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16. Abstract This report summarizes the results of pilot-scale bioretention experiments. Five steel boxes of 6 ft (L) × 6 ft (W) × 4 ft (D) were constructed, each of which has a different type of vegetation: (1) shrubs, (2) grass species in Texas Department of Transportation (TxDOT) Bryan District seed mix, (3) native grasses, (4) Bermudagrass, and (5) no vegetation as the control. Vegetation was given 14 months to establish before testing. Synthetic runoff containing predetermined pollutants with target concentrations was used. The results indicate that pilot bioretentions effectively removed zinc (Zn), lead (Pb), total suspended solids (TSS), and ammonia-nitrogen (NH ₃ -N) from stormwater runoff, but exported copper (Cu), nitrate-nitrogen (NO ₃ -N), total nitrogen (TN), and total phosphorus (TP). Vegetation plays an important role on NO ₃ -N and TN removals due to root uptake and the denitrification processes in root zone. However, vegetation could negatively affect the water quality if the soil infiltration rate is significantly increased by its root system. This effect was specifically obvious on the TSS removal, in which the control box with the longest detention time had much better performance than the vegetated boxes. The results suggest that bioretention is useful to treat stormwater runoff from TxDOT highways, but the design specifications developed in other states should be revised to reflect Texas' unique climate and environmental conditions. Challenges and learned lessons are described in the report.					
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BIORETENTION FOR STORMWATER QUALITY IMPROVEMENT IN TEXAS: PILOT EXPERIMENTS

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DISCLAIMER

This research was performed in cooperation with the Texas Department of Transportation (TxDOT) and the Federal Highway Administration (FHWA). The contents of this report reflect the views of the authors, who are responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official view or policies of the FHWA or TxDOT. This report does not constitute a standard, specification, or regulation.

This report is not intended for construction, bidding, or permit purposes. The engineer in charge of the project was Dr. Ming-Han Li, P.E. #91045.

The United States Government and the State of Texas do not endorse products or manufacturers. Trade or manufacturers' names appear herein solely because they are considered essential to the object of this report.

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CHAPTER 1: INTRODUCTION

Bioretention is a stormwater best management practice (BMP) designed to remove pollutants in stormwater runoff. A typical bioretention consists of (from top to bottom) water storage space, vegetation, mulch, soil filter media, and a gravel layer (see Figure 1). It removes pollutants from the runoff via physical, chemical, and biological processes, including sedimentation, filtration, and sorption on mulch and soil layers, plant uptake, and biodegradation by soil microorganisms (Davis et al., 2001). Since first developed in Prince George's County, Maryland, in the late 1980s, bioretention has been established for residential and industrial applications, such as residential gardens, parking lots, streets, and highways. As bioretention has proven to be very effective for treating stormwater runoff, the Environmental Protection Agency (EPA, 2000) recommends bioretention as one of the low-impact development practices.

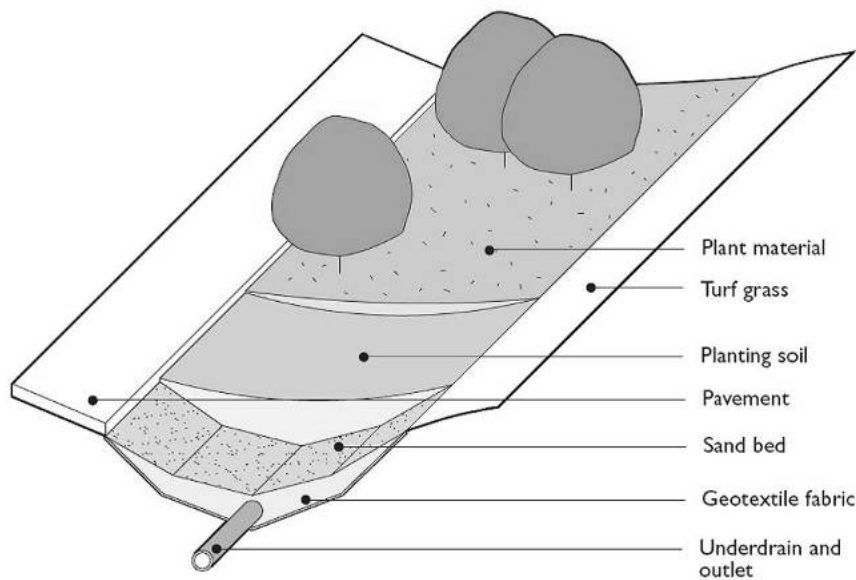


Figure 1. Conceptual Diagram of Bioretention.
(excerpted from Skidmore, Owings & Merrill LLP, 2005)

Despite its popularity nationwide, bioretention is not included in any current TxDOT stormwater management guidelines, such as the Storm Water Management Guidelines for Construction Activities (TxDOT, 2002), the Hydraulic Design Manual (TxDOT, 2004), and the

Landscape and Aesthetics Design Manual (TxDOT, 2007). Existing bioretention manuals may not directly be applicable to TxDOT rights of way and other facilities because they are based on studies in northern states where the climate is significantly different from Texas (e.g., Prince George's County, 2002; Wisconsin Department of Natural Resource, 2003; Puget Sound Action Team, 2005). Considering the hot, arid, and semi-arid climates in Texas, design specifications, such as the types of vegetation, the depth and property of soil filter media, and managerial schemes, must be revised.

This project aims to develop a bioretention design guideline for treating stormwater runoff from TxDOT highways. It proceeds in three major tasks: reviewing existing literature, conducting pilot-scale laboratory experiments, and constructing and monitoring field demonstrations in a real TxDOT highway environment. The existing literature was summarized in the first year report. This second year report describes the findings of the pilot-scale laboratory experiments. The primary goal of this report is to summarize what was learned from the pilot testing results and to adjust design parameters to be experimented through field demonstration projects in the next stage. The pollutant removal performances by selected vegetation were examined using pilot-scale bioretention boxes constructed in a laboratory. This report identifies potential problems learned from the pilot testing and discusses likely solutions for the construction and maintenance of bioretention on TxDOT highways.

CHAPTER 2: MATERIAL AND METHODS

CONSTRUCTION OF PILOT BIORETENTIONS

Five pilot-scale bioretention boxes were constructed on April 24, 2008, at the TxDOT/TTI Hydraulics, Sedimentation, and Erosion Control Laboratory (HSECL) located at Texas A&M University Riverside Campus. The five boxes, each with a dimension of 6-ft long × 6-ft wide × 4-ft deep, were reconstructed from metal garbage dumpsters. To prevent potential corrosion, the inner surfaces of the boxes were first coated with truck bed spray liner (40 percent polyurethane and 60 percent polyurea). After coating, each box was filled with an underdrain polyvinyl chloride (PVC) pipe, followed by an 8-inch gravel layer, a 4-inch pea gravel layer, and a 2-ft depth of compost-amended soil. Grass seeds or nursery shrubs were then applied onto the soil. One foot above the surface of the soil media was the space for detaining stormwater (Figure2).



a. metal garbage dumpsters



b. coated with liner/4 inches PVC underdrain



c. gravel layer (8 inches in depth)



d. pea gravel layer (4 inches in depth)



e. soil/compost filter media (2 feet in depth)



f. vegetation

Figure 2. Construction of Pilot Bioretention.

The 4-inch perforated PVC pipe that served as underdrain was placed laterally through the center of box floor, with one end of the pipe extended to the outside of the box as an outlet. The underdrain pipe was packed with an 8-inch deep gravel layer (particle sizes ranging from 1 to 1½ inches in diameter) and then with a 4-inch deep pea gravel layer (particle size of approximately 3/8 inch in diameter) to separate the PVC pipe from the compost amended soil media atop.

Above the pea gravel layer was 2 ft of the filter media. As recommended by existing literature, sandy soil amended with compost was used to quickly drain water out of the pilot boxes. Although compost provides nutrients that are essential for plant growth, the use of compost has potential adverse impact on effluent water quality if the nutrients in compost soil are leached out when stormwater passes through the pilot boxes. An optimum soil/compost mixing ratio was determined based on the infiltration rates of three different soil/compost mixtures using 1-ft deep columns with a 4-inch diameter. The infiltration rates were 12.0, 10.5, and 9.75 inches/hour for soil/compost soil ratios of 5:5, 6:4, and 7:3, respectively. These three infiltration rates meet the recommended criterion of soil media selection, i.e., the infiltration rate must be 1 inch/hour or greater (Prince George's County, 2002), but are higher than the ranges of infiltration rates in the previous literature. Thus, the mixture with the lowest infiltration rate, i.e., soil/compost ratio equals 7:3, was selected for the filter media of the pilot testing. Although adding compost decreased the infiltration rate, the mixtures with the compost ratio lower than 30 percent were not chosen because compost provides organic matter that is essential to plant growth. The chemical and textural properties of the soil/compost mixture were analyzed by the Soil, Water and Forage Testing Laboratory at Texas A&M University and are listed in Table 1.

Table 1. Characteristics of Soil/Compost Media.

Chemical Analysis	Soil without compost	Soil with 30% compost
pH	8.6	7.6
Conductivity	74 umho/cm	156 umho/cm
NO ₃ -N	4 ppm	14 ppm
P	8 ppm	191 ppm
K	73 ppm	190 ppm
Ca	5500 ppm	4900 ppm
Mg	95 ppm	183 ppm
S	13 ppm	21 ppm
Na	163 ppm	147 ppm
Fe	-	11.5 ppm
Zn	-	9.24 ppm
Mn	-	7.09 ppm
Cu	-	1.16 ppm
Organic matter	0.35%	2.90%
Organic Carbon	0.20%	1.68%
Textural Analysis		
Sand	84%	81%
Silt	4%	2%
Clay	12%	17%
Textural Class	Loamy Sand	Sandy Loam

The top layer of the boxes was vegetation. Each box was randomly assigned to one of five different types of vegetation: shrubs, native grass seedmix, TxDOT seedmix, bermudagrass, and control (no vegetation). Nursery plants were transplanted to the shrub box. For the remaining three boxes, grass seeds obtained from local suppliers were applied. Table 2 lists species names and pure live seed (PLS) rates. All boxes were regularly irrigated with tap water during the whole study period (twice a week in summer and twice a month in other seasons). To maintain bare soil in the control box, all weeds were removed by hand approximately once every two weeks. In the shrub box, weeds taller than the shrubs were regularly removed. Weeds were removed with care to prevent soil compaction and other disturbances.

Table 2. Planted Grass and Shrub Species in Pilot Boxes.

Box	Species		Seeding Rates (lb/acre)	PLS (%)
	Botanical Name	Common Name		
Shrub	<i>Ilex vomitoria</i> 'Stokes Dwarf'	Stroke Dwarf Yaupon Holly	3 counts	-
	<i>Morella cerifera</i>	Wax Myrtle	3 counts	-
	<i>Leucophyllum frutescens</i> 'Bertstar Dwarf'	Texas Sage (Cenizo)	3 counts	-
TxDOT Bryan District seedmix for sand	<i>Cynodon dactylon</i>	Bermudagrass	1.5	95.0
	<i>Eragrostis curvula</i>	Weeping Lovegrass (Ermello)	0.6	92.7
	<i>Eragrostis trichodes</i>	Sand Lovegrass	0.6	88.7
	<i>Leptochloa dubia</i>	Green Sprangletop	0.3	93.0
	<i>Paspalum notatum</i>	Bahiagrass (Pensacola)	7.5	95.0
	<i>Coreopsis lanceolata</i>	Lance Leaf Coreopsis	1	89.6
Native grass seedmix	<i>Bouteloua curtipendula</i>	Sideoats Grama	10	89.6
	<i>Leptochloa dubia</i>	Green Sprangletop	5	93.0
	<i>Schizachyrium scoparium</i>	Little Bluestem	5	67.2
	<i>Eragrostis trichodes</i>	Sand Lovegrass	5	88.7
	<i>Desmanthus illinoensis</i>	Illinois Bundleflower	7	96.9
	<i>Chamaecrista fasciculata</i>	Partridge Pea	5	91.8
Bermudagrass	<i>Cynodon dactylon</i>	Bermudagrass	16.6	95.0
Control	No vegetation		-	-

In April 2009, researchers discovered red imported fire ant (*Solenopsis invicta*) nests in three pilot boxes. The underground channel of networks of fire ant nests creates preferential paths in the filter media that potentially affect the performances of bioretention; hydramethylnon-based insecticide was applied to all boxes to treat fire ants. Approximately one week after treatment, the existing mounds were mechanically demolished and the channel networks of the ant nests were repacked by flooding the boxes for several hours. The flooding treatments were done a total of three times. One of the flooding treatments followed an actual experiment procedure described below. Appendix A provides influent and effluent hydrographs of the flood treatment.

STORMWATER RUNOFF TEST PROCEDURE

Researchers conducted two rounds of tests. The first round (July 14–19, 2009) tested the removal performances of various pollutants (except *E. coli*) listed in Table 3. Synthetic

stormwater runoff that mimics the water quality of the runoff from two Texas highways as reported by Li et al. (2008) was prepared using chemicals listed in Table 3. A stock solution with the chemicals was added into 1600 gallons of tap water in a mixing tank (equipped with a mechanical mixer) for one hour to ensure complete mixing. The second round (November 17–25, 2009) tested *E. coli* removal performance. *E. coli* ATCC10798 was grown in Luria-Bertani Broth (10g tryptone; 5g yeast extract; 10g sodium chloride; per liter adjusted to pH 7.0 with 10N NaOH) at 30°C, 150 rpm overnight. The cell suspension was diluted to reach optical density (600nm) between 0.7~0.9 (Sezonov et al., 2007) and mixed with synthetic runoff at the end of a garden hose. A syringe pump was used to mix the suspension with synthetic stormwater runoff at a constant rate to meet the target concentration. The pumping rates of the syringe pump were 140.8 $\mu\text{L}/\text{min}$ for the first hour, 785.1 $\mu\text{L}/\text{min}$ for the second hour, and 119.6 $\mu\text{L}/\text{min}$ for the third hour.

Table 3. Pollutant Concentrations of Synthetic Stormwater Runoff.

Pollutants	Target Pollutant Concentrations adapted from Li et al. (2008)	Chemicals
Cu	0.02 mg/L	$\text{CuSO}_4 \cdot \text{H}_2\text{O}$
Zn	0.13 mg/L	$\text{ZnSO}_4 \cdot \text{H}_2\text{O}$
Pb	0.08 mg/L	$\text{Pb}(\text{NO}_3)_2$
TSS	98.17 mg/L	Silica
$\text{NO}_2\text{-N}$	0.15 mg/L	NaNO_2
$\text{NO}_3\text{-N}$	0.15 mg/L	NaNO_3
$\text{NH}_4\text{-N}$	0.77 mg/L	NH_4Cl
Organic-N	0.77 mg/L	Glycine
TN	1.84 mg/L	-
TP	0.17 mg/L	Na_2HPO_4

The influent flow rates were determined based on the assumptions that the bioretention box surface area of 36 sq ft was 1 percent of the drainage area, i.e., 3600 sq ft, with a mean 3-hour design storm for Brazos County. Runoff coefficient was assumed to be 0.9. Temporal distribution of rainfall intensity may affect the performance of bioretention. To simulate varying intensities of rainfall within a storm, the influent flow rates varied every hour. The flow rate for each hour was estimated using Soil Conservation Service (SCS) Type III rainfall pattern (Asquith et al., 2006). Although the SCS rainfall pattern specifies rainfall depths for every

30 minutes, we used a simplified version by varying the rainfall depth every hour due to technical difficulties in regulating flow rates. Table 4 shows the influent flow rates calculated from the runoff volume in the hypothetical drainage area. Low flow rates for the first and third hours were generated by gravity through the hose connected to the mixing tank. A water pump was used to increase the flow rate during the second hour of the experiment.

Grab samples of influent were collected once per hour. Effluent was sampled using a Teledyne ISCO 6712 water sampler at a rate of once per 30 minutes until the effluent flow rates became negligible. Flow rate was measured in one-minute intervals by a Global Water WL-14 WaterLogger attached in a 22.5° v-notch weir box. Before each experiment, soil moisture contents were measured at 2.36 inches in depth from the surface at five randomly selected locations using Delta-T Devices ThetaProbe Type ML2x.

Table 4. Design Parameters and Flow Rates for Synthetic Stormwater Runoff.

Design Parameter	
Watershed area	3600 sq ft
Runoff coefficient	0.9
Mean 3 hour storm for Brazos County (Asquith et al., 2006)	0.441 inch
Time (minute)	Influent flow rates (GPM)
0–60	2.58
60–120	10.25
120–180	1.95

WATER QUALITY ANALYSIS

Collected samples were transported to the Environmental Biotechnology Laboratory at Texas A&M University and refrigerated at 4°C until filtering, which was performed within a week. Samples were filtered using a 0.2 µm-pore-diameter membrane filter for the analyses of all pollutants except for total suspended solids (TSS) and *E. coli*. Filtrates were then preserved at –20°C until they were analyzed.

Zinc (Zn), copper (Cu), and lead (Pb) were analyzed by the inductively coupled plasma mass spectrometry (ICP-MS) method using a Perkin Elmer DRCII ICP-MS system (Eaton et al.,

2005). Before the analysis, the filtrates were acidified to a pH of 2 with concentrated trace metal grade nitric acid (HNO_3). A series of standard metal solutions in the range between 2 $\mu\text{g/L}$ and 200 $\mu\text{g/L}$ were used for calibration. The method detection limits were $\mu\text{g/L}$ levels.

TSS was measured as described by Eaton et al. (2005). A 300 mL subsample was taken from a sampling bag and stirred on a magnetic stirring plate using a magnetic stir bar. Then, a 100 mL of subsample was collected from the stirred subsample from mid-depth and midway between the beaker's wall and the vortex created by stirring at a speed of 600 to 700 rpm (Kayhanian et al., 2008). The 100 mL subsample was filtered using a 0.47 mm Whatmann glass fiber filter. The filter was dried at 103–105°C. The filter was weighed before and after drying. The TSS concentration of the sample was calculated by the difference of the filter weights over the volume of filtered sample.

The ammonia-nitrogen ($\text{NH}_3\text{-N}$) concentration was determined using the Phenate method (Eaton et al., 2005). Ammonia interacted with hypochlorite and phenol to form indophenol that was measured as absorbance at a wavelength of 640 nm. Standard curves, constructed with five standard solutions ranging from 0.01 mg/L to 5 mg/L, were used to determine $\text{NH}_3\text{-N}$ concentrations in the samples. The detection limit was 0.01 mg/L. The analysis of the samples was performed in triplicate.

Nitrate-nitrogen ($\text{NO}_3\text{-N}$) and nitrite-nitrogen ($\text{NO}_2\text{-N}$) concentrations were measured using a DX-180 Ion Chromatography (IC) (Dionex Corporation, Sunnyvale, CA). The IC is equipped with an IonPac AS14A-5 μm Analytical Column (3×150 mm) for anion analysis. The effluent solution consisting of 0.16 M sodium carbonate (Na_2CO_3) and 0.02 M sodium bicarbonate (NaHCO_3) was pumped at a flow rate of 1 mL/min. The regeneration solution was 70 mN H_2SO_4 . Standard solutions ranging from 10 $\mu\text{g/L}$ to 5 mg/L were used to develop a calibration curve. The detection limits of $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ were 10 $\mu\text{g/L}$.

Total nitrogen (TN), a summation of organic and inorganic nitrogen, was measured by the perulfate digestion method using a TNT 826 kit (Hach, Product No., TNT826). Because the detection range of TNT 826 is from 1 to 16 mg/L, samples that were expected with high concentrations were diluted to the detection range. Analyses were conducted according to the manufacturer's manual. Both inorganically and organically bonded nitrogen (N) in the sample was oxidized to nitrate by digestion with peroxodisulphate. The digestion was conducted in a Digital Reactor Block 200 (Hach, Product No., DRB200-04) at 100°C for 60 minutes. The

nitrate ions then reacted with 2,6-dimethylphenol in a solution of sulphuric and phosphoric acid to form a nitrophenol. The absorbance of the pale pink colored product was measured at 345 nm.

Total phosphorus (TP), a summation of organic and inorganic phosphorus, was measured by the ascorbic acid method using a TNT 843 kit (Hach, Product No., TNT843). Similarly, samples with expected high TP were diluted to the detection range of TNT 843, ranging from 0.05 to 1.50 mg/L. In this method, persulfate digests organic phosphorus (P) into reactive P. The reactive P then interacted with the molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex. The complex was further reduced by ascorbic acid to form phosphomolybdenum blue, which was measured as absorbance at 800 nm.

The *E. coli* concentration was measured by the real-time polymerase chain reaction (PCR) detection system. A 300 mL sample from the collection bag was stirred on a magnetic stirring plate, and a 100 mL subsample was taken from mid-depth and midway using a wide-bore pipette (Kayhanian et al., 2008). The 100 mL sample was filtered by a 0.45 µm filter. The filters were cut in pieces and placed in the Lysing Matrix E tube provided with Fast DNA Spin Kit for Soil (MP). The whole DNA of *E. coli* was extracted following the manual of the kit. The *E. coli* concentration in a sample was measured by quantifying a 16S rRNA using iCycler iQTM 5 Multicolor Real-Time PCR Detection System (Bio-Rad). Amplification and detection were carried out in 96-well plates with SYBR-Greens PCR 2X Master Mix (QIAGEN, Inc.). A region of 16S rRNA of *E. coli* was amplified using *E. coli* species-specific forward primer (5'-CATGCCGCGTGTATGAAGAA-3', base pairs 395 to 414) and reverse primer (5'-CGGGTAACGTCAATGAGCAAA-3', base pairs 470 to 490) (Huijsdens et al. 2002).

Each reaction was run in a final volume of 25 µL with 1X final concentration of SYBR-Greens PCR 2X Master Mix, 300nM final concentration of each primer, 5µL of each DNA sample. Amplifications were carried out using the following ramping profile: 1 cycle at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 60°C for 1 min (Huijsdens et al., 2002; Furet et al., 2009). Standard curves ranging from 1.47×10^3 to 1.47×10^8 copies of the 16S rRNA gene were estimated using plasmid #931 that carries *E.coli* ATCC10798 partial 16S rRNA. The plasmid DNA was constructed using a TOPO TA Cloning Kit and Wizard Plus SV Minipreps (Promega).

DATA ANALYSIS

Detention times of stormwater runoff in the boxes were estimated using centroid method by the following equations:

$$\text{Detention time} = T_{out} - T_{in}$$

where, T_{in} and T_{out} are times in minutes to centroids from the beginning of influent and effluent hydrographs, respectively (see Figure 3). T_{in} and T_{out} were estimated by:

$$T_{in} = \sum (Influent_t \times StormDuration_t) / \sum Influent_t$$

$$T_{out} = \sum (Effluent_t \times StormDuration_t) / \sum Effluent_t$$

where, $Influent_t$ and $Effluent_t$ are influent and effluent flow rates in gallon/minute (GPM) at t th time intervals, respectively, and $StormDuration_t$ is the time since the beginning of the hydrograph in minutes (Haan et al., 1994).

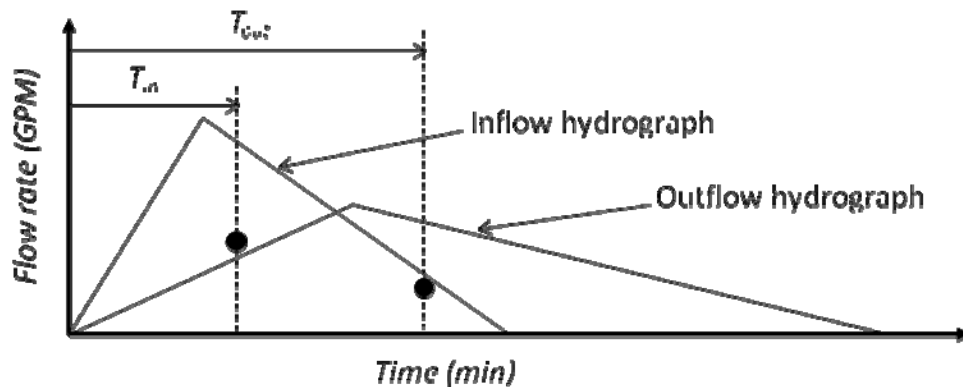


Figure 3. Centroid Method for Estimating Detention Time.
(Black dots represent centroids of triangles of influent and effluent hydrographs.)

Because influent and effluent flow rates vary over time, the event mean concentrations of pollutants do not correctly represent the performance of bioretention. We calculated pollutant removal efficiencies by mass removal percentage as shown in the following equation:

Removal efficiency

$$= 1 - [\sum(C_{out,t} \times Effluent_t \times 1min) / \sum(C_{in,avg} \times Influent_t \times 1min)] \times 3.785 \times 100\%$$

where, $C_{in, avg}$ is an average concentration of all influent samples of the tested box in mg/L, $C_{out, t}$ is effluent concentration at t th time in mg/L, and 3.785 is unit conversion factor in L/gallon.

CHAPTER 3: RESULTS

PEAK FLOW REDUCTION AND DETENTION TIME

Figure 4 shows influent and effluent hydrographs of the five pilot boxes and the detention times for the first experiments (July 14–19, 2009). The result indicates that all boxes reduced the peak flow, but the degree of reduction varied. The control box had better performance in flow reduction than the four vegetated boxes. In the four vegetated boxes, surface ponding occurred only during the second hour, when the influent flow rate reached 10.25 GPM, and quickly disappeared once the flow rate decreased to 1.95 GPM. Effluents were also quickly reduced and merely dripped one hour after the influent ceased (Figures 4a–4d).

In contrast, the control box showed water ponding immediately after the influent began. During the second hour, ponding depth exceeded 1 ft and overflowed over the pilot box. Overflow occurred between 80 and 124 minutes. Effluent lasted for 4 hours after influent ceased, i.e., 7 hours after the beginning of the experiment (Figure 4e). Detention times showed that the control box retained the runoff much longer than the four vegetated boxes. No significant difference in the flow reduction performances among the four vegetation types was observed. Hydrographs for the second experiments showed similar patterns (Appendix B).

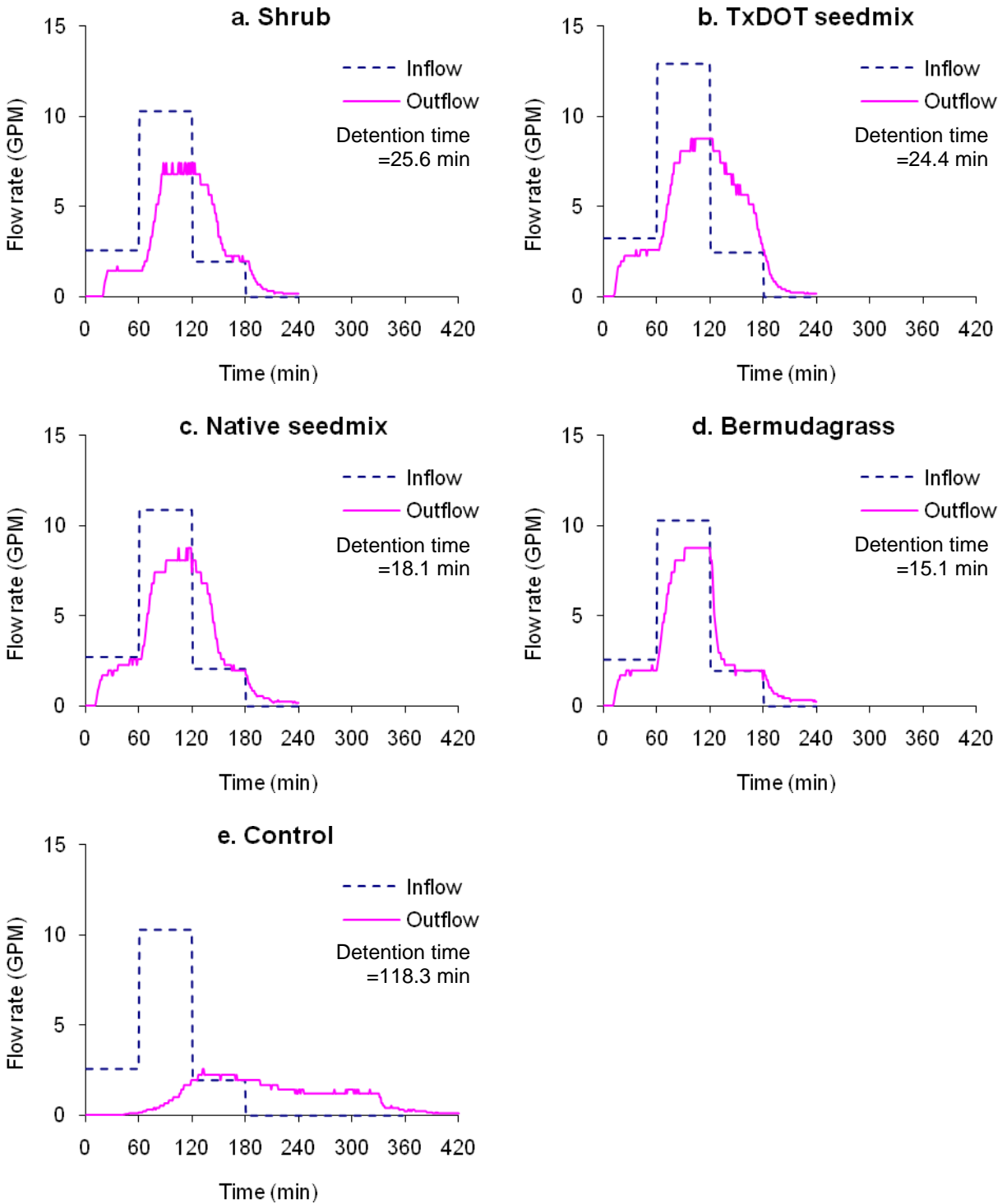


Figure 4. Influent and Effluent Hydrographs of Five Pilot Boxes during the First Round of Experiments.

POLLUTANT REMOVAL PERFORMANCES

Metals

All pilot boxes effectively removed Zn and Pb from the synthetic runoff. On average, removals of Zn and Pb were 61.6 percent and 79.4 percent, respectively (Table 5). The removal efficiencies were similar between the four vegetated boxes and control box. On the other hand, the pilot boxes showed poor performance of Cu removal. Negative removal efficiencies for the vegetated boxes suggest that Cu leached out of the pilot boxes. Only the control box had a positive removal of Cu.

Table 5. Removal Efficiencies of 12 Pollutants by Pilot Boxes.

Pollutants	Shrub	TxDOT seedmix	Native grass seedmix	Bermuda grass	Control*	5 boxes Mean	Range from Previous Studies**
Cu	-25.2%	-43.1%	-13.1%	-19.0%	37.2%	-12.6%	43–99%
Zn	80.9%	82.1%	29.8%	47.0%	68.4%	61.6%	27–98%
Pb	80.0%	76.9%	76.2%	80.0%	84.1%	79.4%	54–95%
TSS	67.8%	36.1%	-6.0%	36.1%	80.5%	42.9%	-170–60%
NO ₂ -N ⁺	-	-	-	-	-	-	-
NO ₃ -N	-3433%	-772%	-425%	-713%	-4139%	-1896%	-5–95%
NH ₃ -N	95.6%	91.7%	87.7%	81.2%	77.2%	86.7%	-1–86%
TN	-438%	-290%	-23%	-48%	-480%	-256%	14–71%
TP	-3251%	-3062%	-1135%	-963%	-954%	-1873%	-240–87%
<i>E. coli</i> ⁺⁺	99.3%	84.9%	79.8%	73.4%	99.0%	87.3%	71%
Antecedent Soil moisture content	14.0%	9.8%	9.2%	11.3%	14.2%		
Detention time	25.6 min	24.4 min	18.1 min	15.1 min	118.3 min		

* Overflow occurred between 80 and 124 minutes.

** Ranges were estimated from the previous studies conducted in field conditions only (Davis et al., 2003; Dietz and Clausen, 2005; Hsieh and Davis, 2005; Sharkey and Hunt, 2005; Dietz and Clausen, 2006; Davis et al., 2006; Hunt et al., 2006; Hunt et al., 2007; Davis, 2007; Dougherty et al., 2007).

⁺ NO₂-N concentrations in all (influent and effluent) samples below detection limit.

⁺⁺ *E. coli* concentrations were measured at the second round experiments (November 17–25, 2009).

Suspended Solids

Suspended solids were also effectively removed by all the pilot boxes, except the native grass box (−6.0 percent). The mean TSS removal was 42.9 percent (Table 5). The control box had the highest TSS removal (80.5 percent), which is approximately twice as high as the average TSS removal of the five boxes.

Nitrogen and Phosphorus

Table 5 summarizes removal performances of N and P. Higher NH₃-N removals were observed for the four vegetated boxes (>81.2 percent) when compared to that of the control box (77.2 percent). NO₂-N concentrations were below detection limits in all influent and effluent samples, suggesting that NO₂-N were rapidly converted to NO₃-N before samples were analyzed in the laboratory. This can also explain why NO₃-N concentrations were higher in the influent samples than the target concentrations. The measured NO₃-N concentrations in the influents were approximately equal to the sum of the target concentrations of NO₂-N and NO₃-N (Appendix B). High NO₃-N concentrations were observed in effluent samples. Removal of NO₃-N was −1896 percent on average. Leaching NO₃-N was the most serious by the control box. The NO₃-N removal by the control box was −4139 percent. TN removal by the control box (−480 percent) was less than those by the four vegetated boxes (ranging from −438 to −23 percent), once again supporting that vegetation mitigates the problem of leaching N out of bioretention.

None of the pilot boxes removed P effectively. The average TP removal was −1873 percent, suggesting that P was leaching from soil media. Unlike TN removal, when compared to the performance of the control (−954 percent), the presence of vegetation resulted in higher P leaching from the soil media.

Pathogen

E. coli was very effectively removed by the pilot boxes. The removal efficiencies were over 70 percent for all pilot boxes (Table 5). Although the control box had the highest removal, the difference between the five boxes was not significant. Note that the removal efficiencies were calculated after excluding one outlier of influent samples because the concentration of this sample was four orders of magnitude higher than the mean influent concentrations.

SUCCESSION OF VEGETATION COMMUNITY

On August 20, 2009 (14 months after the construction), the cover percentage of all vegetation species in the pilot boxes was surveyed (Table 6). In the shrub box, only Texas sage thrived among three shrub species. One wax myrtle survived, but it did not vigorously grow. Two wax myrtles and three dwarf yaupon hollies were dead during the first summer. Vegetation compositions for three grass boxes were similar regardless of original seed mixes. All three boxes were dominant by Johnsongrass and Giant ragweed. Bermudagrass was also found in two of the pilot boxes. Among species in the original seed mixes, only two species (Bermudagrass and Illinois bundleflower) established in the pilot boxes.

Table 6. Species List in Pilot Boxes 14 Months after Construction.

Box	Species		% Cover
	Botanical Name	Common Name	
Shrub	<i>Leucophyllum frutescens</i> 'Bertstar Dwarf'	Texas Sage (Cenizo)*	75
	<i>Cyperus esculentus</i>	Yellow Nutsedge	15
	<i>Euphorbia maculata</i>	Prostrate Spurge	5
	<i>Amaranthus albus</i>	Pigweed	4
	<i>Morella cerifera</i>	Wax Myrtle*	1
TxDOT Bryan District seedmix for sand	<i>Ambrosia trifida</i>	Giant Ragweed	70
	<i>Cynodon dactylon</i>	Bermudagrass*	25
	<i>Sorghum halepense</i>	Johnsongrass	5
Native grass seedmix	<i>Ambrosia trifida</i>	Giant Ragweed	50
	<i>Sorghum halepense</i>	Johnsongrass	35
	<i>Desmanthus illinoensis</i>	Illinois Bundleflower*	15
Bermudagrass	<i>Sorghum halepense</i>	Johnsongrass	70
	<i>Ambrosia trifida</i>	Giant Ragweed	25
	<i>Cynodon dactylon</i>	Bermudagrass*	5
Control	<i>No vegetation</i>		-

* Species originally seeded/planted

CHAPTER 4: DISCUSSION

WATER QUALITY PERFORMANCE

The removal efficiency of the pilot boxes varied by the type of pollutant. The results show that Zn, Pb, TSS, NH₃-N, and *E. coli* removals were effective while Cu, NO₃-N, TN, and TP removals were not. Vegetation did not enhance removal of Zn, Pb, or TSS. To the researchers' surprise, vegetated boxes were less effective on TSS removal than the control box (Table 5). The low TSS removal by vegetated boxes might be due to the presence of plant roots that significantly increased the infiltration, reduced the detention time, and in the meantime, perhaps created preferential paths for suspended solids to leave the boxes. A short detention time appears to decrease TSS removal. Following this finding, researchers realized that balancing the need of removing TSS and other pollutants is the key to optimizing the overall performance of bioretention.

Another point of discussion results from the poor Cu removal in the pilot experiments. We observed that Zn and Pb removals were effective, but Cu removal was not. Considering that removal mechanisms for metal pollutants are similar, researchers attribute the low Cu removal to a relatively low Cu concentration in the synthetic runoff (0.02 mg/L target concentration), in comparison with Zn and Pb concentrations used in this study or Cu concentrations reported in previous studies. For instance, Davis et al. (2003) used synthetic runoff with 0.08 mg/L of Cu concentration and reported positive removal efficiencies. The stormwater field is now debating the legitimacy of reporting water quality performance in percent removal. According to Strecker et al. (2001), percent removal as typically reported in previous stormwater studies is a strong indicator of influent pollutant concentration; i.e., the higher the percent removal, the higher the influent pollutant concentration. One can relate this conclusion as "the dirtier the water draining into the stormwater (bioretention) BMP, the better the pollutant performance (indicated as percent removal) it will be." Bioretention must contain certain amounts of pollutants, including Cu, in soil media because they are essential to plant growth. Therefore, it is inevitable that certain amounts of pollutants leach out of BMPs. Under a low influent concentration condition, the mass of pollutant leached out of the pilot boxes could be higher than the mass removed by the boxes.

High effluent concentrations of $\text{NO}_3\text{-N}$, TN, and TP were observed in this study. These observations are most likely attributed to leaching from compost amended soil media used in this study. As shown in Table 1, the compost amended soil contained much higher $\text{NO}_3\text{-N}$, TP, and organic contents (might have organically bonded N and P) than the soil without compost. The effect of vegetation is contrasting for N and P in that vegetation increased N removal while decreased P removal.

Although the removal efficiencies of TN were negative for all pilot boxes, four vegetated boxes showed much better performance than the control one, which may be attributed to root uptake. In addition, roots create microenvironments suitable for various microorganisms to convert $\text{NH}_3\text{-N}$ to $\text{NO}_3\text{-N}$, and potentially to N_2 . Despite the beneficial effect of vegetation, effluent TN concentrations were still too high, suggesting that the soil-to-compost ratio used in this experiment (7:3) should be revised.

Previous studies found that a permanent water saturation zone at the bottom of bioretention can further improve the performance of bioretention in $\text{NO}_3\text{-N}$ removal because of the denitrification process in the soil media (Kim et al., 2003). Considering that most N in effluent is present in the form of $\text{NO}_3\text{-N}$, creating a saturation zone to promote denitrification might be able to lower TN concentrations in the effluent. We are preparing to examine the effect of a water saturation zone using the existing pilot bioretention boxes in the next fiscal year.

Vegetation negatively affected TP removal in the pilot boxes. As the synthetic stormwater runoff does not contain P, the high concentration of TP might be contributed from the filter media, particularly the compost used in this study (see Table 1). Active microbial activity in the root zone, such as production of carbon dioxide, might result in acidic conditions that promoted leaching of P from the compost. Alternatively, the TP concentrations in water were highly associated with TSS concentrations because organic P was transported in water by being adsorbed on suspended solids. A previous study also reported that TP is more effectively removed by fine soil media (Zhang et al., 2006). The decrease in TSS removal efficiency by plant roots may reduce the performance of TP removal in the vegetated boxes. The effect of plant uptake is negligible if TP concentration in soil is much higher than the amount needed by vegetation.

ROLES OF VEGETATION

Vegetation plays an important role in peak flow rate because a dense root system could increase the infiltration rate of bioretention filter media. As seen in Figure 4, the vegetated boxes had much higher effluent flow rates than the control box, as roots increased soil infiltration. The potential benefit of an increased infiltration rate by vegetation can be explained in two ways. First, duration of standing water on the bioretention surface can be greatly shortened to reduce the safety concern. Second, more stormwater runoff could be treated by allowing faster flows moving through the filter media instead of bypassing via spillways in the case of high flow events. A contradictory drawback of increased infiltration is the reduction of detention time, known as an important factor for removing pollutants. The question of interest to researchers and engineers is “what is the ideal range of infiltration rate that addresses both runoff quantity to be treated and sufficient detention time?”

Another significant benefit of vegetation is the pollutant uptake by roots and microbial activities in the rhizosphere, an important provision of bioretention BMPs that detention/retention ponds or sand filter basins do not offer. Although the root uptake might not be significant in removing pollutants from stormwater runoff during a short detention time, roots prolong the lifetime of bioretention by continuously removing pollutants, particularly N, from soil media.

CHALLENGES TO BIORETENTION ALONG TEXAS HIGHWAYS

Fire Ant Infestation

As mentioned earlier, red imported fire ants infested three of the pilot boxes. Red imported fire ants could significantly decrease the pollutant removal performance of bioretention by funneling water into a dense channel network. The channel network can extend up to 60 cm in depth and the hollow space occupies about 50 percent of cross-sectional area in the channel network (Green et al., 1999). Bioretentions provide an environment favorable to fire ants. Previous studies showed that fire ants prefer highly disturbed habitats with less tree canopy, such as roadsides, due to direct sunlight that maintains the nests' warm temperature (Stiles and Jones, 1998; Russell et al., 2001; Forys et al., 2002). They also prefer sandy soil and are tolerant to wet conditions (Tschinkel, 1987; Milks et al., 2007). In Texas, mound density can reach up to

400 mounds/ha (Milks et al., 2007). The habitat preference and the vigorous spread make the infestation of fire ants a challenge to bioretention along Texas highways.

Drought and Weeds

Another challenge to bioretention in Texas is drought. Although bioretention is frequently inundated, the drought in hot and semi-arid climates can be a more severe stress to vegetation because bioretention is designed to quickly drain water out of the system. Sandy soil and underdrain are the design elements to keep vegetation from inundation stress. The gravel layer below the soil media also prevents plant roots from reaching groundwater. Despite these characteristics, most bioretention manuals recommend wetland species as suitable vegetation types in bioretention environments. However, researchers' observation during the 14-month growth period strongly suggests that wetland species cannot successfully establish in the Texas climate. Of the three shrub species, only Texas sage, known as a desert species, thrived in the pilot boxes even though they were frequently irrigated. A permanent water saturation zone may provide an additional benefit to bioretention in that it reserves and supplies water to the vegetation during dry periods. The list of vegetation species suitable for bioretention in Texas needs to be revised. The list will be included in the final report.

Pilot bioretention experiments showed that a bioretention environment is favorable to Johnsongrass and Giant ragweed, both of which are common weeds on Texas roadsides (TxDOT, 2009). However, it is unclear that a similar successional change will occur in field conditions. For the entire study period, the pilot bioretention boxes were irrigated. The vegetation community could be different in bioretentions outside of the laboratory where bioretentions will face severe drought. On the other hand, the sidewall of the pilot boxes was exposed to direct sunlight, which may have increased the temperature of the bioretention soil media. Although the thick sidewalls of the boxes provide relatively good insulation, this condition may have increased evapotranspiration and aggravated the drought stress of the vegetation. Field demonstration projects will provide more information on the successional change in vegetation communities of real highway environments. This information will be used to develop the list of vegetation and maintenance schemes for future application of bioretention along Texas highways.

Considerations for Field Application

This section discusses what was learned from the pilot testing process and the current literature, and how the information may be modified and transferred for field application. The following list specifically includes aspects deemed critical for field application by the research team. Such information should be used carefully, as it is inferred based on pilot-scale testing. More conclusive guidelines will become available once the field demonstration in the next phase of the project is completed.

- *Use of Compost in Soil Media.* The ratio of soil to compost for the soil media should be determined based on the constituent contents in the compost, particularly N and P. Leaching of N and P is typical as reported in the literature and again observed in the pilot testing. Knowing Texas' compost carries relatively high P, the research team suggests the use of compost should be kept to a minimum, e.g., 5 percent in the soil media. The compost percentage affects the overall infiltration rate of the soil media, which is an important design parameter related to performance. Learning from the sand and compost used in this study, the relationship between the soil/compost ratio and infiltration rate is that the lower the compost content, the slower the infiltration. The infiltration rate of a low-compost soil media could still be within a suitable range for bioretention.
- *Depth (Thickness) of Soil Media.* Depth of soil media affects the infiltration rate. It could also influence the capacity of runoff detention. If the site condition allows, bioretention cells of a greater thickness provide more detention capacity. Depth of soil media could also affect plants' growth or even survival. The soil media for bioretention is a sandy type of soil and will not hold water for a long time. For enhancing plant survival, the research team suggests that the depth of soil media be less than 24 inches and a saturation zone at the bottom of the bioretention be considered.
- *Saturation Zone.* Having a saturation zone at the bottom of the bioretention could address three issues: drought, denitrification for N removal, and fire ants as described in previous sections. The zone depth is suggested to cover the gravel layers and continue up to approximately 4 inches of the bottom part of the soil media.
- *Plant Selection.* Bioretention's cyclic drought and inundation condition is a critical factor in determining plants. Other factors include nativity, tolerance to mowing or other unexpected

disturbances, context of the surrounding land uses, etc. TxDOT's standard seed mixes provide locally adapted plants that could suit such needs. From the pilot testing, the research team identifies the general challenges but is unable to test many plant species. More studies are needed to identify suitable species that could survive in bioretention environments and improve the performance of bioretention.

- *Supplemental Irrigation.* Supplemental irrigation will be needed if plants or seeds are scheduled for installation during summer. If planting can be scheduled during winter or early spring, irrigation may not be needed. Because unvegetated bioretention, tested as the control box, is proved to perform well, leaving bioretention unvegetated while waiting for ideal seasons for planting can be done to eliminate supplemental irrigation.
- *Mowing and Herbicide Maintenance.* Depending upon the vegetation type specified, bioretention may or may not need mowing. If grasses are used, mowing is needed only once per year, scheduled in late fall. Shrubs do not need mowing; however, weeding will be needed and may be done with herbicide.

CHAPTER 5: CONCLUSION

From the pilot testing, researchers drew the following conclusions:

- Peak flow rate of effluents was significantly reduced compared to that of influents. The control box was the most effective, while the vegetated ones were moderately effective due to plant roots that increased soil infiltration rates.
- TSS, Pb, Zn, NH₃-N, and *E. coli* were effectively removed while Cu, NO₃-N, TN, and TP were not.
- Negative removal performance for N, P, and Cu can be attributed to leaching from the soil/compost media.
- Weeds emerged and dominated in the vegetation community.

The lessons learned from the pilot experiments include:

- An ideal bioretention design should enhance plant roots and increase detention time.
- To eliminate leaching of N and P or even Cu from bioretention, the use of compost should be minimized. A saturation zone at the bottom of the bioretention (not tested in this pilot experiment but suggested by previous studies) may further remove N from soil media.
- Prolonged drought is one of the factors affecting the success of bioretention. The saturation zone could mitigate drought stress.
- Red imported fire ants will continue to prevail on Texas roadsides. The saturation zone may also suppress their infestation.
- After observing the weed growth on the pilot bioretention boxes and the wet/dry conditions, it is clear that vegetation planted in bioretentions must be able to tolerate droughts as well as periodic inundation. Vegetation should also sustain mowing as it is typically applied on highways.
- This research is unable to discriminate the performances (including survival and water quality) between different vegetation types. More studies are needed to identify suitable species that improve the performance of bioretention.

The next stage of the project, the field demonstrations, will further clarify the benefits and challenges of bioretention in Texas highways. The information obtained in the next stage will help develop the bioretention design guidelines for TxDOT.

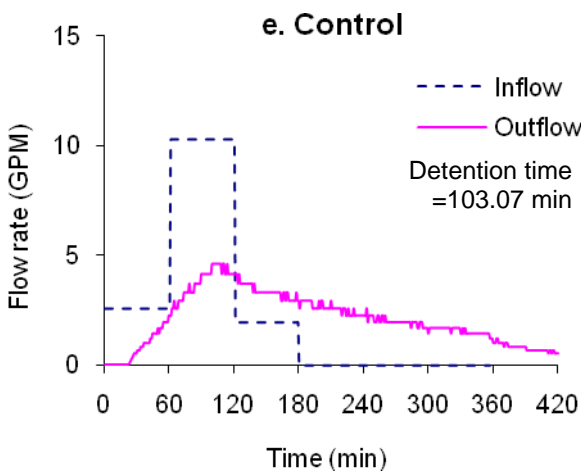
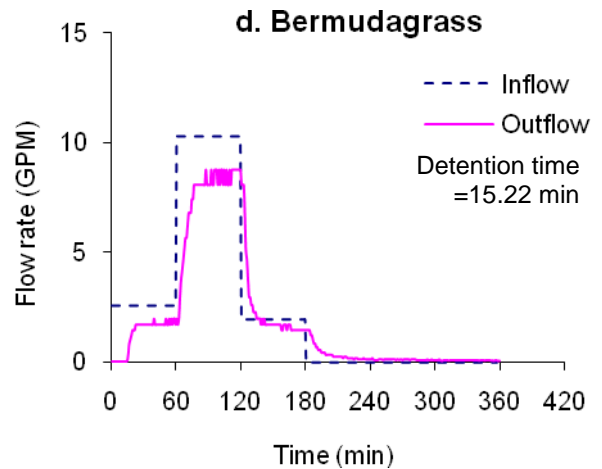
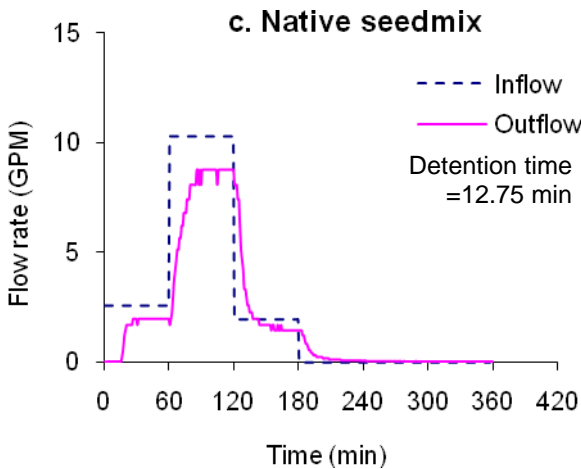
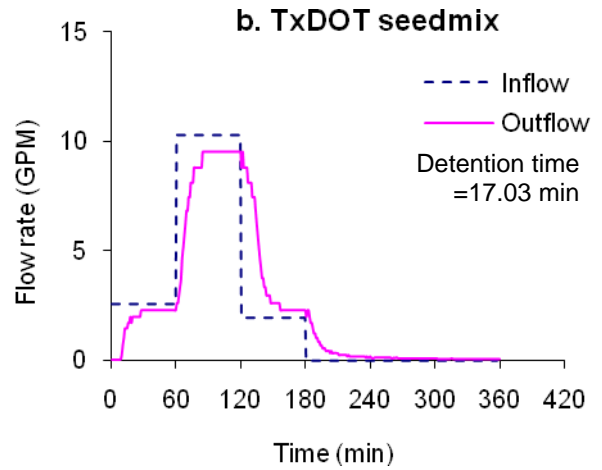
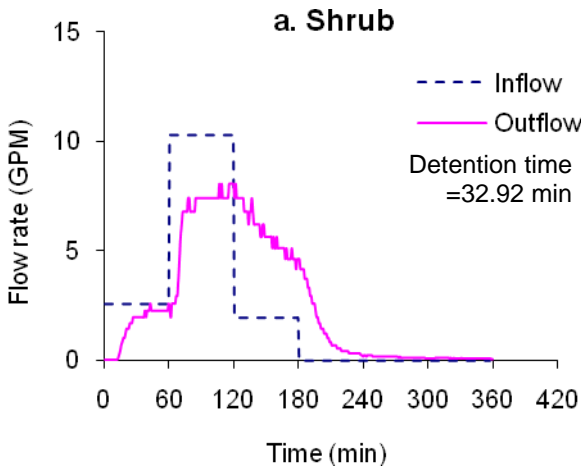
REFERENCES

- Asquith, W. H., Roussel, M. C., Cleveland, T. G., Fang, X., & Thompson, D. B. (2006). *Statistical characteristics of storm interevent time, depth, and duration for Eastern New Mexico, Oklahoma, and Texas* (No. Professional Paper 1725). Austin, TX: U.S. Geological Survey.
- Davis, A. P. (2007). Field performance of bioretention: water quality. *Environmental Engineering Science*, 24, 1048-1064.
- Davis, A. P., Shokouhian, M., Sharma, H., & Minami, C. (2001). Laboratory study of biological retention for urban stormwater management. *Water Environment Research*, 73, 5-14.
- Davis, A. P., Shokouhian, M., Sharma, H., & Minami, C. (2006). Water quality improvement through bioretention media: nitrogen and phosphorus removal. *Water Environment Research*, 78, 284-293.
- Davis, A. P., Shokouhian, M., Sharma, H., Minami, C., & Winogradoff, D. (2003). Water quality improvement through bioretention: lead, copper, and zinc removal. *Water Environment Research*, 75, 73-82.
- Dietz, M. E., & Clausen, J. C. (2005). A field evaluation of rain garden flow and pollutant treatment. *Water, Air, and Soil Pollution*, 167, 123-138.
- Dietz, M. E., & Clausen, J. C. (2006). Saturation to improve pollutant retention in a rain garden. *Environmental Science & Technology*, 40, 1335-1340.
- Dougherty, M., LeBleu, C., Brantley, E., & Francis, C. (2007). *Evaluation of bioretention nutrient removal in a rain garden with an internal water storage (IWS) layer*. Paper presented at the ASABE Annual International Meeting, Minneapolis, Minnesota.
- Eaton, A. D., Clesceri, L.S., Rice, E. W., Greenberg, A. E., & Franson, M. A. H. (2005). *Standard methods for the examination of water and wastewater* (21st ed). American Public Health Association, Washington, DC, 2005.
- Environmental Protection Agency (EPA). (2000). *Low impact development (LID): A literature review* (No. EPA-841-B-00-005). Washington DC: Office of Water, US Environmental Protection Agency.
- Forys, E. A., Allen, C. R., & Wojcik, D. P. (2002). Influence of the proximity and amount of human development and roads on the occurrence of the red imported fire ant in the lower Florida Keys. *Biological Conservation*, 108, 27-33.
- Furet, J.-P., Firmesse, O., Gourmelon, M., Bridonneau, C., Tap, J., Mondot, S., Dore, J., & Corthier, G. (2009). Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR. *FEMS Microbiology Ecology*, 68, 351-362.
- Green, W. P., Pettry, D. E., & Switzer, R. E. (1999). Structure and hydrology of mounds of the imported fire ants in the southeastern United States. *Geoderma*, 93, 1-17.
- Haan, C. T., Barfield, B. J., & Hayes, J. C. (1994). *Design hydrology and sedimentology for small catchments*. San Diego, CA: Academic Press, Inc.
- Hsieh, C.-h., & Davis, A. P. (2005). Evaluation and optimization of bioretention media for treatment of urban storm water runoff. *Journal of Environmental Engineering*, 131, 1521-1531.
- Huijsdens, X. W., Linskens, R. K., Mak, M., Meuwissen, S. G. M., Vandenbroucke-Grauls, C. M. J. E., & Savelkoul, P. H. M. (2002). Quantification of bacteria adherent to gastrointestinal mucosa By Real-Time PCR. *Journal of Clinical Microbiology*, 40, 4423-4427.

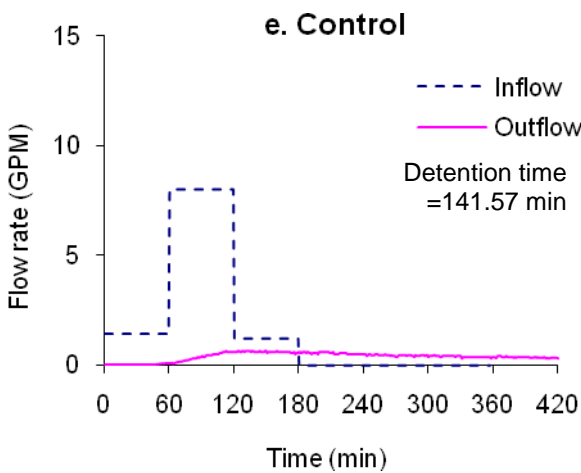
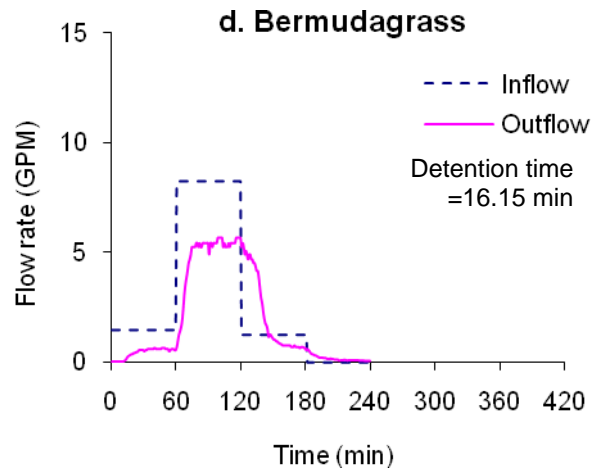
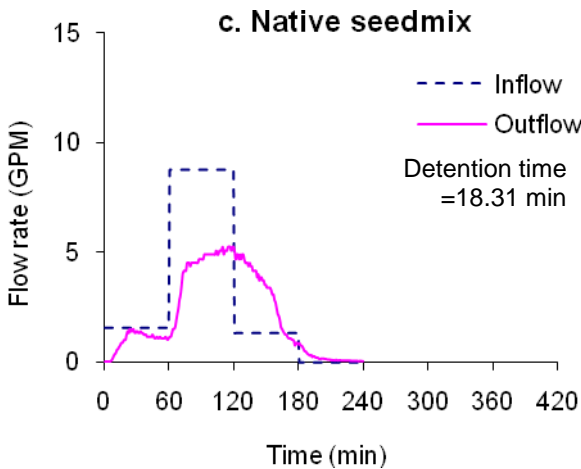
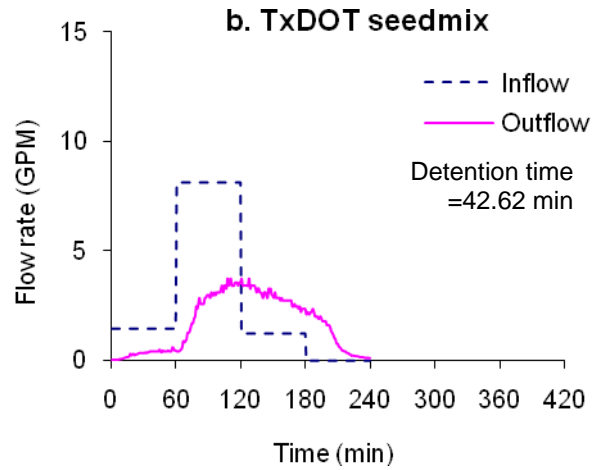
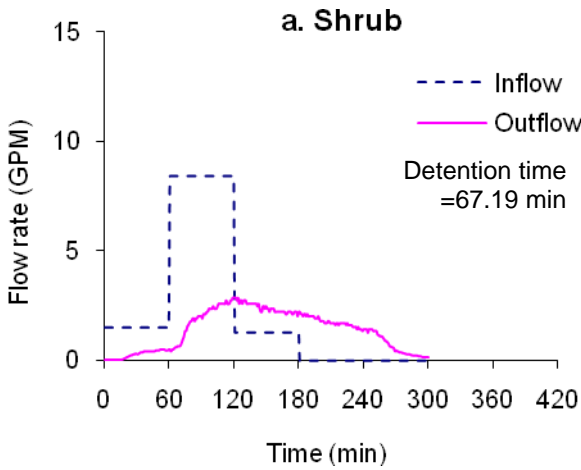
- Hunt, W. F., Jarret, A. R., Smith, J. T., & Sharkey, L. J. (2006). Evaluating bioretention hydrology and nutrient removal at three field sites in North Carolina. *Journal of Irrigation and Drainage Engineering*, 132, 600-608.
- Hunt, W. F., Smith, J. T., & Hathaway, J. (2007). *Hall Marshall bioretention: Final report*. Charlotte, NC: Storm Water Services, City of Charlotte.
- Kayhanian, M., Rasa, E., Vichare, A., & Leatherbarrow, J. E. (2008). Utility of suspended solid measurements for storm-water runoff treatment. *Journal of Environmental Engineering*, 134, 712-721.
- Kim, H., Seagren, E. A., & Davis, A. P. (2003). Engineered bioretention for removal of nitrate from stormwater runoff. *Water Environment Research*, 75, 355-367.
- Li, M.-H., Barrett, M. E., Rammonhan, P., Olivera, F., & Landphair, H. C. (2008). Documenting stormwater quality on Texas highways and adjacent vegetated roadsides. *Journal of Environmental Engineering*, 134, 48-59.
- Milks, M. L., Fuxa, J. R., Richter, A. R., & Moser, E. B. (2007). Multivariate analyses of the factors affecting the distribution, abundance and soil form of Louisiana fire ants, *Solenopsis invicta*. *Insectes Sociaux*, 54, 283-292.
- Prince George's County. (2002). *Bioretention manual*. Landover, Maryland: Department of Environmental Resources, Watershed Protection Branch, Prince George's County, MD.
- Puget Sound Action Team. (2005). *Low impact development: Technical guidance manual for Puget Sound* (No. PSAT 05-03). Olympia, WA: Washington State University.
- Russell, S. A., Thorvilson, H. G., & Phillips, S. A., Jr. (2001). Red imported fire ant (Hymenoptera: Formicidae) populations in Texas highway rights-of-way and adjacent pastures. *Environmental Entomology*, 30, 268-273.
- Sezonov, G., Joseleau-Petit, D., & D'Ari, R. (2007). Escherichia coli Physiology in Luria-Bertani Broth. *Journal of Bacteriology*, 189, 8746-8749.
- Sharkey, L. J., & Hunt, W. F. (2005). *Design implications on bioretention performance as a stormwater BMP: water quality and quantity*. Paper presented at the ASAE Annual International Meeting, Tampa, Florida.
- Skidmore, Owings & Merrill LLP. (2005). Stormwater management design guidelines: for the member institutions of the Texas Medical Center. Retrieved September, 09, 2008, from <http://www.texmedctr.tmc.edu/NR/rdonlyres/292FF18E-4D72-4B80-881F-6CD027251F52/0/StormwaterMasterPlan2005.pdf>
- Stiles, J. H., & Jones, R. H. (1998). Distribution of the red imported fire ant, *Solenopsis invicta*, in road and powerline habitats. *Landscape Ecology*, 335, 335-346.
- Strecker, E. W., Quigley, M. M., Urbonas, B. R., Jones, J. E., & Clary, J. K. (2001). Determining urban storm water BMP effectiveness. *Journal of Water Resources Planning and Management*, 127, 144-149.
- Tschinkel, W. R. (1987). Distribution of the fire ants *Solenopsis invicta* and *S. geminata* (Hymenoptera: Formicidae) in Northern Florida in relation to habitat and disturbance. *Annals of the Entomological Society of America*, 81, 76-81.
- TxDOT. (2002). *Stormwater management guidelines for construction activities*. Retrieved from http://www.txdot.gov/txdot_library/publications/business/contractors_consultants/environmental_resources.htm#storm.
- TxDOT. (2004). *Hydraulic design manual*. Retrieved from <http://onlinemanuals.txdot.gov/txdotmanuals/hyd/hyd.pdf>.

- TxDOT. (2007). *Landscape and aesthetics design manual*. Retrieved from <http://onlinemanuals.txdot.gov/txdotmanuals/lad/lad.pdf>.
- TxDOT. (2009). *Roadside Vegetation Management Manual*. Retrieved from <http://onlinemanuals.txdot.gov/txdotmanuals/veg/veg.pdf>.
- Wisconsin Department of Natural Resources. (2003). *Rain gardens: A how-to manual for homeowners* (No. PUB-WT-776).
- Zhang, W., Brown, G. O., & Storm, D. E. (2006). *Enhancement of phosphorus removal in bioretention cells by soil amendment*. Paper presented at the ASABE Annual International Meeting, Portland, Oregon.

APPENDIX A: INFLUENT AND EFFLUENT HYDROGRAPHS DURING FLOOD TREATMENT



APPENDIX B: INFLUENT AND EFFLUENT HYDROGRAPHS DURING FLOOD TREATMENT



**APPENDIX C:
POLLUTANT CONCENTRATIONS IN INFLUENT AND EFFLUENT
WATER SAMPLES**

Cu

Unit: (mg/L)

Target concentration: 0.002 mg/L

Sample	Time (hr)	Shrub	TxDOT	Native	Bermuda	Control*
Influent	0.0	0.020	0.023	0.024	0.025	0.025
	1.0	0.025	0.023	0.039	0.023	0.023
	2.0	0.027	0.024	0.028	0.025	0.024
	3.0	0.027	0.024	0.040	0.024	0.022
Total mass in Influent (mg)		86.39	109.09	91.55	86.39	86.39
Effluent	0.5	0.068	0.056	0.052	0.049	NF**
	1.0	0.049	0.043	0.049	0.035	0.022
	1.5	0.052	0.052	0.043	0.039	0.031
	2.0	0.034	0.034	0.044	0.030	0.028
	2.5	0.033	0.032	0.033	0.030	0.028
	3.0	0.031	0.028	0.044	0.028	0.030
	3.5	0.033	0.040	0.038	0.036	0.037
	4.0	0.037	0.037	0.062	0.046	0.036
	5.0	-	-	-	-	0.030
	6.0	-	-	-	-	0.048
7.0	-	-	-	-	0.055	
Total mass in Effluent (mg)		103.72	142.15	131.12	96.85	50.00
Total mass removal		-20.06%	-30.31%	-43.21%	-12.11%	42.13%

* Overflow occurs between 80 min and 124 min

** No flow occurs at this time

Zn

Unit: (mg/L)

Target concentration: 0.132 mg/L

Sample	Time (hr)	Shrub	TxDOT	Native	Bermuda	Control*
Influent	0.0	0.082	0.117	0.115	0.117	0.117
	1.0	0.109	0.123	0.130	0.118	0.135
	2.0	0.126	0.122	0.129	0.130	0.137
	3.0	0.130	0.123	0.119	0.133	0.127
Total mass in influent (mg)		409.78	517.44	434.28	409.78	409.78
Effluent	0.5	0.034	0.028	0.038	0.097	NF**
	1.0	0.028	0.034	0.148	0.085	0.083
	1.5	0.028	0.033	0.122	0.096	0.132
	2.0	0.027	0.022	0.078	0.059	0.069
	2.5	0.029	0.018	0.106	0.060	0.072
	3.0	0.032	0.027	0.124	0.084	0.073
	3.5	0.043	0.021	0.027	0.095	0.108
	4.0	0.024	0.025	0.023	0.109	0.106
	5.0	-	-	-	-	0.080
	6.0	-	-	-	-	0.116
7.0	-	-	-	-	0.151	
Total mass in effluent (mg)		71.76	92.03	308.25	221.70	136.77
Total mass removal		82.49%	82.21%	29.02%	45.90%	66.63%

* Overflow occurs between 80 min and 124 min

** No effluent at this time

Pb

Unit: (mg/L)

Target concentration: 0.080 mg/L

Sample	Time (hr)	Shrub	TxDOT	Native	Bermuda	Control*
Influent	0.0	0.035	0.048	0.047	0.053	0.040
	1.0	0.057	0.056	0.064	0.067	0.052
	2.0	0.060	0.056	0.064	0.070	0.053
	3.0	0.063	0.053	0.057	0.066	0.054
Total mass in influent (mg)		187.420	236.66	198.62	187.42	187.42
Effluent	0.5	0.016	0.015	0.018	0.018	NF**
	1.0	0.014	0.015	0.016	0.016	0.026
	1.5	0.015	0.015	0.015	0.016	0.018
	2.0	0.014	0.014	0.018	0.015	0.016
	2.5	0.014	0.014	0.015	0.015	0.017
	3.0	0.014	0.016	0.015	0.016	0.017
	3.5	0.014	0.014	0.015	0.015	0.017
	4.0	0.016	0.014	0.014	0.015	0.017
	5.0	-	-	-	-	0.017
	6.0	-	-	-	-	0.016
7.0	-	-	-	-	0.015	
Total mass in effluent (mg)		36.20	52.38	49.33	42.92	26.53
Total mass removal		80.69%	77.87%	75.16%	77.10%	85.85%

* Overflow occurs between 80 min and 124 min

** No effluent at this time

TSS

Unit: (mg/L)

Target concentration: 98.167 mg/L

Sample	Time (hr)	Shrub	TxDOT	Native	Bermuda	Control*
Influent	0.0	50	60	60	70	50
	1.0	150	100	10	50	60
	2.0	40	70	30	50	50
	3.0	60	50	70	60	40
Total mass in influent (mg)		198,097	250,140	209,939	198,097	198,097
Effluent	0.5	40	80	70	50	NF**
	1.0	50	50	80	40	20
	1.5	50	60	40	60	90
	2.0	10	50	40	30	30
	2.5	40	40	70	30	20
	3.0	20	60	60	50	20
	3.5	20	70	70	50	0
	4.0	50	70	80	50	10
	5.0	-	-	-	-	20
	6.0	-	-	-	-	20
7.0	-	-	-	-	20	
Total mass in effluent (mg)		81,117	189,684	160,244	123,410	32,766
Total mass removal		59.05%	24.17%	23.67%	37.70%	83.46%

* Overflow occurs between 80 min and 124 min

** No effluent at this time

NO₃-N

Unit: (mg/L)

Target concentration: 0.148 mg/L

Sample	Time (hr)	Shrub	TxDOT	Native	Bermuda	Control*
Influent	0.0	0.236	0.290	0.315	0.301	0.279
	1.0	0.257	0.263	0.347	0.296	0.277
	2.0	0.252	0.291	0.318	0.299	0.277
	3.0	0.262	0.285	0.354	0.305	0.276
Total mass in influent (mg)		979.91	1224.81	1027.97	979.91	979.91
Effluent	0.5	46.820	12.621	4.120	5.492	NF**
	1.0	7.340	4.191	2.234	2.273	14.151
	1.5	27.670	3.919	2.870	4.567	22.866
	2.0	2.068	1.190	1.347	1.771	36.888
	2.5	1.618	1.051	1.128	1.191	35.934
	3.0	1.426	0.887	1.072	0.902	32.828
	3.5	1.537	1.083	1.391	1.413	26.789
	4.0	1.746	1.231	1.370	1.990	19.480
	5.0	-	-	-	-	8.642
	6.0	-	-	-	-	17.243
7.0	-	-	-	-	9.031	
Total mass in effluent (mg)		29843.01	10438.28	6226.07	8190.56	39432.42
Total mass removal		-2945.47%	-752.24%	-506.65%	-735.85%	-3924.07%

* Overflow occurs between 80 min and 124 min

** No effluent at this time

NH₃-N

Unit: (mg/L)

Target concentration: 0.770 mg/L

Sample	Time (hr)	Shrub	TxDOT	Native	Bermuda	Control*
Influent	0.0	0.648	0.851	0.943	0.890	0.756
	1.0	0.771	0.851	0.815	0.877	0.794
	2.0	0.878	0.877	0.985	0.865	0.794
	3.0	0.836	0.883	0.791	0.840	0.790
Total mass in influent (mg)		2854.07	3547.64	2977.48	2854.07	2854.07
Effluent	0.5	0.039	0.056	0.051	0.157	NF**
	1.0	0.047	0.058	0.101	0.145	0.274
	1.5	0.037	0.096	0.167	0.222	0.219
	2.0	0.035	0.100	0.134	0.245	0.377
	2.5	0.073	0.083	0.113	0.158	0.448
	3.0	0.072	0.086	0.113	0.133	0.409
	3.5	0.026	0.045	0.063	0.095	0.358
	4.0	0.039	0.040	0.019	0.105	0.394
	5.0	-	-	-	-	0.445
	6.0	-	-	-	-	0.180
7.0	-	-	-	-	0.108	
Total mass in effluent (mg)		115.01	303.35	385.24	546.86	600.81
Total mass removal		95.97%	91.45%	87.06%	80.84%	78.95%

* Overflow occurs between 80 min and 124 min

** No effluent at this time

TN

Unit: (mg/L)

Target concentration: 1.836 mg/L

Sample	Time (hr)	Shrub	TxDOT	Native	Bermuda	Control*
Influent	0.0	1.262	1.454	1.836	2.419	1.523
	1.0	2.078	1.272	1.895	3.341	1.728
	2.0	2.163	1.604	1.809	2.624	1.992
	3.0	1.970	1.342	1.838	2.008	1.728
Total mass in influent (mg)		6360.53	0831.51	6740.73	6360.53	6360.53
Effluent	0.5	43.774	17.045	5.726	10.455	NF**
	1.0	12.812	8.705	3.567	5.973	15.647
	1.5	27.774	9.152	2.754 ⁺	4.214	22.411
	2.0	5.328	3.990	1.941	3.637	31.359
	2.5	2.635	3.787	1.899	3.668	20.528
	3.0	1.996	3.572	1.894	3.672	27.297
	3.5	2.265	4.227	2.916	2.905	24.307
	4.0	3.899	4.978	4.856	4.003	20.742
	5.0	-	-	-	-	11.235
	6.0	-	-	-	-	20.149
7.0	-	-	-	-	19.684	
Total mass in effluent (mg)		33772.00	23447.58	8083.09	12886.57	33953.54
Total mass removal		-430.96%	-191.94%	-19.91%	-102.60%	-433.82%

* Overflow occurs between 80 min and 124 min

** No effluent at this time

⁺ Sample missed, estimated by averaging the concentrations for 1.0 hr and 2.0 hr samples

TP

Unit: (mg/L)

Target concentration: 0.173 mg/L

Sample	Time (hr)	Shrub	TxDOT	Native	Bermuda	Control*
Influent	0.0	0.505	0.275	1.025	0.387	0.488
	1.0	0.668	0.598	0.936	0.794	0.480
	2.0	0.428	0.676	1.287	0.844	0.492
	3.0	0.504	1.247	1.032	0.743	0.408
Total mass in influent (mg)		2319.66	2929.06	2458.32	2319.66	2319.66
Effluent	0.5	15.098	21.425	12.438	13.490	NF**
	1.0	35.137	29.020	14.961	10.026	28.542
	1.5	32.497	29.797	19.150	7.595	92.980
	2.0	18.863	27.288	14.007	9.752	7.514
	2.5	18.320	25.758	15.503	8.007	1.418
	3.0	17.582	19.490	12.542	6.771	4.898
	3.5	23.216	19.529	11.418	8.039	5.878
	4.0	14.993	23.941	10.510	8.418	5.303
	5.0	-	-	-	-	8.050
	6.0	-	-	-	-	16.841
7.0	-	-	-	-	13.560	
Total mass in effluent (mg)		58238.72	93679.38	47001.58	16521.27	24694.68
Total mass removal		-2453.76%	-3098.27%	-1811.92%	-964.58%	-612.23%

* Overflow occurs between 80 min and 124 min

** No effluent at this time

E. coli

Unit: (No. of 16S rRNA gene copy/100ml)

Sample	Time (hr)	Shrub	TxDOT	Native	Bermuda	Control*
Influent	0.0	213	63001	30281	33123	58463
	1.0	558988	128172	39930	33123	41698**
	2.0	518722	29170	91556	4850	24932
	3.0	298315	13108	13010	10166	68401
Total mass in influent (mg)		261132722	253337716	272825232	257235219	249440212
Effluent	0.5	99	19774	6789	7371	NF ⁺
	1.0	882	13305	13108	8689	1341
	1.5	373	16160	11372	10015	1849
	2.0	4434	12533	15802	8246	1768
	2.5	4274	17285	11372	1392	1445
	3.0	4114	12722	11037	2475	936
	3.5	3288	9648	5507	806	2037
	4.0	8370	5385	6115	2627	2768
	4.5	3362	-	-	-	5226
	5.0	2608	-	-	-	3846
	5.5	-	-	-	-	3263
	6.0					3846
Total mass in effluent (mg)		5751951	21634198	23426163	13487378	1177604
Total mass removal		97.80%	91.46%	91.41%	94.76%	99.53%

* Overflow occurs between 80 min and 124 min

** Originally an outliers (2.65×10^9); replaced by the average of the concentrations of 0 and 2nd hour samples.

⁺ No effluent at this time

