		C	D	1	60	1.23	0.272
Fung*Plan*Trans		C	F	1	60	0.22	0.637
Fung*Plan*Trans		P	D	1	60	0.26	0.613
Fung*Plan*Trans		P	F	1	61.2	0.79	0.379
Fung*Plan*Trans	N		D	1	60	32.57	<0.001
Fung*Plan*Trans	N		F	1	60	26.62	<0.001
Fung*Plan*Trans	V		D	1	60	46.66	<0.001
Fung*Plan*Trans	V		F	1	60.8	26.68	<0.001

Table 18c: Test of fixed effects nitrate load

Effect	Num DF	Den DF	F Value	Pr > F
Plant	1	183	138.38	<0.001
Fungus	1	183	0.03	0.855
Fung*Plan	1	183	0.11	0.742
Transpiration	1	183	5.42	0.021
Plant*trans	1	183	4.12	0.044
Fung*trans	1	183	1.03	0.312
Fung*plan*trans	1	183	1.3	0.257

Table 19c: Least square means nitrate load

Effect	Fungus	Plant	Trans	Estimate	Standard Error	DF	t Value	Pr > |t|
Fung*Plan*Trans	N	C	D	17.2914	2.0669	183	8.37	<0.001
Fung*Plan*Trans	N	C	F	14.596	2.0669	183	7.06	<0.001
Fung*Plan*Trans	N	P	D	1.4236	1.3935	183	1.02	0.406
Fung*Plan*Trans	N	P	F	0.907	1.3935	183	0.65	0.006
Fung*Plan*Trans	V	C	D	19.2964	1.8868	183	10.23	<0.001
Fung*Plan*Trans	V	C	F	11.3393	1.8868	183	6.01	<0.001
Fung*Plan*Trans	V	P	D	1.452	1.4615	183	0.99	0.386
Fung*Plan*Trans	V	P	F	1.2372	1.4865	183	0.83	0.003

Table 20c: Test of effect slices nitrate load

Effect	Fungus	Plant	Trans	Num DF	Den DF	F Value	Pr > F
Fung*Plan*Trans	N	C		1	183	0.85	0.358
Fung*Plan*Trans	N	P		1	183	0.07	0.794
Fung*Plan*Trans	V	C		1	183	8.89	0.003
Fung*Plan*Trans	V	P		1	183	0.01	0.918
Fung*Plan*Trans		C	D	1	183	0.51	0.475
Fung*Plan*Trans		C	F	1	183	1.35	0.246
Fung*Plan*Trans		P	D	1	183	0	0.989
Fung*Plan*Trans		P	F	1	183	0.03	0.872
Fung*Plan*Trans	N		D	1	183	40.52	<0.001
Fung*Plan*Trans	N		F	1	183	30.16	<0.001
Fung*Plan*Trans	V		D	1	183	55.9	<0.001
Fung*Plan*Trans	V		F	1	183	17.69	<0.001

Table 21c: Test of effect slices ammonium load

Effect	Num DF	Den DF	F Value	Pr > F
Plant	1	28.1	0.6	0.446
Fungus	1	28.1	1.11	0.301
Fung*Plan	1	28.1	0.01	0.907
Transpiration	1	155	197.83	<0.001
Plant*trans	1	155	1.27	0.261
Fung*trans	1	155	0.91	0.343
Fung*plan*trans	1	155	1	0.319

Table 22c: Least square means ammonium load

Effect	Fungus	Plant	Trans	Estimate	Standard Error	DF	t Value	Pr > |t|
Fung*Plan*Trans	N	C	D	0.2023	0.01594	87.5	12.69	<0.001
Fung*Plan*Trans	N	C	F	0.06601	0.01594	87.5	4.14	<0.001
Fung*Plan*Trans	N	P	D	0.1971	0.01075	87.5	18.34	<0.001
Fung*Plan*Trans	N	P	F	0.0585	0.01075	87.5	5.44	<0.001
Fung*Plan*Trans	V	C	D	0.1754	0.01455	87.5	12.05	<0.001
Fung*Plan*Trans	V	C	F	0.07481	0.01455	87.5	5.14	<0.001
Fung*Plan*Trans	V	P	D	0.18862	0.01127	87.5	16.52	<0.001
Fung*Plan*Trans	V	P	F	0.04671	0.01146	87.5	4.08	<0.001

Table 23c: Test of effect slices ammonium load

Effect	Fungus	Plant	Trans	Num DF	Den DF	F Value	Pr > F
Fung*Plan*Trans	N	C		1	155	38.75	<0.001
Fung*Plan*Trans	N	P		1	155	88.2	<0.001
Fung*Plan*Trans	V	C		1	155	25.31	<0.001
Fung*Plan*Trans	V	P		1	155	79.79	<0.001
Fung*Plan*Trans		C	D	1	87.5	1.56	0.216
Fung*Plan*Trans		C	F	1	87.5	0.17	0.684
Fung*Plan*Trans		P	D	1	87.5	0.49	0.485
Fung*Plan*Trans		P	F	1	89.1	0.56	0.455
Fung*Plan*Trans	N		D	1	87.5	0.07	0.788
Fung*Plan*Trans	N		F	1	87.5	0.15	0.697
Fung*Plan*Trans	V		D	1	87.5	0.35	0.558
Fung*Plan*Trans	V		F	1	88.7	2.3	0.133

Table 24c: Test of fixed effects Ortho-phosphate load

Effect	Num DF	Den DF	F Value	Pr > F
Plant	1	28	63.85	<0.001
Fungus	1	28	0.19	0.670
Fung*Plan	1	28	1.17	0.289
Transpiration	1	155	179.78	<0.001
Plant*trans	1	155	12.78	0.001
Fung*trans	1	155	0.08	0.778
Fung*plan*trans	1	155	0.11	0.738

Table 25c: Least square means Ortho-phosphate load

Effect	Fungus	Plant	Trans	Estimate	Standard Error	DF	t Value	Pr > |t|
Fung*Plan*Trans	N	C	D	4.9451	0.3647	60.3	13.56	<0.001
Fung*Plan*Trans	N	C	F	1.8374	0.3647	60.3	5.04	<0.001
Fung*Plan*Trans	N	P	D	2.0009	0.2459	60.3	8.14	<0.001
Fung*Plan*Trans	N	P	F	0.2739	0.2459	60.3	1.11	<0.001
Fung*Plan*Trans	V	C	D	4.4602	0.3329	60.3	13.4	<0.001
Fung*Plan*Trans	V	C	F	1.5706	0.3329	60.3	4.72	<0.001
Fung*Plan*Trans	V	P	D	2.172	0.2579	60.3	8.42	<0.001
Fung*Plan*Trans	V	P	F	0.4263	0.2611	60.3	1.63	<0.001

Table 26c: Test of effect slices Ortho-phosphate load

Effect	Fungus	Plant	Trans	Num DF	Den DF	F Value	Pr > F
Fung*Plan*Trans	N	C		1	155	54.16	<0.001
Fung*Plan*Trans	N	P		1	155	36.8	<0.001
Fung*Plan*Trans	V	C		1	155	56.18	<0.001
Fung*Plan*Trans	V	P		1	156	33.55	<0.001
Fung*Plan*Trans		C	D	1	60.3	0.96	0.330
Fung*Plan*Trans		C	F	1	60.3	0.29	0.591
Fung*Plan*Trans		P	D	1	60.3	0.23	0.633
Fung*Plan*Trans		P	F	1	61.5	0.18	0.672
Fung*Plan*Trans	N		D	1	60.3	44.82	<0.001
Fung*Plan*Trans	N		F	1	60.3	12.64	0.001
Fung*Plan*Trans	V		D	1	60.3	29.53	<0.001
Fung*Plan*Trans	V		F	1	61.2	7.32	0.009

Table 27c: Test of fixed effects Total-phosphate load

Effect	Num DF	Den DF	F Value	Pr > F
Plant	1	28	66.7	<0.001
Fungus	1	28	0.19	0.668
Fung*Plan	1	28	0.84	0.368
Transpiration	1	155	111.18	<0.001
Plant*trans	1	155	6.51	0.012
Fung*trans	1	155	0.06	0.805
Fung*plan*trans	1	155	0.01	0.929

Table 28c: Least square means Total-phosphate load

Effect	Fungus	Plant	Trans	Estimate	Standard Error	DF	t Value	Pr > |t|
Fung*Plan*Trans	N	C	D	4.5098	0.3693	66.2	12.21	<0.001
Fung*Plan*Trans	N	C	F	2.0207	0.3693	66.2	5.47	<0.001
Fung*Plan*Trans	N	P	D	1.8232	0.249	66.2	7.32	<0.001
Fung*Plan*Trans	N	P	F	0.2588	0.249	66.2	1.04	0.302
Fung*Plan*Trans	V	C	D	4.1499	0.3371	66.2	12.31	<0.001
Fung*Plan*Trans	V	C	F	1.7202	0.3371	66.2	5.1	<0.001
Fung*Plan*Trans	V	P	D	1.8777	0.2611	66.2	7.19	<0.001
Fung*Plan*Trans	V	P	F	0.4396	0.2646	68.7	1.66	0.101

Table 29c: Test of effect slices Total-phosphate load

Effect	Fungus	Plant	Trans	Num DF	Den DF	F Value	Pr > F
Fung*Plan*Trans	N	C		1	155	30.7	<0.001
Fung*Plan*Trans	N	P		1	155	26.68	<0.001
Fung*Plan*Trans	V	C		1	155	35.11	<0.001
Fung*Plan*Trans	V	P		1	156	20.13	<0.001
Fung*Plan*Trans		C	D	1	66.2	0.52	0.474
Fung*Plan*Trans		C	F	1	66.2	0.36	0.550
Fung*Plan*Trans		P	D	1	66.2	0.02	0.880
Fung*Plan*Trans		P	F	1	67.5	0.25	0.620
Fung*Plan*Trans	N		D	1	66.2	36.39	<0.001
Fung*Plan*Trans	N		F	1	66.2	15.65	<0.001
Fung*Plan*Trans	V		D	1	66.2	28.4	<0.001
Fung*Plan*Trans	V		F	1	67.1	8.93	0.004

Appendix (d): Standard Operation Procedures

Cleaning sample containers

Before Effluent samples were taken each effluent sample container and lid was washed with phosphorus free soap and rinsed with 30% HCl then rinsed with DI water. Phosphate free soap was added to each container and lid then scrubbed with a bottlebrush until no residue remained on the surface. Containers were then rinsed with tap water six times. Once rinsed, 10 ml of 30% HCl solution was added to each container and shaken with the lid tightened for 10 seconds. Lids were removed and containers and lids were rinsed with DI water six times. Containers and lids were allowed to air dry and were stored in plastic bags until ready for effluent collection.

Synthetic Stormwater (SSW) Preparation

Four 18L solutions were prepared for each SSW application with the three respective constituents in each solution: sodium nitrate (2 mg/L as N), phosphorus (2 mg/L as P), and ammonium chloride (2 mg/L as N). The pH of each solution was noted but not adjusted if found to be in the range of 6.5-6.8, no adjustment was necessary for any of the applications.

Sodium nitrate (561.173 mg/L) was added to the 18L of DI water and allowed to dissolve. Sodium phosphate (110.69 mg/L) was added to the 18L of DI water and

allowed to dissolve. Ammonium chloride (353.193 mg/L) was added to the 18L of DI water and allowed to dissolve. Once all three constituents were placed into the 18L of water the water was transferred to an empty 18L container and then back into the original container, this was repeated three times for each mixture to better dissolve the chemical constituents.

Determination of Percent Colonization

The extent of colonization of plant roots by mycorrhizal fungi was evaluated using " Quantification of Vesicular-Arbuscular Mycorrhizal Colonization in Roots" found in chapter 18 of SSSA Book Series: 5, Methods of Soil Analysis: Part 2- Microbial and Biochemical Properties.

Inoculum and Control Inoculum

Vesicular Arbuscular Mycorrhizae (VAM) used for this experiment was acquired from the International Culture Collection of (Vesicular) Mycorrhizal Fungi (INVAM). This non-profit research organization is located on the Evansdale Campus of West Virginia University. Through INVAM Dr. Joseph B. Morton, Professor & Curator and Dr. Ranamalie Amarasinghe, Assoc. Curator "Acquire, propagate, characterize, and maintain germplasm of arbuscular mycorrhizal fungi in living cultures for preservation and distribution to any person or institution."

Vesicular Arbuscular Mycorrhizae obtained from INVAM was used to prepare a 1:35 ratio of bulk inoculum to topsoil mixture by adding 1.2 liters of bulk inoculum containing 1:1:1 (v/v/v) of *Glomus clarum, G. intraradices,* and *G. mosseae*. This procedure was also used to prepare a 1:35 ratio sterile control inoculum known to be devoid of VAM. Inoculum was delivered in a perlite medium with viable VAM spores and colonized roots of Switchgrass. The control inoculum was an identical medium guaranteed to be devoid of VAM spores and with non-colonized roots from Switchgrass.

Appendix (e): Acronyms

- Oklahoma Water Resources Board (OWRB)
- Low Impact Development (LID)
- Urbanized Area (UA)
- Concentrated Animal Feeding Operations (CAFOs)
- National Pollutant Discharge Elimination System (NPDES)
- Environmental Protection Agency (EPA)
- Clean Water Act (CWA),
- Non-point Sources of Pollution (NPS)
- Point Source Pollution (PS)
- Vesicular Arbuscular Mycorrhizal Fungus (VAM)
- Ectomycorrhizal Fungus (EM)
- Municipal Separate Storm Sewer Systems (MS4s)

VITA

Steven Eric Bond

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF DORMANT AND FOLIAGED PLANTS ON NITRATE
            AND PHOSPHATE RETENTION IN BIORETENTION CELLS

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Title of Study: EFFECT OF DORMANT AND FOLIAGED PLANTS ON NITRATE
                AND PHOSPHATE RETENTION IN BIORETENTION CELLS

Pages in Study: 137   Candidate for the Degree of Master of Science

Major Field: Environmental Sciences – Watershed Resources Management

Scope and Method of Study: This study is an assessment of the effect of plants, vesicular
        arbuscular mycorrhizal fungi (VAM), and seasonality on Bioretention Cell
        performance. Performance was evaluated as retention of nitrate, ammonium, and
        phosphate during foliaged and dormant seasons. This study is intended to
        quantify the significance of plant presence and VAM interaction in laboratory-
        scale Bioretention Cells by comparing percent changes in effluent volume and
        nutrient concentration and load following two-liter applications of a synthetic
        storm water (SSW) containing 2mg/L nitrate, ammonium, and phosphate (as N
        and P). Bioretention Cells were leached with deionized water (DI) for 33-weeks
        to bring leachate concentration below SSW levels. In week thirty-four DI was
        substituted with SSW weekly for three weeks while plants were dormant
        (12_Jan_08 through 2_Feb_08) and while plants were foliaged (19_Apr_08
        through 10_May_08).

Findings and Conclusions: Plants have a significant role in the process of bioretention.
        Vesicular arbuscular mycorrhizal fungi where not initially present in the roots of
        plants used for this study and failed to colonize the roots of these plants during
        this experiment, VAM was not significant in any of the results. Bioretention
        Cells with dormant and foliaged plants infiltrated and released effluent
        significantly faster than Bioretention Cells without plants. Foliaged plants
        significantly reduced total effluent volume. Very little ammonium was released
        by any Bioretention Cell, with or without plants. Dormant and foliaged plants
        overall reduced effluent concentration and load of nitrates and phosphates, but
        controls only reduced phosphates. Bioretention cells with foliaged plants
        increased nitrate effluent concentration but reduced load. Plants significantly
        influence infiltration rates, effluent concentration and loading of nitrates,
        phosphates, and ammonium; seasonality (dormant/foliaged) significantly
        influence these results.