Final Report

Bio-sorption Activated Media for Nitrogen Removal

In a Rapid Infiltration Basin – Monitoring Project

Florida Department of Environmental Protection:

Project Agreement No. NS 003

Submitted by:

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City of DeLand and University of Central Florida Project Team

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Executive Summary

The purpose of this report is to present background information for the project, data collected, analysis of data, and conclusions for the fate of nitrogen in a stormwater pond modified to discharge reclaimed water and stormwater to the ground. Funding was made possible for this project from the State Department of Environmental Protection under the FY 16-17 general appropriations act in partnership with the St John's River Water Management District and the City of Deland.

The Bent Oak City of DeLand pond (see Appendix A for the Environmental Resource Permit #76187-5) was increased in size and permitted in March of 2016 so that it could function to discharge both excess reclaimed water and excess stormwater to the ground. It was called in the permit a reclaimed water storage and recovery modification. Because of the discharge of treated sewage, it can be referred to as a rapid infiltration basin (RIB). However, it is used for the disposal of both reclaimed water and excess stormwater. The RIB was again modified in the fall of 2016 for additional removal of nitrogen and phosphorus using Bio-sorption Activated Media (BAM) to determine if additional nitrogen can be removed. The information in this report is used to evaluate the effectiveness in the removal of nitrogen and phosphorus and to supply technical data to support the removal when BAM is used.

Two areas within the northern part of the Deland RIB were divided into two RIBs so that one RIB had two feet of BAM added to the bottom and the other RIB did not have BAM and is called the Control RIB. The Control RIB soils had about 2% clay with sand. This type of soil is believed to remove nitrogen and phosphorus, as opposed to sand which produces minor removal. Thus, it was chosen to be compared to BAM. Both the BAM RIB and the Control RIB were dosed with the same water. For two months at the end of 2016, input flow volume monitoring was conducted to balance unit volume of input flow (gallons per acre per day) to the two RIBs. Lysimeters were installed two feet under the BAM in the BAM RIB as well as two feet under the Control RIB. From January 2017 through the end of August 2017 loading was done using reclaimed water; some of which included river water augmentation. In September, Hurricane Irma provided excess stormwater to load the RIBS. Thus, two loading events and water samples from the six lysimeters were obtained to document water quality conditions during this time. In January and February 2018, loading of the RIBS continued with reclaimed water and this time in a high-water table condition. The high-water table was caused by the rain from Hurricane Irma. Thus, a total of 12 loading events of the RIBS were completed. Ten (10) events were monitored using reclaimed water from the DeLand Water Reclamation Facility (WRF) and two (2) events using excess stormwater from Hurricane Irma.

The results show increased removal of nitrogen and phosphorus when using BAM versus the removal using on-site Taveras soil. The on-site soil is expected to remove nitrogen because of a low infiltration rate (½ - 1 inch/hr.) and the high clay composition. Natural soils normally are not consistent in clay content and thus removal is not consistent across an area. It should be noted that normally the soils beneath a RIB are primarily sand and thus little removal of nitrogen is expected.

The BAM is mixed to provide consistency in the percentage of constituents. Thus, removal across an area for nitrate and nitrite species (NOx) should be relatively more consistent than in natural soils and greater using BAM. The BAM was not formulated to remove organic and ammonia species of nitrogen but can be if needed. The history of reclaimed water quality for the Deland Water Reclamation Facility showed a high percentage of nitrates relative to total nitrogen. For this report, the term nitrate will be used as most of the reported NOx is in the form of nitrates. This also recognizes that nitrite rapidly go to nitrate in surface and surficial aquifers.

Comparisons based on measurements below detection and average removal are summarized as:

Loading with Reclaimed Water: Ten loading events were sampled. There was a total of 30 lysimeter samples under the BAM RIB and 29 under the Control RIB. There was one lysimeter in the Control RIB that did not yield sufficient volume of water for analysis. For nitrate measures less than detection, the percent removal was calculated based on half the detection limit.

	BAM RIB				Control RIB			
	% <	%	Average m/L		% <	%	Average mg/L	
	detection	removal	In	Out	detection	removal	In	Out
Nitrate	73	83	3.61	0.72	38	41	3.61	2.42

The median % removal for the BAM RIB and Control RIB were 97% and 49% respectively.

Loading with Excess Stormwater: There were 6 lysimeter samples per RIB. The detection limit for nitrates was lowered resulting in no exceedance.

	BAM RIB				Control RIB			
	% <	%	Average (mg/L)		% <	%	Average (mg/L)	
	detection	removal	In	Out	detection	removal	In	Out
Nitrate	0	95	0.303	0.014	0	90	0.303	0.031

Using both the excess reclaimed and excess stormwater loading, and weighting the removals by the number of samples, the removal of nitrate was 85% in the BAM RIB and 49% in the Control RIB.

Total phosphorus removal was also calculated when loading with reclaimed as 66% and 37% for the BAM RIB and the Control RIB respectively. Other water quality measures are also listed in the report and include pH, turbidity, conductivity, chlorides, fecal coliforms, TKN, and total nitrogen. For fecal coliforms, there were 36 measurements for each of the Control and BAM lysimeters for all loading water and includes the blended river, reclaimed and stormwater. There were five measured values that were too numerous to count in the Control and none in the BAM lysimeters. The average fecal coliforms were 31 and 609 CFU/100 ml in the BAM and Control lysimeters respectively.

The nitrate removal was further supported using microbial assessment using DNA technologies. The populations of microbiological organisms demonstrated that the BAM RIB provided an environment for the cultivation of more of the appropriate bacteria compared to the Control RIB. The Control RIB itself provided potential for removal, presumably because of the 2-4% clay content, but the organisms were not as great in number and the removal was less. The micro biological analysis yielded information regarding the use of 2 feet of media as an appropriate depth and location for which the greater population were established. Further, the quantity of bacteria population can be closely related to the increased microbiological activity and consequently improved nitrate removal. The physiochemical properties of the BAM and Control RIB soil, also affected nitrate removal through the encouragement or inhibition of microbial growth aiding or preventing the removal of nitrate by the bacteria.

ACKNOWLEDGEMENT

The authors at the University of Central Florida and at the City of DeLand appreciate funding and technical guidance from the State Department of Environmental Regulation. The funding provided the opportunity to support students and faculty in their pursuit of information related to the use of BAM for water quality improvement. Also, the information provided another option for the City of DeLand and others to lower the discharge of nitrogen to the aquifer.

In addition, the authors recognize the assistance and funding from the City of DeLand that was used for laboratory water quality analysis, monitoring of water levels, loading of the RIBs, and construction. The St. Johns River Water Management District also provided funding for the construction of the RIBs and initial encouragement to document performance. Environmental Conservation Solutions provided media installation and evaluation of soil properties. The University of Central Florida faculty provided work space, field data collection, analysis of results and research coordination using a team of faculty with water quality, geotechnical, and water resources backgrounds.

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Chapter 1: Project Background and Description

Background

The reduction of nitrates entering aquifers that discharge to springs and estuaries are the focus for water quality and quantity investigations in the State. The results in this report show how a Rapid Infiltration Basin (RIB) can be modified to reduce nitrates as well as to increase the quantity of water entering the aquifer. The Saint Johns River Water Management District with the City of DeLand invested $400,000 in installing a pollution control media in the bottom of a RIB. The modified RIB was monitored and information in this report was generated using an FDEP contract at a cost of $119,305. The FDEP funding was used for collecting and analyzing hydrologic and water quality data.

The sources of water in this report are from the City of DeLand's Wiley M. Nash Water Reclamation Facility (WRF) and from excess stormwater. The WRF has a design treatment capacity of 6 million gallons per day. In 2004 there was an addition of an above ground storage tank and modification of an existing onsite stormwater pond. This allowed storage of excess reclaimed effluent and storm water from around the city. The tank and pond have a capacity of 6.4 million gallons and brings the total storage to just over 12 million gallons onsite. The additional storage allows the facility to discharge less to the St. Johns River. Approximately 99 percent of the reclaimed effluent is now reused for irrigation with the excess treated water discharged to a RIB. The RIB is located just west of the WRF's pond. The water in the storage tank/pond is discharged to the RIB for groundwater recharge. Excess reclaimed water and excess stormwater normally would go to direct discharge to the River if not for the use of the RIB. The RIB allows the opportunity to recharge the surficial aquifer that feeds local springs in the area. Blue spring is located about 5 miles southwest of the RIB.

Rapid Infiltration Basins (RIBs) have been widely used for decades with more than 350 domestic wastewater treatment plant sites utilizing them across the United States and around the world (USEPA, 2003). The primary focus of their use has been for groundwater recharge, effluent disposal, or a combination of both goals. More than 30 sites utilizing RIBs are in the state of Florida (FDEP, 2017). Figure 1 shows a map including the 10 largest RIB sites by permitted daily capacity. Table 1 lists those 10 WWTP sites by name, location, and permitted daily flow capacity. The RIBs at the DeLand WRF is the fifth largest.

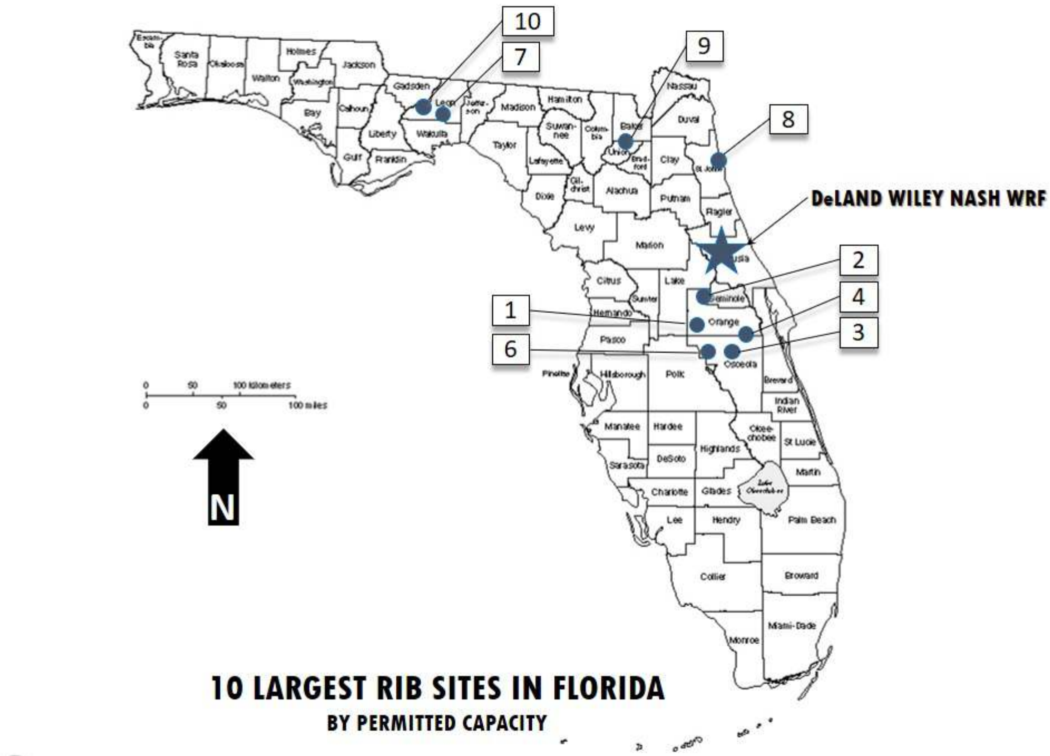


Figure 1 10 Largest WWTP sites utilizing RIBs in the state of Florida

Table 1 Ten (10) Largest WWTP sites utilizing RIBs in the state of Florida

	Plant Name	Location	Permitted ADF (million gallons/day)
1	Orlando Conserv II	West Orange County	80.9
2	Northwest WRF	Northwest Orange County	10.3
3	Toho Water Auth. South Bermuda	Kissimmee/Osceola County	8.5
4	Orlando Conserv I	Southeast Orange County	7.5
5	**Wiley Nash WRF**	**DeLand/Volusia County**	**6.0**
6	Toho Water Auth. Sand Hill Road	Kissimmee/Osceola County	6.0
7	Killearn Lakes WWTP	Quincy/Leon County	0.7
8	North Beach Utilities WWTP	St. Augustine/St. Johns County	0.3
9	Baker Correctional Inst. WWTP	Sanderson/Baker County	0.276
10	Sandstone Ranch WWTP	Tallahassee/Leon County	0.250

DeLand WRF Operations Process

Following is a schematic of the DeLand WRF treatment process train (Figure 2). It is operated to produce reclaimed water for irrigation. In the process design, the DeLand WRF reduces nitrogen using a combination of aerobic and anoxic treatment zones. Currently, it is operated at about half the hydraulic capacity, thus providing an opportunity for additional pollutant removal. When there is not sufficient reclaimed water to meet irrigation demands, makeup water is delivered from the River. When there is excess reclaimed water in the storage tank, it is discharged to the RIBS. During flood conditions, the storage water can be a blend of reclaimed, river and stormwater. Thus, the RIBS while usually only disposing of excess reclaimed water can also dispose of a blend of water including excess stormwater.

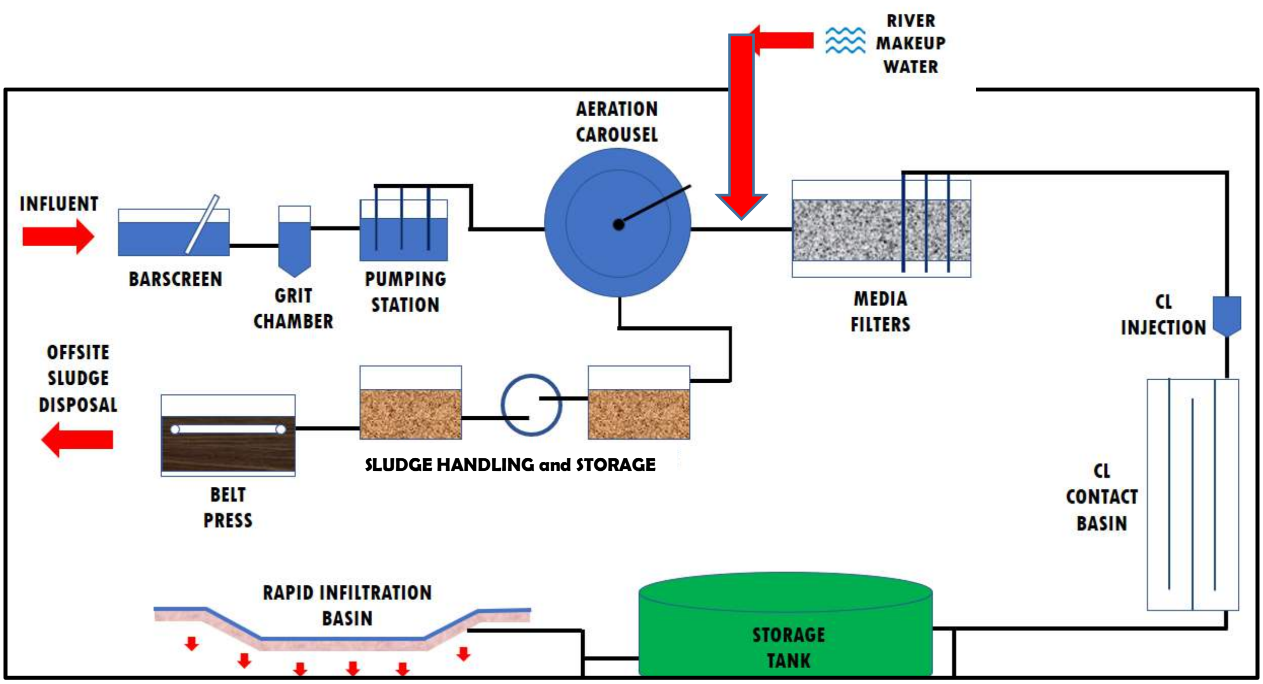


Figure 2 Schematic process flow diagram for the DeLand WRF

Pre RIB Modification Water Quality Conditions

The selection of a Bio-sorption Activated Media (BAM) mix is based on the water quality conditions before the installation of BAM (see Tables 2 and 3). Nitrogen was primarily in the form of nitrates and nitrate is related to springs deterioration. Thus, a BAM blend to remove nitrates was chosen.

Table 2 DeLand WRF Effluent sampling reports: years 2013-2015
Source: City of DeLand Wiley Nash WRF Staff

	TKN mg/L	**NO_x** mg/L	**TN** mg/L	**TP mg/L**
		2013		
8/7-8	0.934	14	14.934	4.16
8/14-15	1.17	9.84	11.010	3.06
8/20-21	1.00	18.1	19.100	4.44
8/28-29	0.12	16.3	16.420	0.426
9/4-5	0.17	14.5	14.670	0.218
9/11-12	3.87	1.31	5.180	0.26
9/18-19	1.1	6.48	7.580	0.751
9/25-26	1.02	2.96	3.980	1.1
10/2-3	1.09	5.72	6.810	2.39
10/9-10	1.38	4.68	6.060	2.01
10/16-17	1.15	3.54	4.690	3.08
10/23-24	1.14	8.5	9.640	2.71
10/30-31	0.758	9.1	9.858	3.19
11/5-6	0.602	10.8	11.402	3.53
11/13-14	4.98	0.572	5.552	3.57
11/20-21	14.0	0.644	14.644	2.66
11/26-27	0.967	7.98	8.947	3.99
12/4-5	0.702	8.24	8.942	3.07
12/11-12	6.22	3.16	9.380	3.39
12/17-18	5.91	2.9	8.810	3.39
12/25-26	1.42	2.9	4.320	3.00
		2014		
1/1-2	0.98	3.5	4.480	2.6
1/8-9	0.90	3.8	4.700	3.2
1/15-16	3.3	5.6	8.900	3.5
1/22-23	2.99	4.82	7.810	3.06
1/29-30	2.03	5.1	7.130	2.84
2/5-6	6.44	1.28	7.720	3.28
2/12-13	4.92	1.57	6.490	3.00
2/19-20	3.73	3.44	7.170	0.88
2/26-27	10.6	2.84	13.440	0.239
3/5-6	11.2	3.34	14.540	0.16
3/12-13	12.1	1.82	13.920	0.431
3/19-20	13.7	2.04	15.740	0.649
3/26-27	13.6	2.36	15.960	0.266
4/2-3	11.3	2.84	14.140	2.12
4/8-9	12.1	1.37	13.470	2.72

4/16-17	9.42	4.52	13.940	3.12
4/23-24	2.39	6.28	8.670	3.45
4/30-5/1	1.13	3.14	4.270	3.39
5/7-8	1.26	3.4	4.660	3.68
5/14-15	1.25	0.282	1.532	3.14
5/21-22	0.987	3.48	4.467	3.48
5/28-29	1.16	4.54	5.700	4.3
6/4-5	0.935	5.95	6.885	4.49
6/11-12	1.14	1.51	2.650	3.36
6/18-19	0.729	1.4	2.129	3.25
6/25-26	0.830	1.77	2.600	3.15
7/2-3	1.2	4.11	5.310	4.57
7/9-10	1.96	2.72	4.680	3.78
7/16-17	1.23	1.79	3.020	3.05
7/23-24	6.98	0.436	7.416	3.83
7/30-31	0.962	2.03	2.992	2.69
8/6-7	0.895	4.53	5.425	4.34
8/13-14	0.823	5.64	6.463	4.63
8/20-21	0.920	2.37	3.290	3.92
8/27-28	3.76	1.05	4.810	4.1
9/3-4	0.824	5.27	6.094	3.35
9/10-11	0.555	12.4	12.955	4.4
9/17-18	0.470	9.26	9.730	4.71
9/24-25	0.838	5.44	6.278	4.45
10/1-2	0.813	1.71	2.523	3.75
10/8-9	2.26	1.83	4.090	4.19
10/15-16	2.15	1.01	3.160	4.49
10/22-23	1.99	2.36	4.350	4.04
10/29-30	1.16	6.13	7.290	4.99
11/5-6	1.30	4.63	5.930	3.53
11/12-13	2.78	3.58	6.360	3.74
11/19-20	3.41	3.18	6.590	3.91
11/25-26	6.77	1.33	8.100	3.29
12/3-4	4.47	2.29	6.760	3.38
12/10-11	0.482	11.9	12.382	2.89
12/17-18	0.647	17	17.647	5.38
12/25-26	2.54	1.4	3.940	3.52
2015				
1/1-2	1.43	2.24	3.670	3.69
1/7-8	1.70	2.13	3.830	3.49
1/14-15	3.60	0.694	4.294	2.59
1/21-22	1.9	1.65	3.550	3.1
1/28-29	1.17	4.37	5.540	2.82
2/4-5	1.95	4.87	6.820	3.55

2/11-12	2.27	3.83	6.100	3.73
2/18-19	2.12	2.36	4.480	3.06
2/25-26	2.04	2.84	4.880	3.73
3/4-5	1.59	3.31	4.900	3.75
3/11-12	1.34	3.36	4.700	3.36
3/18-19	4.02	1.56	5.580	3.95
3/25-26	5.01	2.83	7.840	2.59
4/1-2	4.31	1.89	6.200	3.11
4/8-9	7.96	0.89	8.850	2.98
4/15-16	5.85	1.01	6.860	2.59
4/22-23	5.94	2.14	8.080	3.54
4/29-30	3.86	1.36	5.220	3.5
5/6-7	1.85	1.25	3.100	3.13
5/13-14	1.18	0.968	2.148	3.02
5/20-21	1.5	0.69	2.190	2.73
5/27-28	1.18	1.16	2.340	2.28
6/3-4	1.07	0.997	2.067	3.14
6/10-11	1.78	0.895	2.675	3.6
6/17-18	2.84	0.343	3.183	2.21
6/24-25	1.29	1.37	2.660	2.74
7/1-2	1.18	0.726	1.906	3.01
7/8-9	1.26	0.845	2.105	3.86
7/15-16	1.01	0.327	1.337	2.72
7/22-23	1.89	0.363	2.253	2.39
7/29-30	1.75	0.734	2.484	1.36
8/5-6	2.22	0.541	2.761	2.91
8/12-13	1.81	0.502	2.312	3.37
8/19-20	2.02	0.883	2.903	2.59
8/26-27	2.1	1.28	3.380	2.99
9/2-3	1.09	1.5	2.590	1.92
9/9-10	1.05	2.89	3.940	3.73
9/16-17	0.933	1.68	2.613	2.23
9/23-24	0.953	2.84	3.793	2.96
9/30-10/1	2.74	5.51	8.250	2.95
10/7-8	18.1	0.887	18.987	4.08
10/13-14	13.5	0.805	14.305	1.92
10/21-22	4.08	1.15	5.230	2.86
10/28-29	13.2	0.746	13.946	6.68
11/4-5	5.28	1.62	6.900	4.00
Average	3.22	3.61	6.83	3.10
Maximum	18.1	18.1	19.1	6.68
Minimum	0.12	0.282	1.337	0.16
std dev	3.647	3.624	4.252	1.117
	TKN mg/L	NO_x mg/L	TN mg/L	TP mg/L

The data in Table 2 are provided to document pre-water quality conditions and in Table 3 water quality conditions while loading the BAM RIB and Control RIB.

Table 3 Effluent sampling reports: 7/6/2016 through 8/31/2017 and 1/17/2018 through 2/22/2018
Source: City of DeLand Wiley Nash WRF Staff

Date	TKN mg/L	NO_x mg/L	TN mg/L	TP mg/L
7/6/2016-7/7/16	4.76	0.936	5.70	2.70
7/13/16-7/14/16	3.36	0.888	4.25	1.46
7/20/21/16-7/21/16	5.78	1.21	6.99	2.40
7/27/16-7/28/16	6.96	1.7	8.66	5.34
8/3/16-8/4/16	3.09	1.71	4.80	4.66
8/10/16-8/11/16	3.26	1.48	4.74	3.60
8/17/16-8/18/16	2.46	2.12	4.58	3.40
8/24/16-8/25/16	5.91	1.3	7.21	3.50
8/31/16-9/1/16	6.47	1.34	7.81	2.86
9/7/16-9/8/16	5.33	1.36	6.69	3.96
9/14/16-9/15/16	1.76	1.33	3.09	2.74
9/21/16-9/22/16	1.77	1.76	3.53	3.50
9/28/16-9/29/16	1.57	1.52	3.09	4.12
10/5/16-10/6/16	1.57	0.574	2.14	3.02
10/12/16-10/13/16	2.32	0.763	3.08	1.46
10/19/16-10/20/16	3.03	1.98	5.01	3.12
10/26/16-10/27/16	2.03	2.68	4.71	3.34
11/2/16-11/3/16	1.56	2.30	3.86	3.06
11/9/16-11/10/16	4.62	1.53	6.15	2.66
11/16/16-11/17/16	2.57	2.22	4.79	2.26
11/22/16-11/23/16	1.44	2.06	3.50	2.58
11/30/16-11/31/16	1.91	4.44	6.35	3.84
12/7/16-12/8/16	1.14	3.49	4.63	4.04
12/14/16-12/15/16	1.23	1.81	3.04	3.60
12/21/16-12/22/13	1.02	1.52	2.54	3.68
12/28/16-12/29/16	1.04	8.24	9.28	4.14
1/4/17-1/5/17	0.987	3.38	4.37	4.16
1/11/17-1/12/17	1.00	6.32	7.32	3.84
1/18/17-1/19/17	1.14	6.15	7.29	3.86
1/25/17-1/26/17	0.988	9.46	10.4	3.96
2/1/17-2/2/17	1.97	7.31	9.28	3.54
2/8/17-2/9/17	0.714	8.14	8.85	4.10
2/15/17-2/16/17	1.01	7.40	8.41	4.10
2/22/17-2/23/17	1.01	9.34	10.4	5.36
3/1/17-3/2/17	0.952	8.20	9.15	5.19
3/8/17-3/9/17	0.464	8.48	8.94	5.24
3/15/17-3/16/17	1.20	6.76	7.96	4.50
3/22/17-3/23/17	1.09	4.28	5.37	3.98
3/29/17-3/30/17	3.78	4.20	7.98	5.40

4/5/17-4/6/17	1.18	3.30	4.48	4.06
4/12/17-4/13/17	1.60	5.58	7.18	3.88
4/19/17-4/20/17	1.59	4.54	6.13	2.68
4/26/17-4/27/17	1.94	4.74	6.68	4.62
5/3/17-5/4/17	1.79	0.268	2.06	4.75
5/10/17-5/11/17	1.17	0.788	1.96	2.13
5/17/17-5/18/17	1.90	1.31	3.21	3.57
5/27/17-5/25/17	3.44	1.09	4.53	2.90
6/7/17-6/8/17	1.08	6.49	7.57	3.62
6/14/17-6/15/17	1.02	2.02	3.04	3.73
6/21/17-6/22/17	1.17	0.773	1.94	3.14
6/28/17-6/29/17	1.01	0.079	1.09	2.70
7/5/17-7/6/17	0.282	0.825	1.11	3.12
7/12/17-7/13/17	0.282	1.32	1.60	3.98
7/19/17-7/20/17	0.282	1.78	2.06	3.88
7/26/17-7/27/17	0.282	0.883	1.17	3.61
8/2/17-8/3/17	0.742	0.079	0.821	1.97
8/9/17-8/10/17	1.99	0.079	2.07	2.77
8/16-17/8/17/17	0.706	1.22	1.93	3.65
8/23-17/8/24/17	0.764	0.079	0.843	2.70
8/30/17-8/31/17*	1.52	1.16	2.68	3.18
1/17/18-1/18/18	0.463	2.21	2.67	3.75
1/24/18-1/25/18	0.463	2.80	3.26	3.67
1/31/18-2/1/18	0.463	2.51	2.97	3.81
2/7/18-2/8/18	0.463	2.40	2.86	4.15
2/14/18-2/15/18	0.463	2.00	2.46	4.25
2/21/18-2/22/18	0.463	1.79	2.25	3.89
Average	1.86	2.94	4.80	3.58
Maximum	6.96	9.46	10.4	5.4
Minimum	0.282	0.079	0.821	1.46
std dev	1.676	2.662	2.724	0.913
	TKN mg/L	NO_x mg/L	TN mg/L	TP mg/L

*Discontinued reclaimed loading as excess stormwater from hurricane Irma was used to dose the RIB. Reclaimed water used for dosing continued January 22, 2018.

It is important to note that during the months of July and August while loading the BAM and Control RIBs, the nitrogen levels had been decreasing presumably because of a change in the operation of the DeLand WRF and the addition of make-up water from the river. This is considered helpful in documenting performance with a lower Nitrogen influent concentration.

Chapter 2: RIB Modifications for Additional Pollution Removal

The RIBs for disposal are located approximately 1/2 mile east of the DeLand WRF (see Figure 3).

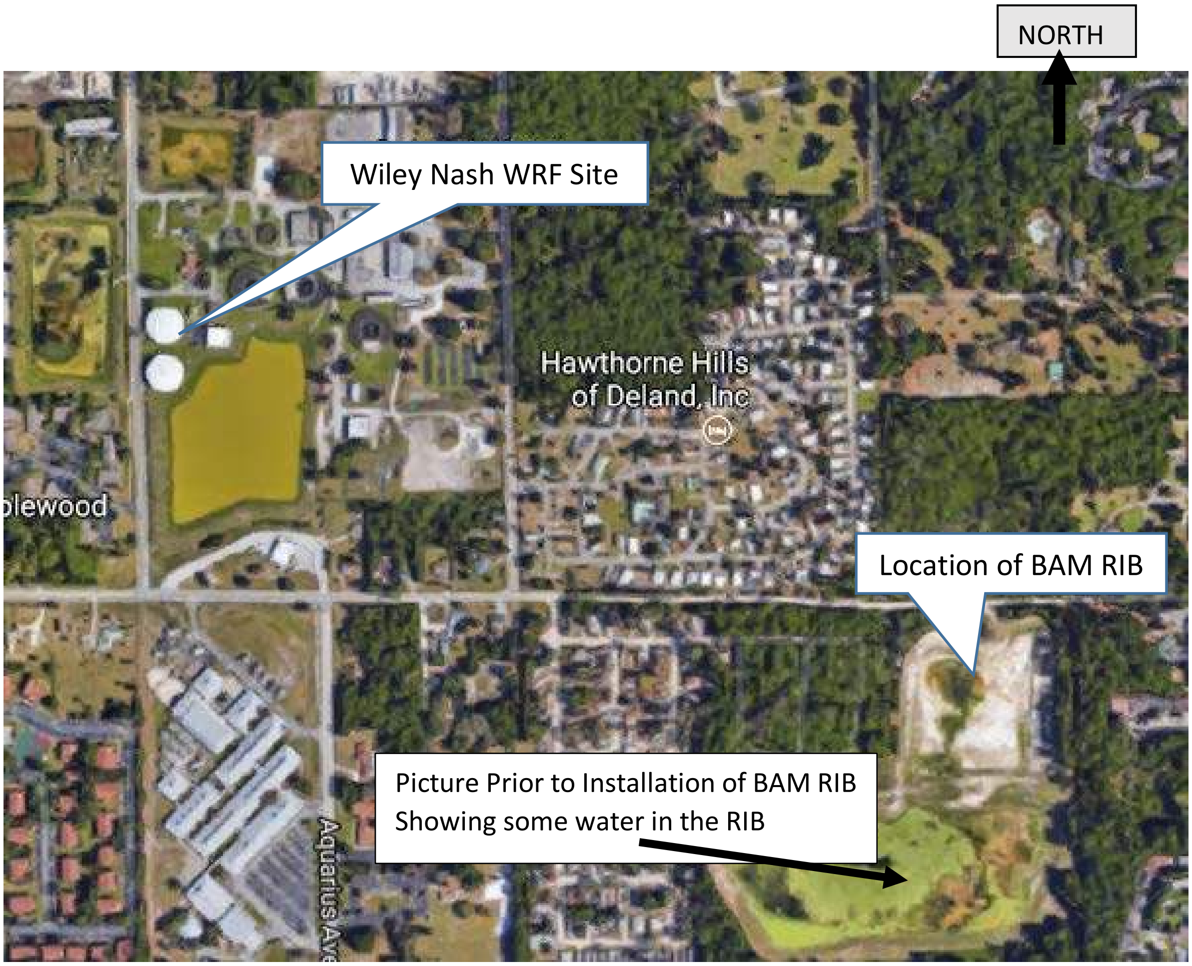


Figure 3 Aerial Photograph Depicting Main WRF Site and RIB Site Prior to Installation of BAM. Source: Google Maps (reference time is 2016)

The RIB to be modified with BAM is part of a larger RIB site. The larger and smaller part of the RIB can be loaded independent of each other. Thus, minimal modification of the RIB plumbing was necessary. All the RIB areas are used by the City of DeLand for reclaimed and excess stormwater disposal when the water is not used for irrigation. Reclaimed water, river water and excess stormwater are pumped to the RIBs. The existing northern RIB is approximately 3 Acres and is shown in Figure 4. The location of the berm separating the BAM RIB from the Control RIB and the lysimeters is shown in Figure 4 (b).

(a)

(b)

Figure 4 Aerial Photographs of (a) North (pre-study) RIB without separation berm and (b) BAM RIB (North) and Control RIB (South) with Lysimeter Locations
Source: Google Maps (reference time is May 2016 and March 2017)

The existing RIB was modified with a dividing berm. The dividing berm separates the RIB with BAM from the Control RIB (see Figure 4). The BAM RIB was constructed on the north side of the RIB and has 2 feet of BAM under an area of about 1 acre (43,600 SF). The Control RIB remained with the existing soil condition and is about 1.68 acres (73,200 SF). Figure 5 is the "as-built" plan. Reclaimed water from the DeLand WRF is equally distributed on an area basis to both the Control RIB and the BAM RIB.

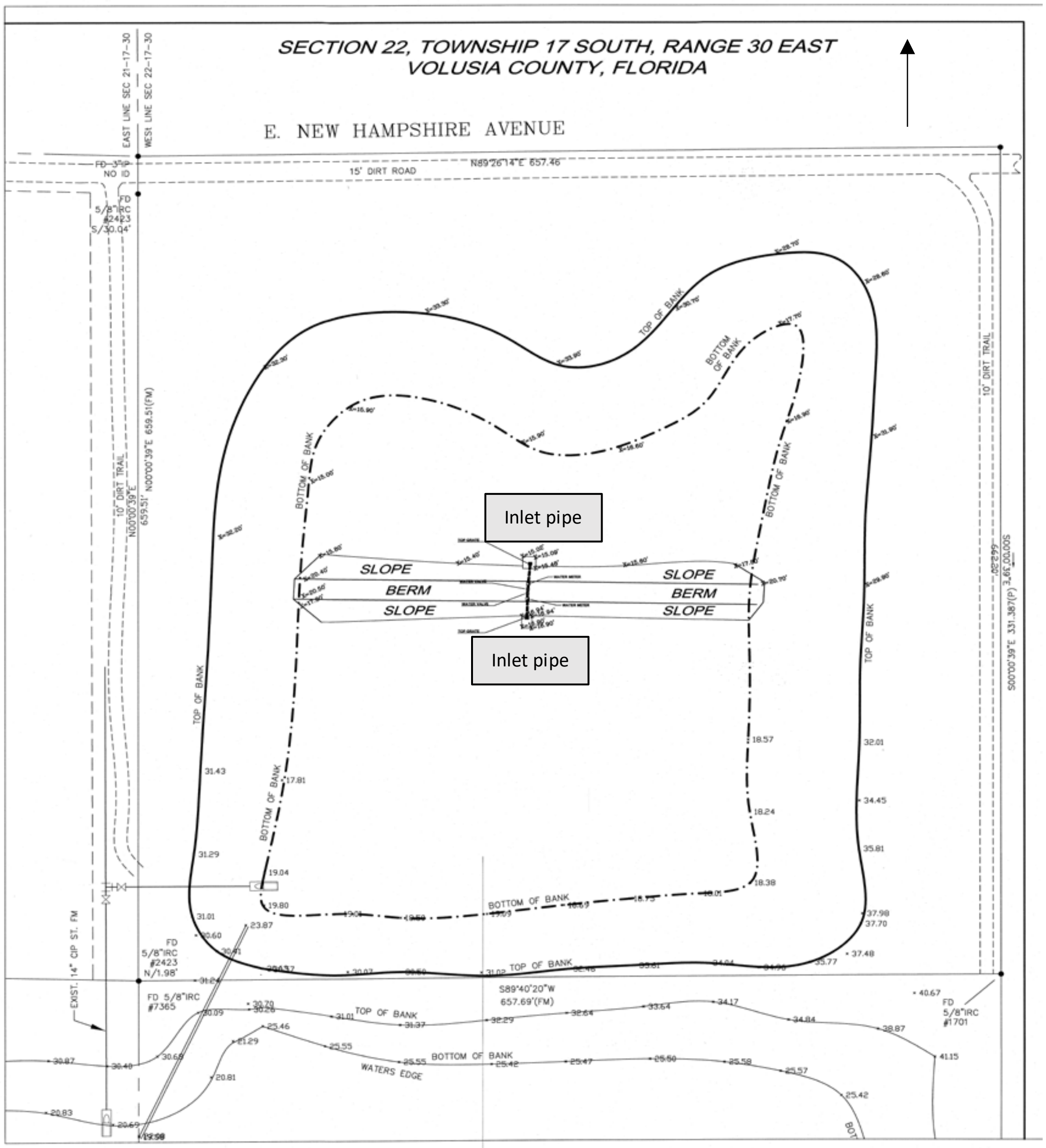


Figure 5 As-Built BAM and Control RIBS with Separating Berm (EFIRD Surveying Group, November 11, 2016, provided by the City of DeLand)

Staff gages were used to directly measure the water levels in both RIBs. Shown in Figure 6 are the staff gages for the BAM RIB and the Control RIB. Also shown are the inlet pipes that discharge water into the RIBS. The inlet pipes include vales and meters to distribute equally (on a RIB area basis) the same input flow rate from the reclaimed water reservoir of the Deland WRF.

(a) BAM RIB looking northeast (b) Control RIB looking southwest

Figure 6 Inlet Pipes and Staff Gages for Depth Measures

Lysimeters were located two feet below the BAM RIB and two feet below the Control RIB at three different locations (see Figures 4 and 7). These lysimeters were used to collect samples of percolated water through both the BAM in the BAM RIB and through the parent soil in the Control RIB. The locations with respect to the inlet water in the BAM RIB, are identified as 1 (60'N and 90'W of inlet), 2 (30'N and 110'W of inlet), and 3 (45'N and 75' E of inlet); and three sampling lysimeter locations within the Control RIB, identified as 4 (60'S and 90'W of inlet, 5 (30'S and 110'W of inlet), and 6 (45'S and 80'E of inlet). The inlet pipes are 6- inches in diameter with compound flow meters and located approximately 185 feet from the top of west bank or approximately 125 feet from the toe of slope.

The BAM used in this study follows those used in stormwater treatment (Chang, 2011, O'Reilly, 2012, Wanielista, 2008). This is a sorption mineral mix that does not decay over time. Numerous different component mixtures have been tested to achieve optimal removal of a variety of pollutants in a variety of settings. This BAM was specifically formulated to remove nitrates because nitrates was the primary component of nitrogen from the WRF at DeLand. Other formulations to remove all species of nitrogen are available. This mix needs an anoxic condition to remove nitrates and thus the mix retains a large residual moisture content. Mixes that also remove phosphorus are available. For this study a mixture of clay, tire crumb, and sand ("CTS") was selected. The measured dry bulk density was 63 pounds/cubic foot and a porosity of 32% at a dry condition and without compaction. The compacted CTS at the BAM RIB is about 90 pounds/cubic foot. The depth is 2 feet. The BOLD & GOLD (B&G®) CTS media was manufactured in the State of Florida using only Florida sourced materials. The final product size had more than 2% but less than 6% passing the 200 sieve. The mix will be composed of 85 % poorly graded sand and 15% sorption materials by volume. The sorption materials are composed of recycled tire crumb with no metal content and mined clay that has no less than 99% clay content. Percentages were determined by in place volume. The mix was non-flammable as tested up to 482° F. Water passing through the media did not exhibit acute or chronic toxicity and did not change the pH of the filtered water by more than 1.0 unit. The B&G® CTS media material has a water holding capacity (amount of water that the media can hold for crop use) of at least 10%, and total porosity of 35%. The permeability as measured in the laboratory was greater than 1.0 inch per hour at maximum compaction. The B&G® CTS media has a dissolved phosphorus (DP) removal capacity that exceeds 0.2 mg DP/gram of media as measured in the laboratory during normal operating conditions.

The experimental RIBs at the time nearing the end of a loading event are shown in Figure 7. The photos of Figure 7 were taken on February 25, 2017. The sampling lysimeters locations are shown.

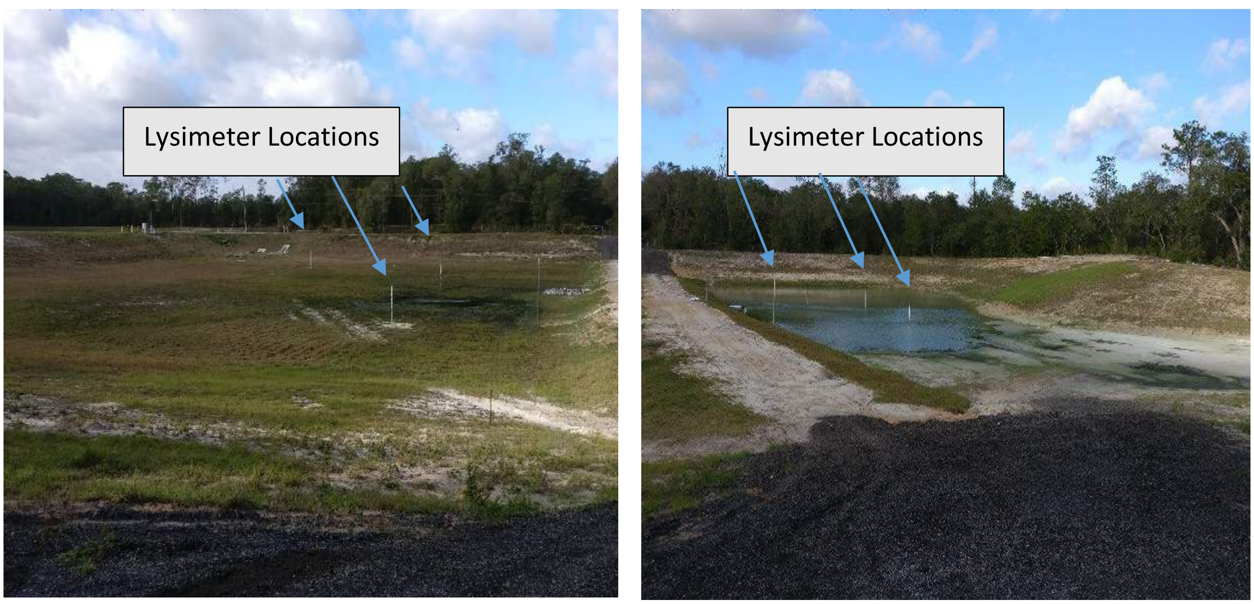


Figure 7 Ground View of RIB (left side is Control RIB, right side is the BAM media RIB) with Lysimeter locations and Separation Berm

Input Water Quality to the RIBS

Sampling of the DeLand WRF effluent indicates changes in nutrient concentration over time. The plant is improving operation to remove nitrogen as shown by the monitoring data starting in July of 2017, and as shown in Figure 8 relative to other times. These changes are positive in terms of documenting the performance of the BAM RIB because the BAM RIB must operate to remove nitrates for a variety of DeLand WRF nutrient water quality conditions.

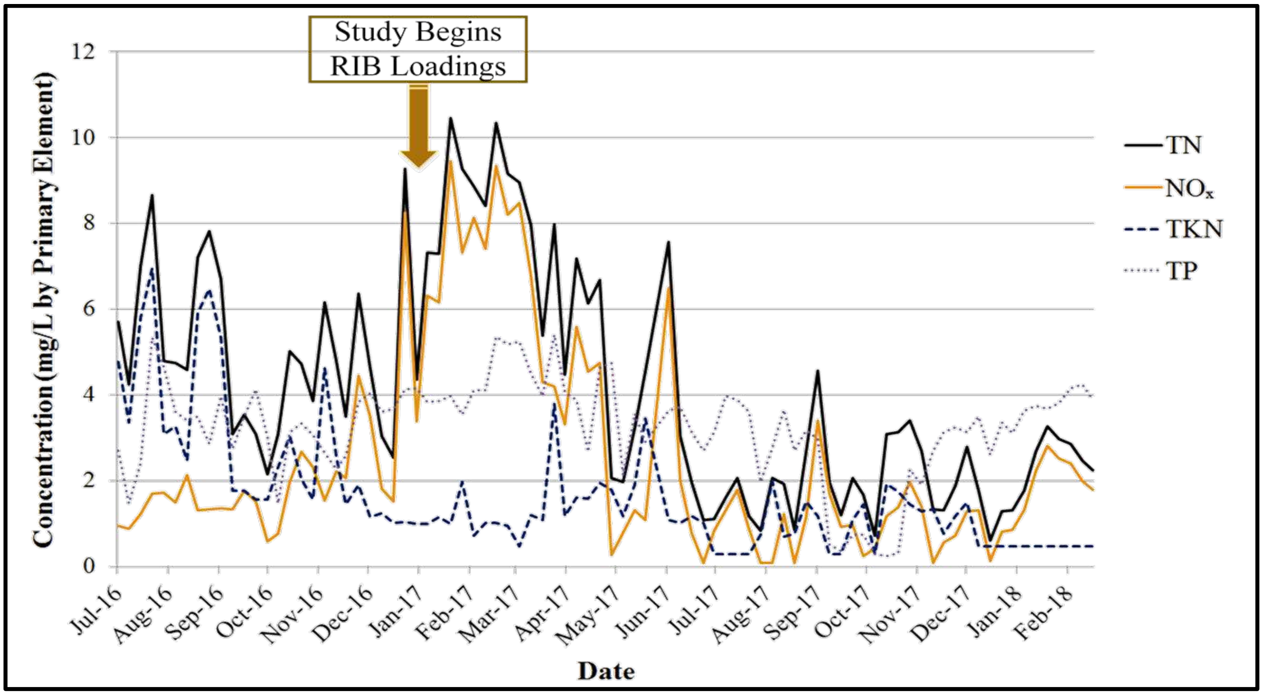


Figure 8 DeLand WRF Effluent Sampling (NOX (primarily nitrate), TKN, TN and TP)
Source: City of DeLand Wiley Nash WRF Staff, September 2017 and March 2018

The RIBS including the experimental RIBs (BAM RIB and Control RIB) received over 40 million gallons of excess stormwater from hurricane Irma during the period September 10-12. The loading was a beneficial use of the RIBS in that flooding in the City was minimized. Also, the stormwater loading of the RIBs provided a sampling “opportunity” not expected. The water delivered to the RIBs was essentially stormwater rather than previously loaded reclaimed water. A photo of the water levels after the stormwater loading is shown in Figure 9. The maximum depth of water in the RIBs was measured at about 12 feet. The water table also had risen and the water infiltrating from the RIBs was a slow rate or on average about 3 inches per day or 1/8 inch per hour. Water infiltrating from the RIBs with no water table restriction is about 1 inch per hour. The soil types at the surface are primarily sandy clay as shown in Figure 9 with clay layers approximately 2-4 feet below the bottom of the BAM and Control RIB areas.

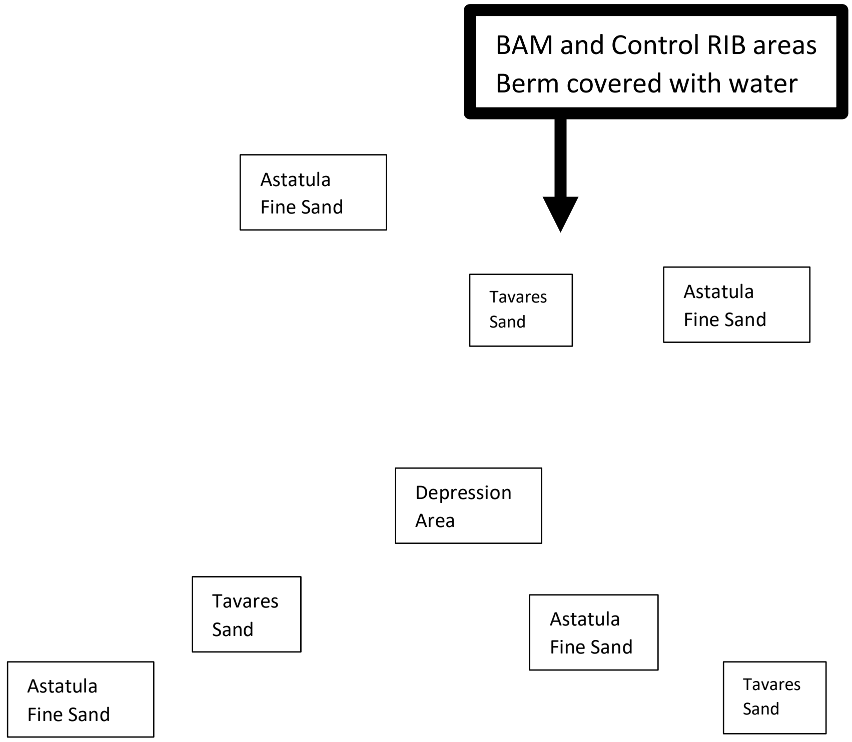


Figure 9 Stormwater Storage Photo with US NRCS Soil Map
Source: U.S. Department of Agriculture Natural Resources Conservation Service website (Retrieved 03 October 2017)

Tavares sand is the on-site natural soil in the RIB area. Within its deeper horizons, the sand particles have clay coatings. A clay content in the upper two feet of the BAM and the Control RIB varies between 2 and 4% determined by wet sieve analysis. The clay content helps provide a longer contact time. This contact time in addition to the clay content usually provides for the removal of nitrates. However, the distribution of these clay particles is not consistent across the soil area or within the profile. From field soil samples, the BAM media and on-site soil saturated hydraulic conductivity are about equal or 372 and 376 mm/hr. respectively. In addition, the moisture holding capacities as reported (CECE, 2017) reflect an environment suitable for bacterial growth. Thus, the RIB with BAM should function to infiltrate similar to the soils in the Control RIB and there is sufficient moisture for bacterial growth under a variety of atmospheric conditions in both the BAM RIB and the Control RIB.

Chapter 3: Installation, Results and Analysis of Data

The following is information related to the BAM RIB installation and the water quality monitoring data. The monitoring data are listed in Appendix B. Water Quality data are in Appendices C and D. Data provided based on the sequence of operation while modifying and dosing the RIB sites.

1. **The installation time for BAM:** The installation was completed in one week. City staff placed 3227 CY of BAM. The BAM was delivered in dump trucks (see Figure 10).

Figure 10 Installation of the BAM in the one-acre RIB

2. **The depth profile of BAM:** The BAM is 2 feet deep and had an initial in-place density of 90 pounds/CF. Density after four months of loading with reclaimed water was 97 pounds/CF. The density of the Control RIB was 94 pounds/CF. The depth of reclaimed water infiltrated in the BAM RIB before the density testing was about 10 feet.

The area of the BAM is 43,600 SF (about 1 Acre). The bottom of the RIB and top of the BAM is barren without vegetation but can develop natural vegetation or be planted (seeded) if desired. A section of the installation is in Figure 11. The Lysimeters for sampling percolating water were placed two feet under the RIB bottom. Thus, for the BAM RIB, it was under the BAM layer as shown in Figure 11.

Figure 11 Cross Section of the BAM installation at DeLand

3. **The dosing and loading of reclaimed water to the BAM RIB and the Control RIB with data to illustrate the amount to each:** The cumulative volume of water from the reclaimed water ponds used for loading the BAM RIB was 5.392 MG/Acre and the volume to the Control RIB was 5.351 MG/Acre. The cumulative depth of loading with reclaimed water was 16.5 feet for the BAM RIB and 16.4 feet for the Control RIB. The storage of excess stormwater from Hurricane Irma in September 2017 was about 4.0 MG/Acre (12 feet deep).

The depth of storage or gage heights shown in Figure 12 reflects the rate of loading over time and infiltration. Infiltration is the slope of the decreasing portion of depth over time. The stage data show no noteworthy difference in the stage and infiltration (slope of line) between the Control and the BAM RIB. The area under the stage curve times the area of water storage is the volume of storage. The time to empty the RIBs was about equal.

Also shown in Figure 12 is a comparison to the water table depth as a measure water in a well. The well was in the separation berm between the BAM RIB and the Control RIB. The RIBs were used to store excess stormwater during and immediately after the Hurricane. The depth of storage was greater than the top of the well-used to measure groundwater and was in a flooded condition up until the end of December. Thus, no data on well depths are available during that time. However, it can be seen that in the months of January and February the water table had risen to within 1-2 feet of the bottom of the RIBS. The rate of infiltration during this period decreased to about 0.125 to 0.25 inch/hour.

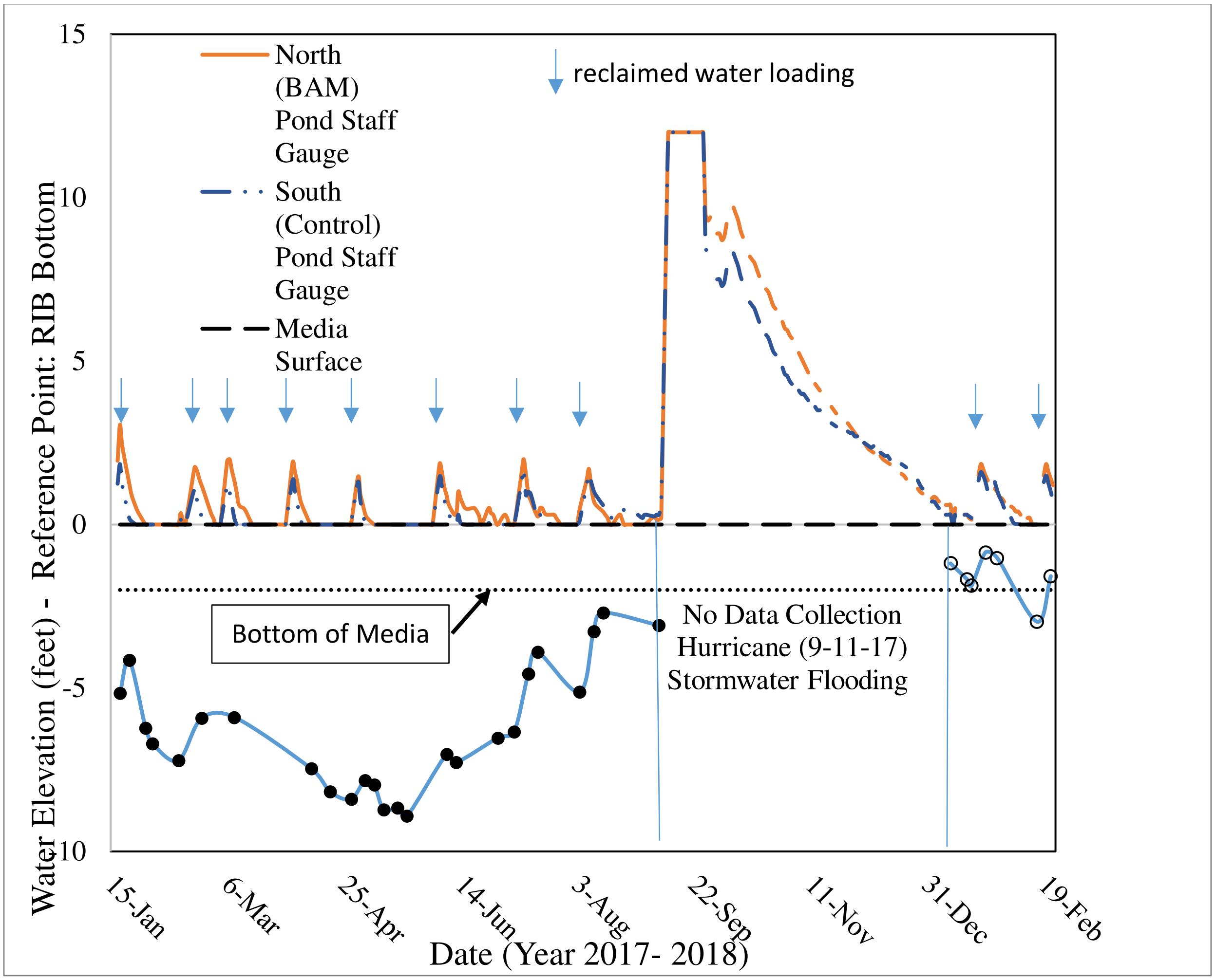
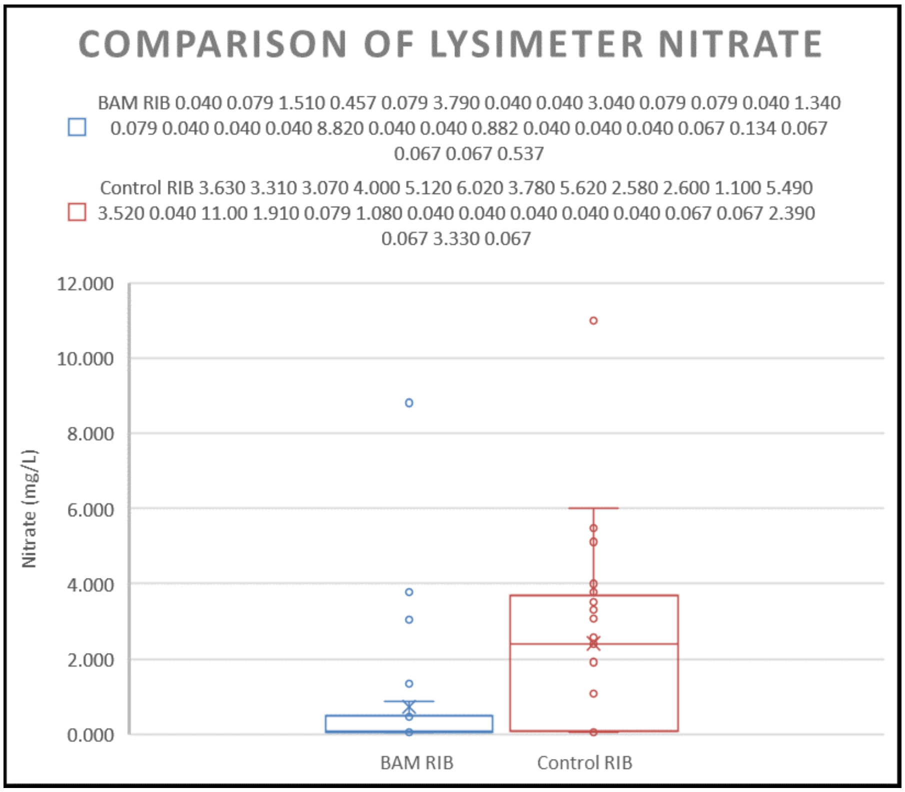


Figure 12 Depth of Water in the BAM RIB and the Control RIB

4. When loading with reclaimed water, the concentration of nitrates in the lysimeters two feet under the surface of the RIBs was measured: The average and median concentrations under the BAM RIB were 0.73 mg/L and 0.079 mg/L respectively (basically the detection limit). There were three (8.82, 3.04 and 3.79) values of nitrates which account for some of the difference between the medium and average. Two measurements were identified as a lysimeter failure when the lid of lysimeter 3 was partially off and sediment (clay soil from the surrounding soil) was found inside. The lid was reset after the March 2017 sampling event. The average and median concentrations under the Control RIB were 2.40 and 2.39 mg/L. Lysimeter 4 within the Control RIB was replaced after the March sampling because it did not yield sufficient sample. It was found to be one foot under the bottom of the Control RIB. It was replaced at a depth of 2 feet.

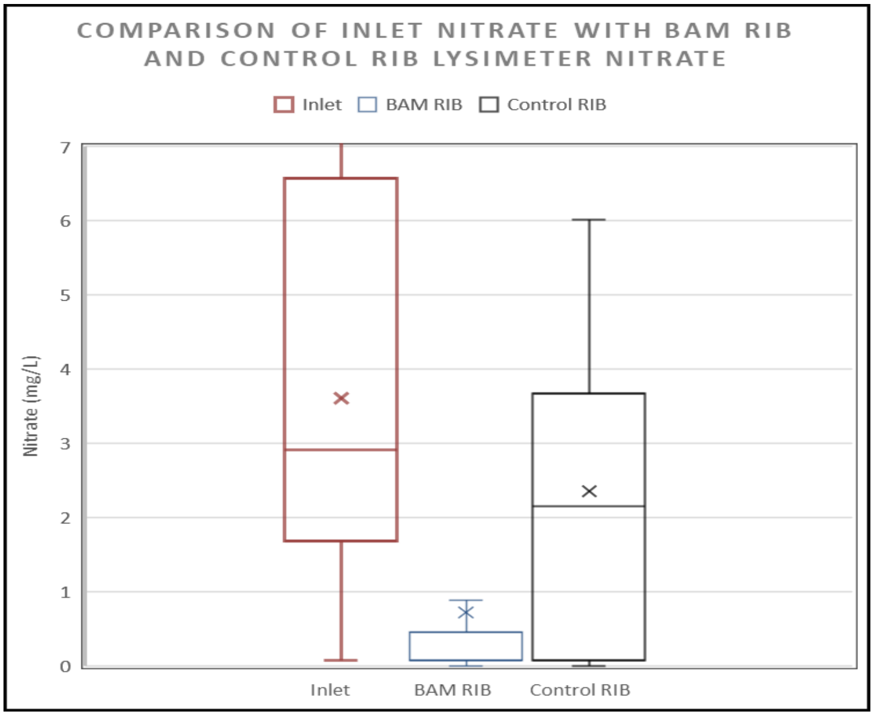
For visual presentation, a comparison of the reclaimed concentration data is shown in a "box" plot of Figure 13. Data used for "box" plots are shown as circles and a listing is in Figure 13. For the BAM RIB, there were 30 samples (3 for each loading event). For the Control RIB there were 29 samples (lysimeter 4 failed to deliver a sample one time). A listing of the data is also in Table 4(a). The reduction in concentration is greatest with the use of BAM.



Legend: Reference EXCEL 2016: average x, minimum ⊥, maximum ⊤, Quartiles ——

Figure 13 Comparison of Nitrate under the BAM and Control RIBs Using Reclaimed Water

5. When loading with reclaimed water, the lysimeter nitrate concentrations are compared to the inlet nitrate concentration: The inlet nitrate concentration was directly measured in the reclaimed reservoir during the loading of the RIBS. However, for two samples (January and May of 2017), the inlet measure was from the discharge from the DeLand WRF. The discharge from the DeLand WRF is into the reclaimed reservoir. The reclaimed reservoir is the storage area (tank and pond) from which the water is used to dose the RIBs. The reclaimed storage area is about 6.4 MG. The DeLand WRF produces about 3 MGD. There were ten loading events with reclaimed water. The comparison of inlet nitrate concentrations to those in the lysimeters is shown in a "box" plot of Figure 14. From Figure 14, the reduction in concentration is greatest with the use of BAM.



Legend: Reference EXCEL 2016: average x, minimum ⊥, maximum ⊤, Quartiles ———

Figure 14 Comparison of Inlet and Lysimeter Nitrate Concentrations Using Reclaimed Water

6. When loading with reclaimed water, the average percent removal for nitrate can be calculated: When using BAM, the average percent removal of nitrate when loading with reclaimed water (inlet nitrogen > 1.51 mg/L) was 89%. When loading with lower concentration reclaimed water (less than 0.723 mg/L) there were 11 of 14 samples less than detection, thus no removal could be estimated. The lower concentration most likely resulted from wastewater treatment process changes and the addition of river water to the irrigation storage reservoirs. Over half the time nitrate removal using BAM exceeds 97% (median removal value).

The average percent removal for the Control RIB when inlet concentrations exceed 1.51 mg/L was 34% and 48% when inlet concentration was below 0.723 mg/L. Percent removal was not calculated for one outlier value in each RIB lysimeter (BAM at 8.82mg/L and Control at 11.0 mg/L). These outlier values could have been caused by sample bottle contamination, human error in coding, local wildlife contaminant at the surface of the sample ports, or other unidentified sampling/analysis problems. The values were more than two standard deviations above the average. The standard deviation of the BAM nitrate values was 1.97 mg/L and for the Control nitrate values it was 2.71 mg/L. The data base used for this analysis is listed in Table 4a with the statistical measures.

For the lower nitrate concentrations measured on July 10 and August 7, detection limits for nitrates would have to be lowered to get more accurate estimates for percent removal. The detection limits for nitrates are used by the City of DeLand water quality laboratory when measuring wastewater from their water pollution reclamation facility. The limit was changed for the stormwater sampling events to 0.002 mg/L and another certified lab (Environmental Research District, ERD) was used.

7. Nitrate values below detection limits were recorded: When loading with reclaimed water, and measurements in the lysimeters below the BAM layer in the lysimeters, the number of nitrate values at or below detection limits was 23 out of 30 readings or 77% of the measurements. In the lysimeters below the Control RIB, the number of nitrate values at or below the detection limits was 11 out of 29 readings, or 38% of the measurements.

When stormwater was discharged to the RIBs, the detection limit was change to a lower number because of the water matrix (not sewage based). There were no measurements below detection limit when using stormwater.

8**. When loading with excess stormwater, the average percent removal for nitrate was calculated:** In the RIBs, the maximum depth of excess stormwater loading from Hurricane Irma was about 12 feet. No more water could be added as 12 feet was the maximum depth. Sampling the lysimeters for water quality continued when the depth of water receded to 2 feet. Thus, a composite sample was taken on December 18 for the initial infiltration of water. A second sample was taken after the lysimeters were evacuated of sample. The sample dates for the six lysimeters were December 18, 2017 and January 10, 2018. The procedure of evacuated between sample dates was the same as when reclaimed water was used. The collected stormwater samples were analyzed by ERD, a certified lab within 24 hours after collection. The parameter analyzed by ERD were nitrate/nitrite (NOx). The results are shown in Figure 15 with the data and statistics shown in Table 4b. The concentration of Nitrate was lower in the BAM RIB lysimeter when compared to the Control RIB lysimeter. This was an unfortunate hurricane event but provided real data for evaluation as there was excess stormwater of sufficient quantity to help load the RIB at a significant depth (12 feet compared the about 16 feet when using reclaimed water).

Table 4 Data Base Used for Nitrate Statistics and Box Plots

(a) Using Reclaimed Water

Sampling		Nitrate (mg/L)	
Date	Inlet	BAM RIB	Control RIB
1/30/2017	6.24	0.040	3.630
		0.079	3.310
		1.510	3.070
2/27/2017	7.59	0.457	4.000
		0.079	5.120
		3.790	6.020
3/13/2017	7.59	0.040	
		0.040	3.780
		3.040	5.620
4/11/2017	3.37	0.079	2.580
		0.079	2.600
		0.040	1.100
5/8/2017	2.47	1.340	5.490
		0.079	3.520
		0.040	0.040
6/12/2017	3.69	0.040	11.00
		0.040	1.910
		8.820	0.079
7/10/2017*	0.96	0.040	1.080
		0.040	0.040
		0.882	0.040
8/7/2017*	0.079	0.040	0.040
		0.040	0.040
		0.040	0.040
1/30/2018#	2.19	0.067	0.067
		0.134	0.067
		0.067	2.390
2/27/2018#	1.92	0.067	0.067
		0.067	3.330
		0.537	0.067
average	3.61	0.72	2.42
median	2.92	0.07	2.39
N	10	30	29

(b) Using Excess Stormwater

	mg/L	Nitrate (mg/L)	
Date	Inlet	BAM RIB	Control RIB
12/18/2017	0.303	0.009	0.010
		0.004	0.007
		0.007	0.027
1/10/2018	0.303	0.024	0.042
		0.014	0.022
		0.024	0.078
average	0.303	0.014	0.031
median	0.303	0.012	0.025
N	2	6	6

Inlet value based on sampling from anotl

* Reclaimed water augmented with river water

Detection limit changed to 0.134 from 0.079 mg/L

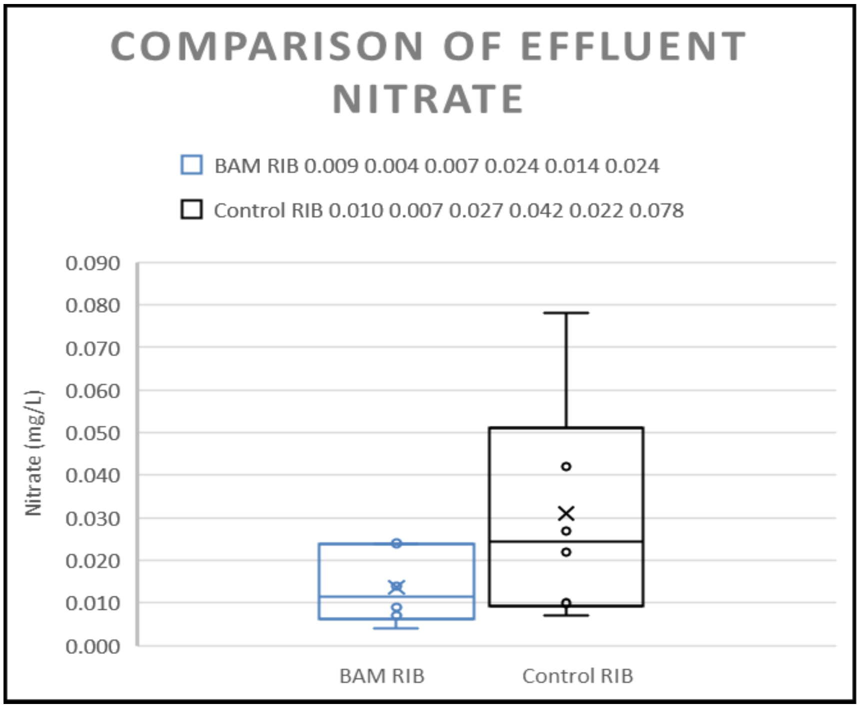
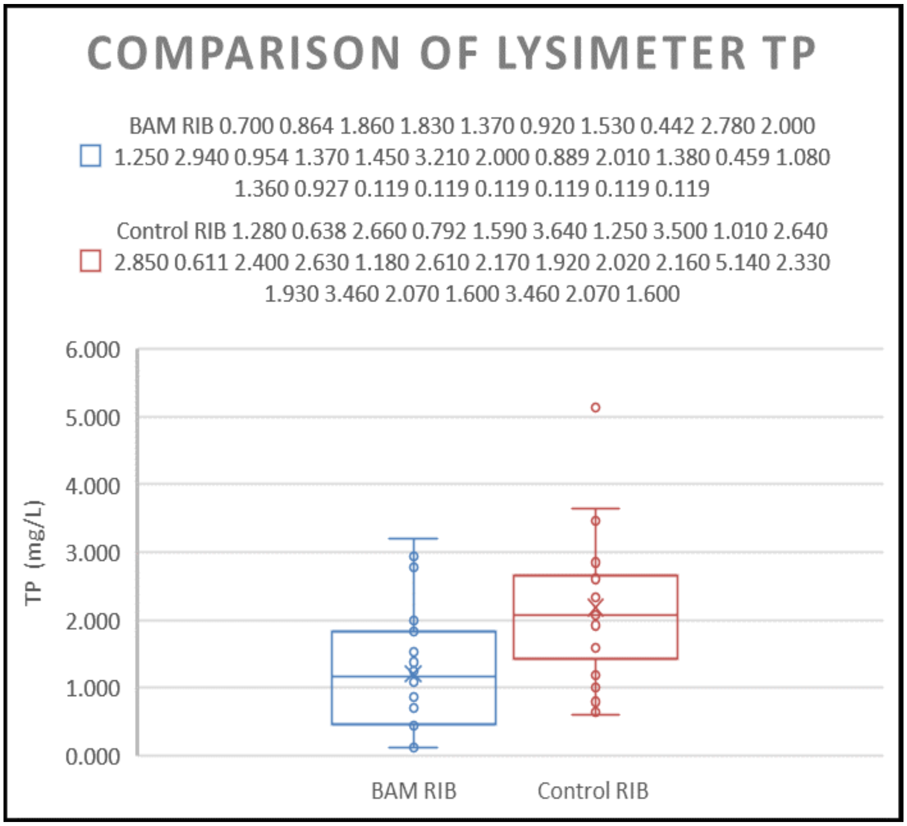


Figure 15 Comparison of Lysimeter Nitrate Concentrations using Excess Stormwater

9. **When loading with reclaimed water, the concentration of total phosphorus (TP) in the lysimeters which are two feet under the surface of the RIBs were recorded and evaluated: T**he average and median nitrate concentrations under the BAM RIB were 1.21 mg/L and 1.17 mg/L. The average and median concentration under the Control RIB were 2.18 and 2.07 mg/L. Lysimeter 4 within the Control RIB was replaced after the March sampling because it did not yield sufficient sample. Upon inspection it was found to be only one foot under the bottom of the Control RIB and thus was replaced at the appropriate depth of 2 feet.

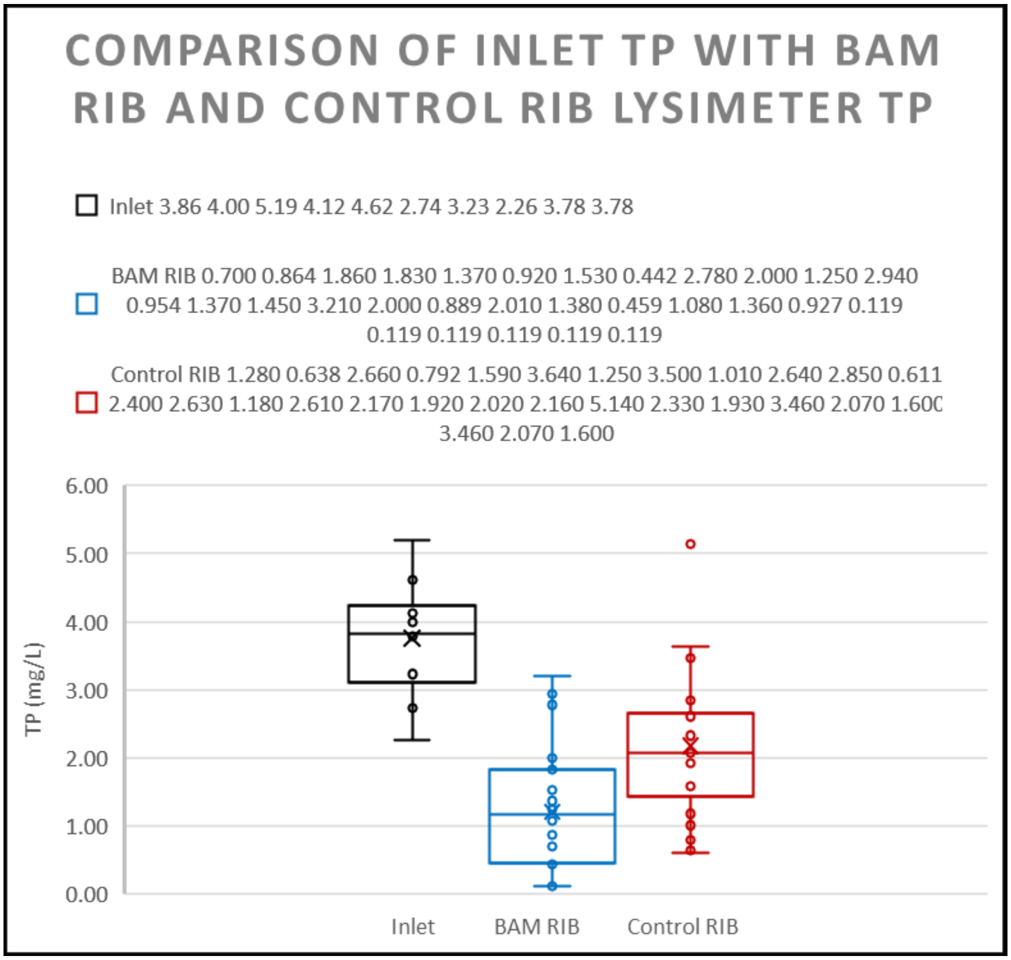
For visual presentations and calculation of statistical measures, a comparison of all the data is shown in a "box" plot of Figure 16. Data used for "box" plots are shown as circles and a listing is in Figure 16. For the BAM RIB, there were 30 samples (3 for each loading event). For the Control RIB there were 29 samples (lysimeter 4 failed to deliver a sample one time). It was reset for the next loading event. A reasonable explanation for the removal of total phosphorus for both the BAM RIB and the Control RIB at 2 feet below the surface is the occurrence of tire crumb in the BAM and clay particles in both. Straining of the particulates and chemical removal of the dissolved species are the most likely removal mechanisms.



Legend: Reference EXCEL 2016: average x, minimum ⊥, maximum ⊤, Quartiles ———

Figure 16 Comparison of Lysimeter Total Phosphorus Concentrations using Reclaimed Water

10. Comparison of the lysimeter total phosphorus concentration to the inlet phosphorus concentration when using reclaimed water was made: The inlet phosphorus concentration was directly measured in the reclaimed reservoir during the loading of the RIBS. However, for three samples (January, March and May 2017), the inlet measure was from the discharge from the DeLand WRF. The discharge from the DeLand WRF is into the reclaimed reservoir. The reclaimed reservoir is the storage area (tank and pond) from which the water is used to dose the RIBs. The reclaimed storage area is about 6.4 MG. The DeLand WRF produces about 3 MGD. There were ten loading events. The comparison of inlet total phosphorus concentrations to those in the lysimeters is shown in a “box” plot of Figure 17. From Figure 17, the reduction in concentration is greatest with the use of BAM, however the Control BAM with clay reduces the total phosphorus, but the reduction is not as great as BAM is used.



Legend: Reference EXCEL 2016: average x, minimum ⊥, maximum ⊤, Quartiles ———

Figure 17 Comparison of Inlet and Lysimeter Total Phosphorus Using Reclaimed Water

11. **The average percent removal for total phosphorus using reclaimed water was calculated:** Using BAM, the average percent removal of total phosphorus was 66%. Using half the value for the detection limit for the calculation of percent removal does not change the average percent removal. There were six samples at or below the detection limit. The average percent removal for the Control RIB was 37%. The removal can be increased with a phosphorus removal BAM mix, while retaining the nitrate removal capabilities. The data base using reclaimed water with statistics used for this analysis is listed in Table 5a.

Table 5 Data Base Used for Total Phosphorus Statistics and Box Plots

(a) Using Reclaimed Water

	TP mg/L	TP (mg/L)	
Date	Inlet	BAM RIB	Control RIB
1/30/2017	3.86	0.700	1.280
		0.864	0.638
		1.860	2.660
2/27/2017	4.00	1.830	0.792
		1.370	1.590
		0.920	3.640
3/13/2017	5.19	1.530	
		0.442	1.250
		2.780	3.500
4/11/2017	4.12	2.000	1.010
		1.250	2.640
		2.940	2.850
5/8/2017	4.62	0.954	0.611
		1.370	2.400
		1.450	2.630
6/12/2017	2.74	3.210	1.180
		2.000	2.610
		0.889	2.170
7/10/2017*	3.23	2.010	1.920
		1.380	2.020
		0.459	2.160
8/7/2017*	2.26	1.080	5.140
		1.360	2.330
		0.927	1.930
1/30/2018#	3.78	0.119	3.460
		0.119	2.070
		0.119	1.600
2/27/2018#	3.78	0.119	3.460
		0.119	2.070
		0.119	1.600
average	3.76	1.21	2.18
median	3.82	1.17	2.07
N	10	30	29

* Reclaimed water augmented with river water

Detection limit was 0.119 mg/L

(b) Using Excess Stormwater

	Lysimeter	TP (mg/L)	
Date	Number	BAM RIB	Control RIB
12/18/2017	1	0.796	3.470
	2	0.819	3.120
	3	1.560	0.119
1/10/2018	4	0.696	3.410
	5	0.630	2.220
	6	1.310	0.701
average		0.97	2.17
median		0.81	2.67
N		6	6

detection limit was 0.119 mg/L

12. When loading with excess stormwater, the concentration of total phosphorus (TP) in the lysimeters which are two feet under the surface of the RIBs were recorded: The data are shown in Table 5(b). Under the BAM, the average and median total phosphorus concentrations were 0.97 and 0.81 mg/L respectively. The total phosphorus standard for discharges from advanced wastewater pollution control facilities in the State of Florida is 1 mg/L. Under the Control RIB, the average and median total phosphorus concentrations were 2.17 and 2.67 mg/L respectively. Based on the limited sampling, BAM removed more phosphorus than the natural soils in the Control RIB. There were no measures for inlet total phosphorus concentrations during Hurricane Irma, no any from other sampling sites in Florida.

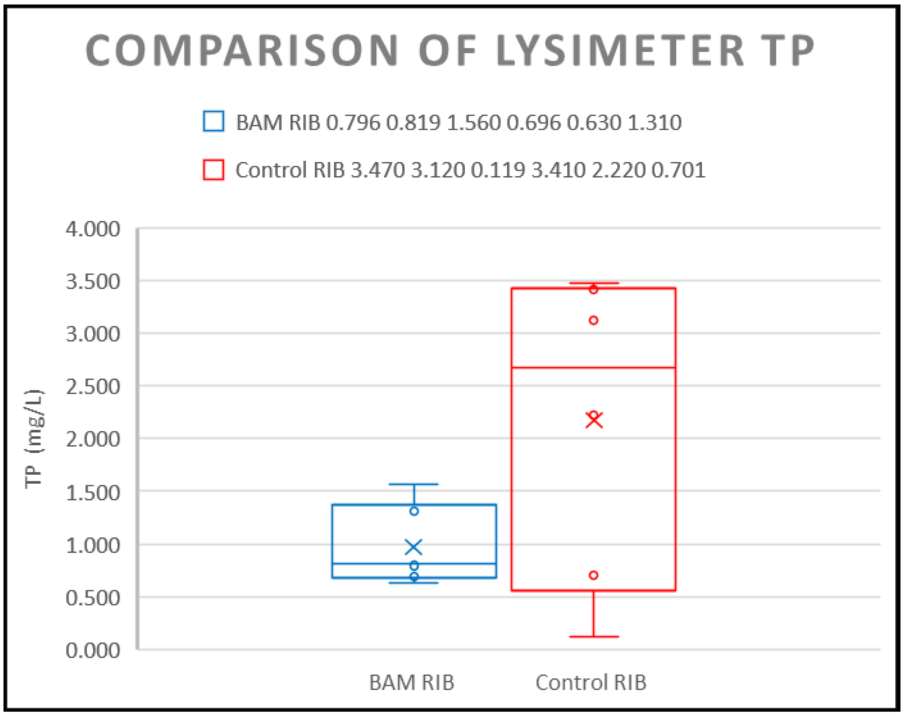


Figure 18 Comparison of Lysimeter Total Phosphorus Concentrations Using Stormwater

Chapter 4: Microbiological Assessment with qPCR Analyses

Introduction:

Nitrate can be eliminated through biological process such as nitrification and denitrification. This is made possible by microbial activity as well as adsorption by green sorption media, such as bio-sorption activated media (BAM) (Xuan, Chang et al. 2010, O'Reilly, Wanielista et al. 2012). Physiochemical and biological process can remove nutrients with the use of green media containing tire crumb, sand, and clay (Chang, Hossain et al. 2010). The use of BAM in stormwater and wastewater treatment for nitrification and denitrification processes enables nutrients in the liquid to sorb to the media (Chang, Hossain et al. 2010). The removal of nitrate in many water bodies is associated with microbial interactions (Santoro, Boehm et al. 2006). The bacteria are capable utilizing the nutrients in the soil, under adequate environments and convert the nutrients through microbial process. As a result, the growth of the microbial organisms is facilitated by the use of green sorption media such as BAM (O'Reilly, Wanielista et al. 2012).

In Rapid Infiltration Basins (RIB), the gene population of Nitrite Oxidizing Bacteria (NOB) and denitrifying bacteria (denitrifier) in the soil of the Control RIB and BAM RIB was analyzed. The quantity of NOB and denitrifier was determined through qPCR to quantify the gene population, and to verify their contribution in nitrate removal. Thus, microbial contribution was analyzed for comparison of nitrate percent removal and NOB and denitrifier population in Control and BAM RIBS.

Soil samples were collected to analyze the Nitrite Oxidizing Bacteria (NOB) and denitrifier microorganisms in the soil of the Control and BAM RIBS to examine and justify their relation to nitrate removal. The NOB are responsible for the conversion of the nitrite to nitrate (Yao and Peng 2017) and utilize nitrite as a food source under aerobic conditions (presence of oxygen) (Mechalas, Allen III et al. 1970). Denitrification is an anaerobic biological process that utilize nitrate in the absence of oxygen (Mechalas, Allen III et al. 1970), in which denitrifying organisms convert nitrate to nitrogen gas (Santoro, Boehm et al. 2006). As a result, microbial denitrification for permanent removal of nitrate can only occur under anaerobic conditions (absence of oxygen) (Xuan, Chang et al. 2010). For effective nitrogen removal denitrification must proceed nitrification. The biological reaction for the conversion of nitrite to nitrate by NOB requires nitrite and oxygen, and for the conversion of nitrate to nitrogen by denitrifier as follow (Mechalas, Allen III et al. 1970):

$$NO_2^- + \frac{1}{2} H_2O \rightarrow NO_3^- + 2H^+ \quad (1)$$

$$4NO_3^- + 4H^+ + 5CH_2O \rightarrow 2N_2 + 5CO_2 + 7H_2O \quad (2)$$

Methods:

Sample Collection

Sampling was conducted at locations near the lysimeters, and at multiple vertical locations in the Control and BAM RIBS. The samples were collected on two separate occasions, on February 2, 2017 and February 16, 2018. For each sample date, 18 samples were collected, leading to a total of 36 collected samples. The method for sample collection is explained below, with sampling locations for BAM RIB and Control RIB in Figure 19, and the depth profile in Figure 20.

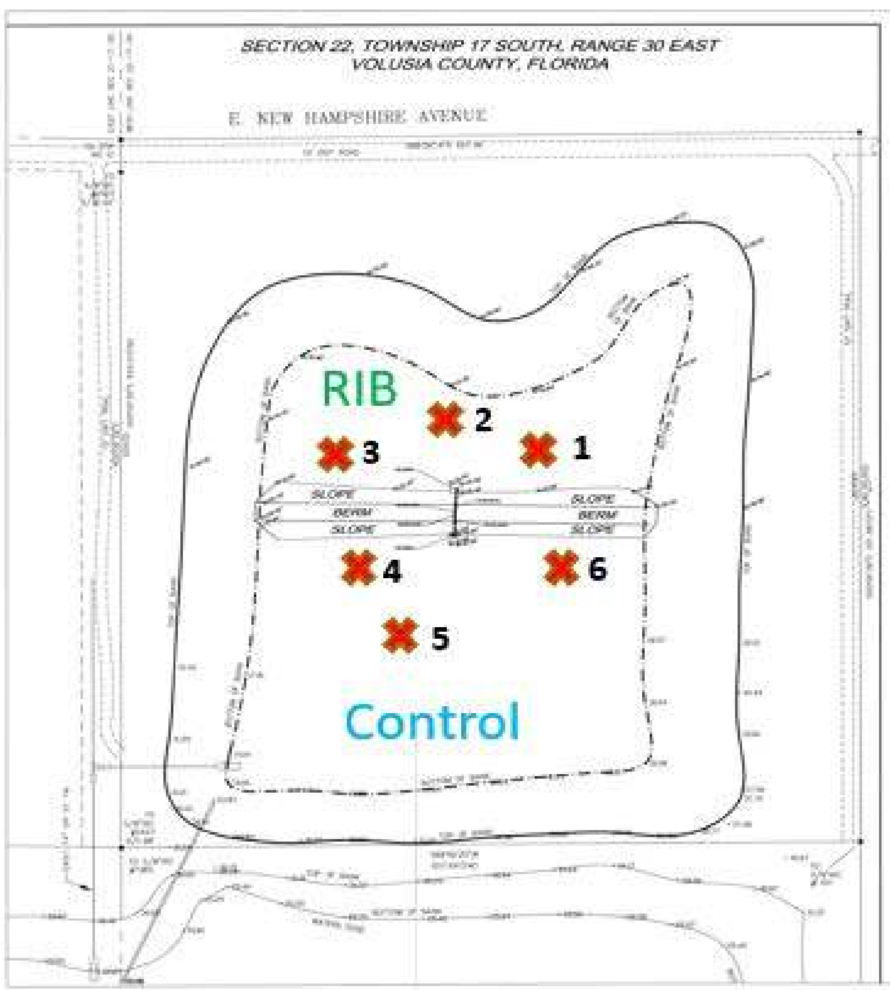


Figure 19 Sampling Locations for BAM RIB and Control RIB

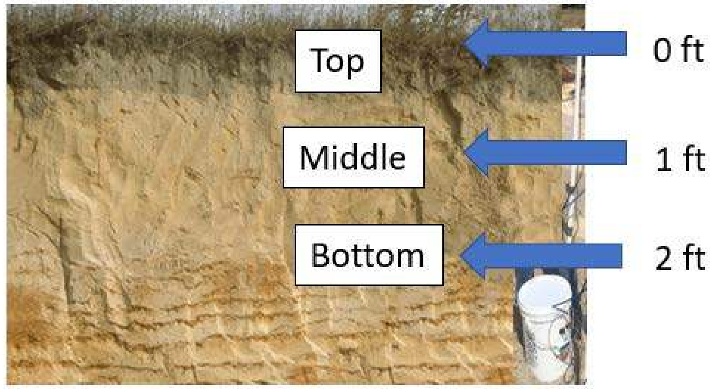


Figure 20 Vertical Locations of BAM and Control Sampling

Method for sample collection:

- Dig 2-feet deep hole at each location in BAM and Control RIBS (2 feet is the depth of BAM).
- Extract samples from the depths as described in Table 1 and 2 for the Control RIB and BAM RIB.
- Place samples in plastic container and store at -80 degrees C.

Table 6 Sample locations for BAM RIB

Sampling Date	Location	Number of Samples per Sampling Date	Sample	Depth (ft)
2/2/2017 and 2/16/2018	1	1	Top	0
		1	Middle	1
		1	Bottom	2
	2	1	Top	0
		1	Middle	1
		1	Bottom	2
	3	1	Top	0
		1	Middle	1
		1	Bottom	2

Table 7 Sample locations for Control RIB

Sampling Date	Location	Number of Samples per Sampling Date	Sample	Depth (ft)
2/2/2017 and 2/16/2018	4	1	Top	0
		1	Middle	1
		1	Bottom	2
	5	1	Top	0
		1	Middle	1
		1	Bottom	2
	6	1	Top	0
		1	Middle	1
		1	Bottom	2

DNA Extraction

DNA extraction is required for final gene expression determination from real-time quantitative PCR or qPCR (quantitative polymerase Chain Reaction analysis). The aim is to determine the concentration of the bacteria present in the media of the Control RIB and BAM RIBS. DNA extraction is the second step for qPCR (Scientific 2014). The initial step consists of sample collection, followed by DNA extraction, denaturation, annealing, extension and DNA amplification (Fig. 21). In DNA extraction, DNA is extracted from samples, in denaturation DNA is incubated at high-temperature to separate double-stranded DNA, in annealing the primers and probe attach to single strand DNA, in extension the fluorescent signal is emitted, and in amplification DNA is replicated (Scientific 2014). In addition, the gene sequences associated with the NOB and denitrifiers are listed in Table 8(a). The forward and reverse primers associated with NOB and denitrifier qPCR analysis are listed in Table 8(b).

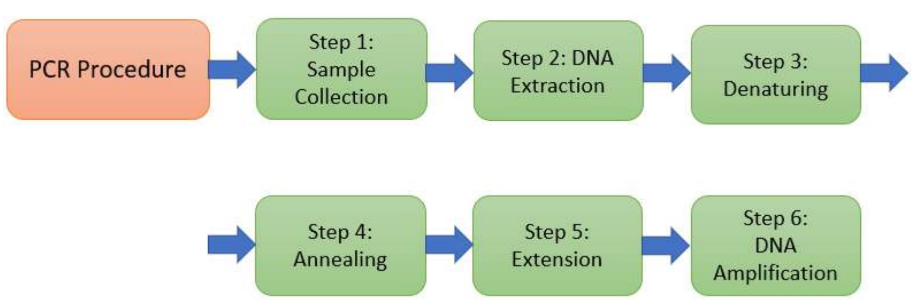


Figure 21 PCR Procedure

Figure 22 qPCR instrument and Accessories used in UCF Lab (1. DNeasy PowerSoil kit, 2. Applied Biosystem StepOne Real-Time PCR System, 3. PCR 48-well plate, 4. Centrifuge, 5. Vortex mixer)

Figure 22 is shown to validate the DNA method of analysis as other lesser accurate instruments can be used. These are the highest-grade instrumentation.

Table 8 (a) Gene Sequences Associated with NOB and denitrifiers

Gene	Gene Sequence	Reference
NOB	Nitrobacteria winogradskyi ATCC25381, ATCC14123 Nitrobacter sp. R6 Nitrobacter hamburgaensis X14 Nitrobacter ATCC 25383, ATCC 25384, ATCC 25385	Teske, Alm et al. (1994)
Denitrifier	narG, nirK, nosZ,	Kandeler, Deiglmayr et al. (2006), Smith, Nedwell et al. (2007)

Table 8(b) List of PCR Primers used for NOB and denitrifier gene

Gene	Primer	Sequence	Reference
NOB	NSR1113f, NSR1242r	5 CCTGCTTT CAGTTGCTACCG 3, 5GTTTGCAGCGCTTTGTACCG 3	Dionisi, ayton et al. (2001), Lo´pez-Gutie´rrez, Henry et al. (2004)
Denitrifier	narG-1960m2f, narG-r 2050m2r	5V-TACTGTGCGGGCAGGAAGAAA CTG-3V, 5VCGT AGA AGC TGG TGC TGT T-3V	Bru, Sarr et al. (2007), Rodrigues Maruyama, Guilger et al. (2015)

Step 1: Sample Collection

- Sample collection for the BAM and Control RIB is performed at the two sampling events as previously described in Table1 and 2.

Step 2: DNA Extraction

The extraction of DNA of the bacteria present in the soil samples collected from the BAM and Control RIBs is performed using qPCR kit. The DNeasy PowerSoil kit was used in the DNA extraction (Fig. 22 (1)), the procedures is as follow:

- Obtain 0.25 g of sample.
- Add 0.25g of sample soil to the PowerBead Tube. Vortex mix.
- Add 60 µL of Solution C1 to tubes. Vortex for 10 minutes at maximum speed.
- Centrifuge tubes for 30 seconds at 10,000 x g.
- Transfer supernatant (400-500 µL) into clean 2 ml collection tube. Label tubes.
- Add 250 µL of solution C2, vortex for 5 seconds. Incubate for 5 minutes at 4^{o}C.
- Centrifuge tubes for 1 minute at 10,000 x g.
- Transfer up to 600 µL of supernatant avoiding pellets to clean 2 mL collection tube.

- Add 200 µL of Solution C3 and vortex briefly. Incubate for 5 minutes at 4^oC.
- Centrifuge tubes for 1 minute at 10,000 x g.
- Transfer up to 750 µL of supernatant avoiding pellets to clean 2 mL collection tube.
- Shake mix solution C4 and add to the 1200 µL of supernatant. Vortex for 5 seconds.
- Load 675 µL onto an MB Spin Column and centrifuge for 1 minute at 10,000 x g. Discard Flow through.
- Repeat step twice, until all of the sample has been processed.
- Add 500 µL of Solution C5, centrifuge for 1 minute at 10,000 x g.
- Discard Flow through. Centrifuge again for 1 minute at 10,000 x g.
- Carefully place the MB Spin Column into a clean 2 mL collection tube, avoid splashing and solution C5 onto the column.
- Add 100 µL of TE Buffer to center of white filter membrane.
- Centrifuge for 30 seconds at 10,000 x g at room temperature. Discard MB Spin. Column. DNA is ready for amplification.

The DNeasy PowerSoil kit (Fig. 22 (1)) was used to perform the DNA extraction for the NOB Gene. This kit can be utilized for the isolation of microbial genomic DNA for all types of soils (Qiagen 2017). The kit includes the all the materials required for DNA extraction such as the Powerbead tubes, Solutions C1-C5, MB Spin Columns, and 2 mL collection tubes.

Steps 3: Denaturing

Denaturing requires high-temperature to separate double-stranded DNA previously extracted (Step 2) from the NOB and denitrifier genes. At high temperature, the DNA separates into two single strands of DNA due to the temperature incubation. This stage occurs inside the qPCR instrument. On average the highest typical temperature that DNA can withstand is 95^0C (Scientific 2014). Denaturing is required to enable the primers to attach to the single stranded DNA in the Annealing step.

Step 4: Annealing

After separation of double-stranded DNA the primers and probes have the opportunity to attach to each single-strand of DNA. In this step the complementary sequences attach to their corresponding target of the DNA template (Scientific 2014). This requires two primers to be used, a forward and backwards primer. This process also occurs in the qPCR instrument where a decrease in temperature is necessary (typically 60^0C).

Step 5: Extension

Once the two primers bind to the DNA strand, they begin to produce nucleotides in the section of the strand that need replication. Each primer is responsible for amplifying their complementary strand, and consequently completing the second single-strand required to complete the template DNA strand (Scientific 2014). In this process the replication of DNA is achieved. This step can often be combined with the annealing step and occurs in the qPCR instrument within a temperature of 70-72^{0}C.

Step 6: DNA Amplification

The amplification of DNA is the final step in qPCR analysis and involves DNA replication. The fluorescence signal that was emitted by the probe, attached to the DNA strand is recorded. The fluorescence signal is plotted versus temperature, and also versus change in fluorescence/ change in temperature to produce a melting curve (Scientific 2014). The results are displayed by StepOne Real-Time PCR System where the quantification of DNA can be obtained. DNA amplification is the product of all the process that occurs within the qPCR instrument, as various copies of a DNA sequence is obtained from one or few DNA segments. Further, the overall method involving the procedure for the analysis of each gene in qPCR is described next.

qPCR Analysis

Real-time quantitative polymerase Chain Reaction (qPCR) employs RNA analysis to determine the gene expression of the bacteria in the soil through DNA amplification. This technology can be utilized to determine the quantity of genes from a known standard (Scientific 2014). The use of qPCR allows for the quantification of desired genes, in this case NOB and denitrifiers. The Applied Biosystems Real-time PCR instrument and StepOne Software v2.3 were utilized for qPCR analysis and data collection. Steps 3-6 (Denaturing, Annealing, Extension, and DNA Amplification) of Fig. 21 occur within the PCR analysis in the Real-time PCR instrument.

The Applied Biosystems Real-time PCR system (Fig. 22 (2)) is a 48-well Real-Time PCR instrument that has a sensitive 3-color optical LED recording system for fluorescent reading. The instrument has temperature range of 4-100^{o}C and utilizes a Peltier thermal cycling system (Scientific 2018). It uses the MicroAmp Fast Optical 48-Well Reaction Plate (Fig. 22 (3)). This well plate is designed specifically for the Applied Biosystems Real-time PCR instrument and is composed of polypropylene (Scientific 2018). In addition, PCR vortex mixer, and centrifuge (Fig. 22 (4, 5)) were employed in PCR analysis. The vortex mixer or vortexer oscillates the tubes in a circular motion. While the centrifuge spins to separate the solids, liquids, and gases in the tubes.

1. *NOB*

In qPCR analysis of NOB gene, the DNA samples and kit components are placed into a 48 well plate. The components are required to be added for one individual well of the plate are shown in Table 9, which includes the assay, sample, and control standards. In addition, the forward and reverse primers used for NOB gene (Nitrospira) are listed in Table 8(b).

Method for qPCR analysis of NOB:

- Prepare assay for the standards and samples.
- Add 110 µL of SYBR Green solution, 8.8 µL of Forward Primer, 8.8 µL Revers Primer (Table 8(b)) and 74.8 µL water into a 1.5 ml collection tube. Briefly vortex, and centrifuge.
- Repeat step for sample assay.
- Fill plate with 48 wells with the five duplicate standards each 1.6 µL in volume, and twelve samples from four locations with triplicates each 5 µL in volume.
- Add 18.4 µL of assay previously prepared for the standards to the 10 wells with the five standards.
- Add 15 µL of assay previously prepared for samples to the proceeding 36 wells.
- Add 5 µL water and sample assay to one well to be used a negative control.
- Seal plate avoiding formation of air bubbles.
- Insert into the qPCR instrument and run analysis.

2. *Denitrifier*

For the qPCR analysis of denitrifier gene, the DNA samples and kit components are placed into a 48 well plate. The components required to be added to one individual well of the plate are shown in Table 4, include the assay, sample, and control standards. While, the forward and reverse primers used for denitrifier (narG) gene are listed in Table 8(b).

Method for qPCR analysis of Denitrifier:

- Prepare assay for the standards and samples.
- Add 407 µL of SYBR Green solution, 32.56 µL of Forward Primer 32.56 µL, Revers Primer (Table 8(b)) and 138.38 µL water into a 1.5 ml collection tube. Briefly vortex, and centrifuge.

- Repeat step for sample assay.
- Fill plate with 48 wells with the five duplicate standards each 1.6 µL in volume, and twelve samples from four locations with triplicates each 5 µL in volume.
- Add 18.4 µL of assay previously prepared for the standards to the 10 wells with the five standards.
- Add 15 µL of assay previously prepared for samples to the proceeding 36 wells.
- Add 5 µL water and sample assay to one well to be used a negative control.
- Seal plate avoiding formation of air bubbles.
- Insert into the qPCR instrument and run analysis.

Table 9 Components for qPCR analysis of NOB and Denitrifies for each well

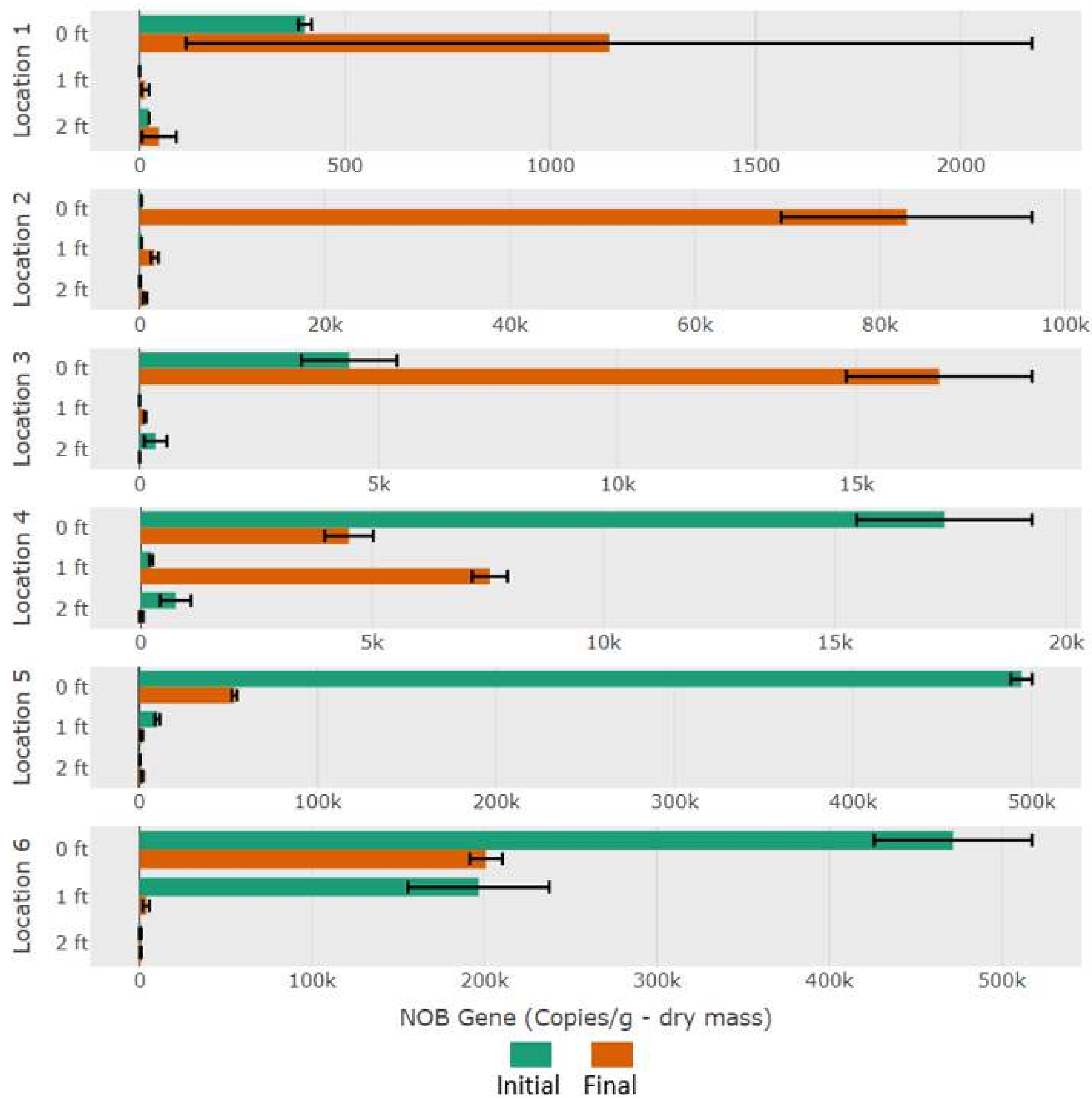
Standard	Component	Quantity (µL)
Assay	Master Mix	10
	Forward Primer	0.8
	Reverse Primer	0.8
	PCR-Water	6.8
	Standard Sample	1.6
NOB Sample	Component	Quantity (µL)
Assay	Master Mix	10
	Forward Primer	0.8
	Reverse Primer	0.8
	PCR-Water	3.4
	DNA Sample	5

Project Results:

NOB

The results obtained from qPCR show that the samples collected at the beginning for the BAM RIB on 2/2/2017 at locations 1, 2 and 3 do not have significant NOB population compared to the samples from the Control RIB at locations 4, 5, and 6 (Fig. 23 (a)). At this stage it can be concluded that the NOB organisms are not fully established into the environment of the BAM RIB as opposed to the Control RIB. However, the second set of samples collected after one year on 2/16/2018, show a growth of NOB gene copies in the BAM RIB for locations 1, 2 and 3, and a decrease in the NOB gene population in locations 4, 5 and 6 of the Control RIB. This explains the lower nitrate removal reported for the Control RIB. This is indicative of the ability of BAM to support the growth and microbial activity of such bacteria as opposed to the Control RIB, and supports the studies performed by Chang, Hossain et al. (2010), O'Reilly, Wanielista et al. (2012). This is supported by the high nitrogen removal (89%) observed for the BAM RIB, and the low concentrations of nitrate in BAM RIB in comparison with Control RIB (Fig.23(b)). An increase in NOB organisms and an abundance in population provides an increase in nutrient uptake and consequently the oxidation of nitrite to nitrate, providing the basis for nitrogen removal. In addition, the soil composition of the Control basin which contains sand and clay layers may result in the inhibition of NOB cultivation compared to BAM, as absence of tire crumb in the soil can decrease adsorption capacity of the soil (O'Reilly, Wanielista et al. 2012).

Results from second sampling event for the NOB show that the optimum depth for nutrient removal was at the surface layer for BAM RIB, and only at locations 5, and 6 for Control RIB (Fig. 23 (a)). The increase presence of NOB, 1 foot below the surface at location 4 can be attributed to ponding effect. The ponding of reclaimed water at this location could have affected adsorption of nutrients in the soil consequently affecting the NOB activity by reducing oxygen in the top layer. The accumulation of nutrient at the surface on the RIB provided the required environment for microbial growth. Ashe quantity of NOB is associated with nutrient availability in the soil and nitrogen removal. The increase in NOB population in the BAM RIB from the first and second sampling event demonstrate the relationship between microbial population and nitrate removal. The more NOB available at the BAM RIB the more microbiological activity present by NOB to convert nitrite to nitrate.


Location 1
0 ft
1 ft
2 ft
0
500
1000
1500
2000
Location 2
0 ft
1 ft
2 ft
0
20k
40k
60k
80k
100k
Location 3
0 ft
1 ft
2 ft
0
5k
10k
15k
Location 4
0 ft
1 ft
2 ft
0
5k
10k
15k
20k
Location 5
0 ft
1 ft
2 ft
0
100k
200k
300k
400k
500k
Location 6
0 ft
1 ft
2 ft
0
100k
200k
300k
400k
500k
NOB Gene (Copies/g - dry mass)
Initial
Final

(a)

(b)

Figure 23 (a) NOB Gene for BAM (Locations 1, 2, and 3) and Control (Locations 4, 5, and 6) in initial (2/2/2017) and final (2/16/2018) sampling, (b) Comparison of nitrate concentration and NOB gene at surface layer for BAM and Control RIB for locations 1-6 for second sampling event for nitrate removal.

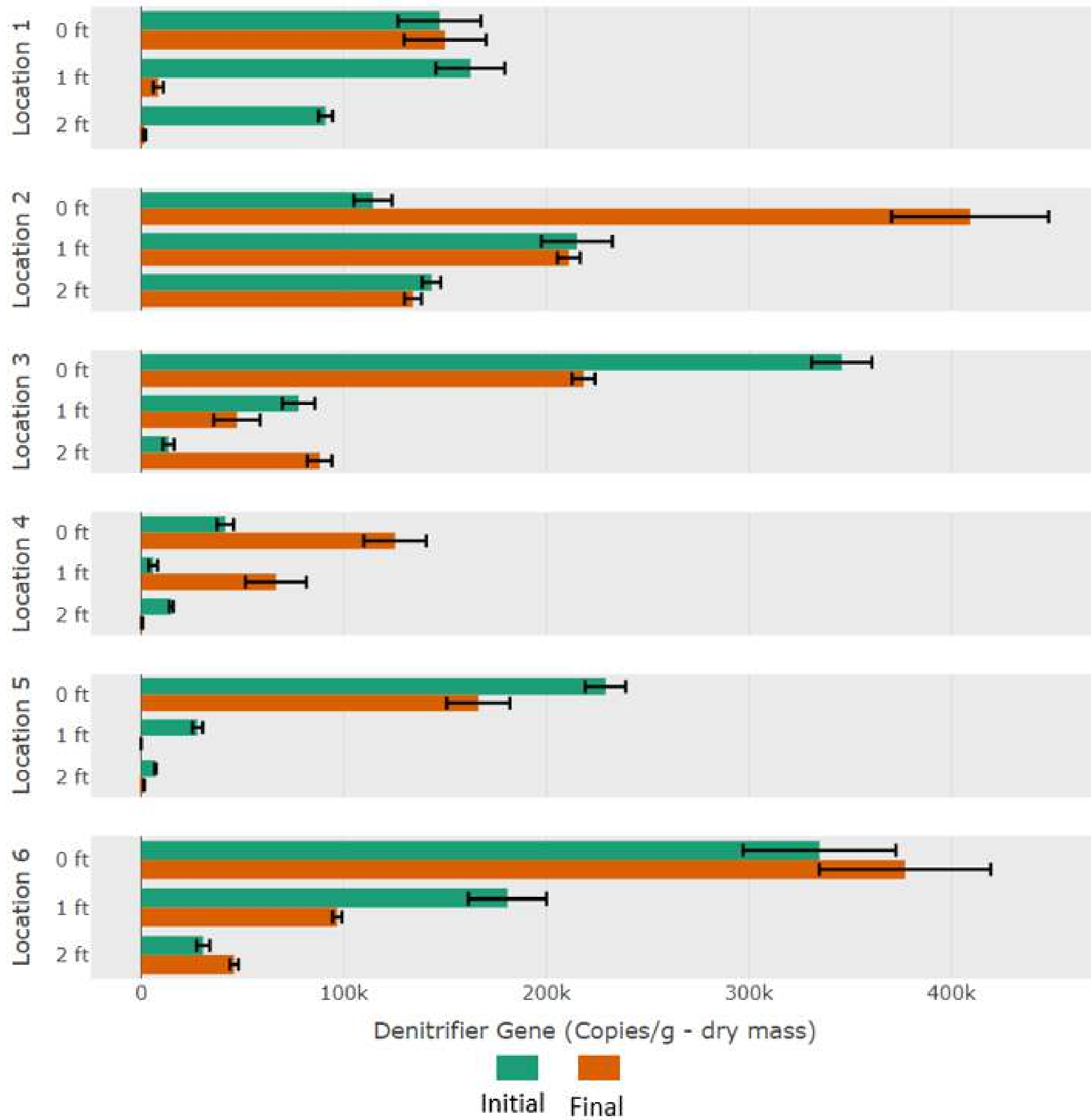
Denitrifier

According to qPCR the vertical location of one foot below the surface (1 ft.) has the highest denitrifier population for the BAM media samples in location 1, and 2 for the first sample date (Fig. 24 (a)). With the only difference being on location 3 which has the top layer of the soil with the most denitrifier genes. This may be the result of ponding, contributing to oxygen depletion causing the denitrifiers to accumulate at the surface. This can be attributed to the position of location 3. Location 3 is positioned the farthest from the inlet and experiences ponding the majority of the time compared to location 1 and 2 which are dry in the BAM RIB. It may be possible that as result of being in contact with the influent for longer time, more nutrients may be available for the denitrifier organisms to utilize and grow. Although, location 3 is an exception the results would imply that the optimum depth for nitrate removal was 1 foot below the surface of the BAM RIB in the initial stage. This is not the same for the Control RIB, the highest denitrifier population at all of the locations was at the surface for the first sampling date.

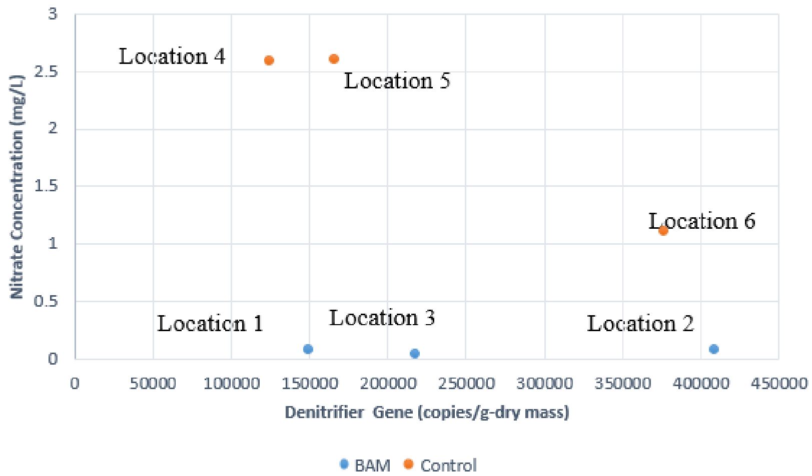
Further, the presence of denitrifier microorganisms also confirms the contribution in nitrate removal. In addition to having an increase in final NOB population, an increase in denitrifier population was observed in the BAM RIB (Fig. 24 (a)). Additionally, denitrifier gene show the largest gene population at the surface of location 2 of BAM RIB (Fig. 24 (a)). This is associated with the previously observed high nitrate removal of BAM (89% overall; 82% with reclaimed water and 95% with stormwater) as opposed by the Control (40% with reclaimed water and 90% with stormwater) (Table 10). This is related with the denitrifier population in the BAM RIB which had high denitrifier gene population opposed to the Control RIB. The largest denitrifier population of approximately 409,330 copies/ g-dry mass occurred on BAM RIB (Table 11(a)), compared to approximately 377,146 copies/ g-dry mass at Control RIB for the second sample event (Fig. 24(a)). The greater presence of denitrifier population in BAM contributes to an increase in nitrate reduction in BAM RIB as these organisms convert nitrate to nitrogen. This is supported by the higher nitrogen removal observed for the BAM RIB even with the low concentrations of nitrate in BAM RIB in comparison with Control RIB (Fig.24(b)). As a result, the denitrifier population can be associated with the observed nitrogen removal. The increase of the denitrifier bacteria quantity in the BAM RIM from the first and second sampling events coincides with nitrogen removal. With an increase denitrifier population, more denitrifiers are available for the conversion of nitrate to nitrogen. The more denitrifiers present in the soil, the greater microbiological activity can be observed.

Table 10 Average Nitrate Percent Removal

RIB	Percent Removal (%)	
	Loading with Reclaimed Water	Stormwater
BAM	82	95
Control	40	90


Location 1
0 ft
1 ft
2 ft
Location 2
0 ft
1 ft
2 ft
Location 3
0 ft
1 ft
2 ft
Location 4
0 ft
1 ft
2 ft
Location 5
0 ft
1 ft
2 ft
Location 6
0 ft
1 ft
2 ft
0
100k
200k
300k
400k
Denitrifier Gene (Copies/g - dry mass)
Initial
Final

(a)



(b)

Figure 24 (a) Denitrifier Gene for BAM (Locations 1, 2, and 3) and Control (Locations 4, 5, and 6) in initial (2/2/2017) and final (2/16/2018) sampling, (b) Comparison of nitrate concentration and denitrifier gene at surface layer for BAM and Control RIB for locations 1-6 for second sampling event for nitrate removal.

Table 11 (a) Comparison of Highest NOB and Denitrifier gene population for BAM and Control for First Sample Date

2/2/2017	NOB (gene copies/g dry mass)	Denitrifier (gene copies/g dry mass)
BAM	4,380	13,519
Control	494,343	5,848

Table 11 (b) Comparison of Highest NOB and Denitrifier gene population for BAM and Control for Second Sample Date

2/16/2018	NOB (gene copies/g dry mass)	Denitrifier (gene copies/g dry mass)
BAM	82,888	409,330
Control	201,019	377,146

Discussion:

Nitrate removal is related to the microbial gene population of NOB and denitrifiers. This removal can be affected by the physiochemical composition of reclaimed water discharged to the basins, and microbiological process of the NOB and denitrifiers. The average percent removal of nitrate for BAM RIB in comparison with the Control RIB can indicate the presence and contribution of NOB and denitrifier organisms. According to the lysimeter data collected (Table 10), the BAM and Control RIBS demonstrated nitrate removal for a range of influent nitrate conditions. It is noted that average percent removal was observed for the inlet nitrate concentration > 2.6 mg/L, indicating the effect the water composition had on the bacteria. Greater nitrogen concentration in the influent provided more nutrient for the NOB and denitrifier. Further, the effect the soil property for each RIB had on the removal is also noted that as the BAM RIB obtained the highest average nitrate removal compared to the Control RIB.

Although, initially the population of NOB was significantly more in the Control RIB compared to the BAM RIB, the population was maintained and increased for BAM RIB as opposed to the Control RIB (Fig. 23(a)). For the Control RIB, the NOB microbial population decreased from the first and second sampling events. The BAM RIB contained more NOB at the surface layer indicating that this depth is the most suitable for NOB to grow compared to the Control RIB. Based on the presence of NOB at the surface of the soil for all locations except for location 4 in the second samples, and its gradual decrease in depth, a decrease in nutrient removal is noted. This indicates the contribution that the microbial NOB had on nitrate reduction and the microbiological effect on nitrate removal.

Similarly, the denitrifier gene population increased from the first and second sampling event, apart from location 3 and location 5 which had a reduction in denitrifier population in the second sample date (Fig. 24 (a)). For the BAM and Control RIBS the top layer remained the location for the major accumulation of denitrifier organisms indicating that this is the appropriate depth for denitrifier growth. The decrease in denitrifier population as depth increases is related with nutrient removal as less nutrient can be removed with a decrease in denitrifiers. As a result, the top layer would provide the most nitrate removal, with removal decreasing vertically.

The difference in NOB and denitrifier population in the distinct depths (0, 1, 2 ft) for both the BAM and Control RIBS for first sample data is due to the initial state of the project. Initially the organisms require time to stabilize before a consistent trend is observed. The inlet water nitrogen concentration varied adding to the required microbial adjustment. A trend of decreasing NOB population with depth is observed at the final stage for all the locations apart from location 4, which has an increase in NOB population at depth of 1 foot before decreasing (Fig. 23(a)). Further, NOB increased at location 2 for second sampling date. The denitrifier follows a similar trend where the population decreases with depth for most of the locations in the final stage (Fig. 24(a)). As a result, the properties of BAM media contributed to NOB and denitrifier growth and microbiological process in nitrate removal (O'Reilly, Wanielista et al. 2012). In contrast with the Control RIB, nutrient removing bacteria cultivation was supported in the BAM RIB, this is related to the physiochemical properties of this media. The efficiency of BAM for nitrate removal is related to microbial activity and quantification, as the highest nitrate removal was performed by the BAM RIB. Figure 7, and 8 summarize the total NOB and denitrifier gene population present in the BAM and Control RIBS, and its relation to nitrate removal.

According to the analysis physiochemical and microbiological properties have significant effects on the nitrate removal observed for BAM and Control RIBS, respectively. The soil property as well as the inlet nutrient concentrations contributed to the nitrate removal by providing the adequate environment for the adsorption of nitrate and increase microbiological process of NOB and denitrifiers. The quantity of NOB and denitrifier population at each RIB was related to the amount of nitrate uptake and consequently overall nitrate removal. In figure 25 and increase in total NOB population per gram of dry mass from the first and second sampling date is observed for BAM and Control RIB. An increase in denitrifier population is noted from the first and second sample dates in the BAM RIB (Fig.26), while there is a slight increase in denitrifier population for the Control RIB. This is indicative that the nitrate removal is affected by microbiological process as well as physiochemical process in the soil. Further, the total gene population should not be solely used for comparison with nitrogen removal as the samples were collected at specific locations in the BAM and Control RIBS.

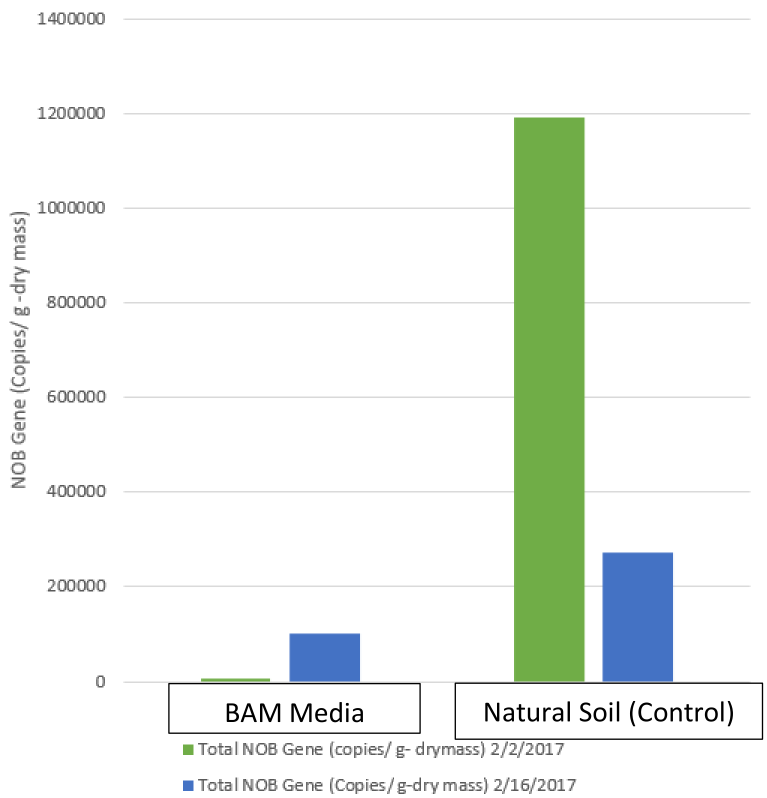


Figure 25 Comparison of total NOB population for BAM and Control RIB for two sampling events and corresponding nitrate removal efficiency.

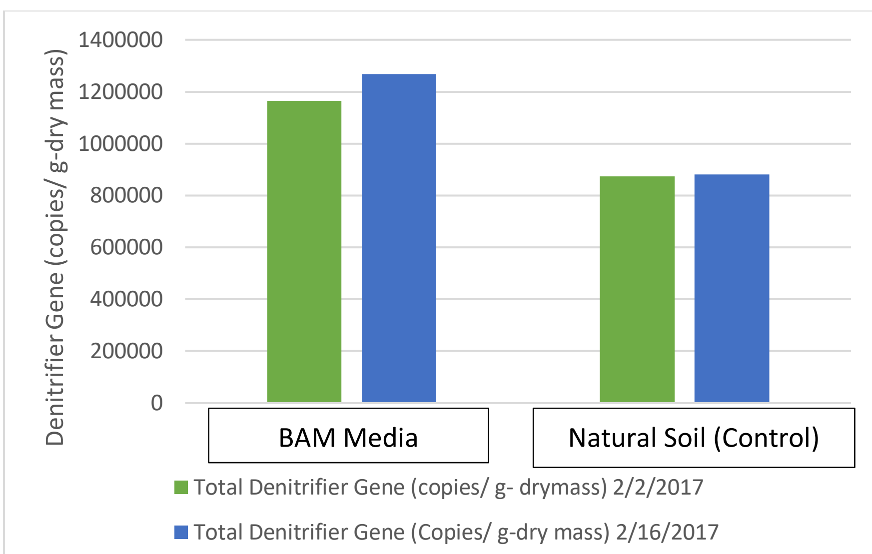


Figure 26 Comparison of total denitrifier population for BAM and Control RIB for two sampling events and corresponding nitrate removal efficiency.

Conclusion using microbiological indicators:

The nitrate removal by chemical analysis and data from the microbial assessment with qPCR results demonstrate that the BAM RIB provided the best environment for the cultivation of NOB and denitrifier bacteria compared to the Control RIB. The qPCR analysis yielded information regarding the appropriate media depths and location for which the greater NOB and denitrifier population were established. Further, the quantity of bacteria population can be closely related to the increased microbiological activity and consequently improved nitrate removal. The physiochemical properties of the BAM and Control RIB soil, also affected nitrate removal through the encouragement or inhibition of microbial growth aiding or preventing the removal of nitrate by the bacteria.

For example, in the initial stage when the first soil sampling event occurred the NOB gene population was more abundant for the Control RIB as opposed to the BAM RIB. A similar relation is observed for the denitrifier gene population in the majority of sample locations for the first sampling event. An explanation for the presence of a large quantity of NOB and denitrifier bacteria in the Control locations for the first sampling event is possible moisture content related with clay in the soil. The moisture provided adequate environment for the bacteria to be sustained. As time progresses and the NOB and denitrifier organisms established in the soil of the BAM and Control RIBs, the population stabilized to the populations observed with the second sampling event, one year after the first sampling took place.

According to the nitrogen concentration for each location at the BAM and Control RIBs. The locations with the lowest nitrate concentration (Location 2 and 6) in the second sampling date were also the locations with the most bacteria population. Further, high nitrate concentrations were observed at the locations with lower bacteria populations (Location 1 and 4) for the same sampling date. This is indicative of the relation between microbiological activity produced by the NOB and denitrifier population and the corresponding nitrogen removal.

The water sample analysis also helped to determine the chemical and microbiological effect on nitrate removal. It was determined that the BAM RIB outperformed the Control RIB in nitrate removal. For BAM an overall removal of 89% was observed, with an 82% removal with reclaimed water, and 95% removal with stormwater. While, for the Control RIB 40% removal with reclaimed water was obtained, and 90% removal with stormwater (Table 10).

Understanding the effect microbial activity from NOB and denitrifier genes have on nitrate removal from reclaimed and stormwater discharge is crucial to deriving nitrogen removal solutions for treating nitrogen impaired groundwater aquifers in the future. The application of green sorption media, such as BAM in modified discharge ponds can aid in the reduction in the impact nutrients have on groundwater aquifers. In addition to the nutrient removal physiochemical properties associated with BAM are of importance as this media enhances microbial growth of bacteria population which can aid in the removal of nitrogen. By applying physiochemical and microbiological activity from green sorption media and bacteria, the reduction of nutrients from groundwater aquifers can be attained through the discharge of water in special RIBs. This reduces the concentration of nutrients that infiltrate to the groundwater aquifer.

In addition, bacteria can also be employed for the removal other types of nutrients present in wastewater discharges and storm water runoff. A long-term investigation with regards to other possible nutrient removals and their effect on groundwater aquifer nutrient concentrations can be investigated. This would provide further support on the application and ability of microbial organisms and sorption media to reduce nutrient concentrations in reclaimed water and stormwater simultaneously prior to transitioning to the groundwater aquifer. Thus, further investigation of the effect of microbial activity in companionship with sorption media on the removal of nutrients is suggested.

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Appendix A

St. Johns River Water Management District

Environmental Resource Permit #76187-5

Dated March 18, 2016

St. Johns River Water Management District
Ann B. Shortelle, Ph.D., Executive Director

4049 Reid Street • P.O. Box 1429 • Palatka, FL 32178-1429 • (386) 329-4500
On the Internet at floridaswater.com.

March 18, 2016

Keith Riger
City of DeLand
1102 S Garfield Ave
Deland, FL 32724-7508

SUBJECT: 76187-5
City of DeLand - Reclaimed Water Storage and Recovery

Dear Sir/Madam:

Enclosed is your individual permit issued by the St. Johns River Water Management District on March 18, 2016. This permit is a legal document and should be kept with your other important documents. Permit issuance does not relieve you from the responsibility of obtaining any necessary permits from any federal, state, or local agencies for your project.

Technical Staff Report:
If you wish to review a copy of the Technical Staff Report (TSR) that provides the District's staff analysis of your permit application, you may view the TSR by going to the Permitting section of the District's website at floridaswater.com/permitting. Using the "search applications and permits" feature, you can use your permit number or project name to find information about the permit. When you see the results of your search, click on the permit number and then on the TSR folder.

Noticing Your Permit:
For noticing instructions, please refer to the noticing materials in this package regarding closing the point of entry for someone to challenge the issuance of your permit. Please note that if a timely petition for administrative hearing is filed, your permit will become nonfinal and any activities that you choose to undertake pursuant to your permit will be at your own risk.

Compliance with Permit Conditions:
To submit your required permit compliance information, go to the District's website at floridaswater.com/permitting. Under the "Apply for a permit or submit compliance data" section, click to sign-in to your existing account or to create a new account. Select the "Compliance Submittal" tab, enter your permit number, and select "No Specific Date" for the Compliance Due Date Range. You will then be able to view all the compliance submittal requirements for your project. Select the compliance item that you are ready to submit and then attach the appropriate information or form. The forms to comply with your permit conditions are available at floridaswater.com/permitting under the section "Handbooks, forms, fees, final orders". Click on forms to view all permit compliance forms, then scroll to the ERP application forms section and

select the applicable compliance forms. Alternatively, if you have difficulty finding forms or need copies of the appropriate forms, please contact the Bureau of Regulatory Support at (386) 329-4570.

Transferring Your Permit:

Your permit requires you to notify the District within 30 days of any change in ownership or control of the project or activity covered by the permit, or within 30 days of any change in ownership or control of the real property on which the permitted project or activity is located or occurs. You will need to provide the District with the information specified in rule 62-330.340, Florida Administrative Code (F.A.C.). Generally, this will require you to complete and submit Form 62-330.340(1), "Request to Transfer Permit," available at http://www.floridaswater.com/permitting/permitforms.html.

Please note that a permittee is liable for compliance with the permit before the permit is transferred. The District, therefore, recommends that you request a permit transfer in advance in accordance with the applicable rules. You are encouraged to contact District staff for assistance with this process.

Thank you and please let us know if you have additional questions. For general questions contact e-permit@sjrwmd.com or (386) 329-4570.

Sincerely,

M. Daniels

Margaret Daniels, Office Director
Office of Business and Administrative Services
St. Johns River Water Management District
4049 Reid Street
Palatka, FL 32177-2529
(386) 329-4570

Enclosures: Permit

cc: District Permit File

Consultant: Andrew Giannini
Quentin L Hampton Associates Inc
PO Box 290247
Port Orange, FL 32129-0247

ST. JOHNS RIVER WATER MANAGEMENT DISTRICT
Post Office Box 1429
Palatka, Florida 32178-1429

PERMIT NO: 76187-5 **DATE ISSUED:** March 18, 2016

PROJECT NAME: City of DeLand - Reclaimed Water Storage and Recovery

A PERMIT AUTHORIZING:
Modification of Permit Number 4-127-76187-4 for Bent Oak Pond Expansion 2009 to include the construction and operation of a 3.97 acre project known as City of DeLand - Reclaimed Water Storage and Recovery, as per plans received by the District on March 17, 2016.

LOCATION:
Section(s): 22 Township(s): 17S Range(s): 30E

Volusia County

Receiving Water Body:

Name	Class
No discharge proposed.	III Fresh

ISSUED TO:
City of DeLand
1102 S Garfield Ave
Deland, FL 32724-7508

The permittee agrees to hold and save the St. Johns River Water Management District and its successors harmless from any and all damages, claims, or liabilities which may arise from permit issuance. Said application, including all plans and specifications attached thereto, is by reference made a part hereof.

This permit does not convey to the permittee any property rights nor any rights or privileges other than those specified herein, nor relieve the permittee from complying with any law, regulation or requirement affecting the rights of other bodies or agencies. All structures and works installed by permittee hereunder shall remain the property of the permittee.

This permit may be revoked, modified or transferred at any time pursuant to the appropriate provisions of Chapter 373, Florida Statutes.

PERMIT IS CONDITIONED UPON:
See conditions on attached "Exhibit A", dated March 18, 2016

AUTHORIZED BY: St. Johns River Water Management District
Division of Regulatory, Engineering and Environmental Services

By: David Dewey

David Dewey
Regulatory Coordinator

"EXHIBIT A"
CONDITIONS FOR ISSUANCE OF PERMIT NUMBER 76187-5
City of DeLand - Reclaimed Water Storage and Recovery
DATED March 18, 2016

1. All activities shall be implemented following the plans, specifications and performance criteria approved by this permit. Any deviations must be authorized in a permit modification in accordance with Rule 62-330.315, F.A.C. Any deviations that are not so authorized may subject the permittee to enforcement action and revocation of the permit under Chapter 373, F.S.

2. A complete copy of this permit shall be kept at the work site of the permitted activity during the construction phase, and shall be available for review at the work site upon request by the District staff. The permittee shall require the contractor to review the complete permit prior to beginning construction.

3. Activities shall be conducted in a manner that does not cause or contribute to violations of state water quality standards. Performance-based erosion and sediment control best management practices shall be installed immediately prior to, and be maintained during and after construction as needed, to prevent adverse impacts to the water resources and adjacent lands. Such practices shall be in accordance with the State of Florida Erosion and Sediment Control Designer and Reviewer Manual (Florida Department of Environmental Protection and Florida Department of Transportation June 2007), and the Florida Stormwater Erosion and Sedimentation Control Inspector's Manual (Florida Department of Environmental Protection, Nonpoint Source Management Section, Tallahassee, Florida, July 2008), which are both incorporated by reference in subparagraph 62-330.050(9)(b)5, F.A.C., unless a project-specific erosion and sediment control plan is approved or other water quality control measures are required as part of the permit.

4. At least 48 hours prior to beginning the authorized activities, the permittee shall submit to the District a fully executed Form 62-330.350(1), "Construction Commencement Notice,"[10-1-13], incorporated by reference herein (http://www.flrules.org/Gateway/reference.asp?No=Ref-02505), indicating the expected start and completion dates. A copy of this form may be obtained from the District, as described in subsection 62-330.010(5), F.A.C. If available, an District website that fulfills this notification requirement may be used in lieu of the form.

5. Unless the permit is transferred under Rule 62-330.340, F.A.C., or transferred to an operating entity under Rule 62-330.310, F.A.C., the permittee is liable to comply with the plans, terms and conditions of the permit for the life of the project or activity.

6. Within 30 days after completing construction of the entire project, or any independent portion of the project, the permittee shall provide the following to the Agency, as applicable:

   a. For an individual, private single-family residential dwelling unit, duplex, triplex, or quadruplex — "Construction Completion and Inspection Certification for Activities Associated With a Private Single-Family Dwelling Unit" [Form 62-330.310(3)]; or

   b. For all other activities — "As-Built Certification and Request for Conversion to Operational Phase" [Form 62-330.310(1)].

   c. If available, an Agency website that fulfills this certification requirement may be used in lieu of the form.

7. If the final operation and maintenance entity is a third party:

    a. Prior to sales of any lot or unit served by the activity and within one year of permit issuance, or within 30 days of as-built certification, whichever comes first, the permittee shall submit, as applicable, a copy of the operation and maintenance documents (see sections 12.3 thru 12.3.3 of Volume I) as filed with the Department of State, Division of Corporations and a copy of any easement, plat, or deed restriction needed to operate or maintain the project, as recorded with the Clerk of the Court in the County in which the activity is located.

    b. Within 30 days of submittal of the as- built certification, the permittee shall submit "Request for Transfer of Environmental Resource Permit to the Perpetual Operation Entity" [Form 62-330.310(2)] to transfer the permit to the operation and maintenance entity, along with the documentation requested in the form. If available, an Agency website that fulfills this transfer requirement may be used in lieu of the form.

8. The permittee shall notify the District in writing of changes required by any other regulatory District that require changes to the permitted activity, and any required modification of this permit must be obtained prior to implementing the changes.

9. This permit does not:

    a. Convey to the permittee any property rights or privileges, or any other rights or privileges other than those specified herein or in Chapter 62-330, F.A.C.;

    b. Convey to the permittee or create in the permittee any interest in real property;

    c. Relieve the permittee from the need to obtain and comply with any other required federal, state, and local authorization, law, rule, or ordinance; or

    d. Authorize any entrance upon or work on property that is not owned, held in easement, or controlled by the permittee.

10. Prior to conducting any activities on state-owned submerged lands or other lands of the state, title to which is vested in the Board of Trustees of the Internal Improvement Trust Fund, the permittee must receive all necessary approvals and authorizations under Chapters 253 and 258, F.S. Written authorization that requires formal execution by the Board of Trustees of the Internal Improvement Trust Fund shall not be considered received until it has been fully executed.

11. The permittee shall hold and save the District harmless from any and all damages, claims, or liabilities that may arise by reason of the construction, alteration, operation, maintenance, removal, abandonment or use of any project authorized by the permit.

12. The permittee shall notify the District in writing:

    a. Immediately if any previously submitted information is discovered to be inaccurate; and

    b. Within 30 days of any conveyance or division of ownership or control of the property or the system, other than conveyance via a long-term lease, and the new owner shall request transfer of the permit in accordance with Rule 62-330.340, F.A.C. This does not apply to the sale of lots or units in residential or commercial subdivisions or condominiums where the stormwater management system has been completed and converted to the operation phase.

13. Upon reasonable notice to the permittee, District staff with proper identification shall have permission to enter, inspect, sample and test the project or activities to ensure conformity with the plans and specifications authorized in the permit.

14. If any prehistoric or historic artifacts, such as pottery or ceramics, stone tools or metal implements, dugout canoes, or any other physical remains that could be associated with Native American cultures, or early colonial or American settlement are encountered at any time within the project site area, work involving subsurface disturbance in the immediate vicinity of such discoveries shall cease. The permittee or other designee shall contact the Florida Department of State, Division of Historical Resources, Compliance and Review Section, at (850) 245-6333 or (800) 847-7278, as well as the appropriate permitting agency office. Such subsurface work shall not resume without verbal or written authorization from the Division of Historical Resources. If unmarked human remains are encountered, all work shall stop immediately and notification shall be provided in accordance with Section 872.05, F.S.

15. Any delineation of the extent of a wetland or other surface water submitted as part of the permit application, including plans or other supporting documentation, shall not be considered binding unless a specific condition of this permit or a formal determination under Rule 62-330.201, F.A.C., provides otherwise.

16. The permittee shall provide routine maintenance of all components of the stormwater management system to remove trapped sediments and debris. Removed materials shall be disposed of in a landfill or other uplands in a manner that does not require a permit under Chapter 62-330, F.A.C., or cause violations of state water quality standards.

17. This permit is issued based on the applicant's submitted information that reasonably demonstrates that adverse water resource-related impacts will not be caused by the completed permit activity. If any adverse impacts result, the District will require the permittee to eliminate the cause, obtain any necessary permit modification, and take any necessary corrective actions to resolve the adverse impacts.

18. A Recorded Notice of Environmental Resource Permit may be recorded in the county public records in accordance with Rule 62-330.090(7), F.A.C. Such notice is not an encumbrance upon the property.

19. This permit for construction will expire five years from the date of issuance.

20. The proposed project must be constructed and operated as per plans received by the District on March 17, 2016.

Appendix B

RIB Loading and Water Quality Data

The information in this Appendix are listed to provide an understanding of methods used for testing as well as the data for effectiveness analyses.

Water Quality Parameters and Methods for Measurement

Parameter	Method/instrument	Detection Limit
Reclaimed Water		-
TKN	EPA Method 351.2	0.463 mg/L
TP	EPA Method 365.4	0.119mg/L
Nitrate	EPA Method 353.2	0.079 mg/L N (0.134 mg/L)*
Chloride	Standard Methods 4500 E	0.444 mg/L
RIB Measurement		
Soil moisture	EC-5 SOIL MOISTURE SENSOR	0 - 100%
Stormwater		
(Nitrates and Nitrites)	Standard Method 4500 F	0.002 mg/L N
Total Organic Carbon	Standard Method 5310 B11	0.23 mg/L
Alkalinity	Standard Method 2320 B	1.4 mg/L N

*Change in detection limit in January 2018, thus two samples with higher detection limit due to change of personnel. Method stayed the same.

References: Standard Methods for the Examination of Water and Wastewater, 21st Edition. 2017. American Public Health Association and American Water Works Association.

U.S. Environmental Monitoring Systems Laboratory Office, (EPA 600/R-93/100). U.S. Environmental Protection Agency, Cincinnati, Ohio, Latest Edition

RIB Loading Volume, RIB Bottom Cover with time and Unit Loading Data

		North (BAM) Pond				South (Control) Pond			
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
1/18/2017	1.32	97	1.95			65	1.25		
1/19/2017	0.743	100	3.05			100	1.85		
1/20/2017	0.26	100	2.4	1,039,500	23.9	65	1.2	1,283,540	17.2
1/21/2017	0								
1/22/2017	0								
1/23/2017	0	80	1.25			20	0.20		
1/24/2017	0	70	0.9			5	0.10		
1/25/2017	0								
1/26/2017	0	30	0.5			0	0.0		
1/27/2017	0	15	0.35			0	0.0		
1/28/2017	0								
1/29/2017	0								
1/30/2017	0	0	0			0	0.0		
1/31/2017	0	0	0			0	0.0		
2/1/2017	0	0	0			0	0.0		
2/2/2017	0	0	0			0	0.0		
2/3/2017	0	0	0			0	0.0		
2/4/2017	0								
2/5/2017	0								
2/6/2017	0	0	0			0	0.0		
2/7/2017	0	0	0			0	0.0		
2/8/2017	0	0	0			0	0.0		
2/9/2017	0	0	0			0	0.0		
2/10/2017	0	0	0			0	0.0		
2/11/2017	0								
2/12/2017	0								
2/13/2017	0	0	0			0	0.0		
2/14/2017	0.52	15	0.35			5	0.15		
2/15/2017	0	1	0.1			0	0.0		
2/16/2017	0	0	0			0	0.0		
2/17/2017	0								
2/18/2017	0								
2/19/2017	0								
2/20/2017	0.98	90	1.75	404,100	9.3	55	1.10	579,750	7.7
2/21/2017	0	85	1.65			35	0.75		
2/22/2017	0	80	1.4			30	0.50		
2/23/2017	0	75	1.2			20	0.25		
2/24/2017	0	70	1			10	0.15		
2/25/2017	0								
2/26/2017	0								
2/27/2017	0	15	0.35			0	0.0		
2/28/2017	0	2	0.2			0	0.0		

*Loading based on the area of RIB bottom, RIB BAM=43,600 Square Feet (SF), RIB Control= 73,200 SF

		North (BAM) Pond				South (Control) Pond			
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
3/1/2017	0	0	0			0	0.00		
3/2/2017	0	0	0			0	0.00		
3/3/2017	0	0	0			0	0.0		
3/4/2017	0								
3/5/2017	0								
3/6/2017	1.24	95	1.95	501,960	11.5	60	1.20	735,380	9.8
3/7/2017	0	95	2			55	1.00		
3/8/2017	0	85	1.65			30	0.60		
3/9/2017	0	75	1.35			15	0.2		
3/10/2017	0	70	1			0	0.0		
3/11/2017	0	40	0.6						
3/12/2017	0								
3/13/2017	0	25	0.5			0	0.0		
3/14/2017	0	20	0.4			0	0.0		
3/15/2017	0	3	0.25			0	0.0		
3/16/2017	0	1	0.1			0	0.0		
3/17/2017	0	0	0			0	0.0		
3/18/2017	0								
3/19/2017	0								
3/20/2017	0	0	0			0	0.0		
3/21/2017	0	0	0			0	0.0		
3/22/2017	0	0	0			0	0.0		
3/23/2017	0	0	0			0	0.0		
3/24/2017	0	0	0			0	0.0		
3/25/2017	0								
3/26/2017	0								
3/27/2017	0	0	0			0	0.0		
3/28/2017	0	0	0			0	0.00		
3/29/2017	0	0	0			0	0.0		
3/30/2017	0	0	0			0	0.0		
3/31/2017	0	0	0			0	0.0		
4/1/2017	0								
4/2/2017	0								
4/3/2017	1.25	95	1.9	613,400	14.1	65	1.35	646,440	8.6
4/4/2017	0	85	1.6			60	1.00		
4/5/2017	0	80	1.3			40	0.65		
4/6/2017	0	70	0.85			20	0.30		
4/7/2017	0	45	0.6			2	0.05		
4/8/2017	0	25				0			
4/9/2017	0								
4/10/2017	0	1	0.1			0	0.0		
4/11/2017	0	0	0			0	0.0		

*Loading based on the area of RIB bottom, RIB BAM=43,600 Square Feet (SF), RIB Control= 73,200 SF

		North (BAM) Pond				South (Control) Pond			
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
4/12/2017	0	0	0			0	0.00		
4/13/2017	0	0	0			0	0.00		
4/14/2017	0	0	0			0	0.00		
4/15/2017	0								
4/16/2017	0								
4/17/2017	0	0	0			0	0.00		
4/18/2017	0	0	0			0	0.00		
4/19/2017	0	0	0			0	0.00		
4/20/2017	0	0	0			0	0.00		
4/21/2017	0	0	0			0	0.00		
4/22/2017	0								
4/23/2017	0								
4/24/2017	0	0	0			0	0.00		
4/25/2017	0	0	0			0	0.00		
4/26/2017	0	0	0			0	0.00		
4/27/2017	0	0	0			0	0.00		
4/28/2017	0	0	0			0	0.00		
4/29/2017	0								
4/30/2017	0	Change in meter recording and flow valve setting							
5/1/2017	0.98	80	1.45	284,952	6.5	65	1.30	695,048	9.3
5/2/2017	0	60	0.95			40	0.75		
5/3/2017	0	50	0.65			30	0.40		
5/4/2017	0	15	0.35			2	0.05		
5/5/2017	0	5	0.2			0	0.00		
5/6/2017	0								
5/7/2017	0								
5/8/2017	0	0	0			0	0.00		
5/9/2017	0	0	0			0	0.00		
5/10/2017	0	0	0			0	0.00		
5/11/2017	0	0	0			0	0.00		
5/12/2017	0	0	0			0	0.00		
5/13/2017	0								
5/14/2017	0								
5/15/2017	0	0	0			0	0.00		
5/16/2017	0	0	0			0	0.00		
5/17/2017	0	0	0			0	0.00		
5/18/2017	0	0	0			0	0.00		
5/19/2017	0	0	0			0	0.00		
5/20/2017	0								
5/21/2017	0								
5/22/2017	0	0	0			0	0.00		
5/23/2017	0	0	0			0	0.00		
5/24/2017	0	0	0			0	0.00		
5/25/2017	0	0	0			0	0.00		
5/26/2017	0	0	0			0	0.00		
5/27/2017	0								
5/28/2017	0								
5/29/2017	0	Holiday							
5/30/2017	0	0	0			0	0.00		
5/31/2017	0	0	0			0	0.00		

*Loading based on the area of RIB bottom, RIB BAM=43,600 Square Feet (SF), RIB Control= 73,200 SF

		North (BAM) Pond				South (Control) Pond			
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
6/1/2017	0	0	0			0	0.00		
6/2/2017	0	0	0			0	0.00		
6/3/2017	0								
6/4/2017	0								
6/5/2017	1.49	95	1.85	510,708	11.7	75	1.45	979,292	13.1
6/6/2017	0	90	1.6			60	1.15		
6/7/2017	0	75	1.15			45	0.75		
6/8/2017	0	65	0.9			35	0.55		
6/9/2017	0	45	0.6			20	0.30		
6/10/2017	0								
6/11/2017	0								
6/12/2017	0	5	0.3			0	0.00		
6/13/2017	0	70	1			20	0.30		
6/14/2017	0	60	0.8			3	0.05		
6/15/2017	0								
6/16/2017	0	30	0.5			0	0.00		
6/17/2017	0								
6/18/2017	0								
6/19/2017	0	30	0.5			0	0.00		
6/20/2017	0	30	0.5			0	0.00		
6/21/2017	0	20	0.4			0	0.00		
6/22/2017	0	5	0.25			0	0.00		
6/23/2017	0	0	0			0	0.00		
6/24/2017	0								
6/25/2017	0								
6/26/2017	0	30	0.5			2	0.05		
6/27/2017	0	20	0.35			0	0.00		
6/28/2017	0	5	0.3			0	0.00		
6/29/2017	0	1	0.1			0	0.00		
6/30/2017	0	0	0			0	0.00		
7/1/2017	0								
7/2/2017	0								
7/3/2017	0	10	0.3			0	0.00		
7/4/2017	0								
7/5/2017	0	0	0			0	0.00		
7/6/2017	0	0	0			0	0.00		
7/7/2017	0	0	0			0	0.00		
7/8/2017	0								
7/9/2017	0	Change in flow valve setting							
7/10/2017	1.66		1.5	574,857	13.2		1.25	1,085,143	14.5
7/11/2017	0	90	2			80	1.70		
7/12/2017	0	80	1.55			70	0.90		
7/13/2017	0	70	0.95			60	1.00		
7/14/2017	0	60	0.75			55	0.95		
7/15/2017	0								
4/16/2017	0								
7/17/2017	0	10	0.25			20	0.20		

*Loading based on the area of RIB bottom, RIB BAM=43,600 Square Feet (SF), RIB Control= 73,200 SF

		North (BAM) Pond				South (Control) Pond			
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
7/18/2017	0	40	0.5			40	0.30		
7/19/2017	0	25	0.5			15	0.20		
7/20/2017	0	15	0.35			5	0.05		
7/21/2017	0	5	0.3			2	0.00		
7/22/2017	0								
7/23/2017	0								
7/24/2017	0	5	0.3			5	0.05		
7/25/2017	0	2	0.2			5	0.10		
7/26/2017	0	1	0.1			5	0.10		
7/27/2017	0	0	0			1	0.00		
7/28/2017	0	0	0			0	0.00		
7/29/2017	0								
7/30/2017	0								
7/31/2017	0	0	0			0	0.00		
8/1/2017	0	0	0			0	0.00		
8/2/2017	0	0	0			0	0.00		
8/3/2017	0	0	0			0	0.00		
8/4/2017	0	20	0.45			10	0.10		
8/5/2017	0								
8/6/2017	0								
8/7/2017	1.41		1.35	454,051	10.4		1.20	955,949	12.8
8/8/2017	0	90	1.7			80	1.55		
8/9/2017	0	70	1.15			70	1.30		
8/10/2017	0	60	0.75			55	1.05		
8/11/2017	0	50	0.6			50	0.90		
8/12/2017	0								
8/13/2017	0								
8/14/2017	0	15	0.4			30	0.60		
8/15/2017	0	5	0.3			20	0.50		
8/16/2017	0	3	0.2			15	0.40		
8/17/2017	0	0	0			10	0.35		
8/18/2017	0	0	0			5	0.20		
8/19/2017	0								
8/20/2017	0								
8/21/2017	0	5	0.3			15	0.40		
8/22/2017	0	3	0.2			15	0.45		
8/23/2017	0	0	0			15	0.45		
8/24/2017	0	0	0			15	0.45		
8/25/2017	0	0	0			15	0.40		
8/26/2017	0								
8/27/2017	0								
8/28/2017	0	0	0			15	0.35		
8/29/2017	0	0	0			10	0.30		
8/30/2017	0								
8/31/2017	0	0	0			10	0.20		

*Loading based on the area of RIB bottom, RIB BAM=43,600 Square Feet (SF), RIB Control= 73,200 SF

		North (BAM) Pond				South (Control) Pond			
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
9/1/2017	0	0	0			15	0.30		
9/2/2017	0								
9/3/2017	0								
9/4/2017	0	Holiday							
9/5/2017	0	5	0.25			20	0.25		
9/6/2017	0	2	0.15			20	0.30		
9/7/2017	0	2	0.15			20	0.30		
9/8/2017	0	3	0.2			35	0.60		
9/9/2017	0								
9/10/2017	0								
9/11/2017	0	100	MAX			100	MAX		
9/12/2017	0	100	MAX			100	MAX		
9/13/2017	0	100	MAX			100	MAX		
9/14/2017	0	100	MAX			100	MAX		
9/15/2017	0	100	MAX			100	MAX		
9/16/2017	0								
9/17/2017	0								
9/18/2017	0	100	MAX			100	MAX		
9/19/2017	0	100	MAX			100	MAX		
9/20/2017	0	100	MAX			100	MAX		
9/21/2017	0	100	MAX			100	MAX		
9/22/2017	0	100	MAX			100	MAX		
9/23/2017	0								
9/24/2017	0								
9/25/2017	0	100	MAX			100	MAX		
9/26/2017	0	100	MAX			100	MAX		

*Loading based on the area of RIB bottom, RIB BAM=43,600 Square Feet (SF), RIB Control= 73,200 SF

Note: Hurricane Irma occurred on September 11, excess stormwater was discharged to the RIBs for three days to prevent flooding. The depth gages were submerged, thus no readings until September 27 when the gages were again visible.

The next pages continue the data on stage depths and loading rates.

		North (BAM) Pond				South (Control) Pond			
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
9/27/2017	0	100	9.9			100	8.50		
9/28/2017	0	100	9.7			100	8.30		
9/29/2017	0.00	100	9.40			100	8.00		
9/30/2017	0.00								
10/1/2017	0.00								
10/2/2017	0.00	100	8.90			100	7.50		
10/3/2017	0.00	100	8.90			100	7.50		
10/4/2017	0.00	100	8.70			100	7.30		
10/5/2017	0.00	100	8.80			100	7.40		
10/6/2017	0.00	100	9.20			100	7.80		
10/7/2017	0.00								
10/8/2017	0.00								
10/9/2017	0.00	100	9.70			100	8.30		
10/10/2017	0.00	100	9.50			100	8.10		
10/11/2017	0.00	100	9.30			100	7.90		
10/12/2017	0.00	100	9.00			100	7.60		
10/13/2017	0.00	100	8.80			100	7.40		
10/14/2017	0.00								
10/15/2017	0.00								
10/16/2017	0.00	100	8.20			100	6.80		
10/17/2017	0.00	100	8.10			100	6.70		
10/18/2017	0.00	100	8.00			100	6.60		
10/19/2017	0.00	100	7.80			100	6.40		
10/20/2017	0.00	100	7.60			100	6.20		
10/21/2017	0.00								
10/22/2017	0.00								
10/23/2017	0.00	100	7.20			100	5.80		
10/24/2017	0.00	100	7.10			100	5.70		
10/25/2017	0.00	100	6.90			100	5.50		
10/26/2017	0.00	100	6.70			100	5.30		
10/27/2017	0.00	100	6.60			100	5.20		
10/28/2017	0.00								
10/29/2017	0.00								
10/30/2017	0.00	100	6.20			100	4.80		
10/31/2017	0.00	100	6.00			100	4.60		
11/1/2017	0.00	100	5.95			100	4.55		
11/2/2017	0.00	100	5.80			100	4.40		
11/3/2017	0.00	100	5.70			100	4.30		
11/4/2017	0.00								
11/5/2017	0.00								
11/6/2017	0.00	100	5.25			100	4.10		
11/7/2017	0.00	100	5.10			100	4.00		
11/8/2017	0.00	100	4.95			100	4.00		
11/9/2017	0.00	100	4.80			100	3.90		
11/10/2017	0.00	100	4.65			100	3.80		
11/11/2017	0.00								
11/12/2017	0.00								

		North (BAM) Pond				South (Control) Pond			
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
11/12/2017	0.00								
11/13/2017	0.00	100	4.30			100	3.60		
11/14/2017	0.00	100	4.20			100	3.50		
11/15/2017	0.00	100	4.10			100	3.50		
11/16/2017	0.00								
11/17/2017	0.00								
11/18/2017	0.00								
11/19/2017	0.00								
11/20/2017	0.00	100	3.60			100	3.10		
11/21/2017	0.00	100	3.50			100	3.05		
11/22/2017	0.00	100	3.40			100	3.00		
11/23/2017	0.00								
11/24/2017	0.00								
11/25/2017	0.00								
11/26/2017	0.00								
11/27/2017	0.00	100	3.10			100	2.85		
11/28/2017	0.00	100	3.00			100	2.80		
11/29/2017	0.00	100	2.90			100	2.80		
11/30/2017	0.00	100	2.80			100	2.70		
12/1/2017	0.00	100	2.70			100	2.70		
12/2/2017	0.00								
12/3/2017	0.00								
12/4/2017	0.00	100	2.50			100	2.50		
12/5/2017	0.00	100	2.40			100	2.45		
12/6/2017	0.00	100	2.30			100	2.40		
12/7/2017	0.00	98	2.25			100	2.35		
12/8/2017	0.00	98	2.20			100	2.40		
12/9/2017	0.00								
12/10/2017	0.00								
12/11/2017	0.00	95	2.10			100	2.20		
12/12/2017	0.00	95	2.00			98	2.15		
12/13/2017	0.00	90	1.95			98	2.10		
12/14/2017	0.00	90	1.90			98	2.10		
12/15/2017	0.00	85	1.85			95	2.00		
12/16/2017	0.00								
12/17/2017	0.00								
12/18/2017	0.00	80	1.70			95	1.90		
12/19/2017	0.00								
12/20/2017	0.00	80	1.55			95	1.85		
12/21/2017	0.00	80	1.50			95	1.80		
12/22/2017	0.00	75	1.40			90	1.75		
12/23/2017	0.00								
12/24/2017	0.00								
12/25/2017	0.00								

		North (BAM) Pond					South (Control) Pond		
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
12/26/2017	0.00								
12/27/2017	0.00	70	1.10			80	1.35		
12/28/2017	0.00	65	1.00			75	1.20		
12/29/2017	0.00	60	0.95			70	1.10		
12/30/2017	0.00								
12/31/2017	0.00								
1/1/2018	0.00								
1/2/2018	0.00	50	0.80			40	0.70		
1/3/2018	0.00	55	0.85			40	0.70		
1/4/2018	0.00	50	0.80			40	0.60		
1/5/2018	0.00	35	0.70			20	0.50		
1/6/2018	0.00								
1/7/2018	0.00								
1/8/2018	0.00	30	0.60			15	0.30		
1/9/2018	0.00	30	0.60			15	0.30		
1/10/2018	0.00	30	0.60			15	0.30		
1/11/2018	0.00								
1/12/2018	0.00	20	0.50			10	0.30		
1/13/2018	0.00								
1/14/2018	0.00								
1/15/2018	0.00								
1/16/2018	0.00	5	0.30			10	0.30		
1/17/2018	0.00	5	0.30			10	0.30		
1/18/2018	0.00	3	0.20			5	0.25		
1/19/2018	0.00	1	0.15			3	0.20		
1/20/2018	0.00								
1/21/2018	0.00								
1/22/2018	1.44	70	1.50	454,112	10.42	60	1.35	990,087	13.53
1/23/2018	0.00	90	1.85			90	1.60		
1/24/2018	0.00	85	1.70			85	1.50		
1/25/2018	0.00	65	1.50			60	1.30		
1/26/2018	0.00	65	1.35			55	1.00		
1/27/2018	0.00								
1/28/2018	0.00								
1/29/2018	0.00	60	1.10			60	1.30		
1/30/2018	0.00	60	1.00			55	1.05		
1/31/2018	0.00	55	0.85			50	0.85		
2/1/2018	0.00	50	0.80			45	0.70		
2/2/2018	0.00	40	0.70			30	0.50		
2/3/2018	0.00								
2/4/2018	0.00								
2/5/2018	0.00	20	0.55			10	0.20		

		North (BAM) Pond				South (Control) Pond			
	Volume	Coverage	Staff Gauge	Volume	Loading	Coverage	Staff Gauge	Volume	Loading
Date	MG	%	Ft.	Gal.	G/SF*	%	Ft.	Gal.	G/SF*
2/6/2018	0.00	15	0.50			5	0.05		
2/7/2018	0.00	10	0.40			3	0.02		
2/8/2018	0.00	10	0.40			3	0.01		
2/9/2018	0.00	5	0.30			1	0.00		
2/10/2018	0.00								
2/11/2018	0.00								
2/12/2018	0.00	1	0.20			1	0.00		
2/13/2018	0.00	1	0.20			1	0.00		
2/14/2018	0.00	0	0.00			1	0.00		
2/15/2018	0.00	0	0.00			1	0.00		
2/16/2018	0.00	0	0.00			1	0.00		
2/17/2018	0.00								
2/18/2018	0.00								
2/19/2018	1.60	95	1.50	558,965	12.82	95	1.30	1,041,006	14.22
2/20/2018	0.00	85	1.85			85	1.50		
2/21/2018	0.00	80	1.60			75	1.25		
2/22/2018	0.00	70	1.40			65	1.00		
2/23/2018	0.00	60	1.20			40	0.70		
2/24/2018	0.00								
2/25/2018	0.00								
2/26/2018	0.00	30	0.6			2	0.05		
Average Loading Rate (G/SF)					12.4				12.1
Cumulative Volume (Million Gallons) =				5.397				8.992	
Cumulative Volume (Million Gallons/Acre) =				5.392				5.351	
	Depth of loading before water quality testing (feet) =					6.4			
	Depth of Cumulative loading (feet) =					16.5	BAM RIB	Control =	16.4
	Depth of loading (calibration + water quality loading)					23	BAM RIB		
	Depth of loading at soil density testing (feet)					9.6	on Feb 3		
	*Loading based on total bottom area of RIB								
	area of BAM RIB (SF) =			43,600		area of Control RIB (SF)=			73,200

RIB Water Quality Data

sample date			1/30/2017					
sample location		RIB Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6
Conductivity	µmhos/cm		865	721	811	924	864	820
Turbidity	NTU		3.41	37.7	4.16	18.5	0.82	2.15
Fecal Coliform	CFU/100ml		15	10	<5	50	815	100
TKN	mg/L		2.01	1.58	1.23	1.52	1.25	1.02
Nitrate/Nitrite	mg/L		<0.079*	0.079*	1.51	3.63	3.31	3.07
TN	mg/L		2.05	1.66	2.74	5.15	4.56	4.09
Cl-	mg/L		126	122	124	126	127	120
TP	mg/L		0.700	0.864	1.86	1.28	0.638	2.66
pH			7.87	7.96	7.74	7.35	7.63	7.89

* Detection limit is 0.079 mg/L no sample

sample date		2/20/2017	2/27/2017					
sample location		RIB Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6
Conductivity	µmhos/cm		867	658	874	816	689	850
Turbidity	NTU		3.21	102	20.6	57.3	1.12	1.40
Fecal Coliform	CFU/100ml		10	2	10	63	643	17
TKN	mg/L	1.64	2.29	1.80	1.64	2.19	1.63	1.72
Nitrate/Nitrite	mg/L	6.04	0.457	0.079*	3.79	4.00	5.12	6.02
TN	mg/L	7.68	2.75	1.88	5.43	6.19	6.75	7.74
Cl-	mg/L		126	117	125	140	109	126
TP	mg/L	4.00	1.830	1.370	0.920	0.792	1.59	3.64
pH			7.81	8.78	7.35	7.18	7.40	7.71

* Detection limit is 0.079 mg/L no sample

sample date		3/6/2017	3/13/2017					
sample location		RIB Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6
Conductivity	µmhos/cm		922	583	782		551	790
Turbidity	NTU		3.13	50	74.2		5.35	1.98
Fecal Coliform	CFU/100ml		5	9	22		3224	6
TKN	mg/L	1.15	2.60	1.43	1.38		1.57	1.17
Nitrate/Nitrite	mg/L	6.08	<0.079*	<0.079*	3.04		3.78	5.62
TN	mg/L	7.23	2.64	1.47	4.42		5.35	6.79
Cl-	mg/L		133	98.0	113		80.0	112
TP	mg/L	4.68	1.530	0.442	2.780		1.25	3.50
pH			8.73	10.04	7.51		7.39	7.77

* Detection limit is 0.079 mg/L no sample

sample date		4/3/2017	4/11/2017					
sample location		RIB Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6
Conductivity	µmhos/cm		859	710	880	1157	741	759
Turbidity	NTU		7.00	12.8	20.3	138	1.28	0.83
Fecal Coliform	CFU/100ml		<1	<1	27	36	870	<1
TKN	mg/L	2.64	1.65	1.48	2.96	2.09	1.46	1.54
Nitrate/Nitrite	mg/L	2.60	0.079*	0.079*	<0.079*	2.58	2.60	1.10
TN	mg/L	5.24	1.73	1.56	3.00	4.67	4.06	2.64
Cl-	mg/L		118	132	138	228	122	122
TP	mg/L	4.12	2.00	1.25	2.94	1.01	2.64	2.85
pH		7.64	6.99	7.48	7.46	7.33	7.38	7.51

* Detection limit is 0.079 mg/L no sample

sample date			5/8/2017						
sample location		RIB Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6	Well
Conductivity	µmhos/cm		925	887	1064	1223	949	983	730
Turbidity	NTU		144	36.1	2.72	10.3	0.79	0.52	33.1
Fecal Coliform	CFU/100ml		9	4	10	14	1110	5	<1
TKN	mg/L		1.82	1.16	2.95	1.80	1.51	1.01	0.387
Nitrate/Nitrite	mg/L		1.34	0.079*	<0.079*	5.49	3.52	0.079*	5.52
TN	mg/L		3.16	1.24	2.99	7.29	5.03	1.04	5.91
Cl-	mg/L		151	178	180	238	173	172	108
TP	mg/L		0.954	1.37	1.45	0.611	2.40	2.63	0.248
pH			6.91	7.25	7.24	7.03	7.24	7.62	6.35
	* Detection limit is 0.079 mg/L				no sample				

sample date		6/5/2017	6/12/2017						
sample location		RIB Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6	Well
Conductivity	µmhos/cm		762	408	904	920	637	614	719
Turbidity	NTU		37.7	135	16.9	20.5	0.78	0.23	35.1
Fecal Coliform	CFU/100ml		280	140	9	confluent	1500	65	1
TKN	mg/L	0.282	2.86	1.36	1.84	1.64	1.45	0.282	0.282
Nitrate/Nitrite	mg/L	3.72	<0.079*	<0.079*	8.82	11.00	1.91	0.079*	6.28
TN	mg/L	4.00	2.90	1.40	10.7	12.6	3.36	0.361	7.56
Cl-	mg/L	144	108	57.9	118	136	95.9	94.8	114
TP	mg/L	2.74	3.21	2.00	0.889	1.18	2.61	2.17	0.243
pH		7.37	7.06	7.50	6.82	7.03	7.09	7.49	6.43
	* Detection limit is 0.079 mg/L				no sample	confluent	too many to count		

sample date		7/10/2017	7/17/2017						
sample location		RIB Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6	Well
Conductivity	µmhos/cm		638	677	340	379	714	830	
Turbidity	NTU		14.0	35.4	10.5	245	2.15	0.62	
Fecal Coliform	CFU/100ml		133	10	3	258	1680	260	
TKN	mg/L	1.02	6.21	1.58	1.27	2.56	1.23	2.67	
Nitrate/Nitrite	mg/L	0.723	<0.079*	<0.079*	0.882	1.08	<0.079*	<0.079*	
			normal analyzed time period						
TN	mg/L	1.74	6.25	1.62	2.09	3.55	1.27	2.71	
Cl-	mg/L	120	68.6	111	12.8	50.2	86.2	116	
TP	mg/L	3.23	2.01	1.38	0.459	1.92	2.02	2.16	
pH		7.62	6.96	7.17	6.95	6.43	7.17	8.34	
	* Detection limit is 0.079 mg/L				no sample				
Note: Check for holding time affects on the Nitrate value, it was minimal to none in the period of analysis									
Analyzed day of collection			<0.079*	<0.079*	0.815	0.987	<0.079*	<0.079*	

sample date		8/7/2017	8/14/2017						
sample location		RIB Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6	Well
Conductivity	µmhos/cm		266	626	149	595	561	617	1081
Turbidity	NTU		114	5.03	39.8	78.1	1.62	12.1	15.4
Fecal Coliform	CFU/100ml		45	680	77	confluent	confluent	644	0
TKN	mg/L	1.00	2.53	1.62	0.371	3.02	0.780	1.56	0.388
Nitrate/Nitrite	mg/L	0.079*	<0.079*	<0.079*	<0.079*	<0.079*	<0.079*	<0.079*	3.42
TN	mg/L	1.08	2.57	1.66	0.411	3.06	0.820	1.60	3.81
Cl-	mg/L	105	17.1	95.1	1.93	70.0	66.4	78.0	122
TP	mg/L	2.26	1.08	1.36	0.927	5.14	2.33	1.93	0.161
pH		7.40	6.86	7.48	6.86	6.79	7.35	7.95	6.93
	* Detection limit is 0.079 mg/L				no sample	confluent	too many to count		

The following two samples were for stormwater loading conditions resulting from Hurricane Irma

	sample date	12/18/2017							
	sample location	BAM Pond	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6	Control Pond
Conductivity	µmhos/cm		230	236	280	512	514	261	
Turbidity	NTU		23.7	2.69	28.3	36.4	17.9	27.0	
Fecal Coliforn	CFU/100ml		<1	1	<1	<2	<10	<1	
TKN	mg/L		2.29	<0.463	4.68	2.95	<0.463	2.16	
Nitrate/Nitrite	mg/L	0.303**	.009	.004	.007	.010	.007	.027	
TN	mg/L		2.36	<0.530	4.75	3.02	<0.530	2.23	
Cl-	mg/L		24.2	24.7	22.8	56.3	56.4	27.2	
TP	mg/L		0.796	0.819	1.56	3.47	3.12	0.119	
pH	SU		6.83	7.04	6.86	6.67	6.86	7.09	

* Detection limit is 0.002 mg/L, a change from previous dates.
** From three locations in Ocala in the same time period as the hurricane that produced the stormwater, average concentration of Nitrate/Nitrite in runoff water was 0.303 mg/L. The total number of samples was Fifteen (15). These are asumed input values to the RIBs

	sample date	1/10/2018							
	sample location		RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6	
Conductivity	µmhos/cm		233	211	258	466	456	378	
Turbidity	NTU		5.06	3.04	2.92	18.8	11.6	1.46	
Fecal Coliform	CFU/100ml		<1	1	196	116	74	<1	
TKN	mg/L		2.01	2.03	3.57	3.64	<0.463	<0.463	
Nitrate/Nitrite*	mg/L	0.303**	0.024	0.014	0.024	0.042	0.022	0.078	
TN	mg/L		2.08	2.10	3.64	3.71	<0.530	<0.530	
Cl-	mg/L		23.5	23.4	21.9	48.2	49.0	38.8	
TP	mg/L		0.696	0.630	1.31	3.41	2.22	0.701	
pH	SU		6.74	7.30	6.86	6.66	6.83	9.51	

* Detection limit is 0.002 mg/L, a change from previous dates.
** From three locations in Ocala in the same time period as the hurricane that produced the stormwater average concentration of Nitrate/Nitrite in runoff water was 0.303 mg/L. The total number of samples was Fifteen (15). These are asumed input values to the RIBs

The next two sampling dates were for reclaimed water loading conditions.

	sample date	1/22/2018	1/30/2018						
	sample location	Rib Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6	Well
Conductivity	µmhos/cm		342.2	230.4	278.9	431.1	420	834.2	360.5
Turbidity	NTU		3.64	16.1	2.79	8.11	10.6	3.35	120
Fecal Coliform	CFU/100ml		<1	<1	<1	TNTC	10	41	<1
TKN	mg/L	2.4	0.463	0.463	0.463	2.73	0.463	0.463	<0.463
Nitrate/Nitrite	mg/L	1.55	<0.134	0.134	<0.134	<0.134	<0.134	2.39	<0.134
TN	mg/L	3.95	0.53	0.597	0.53	2.8	0.53	2.85	0.299
Cl-	mg/L	99.8	30.1	24.5	34.8	47.3	50.6	98.8	38.5
TP	mg/L	3.78	0.119	0.119	0.119	3.46	2.07	1.6	1.19
pH	SU	7.35	6.85	7.03	6.84	6.71	6.67	7.62	6.92
TNTC = too numerous to count					no sample				

	sample date	2/19/2018	2/27/2018						
	sample location	Rib Loading	RIB BAM #1	RIB BAM #2	RIB BAM #3	RIB Control #4	RIB Control #5	RIB Control #6	Well
Conductivity	µmhos/cm		547	834	624	481	449	773	360
Turbidity	NTU		4.58	8.29	0.78	11.0	1.61	0.20	57.9
Fecal Coliform	CFU/100ml		6	<1	<1	625	880	<1	<1
TKN	mg/L	0.463	2.70	0.463	0.463	3.20	0.463	0.463	<0.463
Nitrate/Nitrite	mg/L	1.92	<0.134	<0.134	0.537	<0.134	3.33	<0.134	<0.134
TN	mg/L	2.83	2.77	0.530	1.00	3.27	3.79	0.530	0.299
Cl-	mg/L	95.8	60.6	90.7	82.1	52.6	57.2	100	37.3
TP	mg/L	3.92	0.952	0.520	1.10	1.89	3.06	1.06	0.119
pH	SU	6.73	6.89	7.12	7.05	6.70	6.78	8.37	6.83

The following are the calculated average nitrate removal and the removal per event when reclaimed water is used (a) and when stormwater is used (b). The inlet values were averages of three measurements; before and after the loading date including a measurement the day of loading.

(a) using reclaimed water (b) using stormwater

Nitrate Use 1/2 the detection limit for % removal

Sampling Date	Inlet	Nitrate (mg/L) BAM RIB	Nitrate (mg/L) Control RIB	% Removal BAM RIB	% Removal Control
1/30/2017	6.24	0.040	3.630	99	42
		0.079	3.310	99	47
		1.510	3.070	76	51
2/27/2017	7.59	0.457	4.000	94	47
		0.079	5.120	99	33
		3.790	6.020	50	21
3/13/2017	7.59	0.040		99	
		0.040	3.780	99	50
		3.040	5.620	60	26
4/11/2017	3.37	0.079	2.580	98	23
		0.079	2.600	98	23
		0.040	1.100	99	67
5/8/2017	2.41	1.340	5.490	46	-125
		0.079	3.520	97	-45
		0.040	0.040	98	96
6/12/2017	3.69	0.040	11.00	99	
		0.040	1.910	99	48
		8.820	0.079		98
7/10/2017*	0.96	0.040	1.080	96	-13
		0.040	0.040	96	96
		0.882	0.040	8	96
8/7/2017*	0.079	0.040	0.040	49	49
		0.040	0.040	49	49
		0.040	0.040	49	49
1/30/2018#	2.19	0.067	0.067	97	97
		0.134	0.067	94	97
		0.067	2.390	97	-9
2/27/2018#	1.92	0.067	0.067	97	97
		0.067	3.330	97	-73
		0.537	0.067	72	97
average	3.60	0.72	2.42	83	41
median	2.89	0.07	2.39	97	49
N	10	30	29	29	28

* Reclaimed water primarily augmented with river water
# Detection limit changed to 0.134 from 0.079 mg/L

Date	mg/L Inlet	Nitrate (mg/L) BAM RIB	Nitrate (mg/L) Control RIB	% Removal BAM RIB	% Removal Control
12/18/2017	0.303	0.009	0.010	97	97
		0.004	0.007	99	98
		0.007	0.027	98	91
1/10/2018	0.303	0.024	0.042	92	86
		0.014	0.022	95	93
		0.024	0.078	92	74
average	0.303	0.014	0.031	95	90
median	0.303	0.012	0.025	96	92
N	2	6	6	6	6

Inlet value based on sampling from another watershed

The following are the calculated average total phosphorus removal and the removal per event when reclaimed water is used. During the stormwater loading, there was no estimate for inlet total phosphorus:

removal based on 1/2 the detection limit					
	TP mg/L	TP (mg/L)		% Removal	
Date	Inlet	BAM RIB	Control RIB	BAM RIB	Control
1/30/2017	3.86	0.700	1.280	82	67
		0.864	0.638	78	83
		1.860	2.660	52	31
2/27/2017	4.00	1.830	0.792	54	80
		1.370	1.590	66	60
		0.920	3.640	77	9
3/13/2017	5.19	1.530		71	
		0.442	1.250	91	76
		2.780	3.500	46	33
4/11/2017	4.12	2.000	1.010	51	75
		1.250	2.640	70	36
		2.940	2.850	29	31
5/8/2017	4.62	0.954	0.611	79	87
		1.370	2.400	70	48
		1.450	2.630	69	43
6/12/2017	2.74	3.210	1.180	-17	57
		2.000	2.610	27	5
		0.889	2.170	68	21
7/10/2017*	3.23	2.010	1.920	38	41
		1.380	2.020	57	37
		0.459	2.160	86	33
8/7/2017*	2.26	1.080	5.140	52	-127
		1.360	2.330	40	-3
		0.927	1.930	59	15
1/30/2018#	3.78	0.060	3.460	98	8
		0.060	2.070	98	45
		0.060	1.600	98	58
2/27/2018#	3.78	0.060	3.460	98	8
		0.060	2.070	98	45
		0.060	1.600	98	58
average	3.76	1.198	2.180	66	37
median	3.82	1.165	2.070		
N	10	30	29	30	29

Appendix C

NOB qPCR DATA and Denitrifier qPCR Data

NOB qPCR DATA (following two tables)

2/2/2017		Gene Copies	Gene Copies	Gene Copies	Gene Copies	Mean (copy/g)	SD
	Location 1	TOP	390.9833	413.8616		402.4224401	16.177417
		MID					
		BOT	22.18098			22.18097561	
	Location 2	TOP	179.9147	186.978		183.446365	4.994471
		MID	181.4039	176.8545		179.1291762	3.2169417
		BOT	1.379861	49.59302		25.48644154	34.091853
	Location 3	TOP	4631.359	3278.333	5232.379	4380.690081	1000.8495
		MID	0.726449			0.726448868	
		BOT	201.1101	188.6442	606.1635	331.9726038	237.53804
	Location 4	TOP	18758.69	18145.84	15216.53	17373.68499	1893.1175
		MID	253.2176	202.9868		228.1022252	35.518524
		BOT	1146.176	556.3693	578.1665	760.2373395	334.41054
	Location 5	TOP	490666.3	501173.4	491340.7	494393.4577	5881.3013
		MID	11098.97	10868.7	8727.104	10231.59069	1308.0003
		BOT	385.3879	353.1759	421.919	386.8275991	34.394175
	Location 6	TOP	491927	419531.5	504194.7	471884.3937	45751.962
		MID	237628.7	196462.1	155756.4	196615.7387	40936.361
		BOT	940.1953	243.826	280.3204	488.1139164	391.93891

2/16/2018			Gene Copies	Gene Copies	Gene Copies	Mean (copy/g)	SD
	Location 1	TOP	2300.4483	321.84989	808.60906	1143.635756	1030.968
		MID	6.5884029	19.964426		13.27641454	9.458277
		BOT		76.883674	17.758805	47.32123962	41.8076
	Location 2	TOP	98196.981	78037.698	72430.837	82888.50546	13550.69
		MID	1584.0701	2005.8569	1200.0565	1596.661155	403.0477
		BOT	679.49349	669.06695	329.97098	559.5104734	198.8554
	Location 3	TOP	18746.364	14872.75	16560.862	16726.65884	1942.122
		MID	118.92067	98.075381		108.4980255	14.73985
		BOT					

	Location 4	TOP	3936.4265	4602.999	4975.9907	4505.138733	526.6459
		MID	7709.2487	7829.3175	7110.7272	7549.764462	384.9279
		BOT	62.501015	0	0	20.83367157	36.08498
	Location 5	TOP	54820.693	53531.469	51969.617	53440.59288	1427.709
		MID	1398.2567	856.13195	2077.7679	1444.052196	612.1042
		BOT	1218.8598	2216.1542	1969.9982	1801.670715	519.5186
	Location 6	TOP	202520.57	209608.7	190929.32	201019.5288	9429.721
		MID	5622.9111	2025.8186	3584.2425	3744.324072	1803.881
		BOT	362.31648	535.75429	976.10885	624.7265401	316.4211

Denitrifier qPCR DATA (following two tables)

2/2/2017			Gene Copies	Gene Copies	Gene Copies	Mean (copy/g)	SD
	Location 1	TOP	170877.5	133766.6	137242.8	147295.6578	20496.32
		MID	173003.8	171786.2	142934.1	162574.7002	17020.16
		BOT	90976.05	94566.26	87613.78	91052.03206	3476.866
	Location 2	TOP	103818.4	121850.4	117625.8	114431.5138	9430.853
		MID	233032.4	198048.5	214452.8	215177.9183	17503.22
		BOT	139135.9	148264.9	142732.6	143377.7667	4598.569
	Location 3	TOP	363205.3	336959.3	337591.1	345918.5631	14974.09
		MID	73358.85	72926.27	87005.19	77763.44081	8006.515
		BOT	16609.91	12763.69	11185.7	13519.76773	2790.024
	Location 4	TOP	44475.52	43424.58	36804.51	41568.20391	4158.814
		MID	3492.451	7838.142	6214.062	5848.21853	2195.823
		BOT	15741.33	13805.11	14781.92	14776.12055	968.1279
	Location 5	TOP	228112.3	239791.5	219910.9	229271.5563	9990.835
		MID	25028.35	28750.89	29653.01	27810.74961	2451.479
		BOT	7478.793	6928.182	6746.167	7051.047626	381.4541
	Location 6	TOP	376362.6	326057.5	302405.7	334941.9461	37770.39
		MID	198484.9	160286.6	183833.5	180868.3022	19271
		BOT	27582.73	30441.64	33853.98	30626.11814	3139.691

2/16/2018			Gene Copies	Gene Copies	Gene Copies	Mean (copy/g)	SD
	Location 1	TOP	167627.2	154797.3	127896.1	150106.871	20276.56
		MID	8321.409	6104.347	10961.18	8462.312634	2431.481
		BOT	2302.44	1266.923	1117.894	1562.419122	645.1946
	Location 2	TOP	445828.2	413490.4	368672.7	409330.4246	38745.57
		MID	217019.6	210578.9	205715.3	211104.6074	5670.463
		BOT	132260.8	131389.9	139006.1	134218.9169	4168.619
	Location 3	TOP	222065.6	221354.8	211903.4	218441.2552	5673.136
		MID	59178.77	46244.58	36436.33	47286.56211	11406.97
		BOT	93173.56	81349.84	89930.8	88151.39811	6109.403
	Location 4	TOP	142098.2	122280.1	111740.2	125372.8377	15413.51
		MID	83491.01	61633.39	54560.08	66561.49318	15081.92
		BOT	496.226	698.8301	267.9143	487.6568245	215.5857
	Location 5	TOP	174445.6	176551.2	148490.2	166495.6649	15628.66
		MID	63.70664			63.70663723	
		BOT	1200.44	1569.22	1380.6	1383.419933	184.4062
	Location 6	TOP	411718.3	389763.5	329958	377146.5819	42315.21
		MID	97418.36	94345.94	98602.3	96788.86712	2196.894
		BOT	44227.67	48410.47	45019.28	45885.80676	2221.962

Appendix D

Additional Field Collected Water Quality Data at the RIBs

The following tables display water quality from field sampling for pH, temperature, and laboratory analysis for dissolved organic carbon, alkalinity, boron and its isotope. These data help support the removal of nitrate nitrogen. The data may be used for future correlative and investigative studies. Lysimeter 4 did not have a water sample for 3/13/17 or 411/17 due to insufficient volume. Note the different source water types: Reclaimed water from the city of DeLand WRF and excess stormwater due to hurricane Irma loading of the RIBs with water in the RIBs lasting from September 2017 – January 2018.

pH (Standard pH units).
Collected from the RIB Lysimeters and Input water January 2017 to February 2018 loading events. Samples taken for each reclaimed water loading event except where noted in January 2018 from stormwater loading. Analysis in field with Hach meter/pH probe.

Loading Event	pH (pH units) in-field							
	Input	**BAM**			**Control**			**Monitoring Well**
		L1	**L2**	**L3**	**L4**	**L5**	**L6**	
January'17	7.28	7.27	7.82	7.51	6.84	7.21	7.6	
February'17	7.38	7.47	8.56	7.17	6.78	7.07	7.48	6.12
March'17	7.51	8.2	9.68	7.28		7.23	7.52	6.10
April'17	7.4	6.83	7.38	7.27		7.18	7.32	
May'17	7.31	6.84	6.99	7.13	6.98	7.29	7.41	6.15
June'17	7.47	6.94	7.4	6.71	7.01	6.95	7.3	6.10
July'17	7.58	6.92	7.1	6.81	6.7	7.13	8.25	
August'17	7.72	6.72	7.28	6.68	6.72	7.22	7.72	6.54
Hurricane Impact: Stormwater Loading with sampling in January, 2018								
January'18 - Stormwater	-	6.50[i]	7.18[i]	6.67[i]	6.53[i]	6.67[i]	9.43[i]	
January'18	7.48	6.78	7.00	6.78	6.73	6.53	7.52	6.67
February'18	7.59	6.65	7.07	6.97	6.71	6.64	8.24	6.60

Temperature (°C). For the RIB Lysimeters and Input water January 2017 to February 2018 loading events. Sample analysis in field with Hach meter/pH probe.

Loading Event	Temperature (°C) in-field							
	Input	**BAM**			**Control**			**Monitoring Well**
		L1	**L2**	**L3**	**L4**	**L5**	**L6**	
January'17	20.9	16.3	18.1	17.8	10.8	17.6	16.9	
February'17	10.3	21.5	22.0	22.4	20.8	22.6	21.5	21.4
March'17	11.9	21.4	20.7	22.5		21.5	21.7	21.0
April'17	13.2	23.5	24.1	23.2		25.6	24.6	
May'17	22.8	24.9	26.6	26.2	25.4	26.5	27.1	25.7
June'17	16.8	28.1	28.4	28.7	27.5	27.7	27.9	25.0
July'17	16.3	29.6	31.8	30.4	30.4	30.0	30.9	
August'17	14.8	29.4	30.9	30.0	29.8	28.4	29.0	26.6
Hurricane Impact: Stormwater Loading with sampling in January, 2018								
January'18 - Stormwater	-	17.6[i]	17.8[i]	19.9[i]	17.5[i]	17.4[i]	12.7[i]	
January'18	15.5	18.1	18.8	24.8	17.7	18.6	17.7	20.5
February'18	15.5		25.2	23.8	23.2	22.7	24.2	22.1

Non-Purgable Dissolved Organic Carbon (mg/L).
For RIB Lysimeters and Input water January 2017 to February 2018 loading events. Samples taken for each loading event and analysis at UCF except those noted with an (*) were analyzed by ERD. Input samples represent Thursday composite after the Monday loading.

Loading Event	NPDOC (mg-C/L)							
	Input	**BAM**			**Control**			**Monitoring Well**
		L1	**L2**	**L3**	**L4**	**L5**	**L6**	
January'17	7.30	11.50	7.84	8.06	9.96	8.49	7.36	3.99
February'17	7.19	10.83	6.60	9.45	9.08	11.53	7.32	3.50
March'17	7.46	12.16	7.09	6.42	-	8.55	6.23	
April'17	6.77	10.40	7.81	15.79	-	9.62	6.92	
May;17	7.76	-	7.33	14.61	-	10.72	6.65	3.09
June'17	6.39	17.34	6.01	21.65	13.46	10.08	5.87	3.07
July'17	2.64	25.75	7.04	12.52	6.27	7.01	10.76	
August'17	6.16	12.52	7.93	4.12	8.71	6.06	8.76	3.82
December'17 - Stormwater	6.10[i]*	5.2[i]*			10.6[i]*			
January'18 - Stormwater	-	5.34[i]	6.99[i]	5.72[i]	6.85[i]	5.86[i]	5.13[i]	
January'18	6.87	7.53	6.80	6.71	6.57	5.51	7.74	1.96
February'18	6.61	11.36	9.04	7.31	5.06	5.39	6.73	1.89

Alkalinity (mg/L as CaCO3).
For the RIB Lysimeters and Input water January 2017 to February 2018 loading events. Samples analysis at UCF, except those noted by (*) were analyzed by ERD. Input samples represent Thursday composite after the Monday loading event.

Loading Event	Alkalinity (mg/L as $CaCO_3$)							
	Input	**BAM**			**Control**			**Monitoring Well**
		L1	**L2**	**L3**	**L4**	**L5**	**L6**	
January’17	150	197	113	145	138	227	148	160
February’17	135	198	99	153	94	86	143	148
March’17	140	212	108	153	-	86	150	212
April’17	158	197	80	162	-	103	122	
May;17	147	-	85	181	-	99	153	132
June’17	136	169	80	148	162	92	99	103
July’17	150	172	96	103	52	153	172	
August’17	135	95	109	71	157	162	133	260
December’17 - Stormwater	100[i]*	82[i]*			128[i]*			
January’18 - Stormwater	-	69[i]	60[i]	83[i]	139[i]	134[i]	95[i]	
January'18	139	102	69	83	120	111	180	93
February'18	162	136	116	153	125	102	162	106

Boron (mg/L).
For the RIB Lysimeters and Input water January 2017 to February 2018 loading events. Samples taken for each reclaimed water loading event (but no stormwater sample). Sent to Advanced Environmental Laboratory for analysis. Input samples represent Thursday composite after the Monday loading.

Loading Event	Boron(mg/L)							
	Input	**BAM**			**Control**			**Monitoring Well**
		L1	**L2**	**L3**	**L4**	**L5**	**L6**	
January’17	0.21	0.16			0.20			
February’17	0.18	0.17			0.19			0.22
March’17	0.19	0.19	0.13	0.18		0.15	0.19	0.16
April’17	0.18	0.19	0.13	0.16		0.18	0.15	
May’17	0.16	0.19	0.12	0.16	0.20	0.19	0.16	0.18
June’17	0.15	0.16	0.10	0.19	0.17	0.14	0.12	0.20
July’17	0.18	0.22	0.13	0.13	0.12	0.16	0.17	
August’17	0.16	0.12	0.12	0.05	0.13	0.13	0.14	0.22
Hurricane Impact: Stormwater Loading (no sample)								
January'18	0.15	0.05	0.04	0.04	0.08	0.09	0.19	0.09
February'18	0.17	0.10	0.15	0.11	0.09	0.09	0.15	0.08

Stormwater Management Academy

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