DOES IT PAY TO BE MATURE? ASSESSING THE PERFORMANCE OF A BIORETENTION CELL SEVEN YEARS POST-CONSTRUCTION

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Thesis submitted to the Faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
In
Biological Systems Engineering

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September 3rd, 2014
Blacksburg, VA

Key Words: bioretention, nitrogen, phosphorus, fecal indicator bacteria, denitrification

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Abstract

Bioretention cells (BRCs) are low-impact development stormwater management structures that integrate water quantity and quality management. Although BRCs have a predicted design life of about 25 years, most current research focuses on performance of cells less than two years old. This project evaluated the effectiveness of a BRC installed in 2007 to treat a 0.16-ha parking lot in Blacksburg, VA. After installation, this BRC was monitored for five months to determine initial flow reduction and total suspended solids, and nutrient removal. By monitoring for the same parameters, changes in cell performance since installation were quantified. ISCO automated stormwater samplers collected inflow and outflow composite samples from the cell, which were then analyzed for fecal indicator bacteria (total coliforms, *E. coli*, and enterococci), total suspended solids (TSS), total nitrogen (TN), and total phosphorus (TP). To determine if denitrification is occurring within the BRC, media samples taken throughout the cell were analyzed using qPCR. The bioretention media was also sampled to quantify changes in media nutrient content and particle size over the past seven years. Results indicate the bioretention media has not accumulated nitrogen and phosphorus since installation, and that the BRC remains effective at reducing flow volume and peak flow rates, as well as TSS, TN, TP, total coliforms, *E. coli*, and enterococci loads. Bacterial analysis of the media show most of the denitrifiers are present in the top layers of the bioretention media, despite an internal water storage layer and the bottom of the cell designed specifically for denitrification

Acknowledgements

I would like to thank my advisors, Drs. Tess Thompson and Leigh-Anne Krometis, for making this research possible with their continued support and encouragement and their guidance for graduate school and the future. I would also like to thank my committee members, Drs. Greg Evanylo and Brian Badgley, for their support and direction throughout this project, especially in subjects that were new to me.

This project was funded by a College of Agriculture and Life Sciences integrated grant, without which this research would not have been possible.

I would also like to thank Laura Lehman for her assistance in equipment setup and maintenance, and her general ability to solve problems. I would like to thank Tim Neher for his great assistance in qPCR, and Hehuan Liao for all of her training in IDEXX and DNA extraction. I would also like to thank everyone in the Krometis and Thompson research groups as well as countless BSE faculty, staff, and graduate students for their help and support throughout the year. Finally, I'd like to thank my friends and family for all of their encouragement throughout the year.

Dedicated to my mom,

Thank you for everything

Table of Contents

Abstract ii
Acknowledgements iii
Table of Contents iv
LIST OF TABLES vii
LIST OF FIGURES ix
1. Introduction 1
2. Review of Literature 3
2.1 Historical changes in urban stormwater control 3
2.2 Bioretention design 5
2.3 Hydrologic controls 6
2.4 Water quality controls 8
2.4.1 Total Suspended Solids 8
2.4.2 Nutrients 9
2.4.3 Metals 15

2.4.4 Polycyclic aromatic hydrocarbons 17
2.4.5 Indicator bacteria 17
2.4.6 Thermal pollutation 19
2.5 Future recommendations 19
3. Goals and Objectives 20
4. Methods 21
4.1 Site description 21
4.2 Monitoring 28
4.2.1 Bioretention media monitoring 28
4.2.2 Stormflow monitoring 29
4.3 Media and Water Quality Analyses 32
4.3.1 Bioretention media analyses 32
4.3.2 Stormwater sample analyses 34
4.3.3 Data analyses 34
4.3.4 Statistical analyses 35
5. Results and Discussion 37
5.1 Bioretention Media 37
5.1.1 Particle size distribution 37
5.1.2 Nutrients 40
5.1.3 Fecal indicator bacteria 44
5.1.4 Denitrifying bacteria 50
5.2 Precipitation 58
5.2.1 Hydrology 63
5.2.2 Peak Flow Rate 63
5.2.3 Flow Volume 66
5.2.4 Hydrology Summary 71
5.3 Impacts on Water Quality 72
5.3.1 Total suspended sediment 72
5.3.2 Nitrogen 75
5.3.3 Phosphorus 78
5.3.4 Total coliforms 81

5.3.5 Escherichia coli (*E. coli*).....83
5.3.6 Enterococci 86
5.3.7 Water Quality Summary 89
6. Conclusions.....91
7. Future Research 95
8. References.....96
Appendix A. Original planting list and layout.....102
Appendix B. Particle size distribution data.....103
Appendix C. Nutrient content of bioretention media.....104
Appendix D. Fecal indicator bacteria in BRC 105
Appendix E. Denitrifying genes in BRC 106
Appendix F. Storm data 107
Appendix G. Hydrologic data 109
Appendix H. Total suspended solids, total nitrogen, and total phosphorus storm data..111
Appendix I. Fecal indicator bacteria storm load and reduction data 113

LIST OF TABLES

Table Page

Table 2.1. Total Suspended Solids (TSS) mass reductions observed in previous studies... 9

Table 2.2. Total phosphorus (TP) and orthophosphate (PO_4) mass reductions(%) observed in previous studies. 10

Table 2.3. Total nitrogen (TN) and nitrogen species mass reduction(%) observed in previous studies. 13

Table 2.4. Copper (Cu), Lead (Pb) and Zinc (Zn) mass reductions (%) observed in previous studies. 17

Table 2.5. Indicator bacteria concentration reduction observed in previous studies. 18

Table 4.1 Weir rating curve regression equations and R^2 values..... 30

Table 4.2 Quantitative PCR reaction mixtures and thermal profiles for study target genes.33

Table 4.3. Standard methods used for stormwater analyses 34

Table 5.1. Summary of storms during 2013-2014 study period. A check mark indicates a sample taken at the inflow or outflow of the BRC. 59

Table 5.2. Storms producing outliers during 2013-2014 sampling period. 62

Table 5.3. Negative correlations associated with hydrologic and pollutant % reductions in the Blacksburg BRC. Bolded items indicate a correlation for all storm events and events generating outflow. “PF” refers to peak flow rate. “V” refers to volume. “In” and “out” refer to inflow and outflow, respectively...90

Table B.1. Particle size distribution based on distance from inlet and depth……..103

Table C.1. Total nitrogen and total phosphorus content of BRC during 2007 to 2008 study….104

Table C.2. Total nitrogen, total phosphorus, and total carbon content of BRC in 2013…….104

Table D.1. Fecal indicator bacteria in BRC in July 2013 and April 2014……105
Table E.1. *NirK*, *nirS*, Eubacteria, and fungi copies per gram of soil……...106
Table F.1. Precipitation data of 2007-2008 storms……....……107
Table F.2. Precipitation data of storms during 2013-2014…….108
Table G.1. Volume and peak flow data observed in 2007-2008 study……..109
Table G.2. Volume and peak flow data observed in 2013-2014 study……..110
Table H.1. Mass loads and reductions of TSS, TN, and TP over 2007-2008……....111
Table H.2. Mass loads and reductions of TSS, TN, and TP in 2013-2014……....112
Table I.1. Total loads and reductions of total coliforms, *E. coli*, and enterococci over 2013-2014 study……...…113

LIST OF FIGURES

Figure Page

Figure 2.1. Typical bioretention cell. 6

Figure 2.2. Nitrogen cycle and associated genes for enzymes catalyzing each step. The target genes in this study are highlighted in red. 15

Figure 4.1. Location of impaired reach of Stroubles Creek within Montgomery County, VA and its relation to the town of Blacksburg (DeBusk, 2008). 22

Figure 4.2. Aerial view of BRC and the connected impervious parking lot. 23

Figure 4.3. Aerial view of BRC, indicated by red dot, in Blacksburg (Google, 2014). 24

Figure 4.4 Cross section of the study bioretention cell (DeBusk, 2008). 25

Figure 4.5 Longitudinal cross section of study bioretention cell. 25

Figure 4.6 The bioretention cell immediately after construction (July 2007) (top) (DeBusk, 2008), seven years post-construction (July 2014) (middle) (Willard, 2014), and seven years post-construction after mowing (November 2013) (bottom) (Willard, 2013). 27

Figure 4.7 Example of bioretention media sampling sites 29

Figure 5.1. Bioretention media content by depth and distance from inlet observed in 2013-2014 study 39

Figure 5.2. Soil layer stratification observed in BRC in 2013. The photo on the left shows a partial soil profile. The photo on the right shows the composites collected at each depth and distance from the inlet: Section A (0-2.53 m), Section B (2.53-5.06 m) and Section C (5.06-7.60), respectively. 40

Figure 5.3. Nitrogen content of BRC based on depth and distance from inlet observed in 2013.....41

Figure 5.4. Carbon content of BRC based on depth and distance from inlet observed in 2013.....42

Figure 5.5. Phosphorus content of the BRC based on depth and distance from inlet observed in 2013. 42

Figure 5.6. C:N ratio of BRC based on depth and distance from inlet observed in 2013. 44

Figure 5.7. Total coliforms (MPN/g dry soil) present in BRC in July, 2013 and April, 2014 based on depth and distance from inlet. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m. A full bar indicates that the sample was above the limit of detection for the IDEXX trays. 46

Figure 5.8. *E. coli* content of the BRC in July, 2013 and April, 2014 based on depth and distance from inlet. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m. 47

Figure 5.9. Enterococci content of the BRC in July, 2013 and April, 2014 based on depth and distance from inlet. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m. 48

Figure 5.10. Gene copies of *nirK* per gram of soil present in the BRC at varying distances from the inlet and target depths. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m. 53

Figure 5.11. Gene copies of *nosZ* per gram of soil present in BRC at varying distances from inlet and target depths. Section A corresponds with 0-2.53 m from inlet,

section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m. 54

Figure 5.12. Gene copies of 16S rDNA per gram of soil present in BRC at varying distances from inlet and target depths. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m. 55

Figure 5.13. *NirK* genes present in BRC as a percentage of total 16s rDNA genes. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m. 56

Figure 5.14. *NosZ* present in BRC as a percentage of 16s rDNA. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m 57

Figure 5.15. Monthly precipitation comparison of 2007-2008 study period, partial 2013-2014 study period, and 30 year precipitation (1981-2010) average of Blacksburg, VA.....60

Figure 5.16. Summary of storm duration and precipitation depth during 2013-2014 BRC study period.....60

Figure 5.17. Summary of storm duration and precipitation during 2007-2008 study period. Storms 1-4, 7, 9, 24, and 26 were not used in data analysis (DeBusk, 2008). 61

Figure 5.18. Water-level data for storm 17 (April 7th, 2014) as recorded by HOBO data loggers and ISCO stormwater samplers. The left axis corresponds with piezometer distance from inlet (2.5 m or 5.0 m) and indicates water height above the piezometers, which were located at the bottom of the BRC (1.8 m deep). The right axis corresponds with the flow observed at the inflow and outflow ISCO and data labeled “inflow” and “outflow”. 63

Figure 5.19. Peak inflow and outflow rates for storms producing outflow in the BRC from 2013-2014. 64

Figure 5.20. Quantile box plot comparing peak flow rate reduction in storms producing outflow in 2007-2008 and 2013-2014.....66

Figure 5.21. Inflow and outflow volumes of 2013-2014 BRC storm events.....67

Figure 5.22. Cumulative stormwater reduction plot for BRC. Point labels indicate storm number. 68

Figure 5.23. Quantile box plot comparing volume reduction from storms generating outflow in 2007-2008 and 2013-2014.....70

Figure 5.24. Inflow and outflow sediment load for each storm event. 74

Figure 5.25. Cumulative sediment plot for BRC. 74

Figure 5.26. Influent and effluent total nitrogen mass loads. 77

Figure 5.27. Nitrogen accumulation within BRC during study period.....77

Figure 5.28. Influent and effluent total phosphorus mass loads. 80

Figure 5.29. Accumulation of total phosphorus in BRC due to stormwater runoff.....80

Figure 5.30. Influent and effluent total coliform MPN.....82

Figure 5.31. Total coliform content during 2013-2014 study period based on mass influent and effluent of viable organisms. 83

Figure 5.32. Influent and effluent *E. coli* MPN. 85

Figure 5.33. *E. coli* content during 2013-2014 study period based on mass influent and effluent of viable bacteria. 85

Figure 5.34. Inflow and outflow *E. coli* concentrations of storm events compared to Virginia Water Quality Standards for geometric mean (GM) standards and single sample (SS) standards. 86

Figure 5.35. Influent and effluent enterococci loads. 88

Figure 5.36. Enterococci content over 2013-2014 BRC study period based on mass influent and effluent of viable organisms. 88

Figure 5.37. Influent and effluent enterococci concentrations from storm events compared to Virginia Water Quality geometric mean (GM) standard and single sample (SS) standard. 89

Figure A.1. Planting list and layout for bioretention cell……..……...…102

1. Introduction

Increasing urbanization, and accompanying impervious landcover, has rendered urban stormwater management a critical issue in the United States and beyond (US EPA, 2002). Greater impervious area increases runoff by decreasing infiltration rates. Increased runoff leads to more frequent, higher flows and greater flow variability (Meyer, 2005). As runoff flows over impervious areas, it collects nutrients, bacteria, heavy metals, sediments, and other pollutants which are subsequently transported to receiving waters (US EPA, 2002). Stormwater management has, therefore, increasingly shifted focus from water quantity to prevent flood damage to an integrated quantity and quality management approach, referred to as low-impact development (LID) (Niemczynowicz, 1999). The LID paradigm includes many localized structures distributed throughout the watershed to reduce surface runoff volume and accompanying peak flows, maintain groundwater recharge, and improve the quality of stormwater discharged to local receiving waters (County, 1993). Bioretention cells are increasingly popular LID structures used to treat runoff from small parking lots. Nutrients, suspended solids, bacteria, and hydrocarbons are removed by routing flow through a vegetated cell filled with specialized media. Total suspended solids (TSS) removal is generally adequate, with concentration reduction ranging from 40 – 90% (Davis, 2007; Hatt et al., 2009) and mass removal as high as 90% (DeBusk and Wynn, 2011). Short term phosphorus removal is variable, although a mesocosm study indicated phosphorus removal is largely dependent on media type (Lucas and Greenway, 2008). Nitrogen removal is even more variable than phosphorus removal, depending on both chemical and biological processes within the bioretention cell. Specifically, nitrogen removal depends upon denitrification, which is promoted via inclusion of an anoxic layer within the cell to support denitrifying bacteria (Hunt et al., 2012). Previous research has established that heavy metals are readily retained within the mulch layer of bioretention cells (Davis et al., 2001; UNHSC, 2006; Davis, 2007), and hydraulic conductivity does not significantly diminish over a four year time period (Emerson and Traver, 2008; Le Coustumer et al., 2009).

The US EPA (2000) states that bioretention media should be replaced every 5-10 years, although there are no studies that document cell performance over this time range.

As available data overwhelmingly describe bioretention cells that are less than two years old, current information is not adequate to predict the performance of a mature bioretention cell or overall design life (Davis, 2007; Hatt et al., 2009; Hunt et al., 2006; Passeport et al., 2009). Understanding how bioretention cells function as they mature is necessary to design effective management strategies and evaluate LID installation in terms of cost efficiency.

2. Review of Literature

Given increasing reliance on LID in urban stormwater planning, a number of short-term studies are available documenting the installation of LID structures including bioretention cells and their initial post-construction performance. There are no published data on pollutant removal of bioretention cells older than four years, however, and no studies that compare initial removal rates to removal rates years later.

2.1 Historical changes in urban stormwater control

As urban development progresses, stormwater management infrastructure is critical to route high flows away from structures to prevent damage; traditional stormwater management infrastructure focused nearly entirely on efficiently moving large water quantities away from city centers quickly. This infrastructure includes sewers, channels, pipes, conduits, and street design, all of which are primarily designed to move water away from cities and out of transportation pathways. Under this paradigm, stormwater runoff enters surface waters directly without prior treatment (Burian et al., 1999). In the 1960s, communities recognized that routing urban runoff water quickly downstream resulted in decreased groundwater recharge and increased peak flows, erosion, and pollutant transport, inevitably degrading receiving water quality (Burian et al., 1999). As a consequence of these environmental concerns, stormwater management began to focus on detention, retention, and recharge in the 1970s and 1980s. Water was still routed away from immediate urban area, but was then detained in structures such as detention and retention ponds to provide flood control, channel protection, and pollutant removal (US EPA, 2012b). Throughout the 1980s, stormwater was increasingly recognized as a large contributor to downstream pollution and ecological degradation, and management strategies were developed to treat stormwater as locally as possible (Niemczynowicz, 1999). By the 1990s, development strategies emerged to maintain upland hydrology through small scale, biological treatment of stormwater via a set of best management practices known collectively as low-impact development, or LID (US EPA, 2000).

Under the LID paradigm, stormwater is treated close to the source, instead of routing it to a treatment facility or allowing it to flow directly into surface waters. Stormwater is treated as a resource, not a waste product, and integrated back into the natural landscape. To achieve these functions, LID employs small-scale practices implemented throughout an urban area to capture polluted stormwater for on-site treatment, thus reducing the Effective Impervious Area (EIA) (the impervious area of a watershed directly connected to the storm drain system) of the site (US EPA, 2000). When used as a system, LID practices, which include bioretention cells, permeable pavements, grass swales, vegetated roof covers, rain barrels, and vegetated filter strips, can help maintain the natural hydrology of an area and improve water quality (US EPA, 2000). These techniques are also cost effective, in some cases saving more money over time than traditional stormwater management strategies (US EPA, 2013).

Bioretention cells, also called rain gardens, are LID structures aimed at managing stormwater in urban areas through localized restoration of natural infiltration capacity (US EPA, 2000, 2013b). As they can be installed almost anywhere, including retrofits on parking lots, ultra urban areas, arid climates, cold climates, and in any soil type, bioretention cells are increasingly popular installations in new and renovated urban developments. During development projects, they decrease the need for longer stormwater conveyance pipes while simultaneously removing pollutants from stormwater, decreasing peak flows, and can serve as an aesthetically pleasing landscaped area if managed appropriately. In Maryland, they are estimated to cost about $5,000 to $10,000 per acre drained, but save about half of this amount in stormwater drain pipe cost (US EPA, 2000). The cost-effectiveness, ease of use, and ecological benefits of bioretention cells has contributed to their rising popularity (US EPA, 2013a). In stormwater hotspots, such as gas stations, where runoff is highly polluted, bioretention cells filter out pollutants, minimizing anthropogenic impact on the environment. They can also be installed next to trout streams to reduce nutrient concentrations and thermal pollution before water enters more sensitive ecosystems.

2.2 Bioretention design

Bioretention cells are primarily designed to remove pollutants from urban stormwater runoff, maintain groundwater recharge and base flow, and reduce surface runoff volume and peak flows through localized restoration of natural infiltration capacity (County, 1993). These bioretention cells are generally designed as bowls that allow 15-30 cm of stormwater ponding on the surface with subsequent infiltration to reduce runoff volume and peak flows from urban areas (Davis et al., 2009). As runoff flows into the bioretention cell, it is slowed by vegetation. The runoff infiltrates through a thin mulch layer, which protects the bioretention media from erosion and provides pollutant treatment via infiltration and/or adsorption (Davis et al., 2001). The underlying soil layer, typically 0.7 m to 1 m thick, generally has high sand content with lower levels of silt, clay, and organic matter. The sand allows water to infiltrate rapidly, while the clay and silt promote pollutant sorption within the media (Davis et al., 2009; Davis et al., 2001). In some cases, underdrains are installed at the bottom of the bioretention cell to allow stormwater to drain faster. In areas where native soils have high infiltration rates, their use is controversial with regards to pollutant removal. During large storm events, runoff might percolate quickly through native soils and the bioretention cell with minimal pollutant removal, then flow directly to streams. In these areas, it might be more effective to allow effluent from the bioretention cell to continue infiltrating through surrounding soils (Dietz and Clausen, 2005).

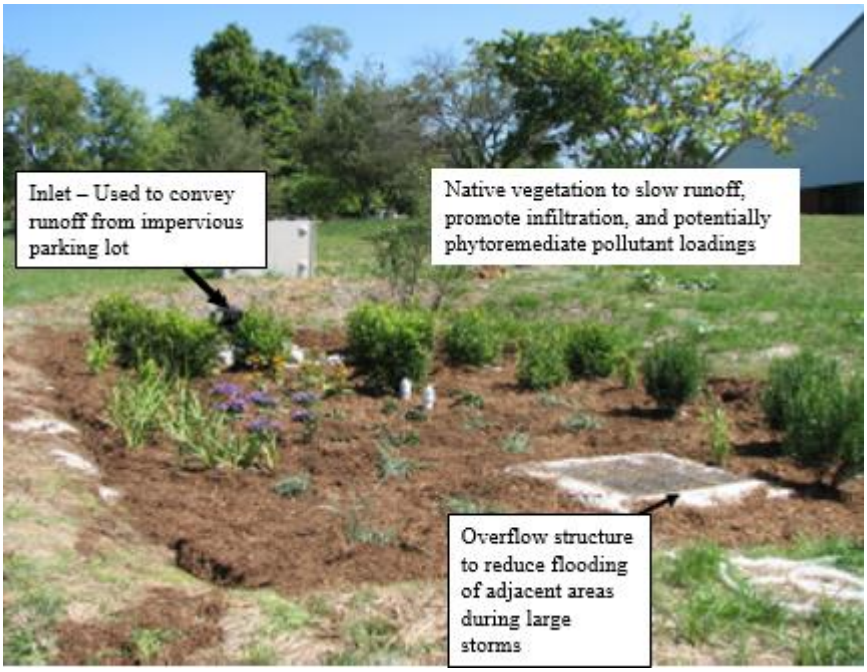

Figure 2.1. Typical bioretention cell.

2.3 Hydrologic controls

While maintenance of groundwater recharge and downstream baseflow is an overall goal of LID, it is not typically a requirement in the design of a specific bioretention cell (Davis et al., 2009). However, to meet this watershed-level goal, municipalities can require that all runoff be converted to either infiltration or evapotransporation (ET) to mimic pre-development hydrologic flow paths as closely as possible. This goal therefore requires the installation of LID structures designed to increase infiltration (NCSU, 2009; PaDEP, 2006). Bioretention structures, when unlined, include several practices to promote recharge: a subsurface base wider than the cell surface (Davis et al., 2009), higher media-to-runoff volume ratios, deeper media depths, internal water storage (IWS) layers for improved prolonged residence times to encourage treatment (particularly denitrification), and the inclusion of vegetation to slow influent and promote ponding (Davis et al., 2012; Li et al., 2009).

The bioretention abstraction volume (BAV), a common design parameter in biorention cell construction, quantifies many variables associated with infiltration and ET. According to Hunt

et al. (2012), BAV is "the available storage volume in the bioretention cell and is calculated as the sum of the storage in the surface bowl and that within available media porosity in the root depth." For example, a cell with an IWS layer and an underdrain would have a BAV as expressed in equation 1:

$$BAV = Bowl\ Vol. + RZMS * (SAT - WP) + LMS * (SAT - FC) \quad (1)$$

Where

BAV = bioretention abstraction volume

RMZS = root-zone media storage volume

SAT = saturation point

WP = wilting point

LMS = lower media-storage volume (deeper than plant roots)

FC = field capacity

The RZMS*(SAT – WP) term refers to increased storage due to porosity of the soil in the root zone, while the LMS*(SAT-FC) term refers to increased storage due to an underdrain system and IWS layer (Hunt et al., 2012).

A greater BAV indicates that there is more storage available as infiltration and evapotransporation. As a result, groundwater recharge should occur, and the hydrology of the immediate area would be more likely to be returned to that of a pre-development state (Davis et al., 2012; Li et al., 2009). Jones and Hunt (2009) observed that between two almost identical bioretention cells (i.e. main difference = 7% vs 11% of the watershed area), the bioretention cell that covered less surface area (7%) produced outflow in 76% of storm events, while the cell that covered 11% of the surface area, and had 2.3 m^3 more overall volume, produced outflow only 27% of the time. Increasing the size of the IWS will also increase BAV by increasing available cell storage volume (Brown and Hunt, 2011). Vegetation with longer roots also promotes infiltration, increasing BAV (Li et al., 2009)

While the primary goal of traditional stormwater management is peak flow reduction, this is not always feasible solely through use of bioretention given the often limited media depths and volumes involved. Shallow cell depths are necessary to promote vegetation growth and decrease

sediment compaction, and are easier to construct. A focus on peak flow reduction is generally discouraged in bioretention cell design as this may inhibit the achievement of other goals, such as pollutant removal (Davis et al., 2009). Consequently, it is often most efficient to pair bioretention cells with other BMPs, e.g. a bioretention cell with an outlet structure opening to a detention basin, to achieve both peak flow reduction and effluent water quality goals (Hunt et al., 2012).

2.4 Water quality controls

Both laboratory and field-scale studies have assessed bioretention cell removal capabilities for those pollutants most commonly associated with stream impairments, i.e. pathogens, metals, nutrients, and sediment. Removal of these pollutants is necessary for many municipalities to achieve goals as set forth in mandated Total Maximum Daily Load plans (TMDLs) (Hunt et al., 2012). While observations of bioretention cell performance are promising, especially regarding the removal of TSS and heavy metals, it is also important to note that percent concentration removals and/or mass load removals reported within studies and among different studies are not always readily comparable, and can therefore be misleading. For example, if inflow water quality already has a low pollutant concentration, the bioretention cell may have a low percent concentration removal despite the fact that the outflow water quality is within acceptable regulatory levels. For reductions in mass loadings, calculated as the product of concentration and flow, there may be considerable variability between flow rates and associated with different storm events; mass loads from hydrologically different events should not be directly compared. Therefore, it is important to note how the research was conducted (i.e. lab or field study) and how the authors present data (Davis et al., 2009).

2.4.1 Total Suspended Solids

Previous research suggests that bioreteion cells are somewhat effective at removing TSS from stormwater runoff (Table 2.1). DeBusk and Wynn (2011) observed over 99% mass removal rates while Davis (2007) and Hatt et al. (2009) observed 76% to 93% mean mass reductions. Bioretention cells remove particulate matter through sedimentation and filtration as

runoff is slowed by vegetation. However, this process can be limited if flows are very turbulent or there is excess sediment in runoff due to nearby construction or erodible ground. Long term field research on general infiltration BMPs indicates that cells with at least minimum vegetation cover, such as grass, experience no overall reduction in infiltration rate due to media clogging (Emerson and Traver, 2008) and that the use of media with an initially high hydraulic conductivity value prevents future decreases in hydraulic conductivity significant enough to affect infiltration and subsequent TSS removal (Le Coustumer et al., 2009).

Table 2.1. Total Suspended Solids (TSS) mass reductions observed in previous studies.

Study	Study Type	Cell Age (yr)	TSS % removal	Notes
Davis, 2007	Field	<1	22	no anaerobic layer
Davis, 2007	Field	<1	41	anaerobic layer with newspaper
DeBusk and Wynn, 2011	Field	<1	>99	
Hatt et al, 2009	Field	2	93	Sand, loam, gravel
Hatt et al, 2009	Field	2	76	sandy loam, vermiculite, perlite, organic matter

2.4.2 Nutrients

Although nitrogen and phosphorus are often targeted in bioretention cell field and laboratory studies, the removal rates for these nutrients are generally lower and more variable as compared to observations of TSS, heavy metals, and hydrocarbons (Hsieh and Davis 2005). Since nitrogen and phosphorus are nutrients, their retention and transportation depend on biological factors as well as chemical reactions. Phosphorus removal can be highly variable over short term studies, possibly due to increased microbial and plant uptake in bioretention cells with vegetation (Henderson, 2009). Long term retention, however, mainly depends on media sorption properties (Lucas and Greenway, 2011).

Bioretention is effective at removing particle-bound phosphorus through filtration; removal of dissolved phosphorus is more complicated (Hunt et al., 2012) (Table 2.2). To effectively remove dissolved phosphorus, the bioretention media must have low initial

phosphorus levels and low organic matter (OM), which can release phosphorus during decomposition (Clark and Pitt, 2009; Hatt et al., 2009; Hunt et al., 2006). Field studies conducted in North Carolina indicate that bioretention media initially high in phosphorus can contribute up to 240% mass addition to outflow waters (i.e. net export). In media with low phosphorus levels, a 65% mass removal was observed (Hunt et al., 2006). As a higher residence time within the media also contributes to phosphorus removal through the provision of additional reaction time, a media depth of ≥0.9 m is generally recommended (Hsieh et al., 2007). Field studies also report that vegetation improves phosphorus removal (Lucas and Greenway, 2008), likely due to increased sorption to ferric iron (present due to ferrous iron oxidized by plant roots) (Mendelssohn et al., 1995), uptake by mycorrhizal fungi (Van Tichelen and Colpaert, 2000), and immobilization by bacteria (Henderson, 2009).

It is important to note that some laboratory studies have suggested that sandy bioretention media may only be able to remove phosphorus from stormwater for five years before saturation of media sorption sites (Hsieh et al., 2007). However, in a recent study, Lucas and Greenway (2011) examined mesocosms seeded with a sandy bioretention media mixed with clays rich in iron and aluminum hydroxides. These media mixes, along with vegetative cover, demonstrated the equivalent of up to 50 years of phosphorus retention (Lucas and Greenway, 2011).

Table 2.2. Total phosphorus (TP) and orthophosphate (PO_4) mass reductions(%) observed in previous studies.

Study	Study Type	Age of cell (yr)	TP % removal	PO_4 % removal	Notes
Davis, 2007	Field	<1	74	-	no anaerobic layer
Davis, 2007	Field	<1	68	-	anaerobic layer with newspaper
DeBusk and Wynn, 2011	Field	<1	>99	-	
Passeport et al., 2009	Field	1-2	63	-	shallow, P-index = 5
Passeport et al., 2009	Field	1-2	58	-	Deeper, P index = 8
Hunt et al., 2006	Field	1-3	-240	-	high P index
Hunt et al., 2006	Field	1-3	65	-	low P index
Lucas and Greenway, 2008	Column	-	68	-	Vegetated (average reduction

Study	Study Type	Age of cell (yr)	TP % removal	PO_4 % removal	Notes
Lucas and Greenway, 2008	Column	-	36	-	nonvegetated
Hatt et al., 2009	Field	2	86	-	Sand, loam, gravel
Hatt et al., 2009	Field	2	-398	-	sandy loam, vermiculite, perlite, organic matter
Lucas and Greenway, 2011	Column	-	-	52	red mud added
Lucas and Greenway, 2011	Column	-	-	87	water treatment residuals added
Lucas and Greenway, 2011	Column	-	-3	1	high clay soil added
Dietz and Clausen, 2006	Field	0-1	-108	-	
Bratieres et al., 2008	Column	-	81	-	
Davis et al., 2001	Column	-	81	-	

Biological nitrification-denitrification (conversion of ammonia to nitrogen gas) reactions are the primary nitrogen removal mechanism in bioretention cells. Nitrification occurs under aerobic conditions when nitrifying bacteria convert ammonia to nitrate. Denitrification, in which nitrate is converted to nitrogen gas and is released to the atmosphere, occurs under anoxic conditions. Therefore, bioretention cells generally include an IWS layer for efficient nitrogen removal. This layer is considered most effective at 0.9 m deep, where the nitrification-denitrification process is buffered against colder atmospheric conditions (Hunt et al., 2012), and the IWS is deep enough that phosphorus sorbed to the bioretention media remains above the anoxic layer (Hatt et al., 2009). The bioretention media within the IWS layer should have a low infiltration rate to give the nitrification-denitrification processes enough time to occur (Dietz and Clausen, 2006; Hunt et al., 2012; Kim et al., 2003). Although a permanently saturated layer may be most effective for denitrification, the process can occur anywhere within the cell where saturation occurs. Inundation within the top layers of soil, although only saturated briefly, may

cause more denitrification because dissolved organic carbon values remain high from leaf litter (Parkin, 1987).

Organic matter (OM) content (i.e. microbial carbon source) also affects observations of nitrogen removal. Clark and Pitt (2009) demonstrated that bioretention media composed purely of organic matter, such as peat and compost, release nitrogen species to the effluent under anaerobic conditions. Hunt et al. (2006) noted similar relative increases in nitrogen species concentrations in the outflow of two similar bioretention cells. The only physical difference between the two was that one contained an IWS with an added carbon source, indicating that the IWS did not contribute to denitrification. Current recommendations suggest an OM content of 5-10% of total media weight to optimize carbon content in the media and the IWS so that denitrification occurs while preventing nitrogen release during anaerobic conditions (Hunt et al., 2012).

Dense vegetation cover fosters nitrogen removal (Lucas and Greenway, 2008), and specific vegetation types can be selected to maximize removal efficiency. In a laboratory scale column study, Bratieres et al. (2008) observed that plants with higher surface area-to-volume ratios in the root system removed more nitrogen because the roots had more area for uptake. In this column study, *Carex*, with a dense, fine root system with high surface area, was effective at removing nitrogen. Similarly, *Melaleuca* showed improved nitrogen uptake with time as roots developed and mycorrhizal fungi systems appeared in the roots. The other three plants in the study, *Microleana stipoides, Dianella revoluta,* and *Leucophyta brownie,* had low nitrogen removal rates similar to the unvegetated columns, likely because their root systems were less complex with lower surface area (Bratieres et al., 2008). Results from previous studies on nitrogen species removal are summarized in Table 2.3.

Table 2.3. Total nitrogen (TN) and nitrogen species mass reduction(%) observed in previous studies.

Study	Study Type	Cell Age (yr)	TN % removal	NOx –N % removal	NHx % removal	Notes
Davis, 2007	Field	<1		79	-	no anaerobic layer
Davis, 2007	Field	<1		86	-	anaerobic layer with newspaper
DeBusk and Wynn, 2011	Field	<1	>99	-	-	
Passeport et al., 2009	Field	1-2	54	33	70	shallow, P-index = 5
Passeport et al., 2009	Field	1-2	54	8	84	Deeper, P index = 8
Hunt et al., 2006	Field	1-3	40	-	-	No IWS layer
Hunt et al., 2006	Field	1-3	40	-	-	IWS layer
Lucas and Greenway, 2008	Column	-	56	-	-	Vegetated (average reduction
Lucas and Greenway, 2008	Column	-	12	-	-	nonvegetated
Hatt et al., 2009	Field	2	37	-	-	Sand, loam, gravel
Hatt et al., 2009	Field	2	-7	-	-	sandy loam, vermiculite, perlite, organic matter
Hsieh et al., 2007	Column	-	-	-16	13	low permeability layer above high permeability layer
Hsieh et al., 2007	Column	-	-	-56	59	high permeability layer over low permeability layer
Dietz and Clausen, 2006	Field	0-1	51	81	82	
Kim et al., 2003	Column	-	75	-	-	
Bratieres et al., 2008	Column	-	70	-	-	
Davis et al., 2001	Column	-	-	24	79	

It is important to recognize that the majority of examinations of nitrogen processing by bioretention cells simply assume denitrification is primarily responsible for the removals observed when comparing influent and effluent loadings or concentrations. Recent advances in molecular biology, particularly the widespread use of quantitative polymerase chain reaction (qPCR), permit the targeting of particular gene sequences associated with specific steps in the de/nitrification cycle (Henry et al. 2004; Henry et al. 2006). The presence and/or relative quantity of specific genes recovered is assumed to correlate with the denitrification potential of the microbial community. Chen et al. (2013) studied nitrogen removal in a bioretention cell by sampling influent and effluent stormwater while also quantifying nitrification and denitrification genes in bioretention media. Since denitrification takes place over four reaction steps, quantitative PCR was used to target functional genes for nitrate reductase (*nirS* and *nirK*), nitric oxide reductase (*norB*), and nitrous oxide reductase (*nosZ*). For nitrification, the ammonia monooxygenase (*amoA*) gene was targeted. The study observed correlations between denitrifiers, nitrifiers, media inundation time, soil pH, nitrate content, and soil organic matter content, suggesting that an IWS layer may only be effective if both pH and organic carbon content are at favorable levels (Chen et al., 2013).

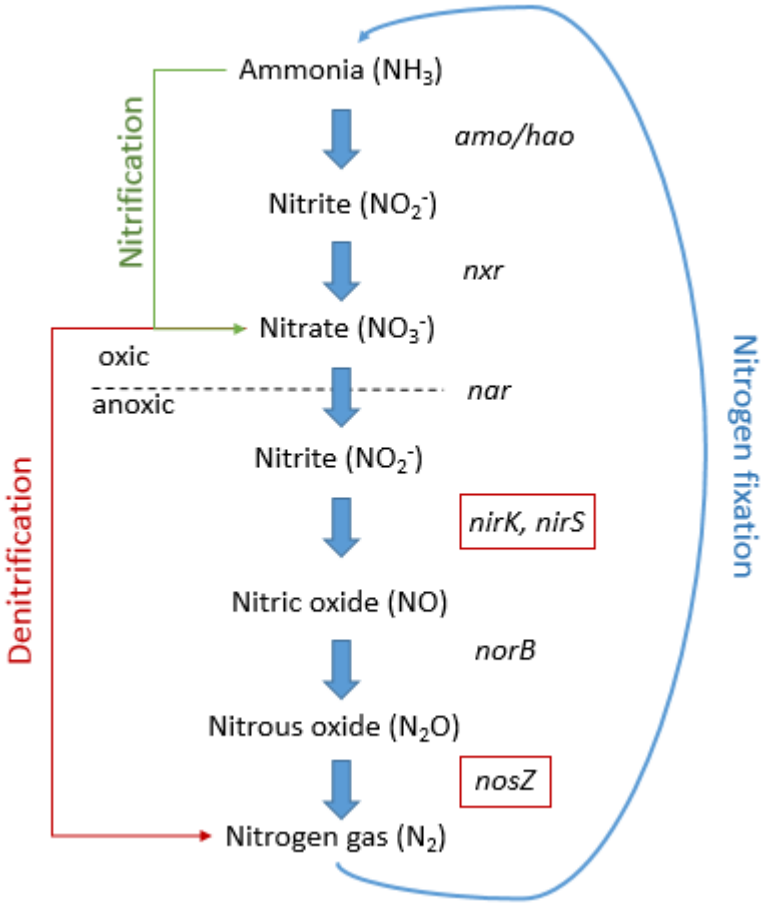

Figure 2.2. Nitrogen cycle and associated genes for enzymes catalyzing each step. The target genes in this study are highlighted in red.

2.4.3 Metals

Heavy metal data collected in both laboratory and field-scale studies demonstrate that bioretention strategies can remove over 50% of copper, lead, zinc, and cadmium (Davis, 2007; Davis et al., 2003; UNHSC, 2006) (Table 2.4). Heavy metal removal appears to occur primarily within the top few centimeters of the cell, and is generally attributed to the mulch layer (Davis et al., 2001) and the use of finer bioretention media which is able to sequester more metals via adsorption (Davis et al., 2003; Li and Davis, 2008a). While high removal rates (50-100%) are promising for downstream water quality, they simultaneously raise concerns regarding

accumulation over time; saturation of sorption sites might result in future release of heavy metals (Davis et al., 2001).

Jones and Davis (2013) monitored a bioretention cell over four years to determine how metals accumulate within the cell. Over the study duration, they mapped the flow paths of water entering the cell and divided it into zones longitudinally. After four years, they determined that considerable metals concentrations (Pb = 64 mg/kg; Cu = 49 mg/kg; Zn = 257 mg/kg) accumulated within the top 5 cm near the cell inlet, with lower accumulations as the water infiltrated and dispersed laterally. Despite evidence of accumulation, the observed media concentrations were still well below regulatory cleanup levels, and at least 87% of each metal examined was in an immobile form. Risks to human health were assessed in this study via the toxicity hazard leaching procedure (TCLP) for toxicity hazard of solid wastes. Waste is considered toxic via direct ingestion at lead concentrations of 5 mg/L; the highest concentration of lead in this study was 76 mg/kg, which corresponds to a maximum TCLP extract concentration of 3.8 mg/L. Sequential extraction revealed that the majority of this lead is not bioavailable, so the actual concentration available to humans would probably be far lower, and unlikely toxic to humans, even under the extreme and somewhat unlikely exposure of direct ingestion of media. Therefore, with proper maintenance of the cell, including a focus on cleaning accumulation hotspots (i.e. the top 5 cm of media that experiences the most exposure to ponding near the inlet), full media replacement may never be necessary due to metal toxicity, decreasing the overall cost of the system (Jones and Davis, 2013).

Table 2.4. Copper (Cu), Lead (Pb) and Zinc (Zn) mass reductions (%) observed in previous studies.

Study	Study Type	Cell Age (yr)	Cu % removal	Pb % removal	Zn % removal	Notes
Davis, 2007	Field	<1	51	79	28	no anaerobic layer
Davis, 2007	Field	<1	57	86	63	anaerobic layer with newspaper
Hunt et al., 2006	Field	1-3	99	81	98	
Hatt et al., 2009	field	2	98	98	99	Sand, loam, gravel
Hatt et al., 2009	field	2	67	80	84	sandy loam, vermiculite, perlite, organic matter
Bratieres et al., 2008	column	-	98	80	98	
Davis et al., 2003	field	1 & 5	43	70	64	
Davis et al., 2001	lab	-	92	>98	>98	

2.4.4 Polycyclic aromatic hydrocarbons

Similar to heavy metal removal, bioretention cells typically removes polycyclic aromatic hydrocarbons (PAHs) and other petroleum-based pollutants via sorption to mulch and media. These contaminants are formed when coal, oil, gas, and garbage are burned, and are common on the surfaces of parking lots. Most fuel-based hydrocarbons adsorb to organic matter at the surface of the cell and eventually undergo natural decomposition given a robust soil microbiome (Hong et al., 2006; Hunt et al., 2012). Therefore, a thin mulch layer, which studies indicate can still remove at least 80% of the hydrocarbon loads, is considered sufficient to meet effluent water quality goals (Hong et al., 2006).

2.4.5 Indicator bacteria

No studies have directly monitored human pathogen removal by bioretention cells, though several have documented the removal of fecal indicator bacteria (FIB, e.g. *E. coli*, coliforms) (Table 2.5). As direct pathogen monitoring is very costly, most water quality monitoring programs target FIB, which are indicative of fecal contamination and generally used

as pathogen surrogates in fate and transport studies (Savichtcheva and Okabe, 2006). Water quality standards to protect public health rely on this indicator paradigm; therefore, TMDL plans focus on reducing FIB loadings. Field and laboratory studies have reported the removal of *E. coli* and fecal coliforms by bioretention cells and, in some cases, suggest *E. coli* removal efficiency may even increase over time (Passeport et al., 2009; Zhang et al., 2011). However, cell design specifications appear to have a significant effect on effluent bacteria concentrations, and can result in poor performance and/or bacteria export from the cell. For example, if the ponding time in the cell is too long, the cell may promote bacterial growth due to increased nutrient availability and decreased desiccation. Conversely, if the infiltration rate is too high, bacterial concentrations may increase in the outflow as bacteria are stripped from the cell media (Bright et al., 2010; Hathaway et al., 2011). Another factor that may influence bacterial loadings is vegetation, which attracts animals that defecate within the cell and may also provide a suitable environment for long-term survival by shading bacteria from UV light exposure (Hunt et al., 2012). Current research also indicates that heavy metal concentrations may increase bacterial attachment to soil particles (Zhang and Olson, 2012).

Table 2.5. Indicator bacteria concentration reduction observed in previous studies.

Study	**Study Type**	**Cell Age (yr)**	**Fecal Coliforms**	***E. coli***	**Enterococci**	**Notes**
Bright et al., 2010	column	-	-	14 - 100	-	
Passeport et al., 2009	Field	1-2	95	-	-	shallow, P-index = 5
Passeport et al., 2009	Field	1-2	85	-	-	Deeper, P index = 8
Bratieres et al., 2008	column	-	-	83	-	
Hathaway et al., 2009	field	unknown	89	92	-	
Hathaway et al., 2009	field	unknown	-	-611	-132	shallow cell
Hathaway et al., 2009	field	unknown	-	60	86	deep cell

2.4.6 Thermal pollutation

Implementation of bioretention cells can also reduce the thermal pollution resulting from runoff warming on impervious areas, which is detrimental to organisms living in receiving waters. The ground acts as a thermal buffer for the cell, with only the upper layers warmed by the sun and exposed to air temperatures. Vegetation also acts as a physical barrier from the sun for the media as well, allowing more time for the runoff to cool (Jones and Hunt, 2009). By increasing the residence time and infiltration of runoff in the cell, the underlying media cools the water and decreases the temperature of effluent leaving the cell. Therefore, bioretention cells that are deeper, with a comparatively larger storage volume per watershed area than recommended in current design specifications, are best for controlling thermal pollution (Jones and Hunt, 2009; Li et al., 2009). Layers for IWS also increase residence time and cool water deeper within the cell (Brown and Hunt, 2010). Jones and Hunt (2009) found that bioretention cells in Asheville, NC could cool influent by as much as 4˚C. Cells that treated a smaller percentage of the contributing watershed were more effective at reducing influent temperatures.

2.5 Future recommendations

While many studies have documented likely pollutant removal mechanisms of bioretention cells and worked to optimize these processes, significant questions related to effective design remain. The majority of past studies only examined bioretention cells for a short time (approximately 6 months to 2 years). The longest studies, conducted by Emerson and Traver (2008) and Jones and Davis (2013) over four years, focused on the infiltration rate of bioretention cells and metal accumulation within the media; these studies provided no data on overall water quality performance. Few studies, if any, have monitored a bioretention cell to quantify pollutant removal capacity over an extended amount of time. Therefore, there is no evidence to indicate that bioretention cells continue to function as designed after four years. If, for example, cell media become saturated with pollutants over time, at a certain point "breakthrough" (i.e. sloughing off of bound pollutants) would be expected, resulting in net exports of nutrients, metals, or bacteria. There is also little information available from field studies of bacteria in bioretention cells regarding the evolution of vegetative cover, or

documenting the primary microbial species present in the cells, and their primary functions and interactions with the larger bioretention cell ecosystem.

3. Goals and Objectives

This research effort aimed to evaluate the pollutant removal and hydrologic performance of the Blackburg Aquatic Center bioretention cell (BRC) seven years post-construction. The project also more thoroughly characterized the microbial community of the BRC with respect to fecal indicator bacteria and denitrifying bacteria. Evaluation of these parameters will assist in assessing whether a projected design life of 10 years for urban BRC is reasonable, and inform maintenance recommendations (US EPA 2000).

To achieve these goals, current (i.e. 2014) mass removal rates of total nitrogen (TN), total phosphorus (TP), and TSS were compared to those observed for the BRC from 2007 to 2008, which have been previously published by DeBusk and Thompson, 2011. Removal rates of fecal indicator bacteria (FIB) (total coliforms, *E. coli*, and *Enterococci*) were also determined as there was limited evaluation of FIB performance following initial construction. Hydrologic performance was evaluated through observation of any changes in peak flow and runoff volume reductions. Changes in the BRC media was characterized through evaluation of changes in soil particle size, organic matter (OM), and nutrient content of the BRC since construction.

4. Methods

4.1 Site description

The project BRC is located in the Stroubles Creek watershed at 37˚ 14' N, 80˚ 24' W (Town of Blacksburg, Montgomery County, Virginia, USA). This area is in the Valley and Ridge physiographic province, between the Alleghany and Blue Ridge mountains and has dolomite and limestone geology. On average, Blacksburg receives 104 cm of precipitation per year. An 8.0-km reach of Stroubles Creek was listed on the Virginia 303(d) list for benthic macroinvertebrate and bacterial impairments. The watershed contributing to the impaired reach contains parts of the Virginia Tech campus and parts of Blacksburg, both of which are highly developed (DeBusk, 2008). Figure 4.1, Figure 4.2, and Figure 4.3 show the location of the BRC and the surrounding development.

The BRC was originally designed to detain 13 mm of runoff from the 1600 m^2 parking lot at the Blacksburg Aquatic center, and to match the pre-development peak flow rates of both a 2-yr and a 10-yr 24-hr storm event (equivalent to 6.99 cm and 10.39 cm of rainfall, respectively). It measures 4.6 m wide, 7.6 m long, 1.8 m deep. At the time of construction, the bioretention media consisted of 88% medium washed sand, 8% clay and silt fines, and 4 % leaf compost by volume (see Figures 4.1 and 4.2 for cross-sections). During construction, the media were overfilled and not compacted to allow future settling. Due to underlying regional karst geology, the BRC includes a 15-cm clay liner and an underdrain system consisting of two sets of two parallel, 10 cm perforated pipes covered with #57 stone and wrapped in filter fabric to prevent clogging by fines. These pipes span the length of the BRC and are connected to an outflow structure that leads to the storm drainage system. The outflow structure also contains a surface overflow drain to limit water ponding depths in excess of 10 cm (DeBusk, 2008).

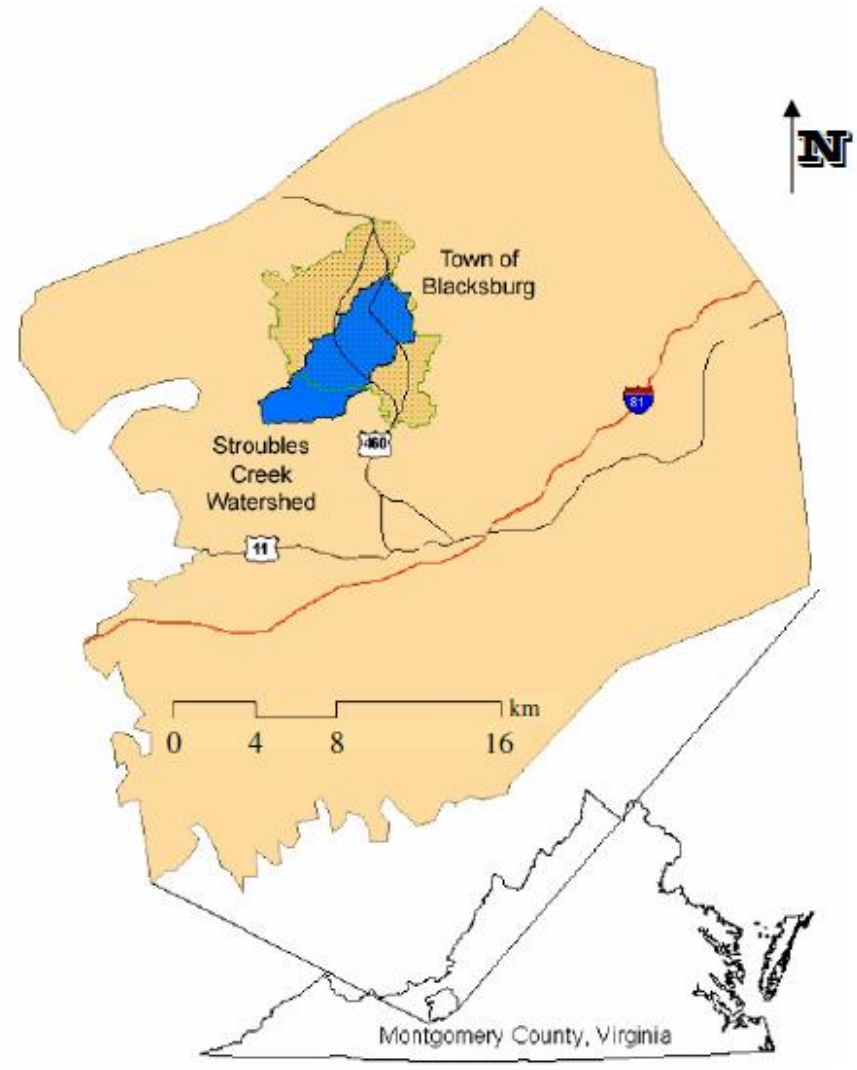

Figure 4.1. Location of impaired reach of Stroubles Creek within Montgomery County, VA and its relation to the town of Blacksburg (DeBusk, 2008).

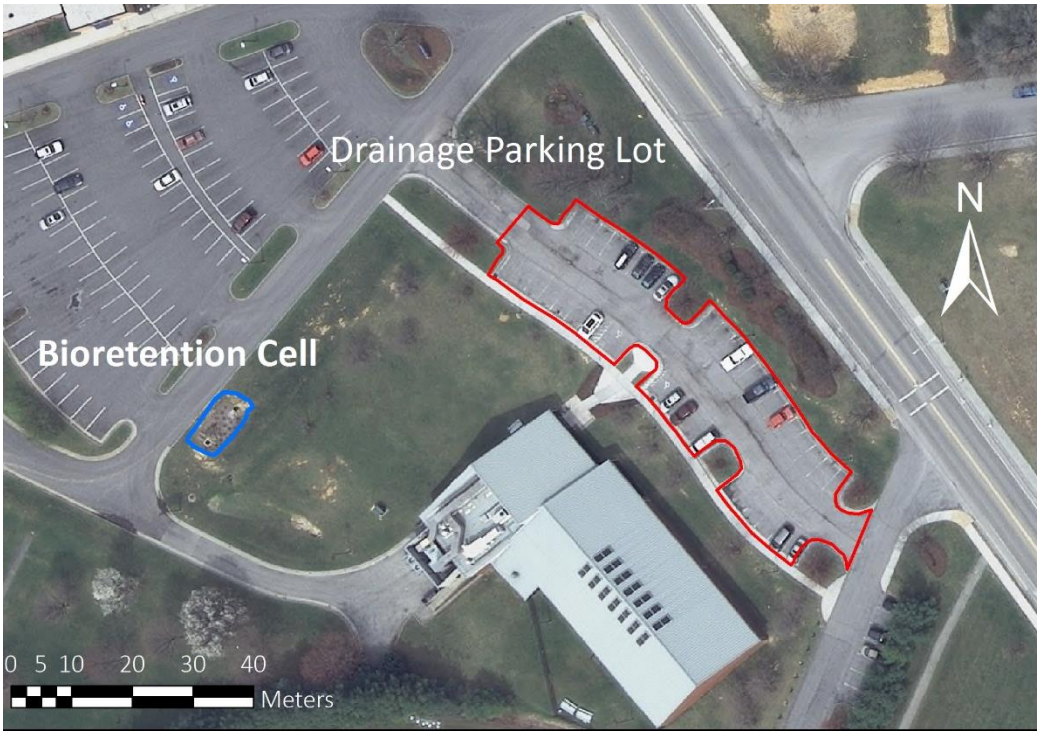

Figure 4.2. Aerial view of BRC and the connected impervious parking lot.

Figure 4.3. Aerial view of BRC, indicated by red dot, in Blacksburg (Google, 2014).

Of particular interest to this study, the BRC was initially designed to promote denitrification through inclusion of a 30-cm ponding depth (IWS layer) at the bottom of the cell to provide an anaerobic region. The clay liner beneath the BRC prevents water from infiltrating into the subsoil and filter fabric lining the sides of the BRC prevents surrounding soil from entering the cell and clogging the media. After construction, the media was covered with 10 cm of mulched wood chips to encourage the growth of the planted vegetation, which included hardy native perennials, shrubs, and trees (Figure A.1). This vegetation was included to promote infiltration and pollutant removal.

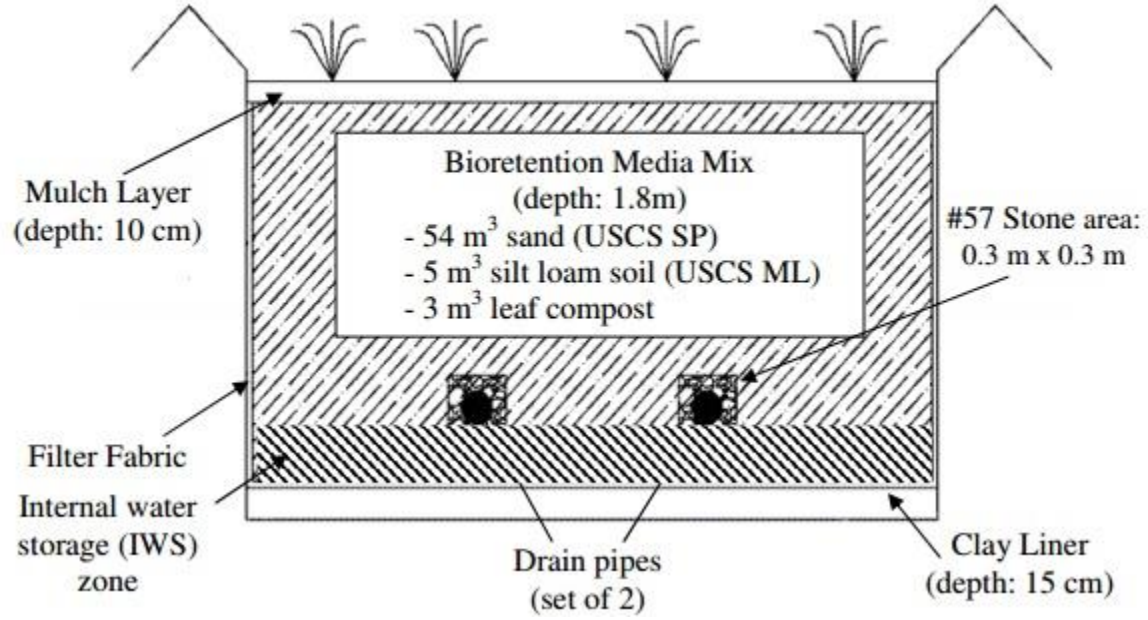

Figure 4.4 Cross section of the study bioretention cell (DeBusk, 2008).

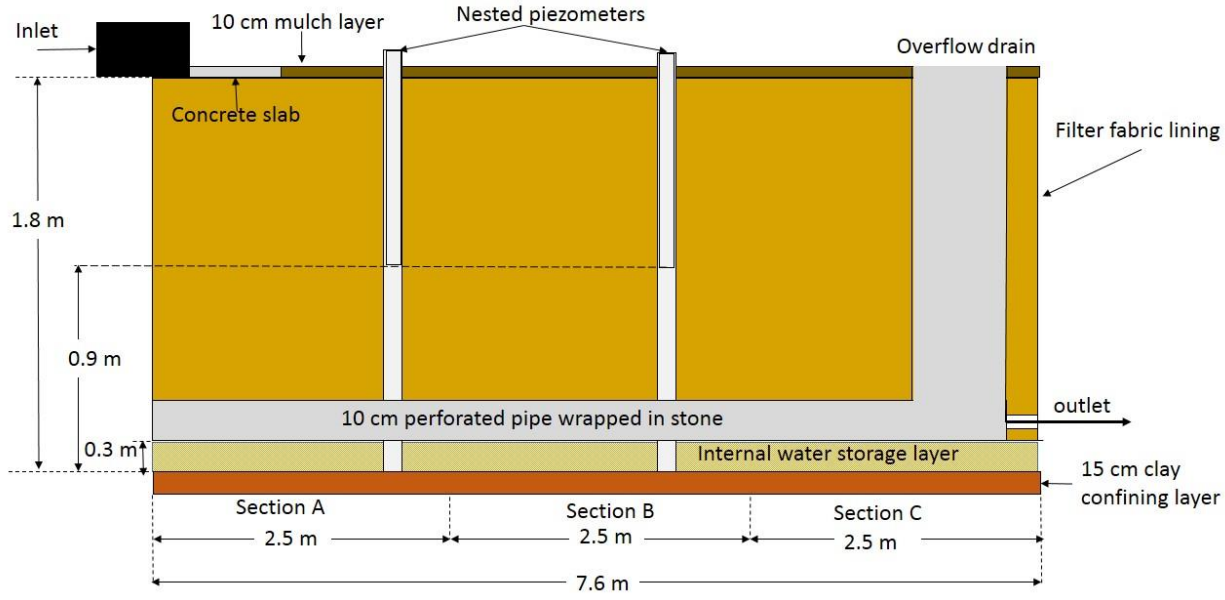

Figure 4.5 Longitudinal cross section of study bioretention cell.

Visual inspection of the BRC indicates a high degree of vegetation maturation over the seven years since initial installation (Figure 4.3). General maintenance has been performed by the Town of Blacksburg, which included mowing the cell at least once per year and possible mulching. At the beginning of the study period, the cell was overgrown with vegetation, suggesting that it had not been mowed or mulched recently. The cell was mowed on November 10th, 2013, prior to storm event data collection. It was not mulched during the study period.

Figure 4.6 The bioretention cell immediately after construction (July 2007) (top) (DeBusk, 2008), seven years post-construction (July 2014) (middle) (Willard, 2014), and seven years post-construction after mowing (November 2013) (bottom) (Willard, 2013).

4.2 Monitoring

4.2.1 Bioretention media monitoring

Media samples were collected on three separate occasions: July 26th, 2013 and October 15th, 2013 to assess long-term changes in BRC media characteristics (i.e. changes during the first seven years post-construction) and April 26th, 2014 to assess short term/seasonal changes in the BRC microbial community occurring from July to April. Samples collected at multiple depths on July 26th and October 15th were combined for each depth (due to high sample volume needed for analyses) and analyzed for TN, TP, total organic carbon (TOC), and particle size distribution. To determine mass nutrient content of the media by depth layer, the media was also sampled for bulk density on April 26th. The July 26th and April 26th samples were also analyzed for retained concentrations of FIB via standard culture-based methods and for markers of denitrifying bacteria via the quantitative polymerase chain reaction (qPCR).

To account for potential changes in media content across the longitudinal distance between inflow and outflow points, the BRC was divided longitudinally into three segments (A, B, and C), each 4.60 m wide x 2.53 m long during all sampling efforts (Figure 4.4). Each of these segments was then divided into six layers of varying depths: 0-2 cm, 2-10 cm, 10-30 cm, 30-80 cm, 80-130 cm, and 130-180 cm. The layers at the top are shallower as more significant changes in nutrient and bacterial content were anticipated at the BRC surface given past experience and a review of the recent literature.

A normally distributed random number generator in Microsoft Excel was used to generate three sets of coordinates in each 4.60 m x 2.53 m section (A, B, C), which was divided into a grid consisting of 1 cm^2 squares. To ensure sufficient volume/mass for analysis, additional samples for the shallower depths (0-2 cm and 2-10 cm) were collected randomly. For each depth and segment combination, a composite sample was created in the field. A portion (roughly 200 mL) of each sample was placed in a sterile Whirl-Pak ® bag (Nasco, Fort Atkinson, WI) for microbiological analyses. Analyses for FIB (total coliforms, *E. coli*, enterococci) were performed within 24 hours of collection. Between 1.0 and 1.5 mL of media of qPCR analysis was aseptically transferred to a cryogenic tube and stored in a -80˚C freezer for subsequent DNA extraction (perfomed within six months). Sub-samples for nutrient and carbon analysis were

stored at 4˚C and processed within six months. Sub-samples for particle size analysis were stored at 4˚C and processed within eight months.

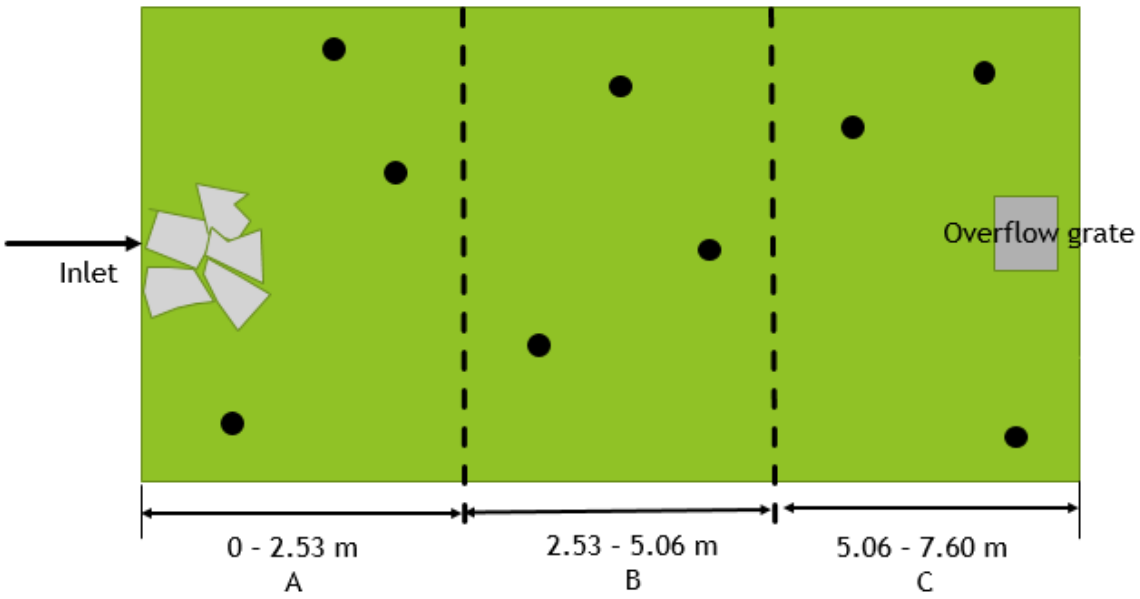

Figure 4.7 Example of bioretention media sampling sites

4.2.2 Stormflow monitoring

Teledyne ISCO (Lincoln, NE, USA) automated stormwater samplers were installed at the inlet and outlet of the BRC to collect flow-weighted samples. At the inlet, the intake tubing was installed in a smooth walled corrugated plastic pipe leading from the culvert in the Blacksburg Aquatic Center parking lot. At the outlet, the intake tubing was installed at the bottom of the overflow drain, a standard corrugated plastic pipe. Teledyne ISCO 730 Bubbler Flow Modules recorded the depth of flow entering and exiting the BRC. The bubbler tubing at the inlet was installed in the pipe along with the intake tubing. At the outlet, the bubbler tubing was installed in a Thel-Mar compound weir (Thel-Mar, LLC, Brevard, NC, USA) to obtain more accurate effluent estimates. A weir was not used in the inlet, due to problems with sediment clogging the weir.

The inlet ISCO was calibrated by sending a known volume of water from a fire hydrant through the BRC over a known time period. The outlet ISCO was calibrated using the Thel-Mar weir rating curve. The inlet ISCO stage discharge relationship was:

$$Q = 0.22h^{1.44} \quad (2)$$

Where Q = flow rate (m/s)

And h = height of influent (m)

The outlet ISCO stage-discharge relationship based on field data was:

$$Q = 0.07h \quad (3)$$

Where Q = flow rate (m/s)

And h = height of effluent (m)

The outlet included a Thel-Mar weir to record discharge; therefore, the rating curve for the weir was developed from a weir discharge table provided by Thel-Mar and used for flow calculations (Table 5.1).

Table 4.1 Weir rating curve regression equations and R^2 values

Head (m)	Trendline Type	Equation	R^2
0.005 - 0.025	Power	$3.0977x^{2.6968}$	0.9978
0.026 - 0.037	Power	$864.83x^{4.1566}$	0.9916
0.038 - 0.055	Power	$4.0681x^{2.5212}$	0.9986
0.056 - 0.081	Power	$0.8895x^{1.9988}$	0.9997
0.082 - 0.099	Exponential	$0.0007e^{26.445x}$	0.9960
0.100 - .185	Linear	0.2048x - 0.0107	0.9999

Since the ISCO could not be programmed with multiple equations, the regression equation based on field data was used for field sample collection, and the rating curve was used for flow volume calculations after the study period.

During the study, the ISCOs were triggered by an inflow/outflow greater than 2.5 cm. When inflow/outflow reached the target depth, the ISCO ran through a tube-rinsing cycle and collected a sample. At the beginning of the study, 100 mL samples were collected for every 3.0

m^3 of flow at the inlet and every 0.5 m^3 at the outlet. However, as the BRC became saturated in February, outlet samples were taken less frequently (every 1.0 – 3.0 m^3) to capture an adequate representation of the outflow (i.e. initial outlet sampling was higher frequency to ensure sufficient volume for nutrient and bacterial analyses, as a lower overall volume was anticipated). Once an ISCO was enabled, it stayed enabled. This ensured that the ISCO would continue to sample if there was a pause within the storm where no runoff was produced, but a researcher was unable to reset the program. The program ended when all 24 bottles had been filled or if it was manually ended by the researcher. If more than six hours occurred between runoff, the events were considered two separate storm events, based on protocol followed in the initial study (DeBusk, 2008).

Water quality samples were analyzed for FIB, TN, nitrate/nitrate, ammonia/ammonium, TP, phosphate, and TSS. The bottles were acid washed and rinsed in DI water to ensure accurate nutrient and TSS analyses for storms 1-5 and storms 19-23. Precautions were also taken to ensure sterility and aseptic technique for all storms. Before placement in the ISCO, each sample bottle and cap was rinsed in a 50% ethanol solution once, then rinsed three times with DI water. Due to lab error, bottles for storms 6-18 were only rinsed with the ethanol solution and DI water. However, when tested, the results from storms 6-18 were not different than those from other storms, so the effects of the lapse in protocol likely had little effect on study results due to bottle preparation for bacterial analysis. Caps were stored in sealed plastic bags between washing and being placed back on bottles during sample collection. All bottles were loaded into the ISCOs and collected wearing latex gloves. Beginning March 3rd, the ISCO intake lines were rinsed between every three storms with 50% ethanol and DI water to discourage microbial growth as recommended in the recently published automatic sampling protocol by Hathaway et al (2014).

Following collection, the multiple samples from both the inlet and the outlet were composited to create one influent sample and one effluent sample, respectively, using a five-gallon bucket that was pre-sterilized with a 50% ethanol and triple DI rinse and stirred with a sterilized rod. To collect a representative sample, subsamples were collected from the composite while it was being stirred. Subsamples include approximately 500 mL for FIB analysis; 250 mL for TSS; and 100 - 800 mL for N and P analyses. Beginning April 28th, half of the samples set

aside for N and P analyses were filtered using a 0.45 µM filter for analyses on nitrogen and phosphorus species at the request of the water quality laboratory for QA/QC.

4.3 Media and Water Quality Analyses

4.3.1 Bioretention media analyses

The BRC media samples were analyzed for TP, TN, TOC, particle size distribution, FIB, and denitrifying bacteria. Total phosphorus was determined using the USEPA SW-846 method (USEPA, SW-846, Test Methods for Evaluating Solid Wastes, Physical/Chemical methods, 3rd Ed. Current revision). TOC and TN amounts were determined using dry combustion with an Elementar vario MAX CNS (Elementar, Germany) high temperature combustion cube following ISO 10694:1995 and ISO 13878:1996 standard methods for TC and TN, respectively. Detection limits range from 0.02 to 30.0 mg for nitrogen and 0.02 to 200 mg for carbon with a specification of 0.5%.+

BRC media bulk density was measured for each longitudinal section A, B, and C for depths of 0-2 cm and 2-10 cm. Samples from deeper media were difficult to collect due to the sandy texture of the soil, and therefore only one sample was used to estimate bulk density for all depths greater than 10 cm. Particle size distribution at each longitudinal section and each depth was assessed using sieve (ASTM D6913-04) and hydrometer (ASTM D422-63) analyses.

To prepare samples for PCR and qPCR amplification, DNA extraction was performed on the media samples collected on July 26th, 2013 and April 26th, 2014 using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). Next, PCR was performed targeting the functional marker genes of microbial denitrification, *nirS*, *nirK*, and *nosZ*. The *nirK* and *nirS* genes encode the enzyme nitrate reductase, which allows for the reduction of nitrite (NO_2) to nitric oxide (NO), an intermediate step in denitrification. The *nosZ* gene encodes the enzyme nitrous oxide reductase, which allows for reduction nitrous oxide (N_2O) to nitrogen gas (N_2), completing the process of denitrification (Canfield et al., 2010). DNA primer sequences and cycle protocols were chosen based on prior studies that have observed positive results. Primer sequences used were nirK876C and nirK1040 for *nirK*, cd3af and R3cd for *nirS*, and nosZ2F and

nosZ2R for *nosZ*. Reaction components and cycle protocols are provided in Table 6.1. All protocols were modelled after Harter et al. (2013), which modified Ollivier et al. (2010) for *nirK* and *nirS* protocols and Babic et al. (2008) for *nosZ* protocols. Each sample was run in triplicate and a triplicate no-template control was included in each cycle.

The PCR amplification indicated that *nirK* and *nosZ* genes were present in the media samples; therefore, further qPCR efforts to quantify relative amounts of nitrification markers focused on these two genes. The qPCR reaction mixtures and thermal profiles were modified from Harter et al., 2013 (Table 6.1). Copy numbers were estimated from known standards of 10^1 to 10^8 gene copies using cloned plasmids.

Table 4.2 Quantitative PCR reaction mixtures and thermal profiles for study target genes.

Target Gene	Reaction Mixture	Volume (µL)	Thermal profile
nirK	iQTM SYBR® Green Supermix	12.5	98 °C - 15 s
	*nirK*876C (5 µM)	0.5	63-58 °C - 30 s
	*nirK*1040 (5 µM)	0.5	72 °C - 30 s
	PCR water	6.5	80 °C – 15s
	Template (5-50 ng/µL)	5	6 cycles
			98 °C - 15 s
			58 °C - 30 s
			72 °C - 30 s
			80 °C – 15s
			40 cycles
nirS	iQTM SYBR® Green Supermix	12.5	98 °C - 60 s
	cd3af (5 µM)	0.5	57 °C - 60 s
	R3cd (5 µM)	0.5	72 °C - 60 s
	PCR water	6.5	40 cycles
	Template (5-50 ng/µL)	5	
nosZ	iQTM SYBR® Green Supermix	12.5	98 °C - 30 s
	*nosZ*2F (5 µM)	0.5	65-60 °C - 30 s
	*nosZ*2R (5 µM)	0.5	72 °C - 30 s
	PCR water	6.5	6 cycles
	Template (5-50 ng/µL)	5	98 °C - 15 s
			60 °C - 15 s
			72 °C - 30 s
			40 cycles

4.3.2 Stormwater sample analyses

Following the completion of inflow and outflow collection by the ISCO autosamplers, sample bottles were promptly removed, transported to the Seitz Water Quality Labs on ice, homogenized, and analyzed for nutrients, sediment, and FIB. Nitrogen and phosphorus species were determined using the techniques listed in Table 6.2 via a SEAL AutoAnalyzer 3 (SEAL Analytical Inc., Mequon, WI, USA).

Table 4.3. Standard methods used for stormwater analyses

Analyte	Method	Detection Limit
Total Nitrogen	G-200-97	0.001 mg/L
Nitrate	G-200-97	0.006 mg/L
Ammonia	G-171-96	0.0007 mg/L
Total Phosphorus	G-175-96	0.001 mg/L
Phosphate	G-175-96	0.001 mg/L

Stormwater samples were analyzed for FIB via the Colilert and Enterolert defined substrate techniques (IDEXX Laboratories, Westbrook, ME, USA). Manufacturer recommendations for incubation were observed, i.e. incubation with Colilert at 37 °C for the detection of coliforms and *E. coli* and incubation with Enterolert at 42 °C for the detection of enterococci. To reduce the variability associated with measures of bacteria concentration in stormwater, all samples were analyzed using two Quanti-tray 2000s in order to increase well sample size (i.e. two trays = 98 large wells and 96 small wells in total). Combinations of positive and negative wells were converted to most probable number concentrations via the Thomas equation (Hurley and Roscoe, 1983).

4.3.3 Data analyses

Bulk density measurements at each section from the inlet and each layer of bioretention media were used to determine the mass of soil within the BRC. The soil mass of each layer was

then multiplied by the concentration of nitrogen, carbon, and phosphorus to determine the total mass of each within the BRC.

Precipitation data for the duration of the study were obtained from the Town of Blacksburg Telog website and used to determine the duration, average and maximum rainfall intensity, duration of preceding dry weather, and total precipitation of each storm.

Water-levels recorded by the inlet and outlet ISCO were used to calculate flow-rate and volume of runoff for each storm event using equation 2 for the inflow and the weir rating curve in Table 5.1 for the outlet. Mass loads of each pollutant were calculated by multiplying the total volume of flow by the composite concentration for each storm event. Cumulative removals were calculated using total mass loads for each pollutant and subtracting the effluent load from the influent load.

Four HOBO U20 water level data loggers (Onset Computer Corporation, Bourne, MA, USA) in two sets of nested piezometers collected water level data within the BRC. Two were installed approximately 2.5 m from the inlet of the BRC, with one installed at half the depth (0.9 m) and one installed at the full depth (1.8 m). A pair was also installed approximately 5.0 m from the inlet at half and full depth of the BRC. One HOBO pressure transducer recorded atmospheric pressure data, which were used to correct the data collected in the piezometers and convert it to centimeters of water. These data were used to estimate the flow path of runoff through the BRC during a storm event.

4.3.4 Statistical analyses

Data sets comprising TN, TP, TSS, NH_3-N, NO_3-N, PO_4-P, and FIB influent and effluent loads, as well as the gene copies per gram of soil for the *nirK* and *nosZ* target genes, were assessed for normality using the Shapiro-Wilk test (Dalgaard, 2002). All data were non-normal. Therefore, a one-sided, nonparametric Wilcoxon Signed-Rank Test was used to test for significant changes in mass loads for each of the nutrients, FIB, and TSS, differences in peak flow rate and runoff volume through the BRC, and differences in *nirK* and *nosZ* genes copies per gram of soil in April and July. The Wilcoxon Signed Rank test was also used to assess differences in pollutant removal from 2007 to 2014.

The nonparametric Spearman's ρ correlation test was used to assess correlations between precipitation data (i.e. storm duration, total precipitation, average intensity, maximum intensity, and duration since previous storm), flow, and pollutant removal (Dalgaard, 2002).

An alpha value of 0.05 was used for all statistical tests to determine significance. Outliers were defined as values that exceeded the median by 1.5 times the interquartile range (the difference between the 75^{th} and 25^{th} percentile), the same definition used in DeBusk, 2008.

5. Results and Discussion

5.1 Bioretention Media

Samples were collected on July 26th, 2013, October 15th, 2013, and April 26th, 2014. Particle size distribution and nutrient analyses were conducted using samples taken July 26th and October 15th. The July 26th and April 26th samples were analyzed for fecal indicator bacteria and denitrifying bacteria. All correlations between particle size, nutrients, and bacteria were conducting using bacteria analysis only from July 26th.

5.1.1 Particle size distribution

Immediately following installation in 2007, the BRC contained 88% sand, 8% clay and silt fines, and 4% organic matter by volume. Simple visual observations of the BRC in 2013 suggested that the media composition had changed, particularly due to the addition of gravel from runoff inflow and the buildup of organic matter. A concrete pad at the inlet had accumulated 8.1 cm of gravel as of July 15th, 2014. Piezometers located 2.5 m from the inlet were buried under gravel and organic matter washed from the parking lot.

Particle size analysis results illustrating the fractions of sand, gravel, silt, and clay in the BRC in 2013 are provided by Figure 5.1. Sand was the dominant particle fraction type by mass in all BRC sections, with sand content ranging from 77.7% (section A1) to 95.9% (section A5). The mean and median sand content were 91.1% and 92.7%, respectively, with a standard deviation of 4.4%. The Spearman's ρ correlation indicated that sand content is significantly positively correlated with depth. It is negatively correlated with TN, TP, TC, *E. coli*, enterococci, and *nirK*.

Gravel content ranged from 3.2% (section A5) to 12.7% (section A1). The mean and median gravel content were 7.0% and 6.3%, respectively, with a standard deviation of 2.5%. Silt content ranged from 0.2% (section C3) to 8.4% (section A1). The mean and median silt contents were 1.3% and 0.5%, respectively, with a standard deviation of 1.3%. Clay content ranged from

0.2% (section A6) to 1.5% (section A2). The mean and median clay contents were 0.6% and 0.4%, respectively, with a standard deviation of 0.4%.

Unlike sand, gravel, silt, and clay content were negatively correlated with depth, although all three were also positively correlated with TN, TP, TC, and enterococci. Silt and clay were also positively correlated with *E. coli* and *nirK*, and silt was positively correlated with *nosZ*. Therefore, as depth increased, sand content increased and gravel, silt, and clay contents decreased. Both the minimum and maximum percentage of sand, gravel, and clay were present in section A, closest to the inlet. This finding indicates that most of the inflow infiltrated near the inlet, deposited particles at the surface, and then flowed longitudinally and downward to deposit particles throughout the BRC. There was no correlation between particle type and distance from inlet for the entire BRC. When only the top layer was considered, gravel content was negatively correlated with distance from the inlet. Given that larger particles settle first, the correlation with gravel makes sense. Sand, silt, and clay contents are likely more variable due to the existence of surface flow paths, which depend on vegetation and media settling. Although the media was unlikely a completely homogenous mixture in 2007, it is now clearly stratified, with most finer particles and gravel settled at the top 10 cm of the media, and sand particles settled below. Figure 5.2 indicates a transition from darker media with more organic matter to lighter, sandy media occurring between 10 and 30 cm.

The nutrient and bacteria correlations observed in the BRC were expected. Small particles, such as silt and clay, and organic matter have higher cation exchange capacity (CEC), meaning that positively charged ions, including nitrogen and phosphorus species, will sorb to fine sediment (McBride, 1994). Several studies have also shown that bacteria are usually associated with settleable particles in the water column (Characklis et al., 2005; Schillinger and Gannon, 1985); therefore, small TSS particles deposited at the top of the BRC likely contribute to higher numbers of bacteria.

In a study of a BRC outside Washington, D.C., Li and Davis (2008b) observed incoming TSS particles settling in the top 20 cm of the BRC, which is similar to observations at the Blacksburg BRC of finer sediment within the top 10-30 cm. Li and Davis (2008a) also observed average decreases in column hydraulic conductivity from 80% to 95%. However, the BRC media studied included 50% sand, 30% top soil, and 20% mulch, meaning the overall pore size of the media was likely smaller than that of the Blacksburg BRC, which had a higher sand

content at installation. A laboratory study by Hatt et al. (2006) indicates that media with larger pore sizes are less susceptible to clogging than those with small pore sizes, but also do not reduce nutrient concentrations. Le Coustumer et al. (2009) observed similar results in a field study, where BRCs with initially high hydraulic conductivities did not experience significant reductions in hydraulic conductivity over time. Observations at the Blacksburg BRC during storms only showed small ponds forming at the inlet of the BRC, where a concrete pad prevents infiltration. The BRC experienced very little, if any, overflow during the study period, and overall seemed to have minimal clogging. As discussed in Li and Davis (2008b), mature vegetation roots may have helped loosen the media to decrease clogging. Based on these findings, if TSS is a target contaminant in a watershed, a BRC with initially high sand content, and subsequent high hydraulic conductivity, would be best for long-term hydraulic performance. Maintenance efforts should focus on replacing up to 30 cm of media, especially near the inlet, to reduce clogging.

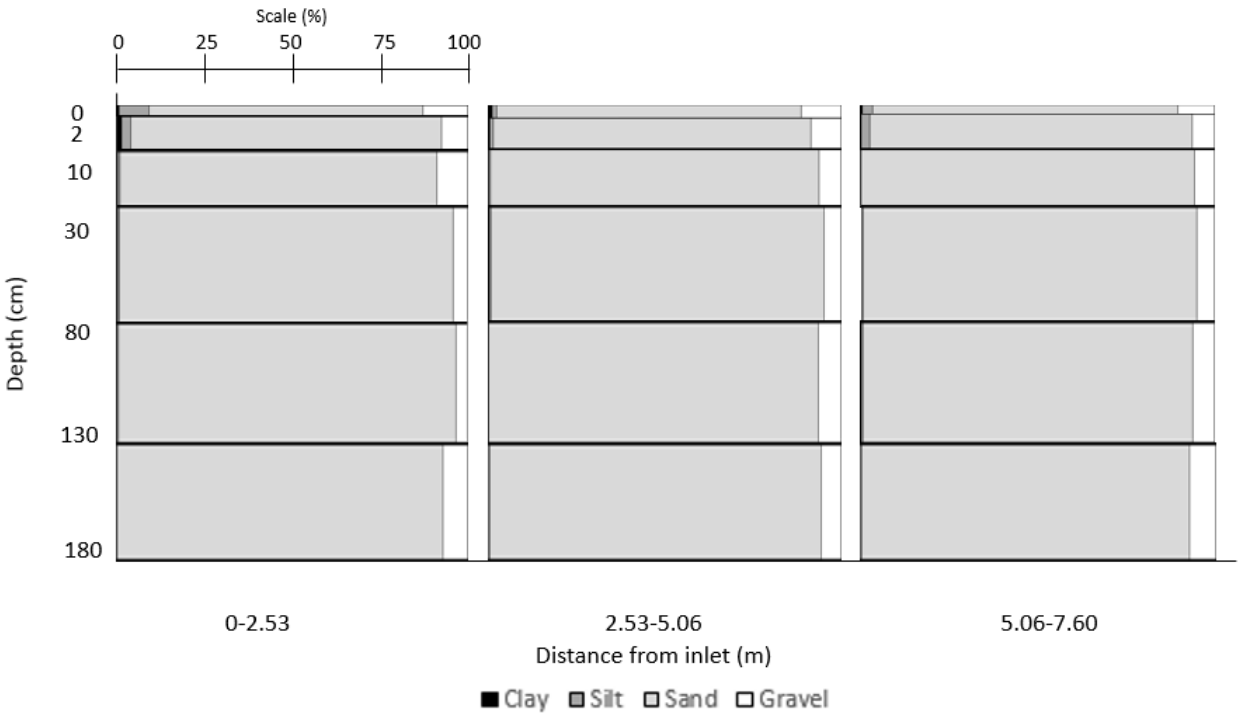

Figure 5.1. Bioretention media content by depth and distance from inlet observed in 2013-2014 study.

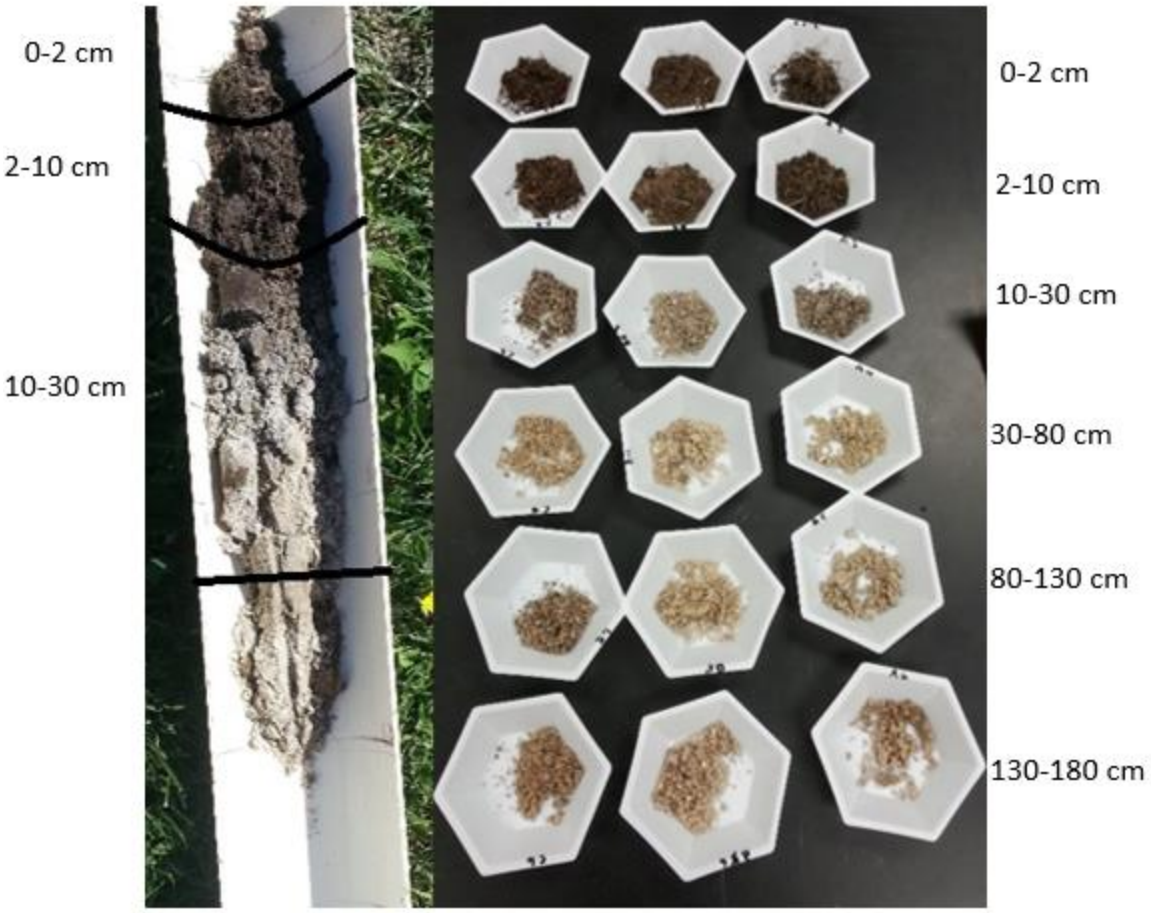

Figure 5.2. Soil layer stratification observed in BRC in 2013. The photo on the left shows a partial soil profile. The photo on the right shows the composites collected at each depth and distance from the inlet: Section A (0-2.53 m), Section B (2.53-5.06 m) and Section C (5.06-7.60), respectively.

5.1.2 Nutrients

Nitrogen content within the BRC media ranged from 182 mg/kg (Section A4) to 7510 mg/kg (Section B1). The mean and median nitrogen content were 1840 mg/kg and 619 mg/kg, respectively. The Spearman's ρ correlation coefficient indicated that nitrogen content was positively correlated with carbon content, phosphorus content, *E. coli*, enterococci, gravel content, silt content, clay content, *nirK*, and *nosZ*. It was negatively correlated with depth and sand content. Figure 5.3 shows the distribution of nitrogen throughout the BRC.

Carbon content within the BRC ranged from 2920 mg/kg (Section A5) to 153,000 mg/kg (Section B1). The mean and median carbon content were 48,000 mg/kg and 15,900 mg/kg respectively. The Spearman's ρ correlation coefficient indicated that carbon content was positively correlated with nitrogen content, phosphorus content, *E. coli*, enterococci, gravel content, silt content, clay content, *nirK*, and *nosZ*. It was negatively correlated with depth and sand content. Figure 5.4 shows the distribution of carbon throughout the BRC.

Phosphorus content within the BRC ranged from less than 50 mg/kg soil (at all depths greater than 10 cm) to 617 mg/kg soil (Section B1). The mean and median phosphorus content were 153 mg/kg and 50 mg/kg, respectively. The Spearman's ρ correlation coefficient indicated that phosphorus content was positively correlated with nitrogen content, carbon content, *E. coli*, enterococci, gravel content, silt content, clay content, *nirK*, and *nosZ*. It was negatively correlated with depth and sand content. Figure 5.5 shows the distribution of phosphorus throughout the BRC.

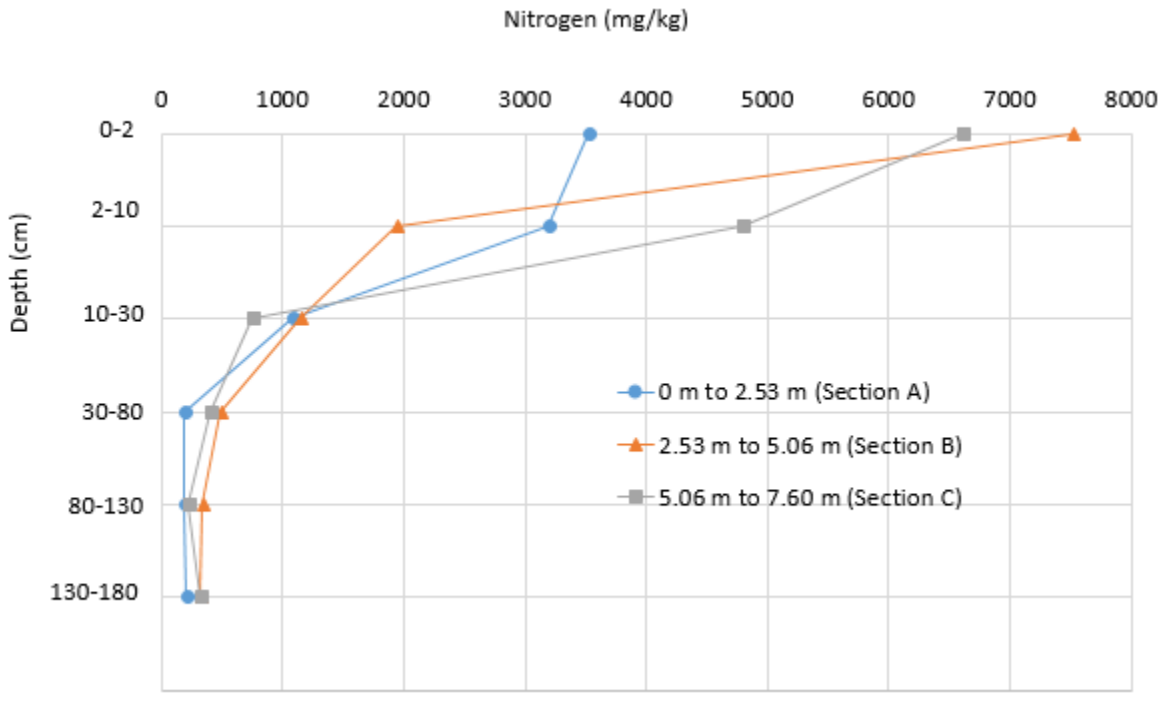

Figure 5.3. Nitrogen content of BRC based on depth and distance from inlet observed in 2013.

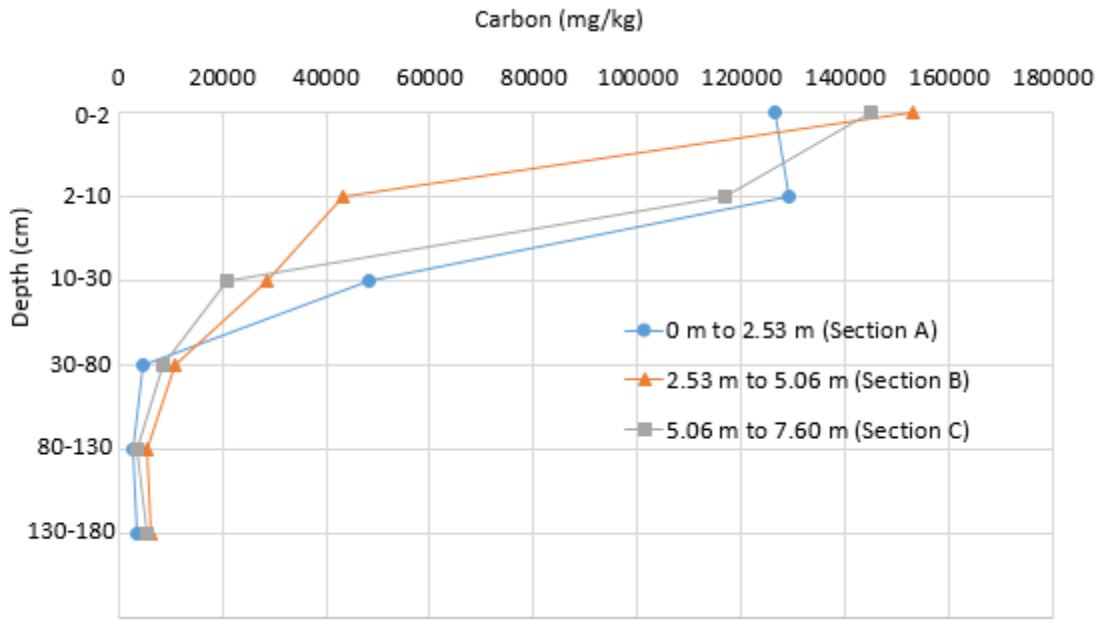

Figure 5.4. Carbon content of BRC based on depth and distance from inlet observed in 2013.

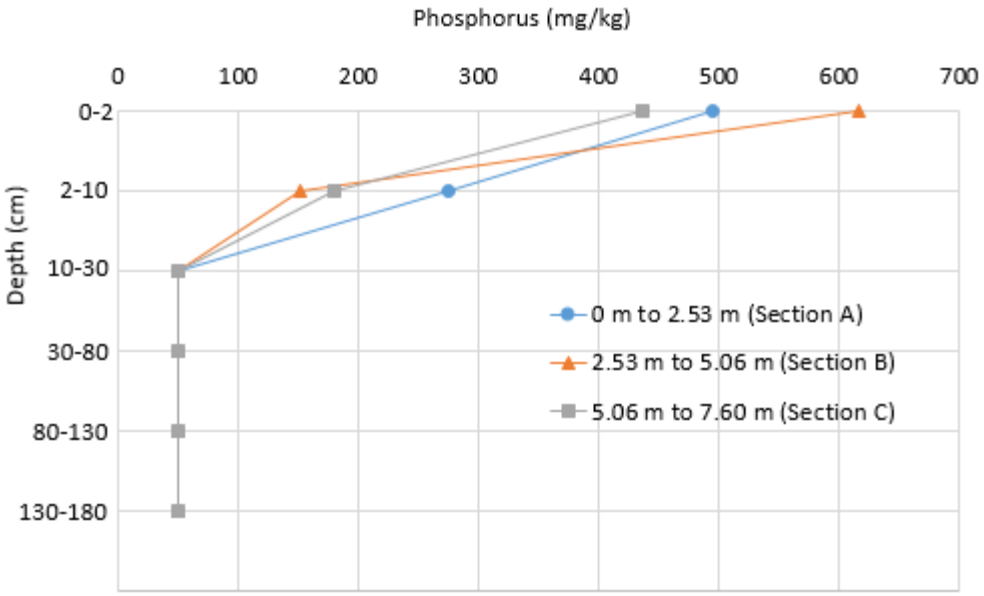

Figure 5.5. Phosphorus content of the BRC based on depth and distance from inlet observed in 2013.

The top 10 cm of BRC media consistently contained the highest TN, TP, and TC concentrations; concentrations were also highest in the center section (i.e. Section B, Figure 4.7), likely because this section had the most vegetative cover (Sections A and C had concrete structures for the inlet and outlet, and growth in Section A was limited by gravel) and a higher percentage of organic substances settled near the inlet due. All nutrient concentrations were negatively correlated with depth, i.e. lower concentrations were at greater depths. Decreases with depth were the most gradual in the section closes to the inlet (Section A, 0 m – 2.53 m). This gradual decrease may be the result of greater infiltration closer to the inlet, as runoff often ponds there during storm events, due to accumulated leave packs and flow resistance from the BRC vegetation. However, nutrient content in the upper layers was also lowest in Section A, possibly due to high gravel content, which limited capacity for nutrient sorption.

Compared to the mass of each nutrient at the installation, during the intervening seven years the overall TN content of the cell has decreased by 94% (from 250 kg to 15.7 kg) and the TP content has decreased by 74% (from 7.6 kg to 2.0 kg). Much of the initial nitrogen in the BRC was associated with the sand in the bioretention media mix and the leaf compost. After construction, excess nitrogen may have been flushed from the BRC during heavy storms. This is especially likely since polymers used to wash the sand probably broke down over time. In addition, the BRC was initially planted with a variety of wood and herbaceous vegetation. The mature root system may be able to remove nitrogen inputs from stormwater runoff, as observed by Lucas and Greenway (2008). A reduction in TP may be the result of the lack of organic matter within the BRC, resulting in a low CEC that does not easily immobilize phosphorus in the soil. Mature vegetation may also reduce TP content within the BRC as phosphorus is incorporated into new plant tissues. To the author's best knowledge, no other studies exist that document TN, TP, or TC accumulation within a BRC.

Figure 5.6 indicates that the carbon-to-nitrogen (C:N) ratio peaked at depths of 10-30 cm, then declined until depths of 130-180 cm, where it briefly increased. This may be due to vegetation utilizing nitrogen at rooting depths (10-30 cm), and potential denitrification occurring in the IWS layer (130-180 cm).

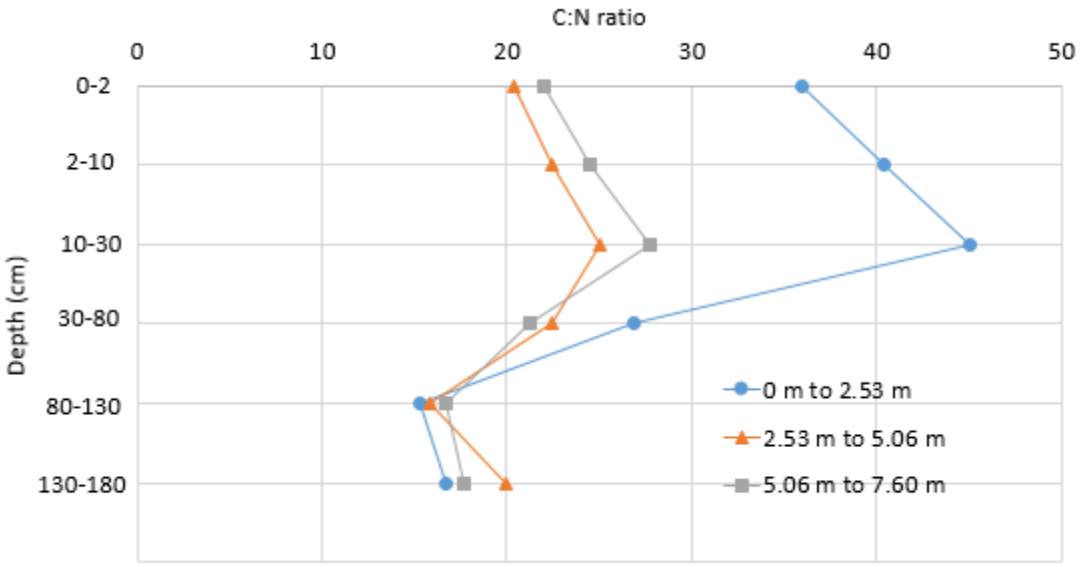

Figure 5.6. C:N ratio of BRC based on depth and distance from inlet observed in 2013.

5.1.3 Fecal indicator bacteria

Total Coliforms

Total coliforms within the BRC in July of 2013 ranged from 602.8 MPN/dry g soil (Section C6) to over the limit of detection (approximately 4000 MPN/dry g soil) (Sections A1-A6, B1-B3, B4, B5, C1-C3, and C4). The mean and median July coliform content were 3647 MPN/dry g soil and over 4000 MPN/dry g soil, respectively. The April 2014 coliform content ranged from below the limit of detection (denoted as 0 MPN/dry g soil) (Section C4) to over the limit of detection of 4000 MPN/dry g soil (Sections A1, A2, B1, B2, C1, and C2). The mean and median April coliform content were 1397 MPN/dry g soil and 58.3 MPN/dry g soil, respectively. Figure 5.7 shows total coliform content in July and April. Although total coliforms are present in the BRC media, they are not necessarily indicative of fecal pollution because they naturally live in soil environments (Leclerc et al., 2001).

The Spearman's ρ correlation coefficient indicated that coliform levels were positively correlated with *E. coli*, enterococci, and *nirK*. The Wilcoxon Signed Rank test indicated that

there was a significant difference ($\alpha = 0.05$, $p = 0.0005$) between coliform content in the BRC between July 2013 and April 2014, with concentrations higher in July.

Escherichia coli

E. coli within the BRC in July of 2013 ranged from below detection (0 MPN/dry g soil (Section B5) to 670.4 MPN/dry g soil (Section C1). The mean and median July coliform content were 88.7 MPN/dry g soil and 8.5 MPN/dry g soil, respectively. The April 2014 *E. coli* levels ranged from below the limit of detection (0 MPN/dry g soil) (Sections A1-A3, A5, A6, B1-B6, and C2-C5) to 4.8 MPN/dry g soil (Sections A1, A2, B1, B2, C1, and C2). The mean and median *April E. coli* content were 0.3 MPN/dry g soil and below the limit of detection, respectively. Figure 5.8 shows *E. coli* levels in July and April.

The Spearman's ρ correlation coefficient indicated that *E. coli* content was positively correlated with TN, TC, TP, coliforms, enterococci, silt, clay, *nirK*, and *nosZ*. It was negatively correlated with depth and sand. The Wilcoxon Signed Rank test showed that there was a significant difference ($\alpha = 0.05$, $p = 0.0001$) between *E. coli* content in the BRC between July 2013 and April 2014, with concentrations higher in July.

Enterococci

Enterococci within the BRC in July, 2013 ranged from 0.52 MPN/dry g soil (Section C6) to 275.5 MPN/dry g soil (Section B1). The mean and median July enterococci levels were 41.7 MPN/dry g soil and 12.6 MPN/dry g soil, respectively. The April 2014 enterococci levels ranged from below the limit of detection (0 MPN/dry g soil) (Sections A5 and B4) to 1579 MPN/dry g soil (Section C1). The mean and median April enterococci levels were 461.5 MPN/dry g soil and 2.2 MPN/dry g soil, respectively. Figure 5.9 shows enterococci concentrations in July and April.

The Spearman's ρ correlation coefficient indicated that enterococci concentration was positively correlated with TN, TC, TP, coliforms, *E. coli*, gravel, silt, clay, and *nirK*. It was negatively correlated with depth and sand. The Wilcoxon Signed Rank test indicated that, unlike total coliforms and *E. coli*, there was no significant difference ($p = 0.4423$) between enterococci content in the BRC between July 2013 and April 2014.

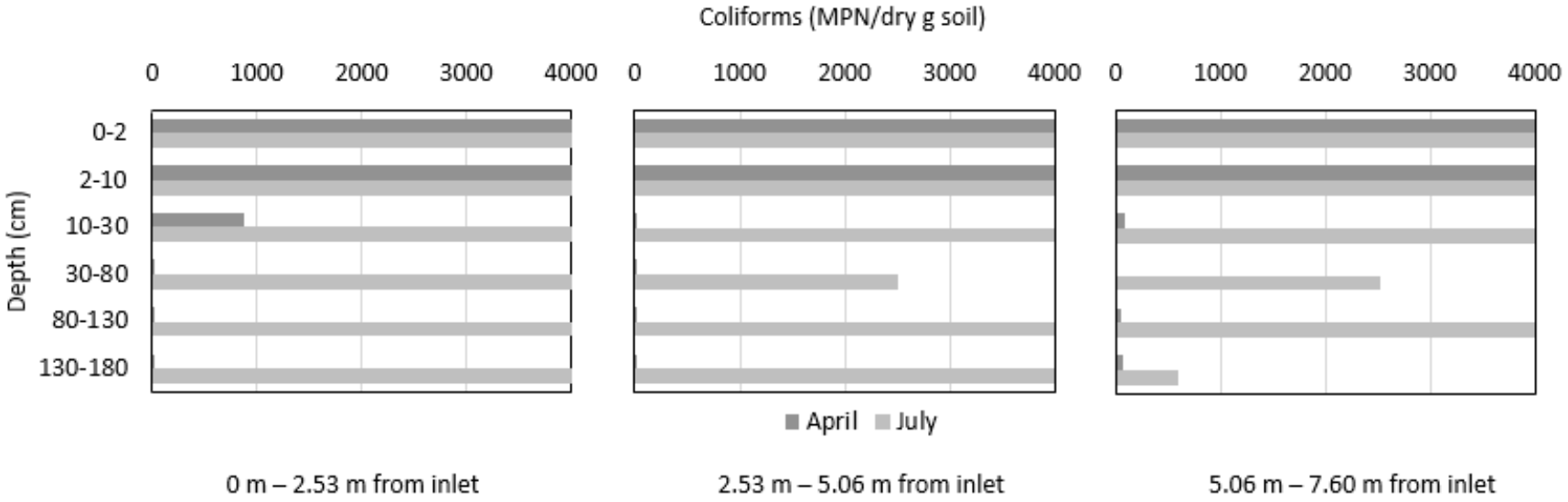

Figure 5.7. Total coliforms (MPN/g dry soil) present in BRC in July, 2013 and April, 2014 based on depth and distance from inlet. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m. A full bar indicates that the sample was above the limit of detection for the IDEXX trays.

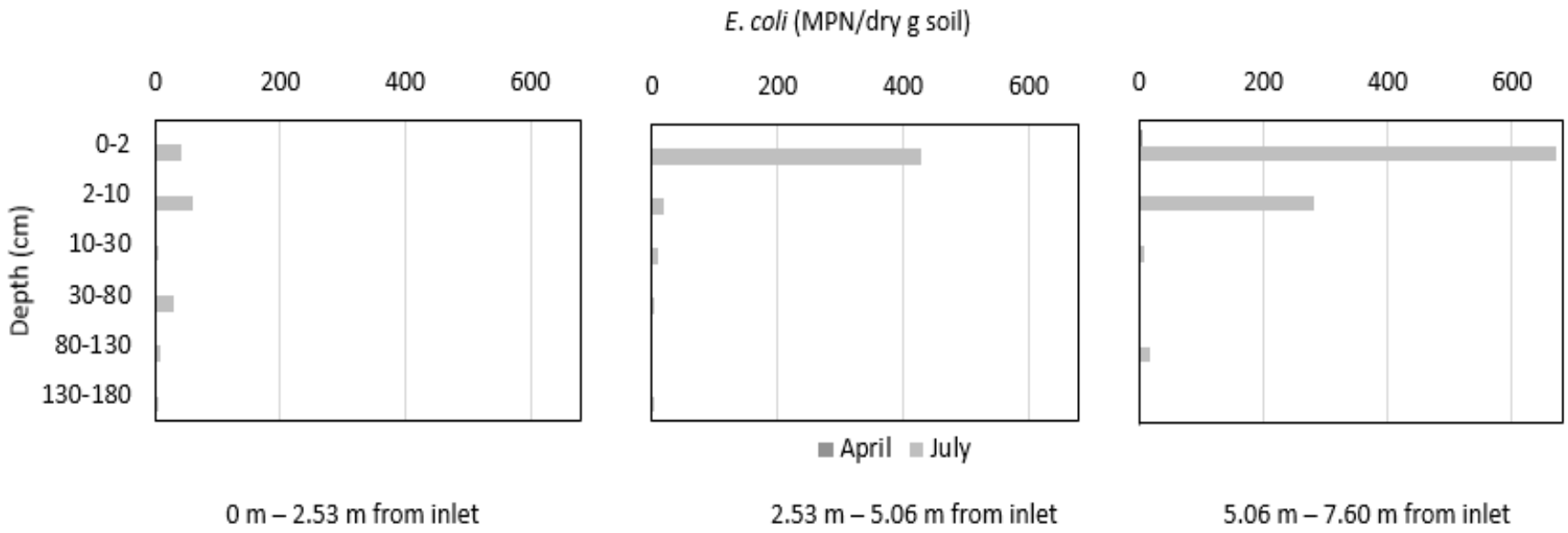

Figure 5.8. *E. coli* content of the BRC in July, 2013 and April, 2014 based on depth and distance from inlet. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m.

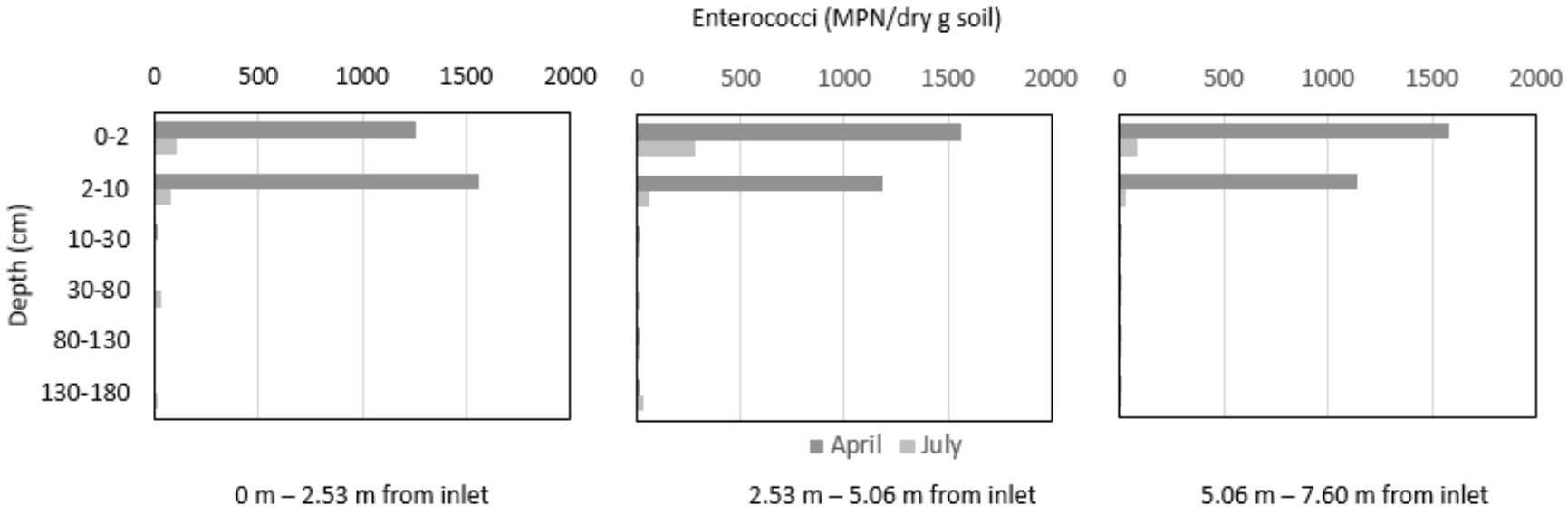

Figure 5.9. Enterococci content of the BRC in July, 2013 and April, 2014 based on depth and distance from inlet. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m.

Although considered human fecal indicator bacteria, many coliforms are native to the soil environment and other places outside the human body, and therefore are not reliable as indicators for recreational water. *E. coli* is more widely used as an indicator of health risk in recreational waters because it is specific to fecal contamination from warm-blooded animals, including humans. Enterococci is a subgroup of bacteria from the fecal streptococcus group, which is also specific to fecal matter of warm-blooded animals. Enterococci are used as indicator bacteria because of their high salt tolerance, which mimics that of many pathogens in recreational salt waters (US EPA, 2012a). Coliforms' omnipresence in the environment may explain why there was no significant correlation between coliforms and other parameters, such as nutrient content, particle size, and depth. Both *E. coli* and enterococci content were positively correlated with each nutrient, silt, and clay and negatively correlated with depth. This may be a result of *E. coli* and enterococci being stormwater inputs as well, unlike total coliforms, which are always in the soil. Microorganisms can also sorb to small particles, such as clay, and therefore would be distributed across the BRC in a similar manner. Also, since any animals would defecate on the surface of the BRC cell, it is not surprising the content is greater in the top layers of the BRC.

Coliforms and *E. coli*, which are gram-negative bacteria, showed significant differences in concentration between July and April, with generally lower concentrations at each depth in April. This decrease could be due to a combination of factors, such as long periods of cold weather before sampling in April that killed the microorganisms and the lack of vegetation in the BRC, leading to UV exposure of microorganisms. In contrast, the soil samples in July were taken during warm weather conditions with vegetation cover shown in the middle tile of Figure 4.6, before the vegetation was mowed. Concentrations of enterococci, which are gram-positive, were not significantly different throughout the entire BRC in April and July, concentrations in the top 30 cm were significantly different ($\alpha = 0.05$, $p = 0.0117$). In this case, the April samples generally had a higher concentration. This increase of enterococci in winter and coliforms in summer is similar to findings by Sinton et al., (2002) where summer inactivation of enterococci and winter inactivation of fecal coliforms was observed. The seasonal differences are apparent in Figure 5.8 and Figure 5.9, where enterococci was visibly more dominant in April while *E.*

coli was more prevalent in July. To the author's knowledge, no other studies quantify FIB within a BRC.

5.1.4 Denitrifying bacteria

Gene copies of *nirK* within the BRC in July of 2013 ranged from $3.66x10^7$ gene copies perg of soil (Section C6) to $1.66x10^9$ gene copies per g soil (Section A3). The mean and median July *nirK* gene copies are $3.36x10^8$ gene copies per g soil and $2.0x10^8$ gene copies per g soil, respectively. The April 2014 *nirK* content ranged from below the limit of detection ($5.65x10^2$ gene copies per g of soil for Section B2 and $3.15x10^3$ gene copies per g of soil for C2) to $9.97x10^8$ gene copies per g soil (Section B2). The mean and median April *nirK* content are $1.71x10^8$ genes per g soil and $3.68x10^7$ gene copies per gram soil, respectively. Excluding samples that registered below the limit of detection, gene copies of *nirK* present per gram of soil generally ranged from $1.1x10^5$ to $1.7x10^9$ copies per gram of soil, which is consistent with prior studies using qPCR to quantify denitrifiers in soils in the United States, Asia, Africa, and Europe (Henry et al., 2004; Qiu et al., 2004; Henry et al., 2006), agricultural and riparian soils (Dandie et al., 2011), and bioretention media (Chen et al., 2013). Figure 5.10 shows the range of *nirK* gene copies present in the BRC in April and July.

The Spearman's ρ correlation coefficient indicated that *nirK* content is positively correlated with TN, TC, TP, coliforms, *E. coli*, enterococci, silt, clay, and *nosZ*. It is negatively correlated with depth and sand. The p-value of 0.06 was very close to 0.05 for the Wilcoxon Signed Rank Test comparing *nirK* in April and July, suggesting that the differences were significant. Visual observations of the data show overall less *nirK* in April, indicating that a lack of vegetative cover or the cold temperatures may have affected levels present.

Gene copies of *nosZ* within the BRC in July, 2013 ranged from $2.43x10^5$ gene copies per g of soil (Section B5) to $3.61x10^6$ gene copies per g soil (Section A3). The mean and median July *nosZ* gene copies are $1.01x10^6$ gene copies per g soil and $6.82x10^5$ gene copies per g soil, respectively. The April 2014 *nosZ* content ranged from below the limit of detection ($2.85x10^3$ gene copies p g soil in Section B1, $5.65x10^2$ gene copies per g soil in B2, and $7.43x10^3$ gene

copies per g soil for section C2) to $3.49x10^6$ gene copies per g soil (Section B2). The mean and median April *nosZ* content are $7.54x10^5$ genes per g soil and $2.72x10^5$ gene copies per gram soil, respectively. Excluding the sections that registered below the limit of detection, gene copies of *nosZ* present per gram of soil ranged from $1.0x10^5$ to $3.5x10^6$ copes per gram of soil, which is similar to most studies that observed nosZ values 1-2 exponential units lower than nirK (Henry et al., 2004;Qiu et al., 2004; Henry et al., 2006). Figure 5.11 shows the range of *nosZ* gene copies observed in the BRC.

The Spearman's ρ correlation coefficient indicated that *nosZ* content is positively correlated with TN, TC, TP, *E. coli*, silt, and *nirK*. It is negatively correlated with depth. There was no significant difference in *nosZ* between July and April samples (alpha = 0.05, test statistic = 0.05999) using the Wilcoxon Signed Rank Test.

The IWS layer in the Blacksburg BRC is located in the bottom 30 cm. Therefore, an increase in *nirK* and *nosZ* genes was expected for depths 130-180 cm. Piezometer data indicated that the bottom of the BRC does not stay completely saturated over time, so the IWS layer is not anoxic. Analysis of water levels between March 13th, 2014 and April 20th, 2014 showed that the bottom 5 cm – 20 cm typically stay inundated in 3-7 day cycles. The media analysis also indicated that carbon levels are low at 130-180 cm. Chen et al. (2013) quantified denitrification genes within a 5-year-old BRC. Data in this study indicate that media saturation time is essential in establishing a microbial system that promotes denitrification, as well as media that is at a good pH and has sufficient carbon.

When comparing the amount of *nirK* and *nosZ* present in the BRC to the total 16s rDNA (a gene present in generally all microorganisms) (Figure 5.12), percentages of *nirK* and *nosZ* are generally higher deeper in the BRC, as indicated in Figure 5.13 and Figure 5.14. This may suggest that these conditions are more favorable for denitrifiers than other bacteria, but it seems unlikely that much denitrification is occurring.

The *nirK* and *nirS* genes encode the enzyme nitrate reductase, which is involved in for the reduction of nitrate (NO_2) to nitric oxide (NO), and intermediate step in denitrification. The *nosZ* gene encodes the enzyme nitrous oxide reductase, which allows for reduction of nitrous oxide (N_2O) to nitrogen gas (N_2), completing the process of denitrification (Figure 2.2) (Henry et al., 2004; Henry et al., 2006). The observations of relatively higher gene copy numbers of *nirK* as compared to *nosZ* is not necessarily surprising, as the *nosZ* gene is not present in a high

percentage of denitrifiers, such as *Agrobacterium tumefaciens* C58 (Wood et al., 2001). The higher concentration of *nirK* indicates that the denitrification process in the BRC may end when nitrogen is converted to NO or N_2O (a greenhouse gas), which are forms unusable by legumes and ultimately other plants. Overall, the relative abundances of both *nirK* and *nosZ* to 16s rDNA were on the low side (0.2%-1% and 0.0%-0.005%, respectively) compared to studies in natural soils (including agricultural lands, a marsh, and the Himalayan mountains), which observed *nirK* at 1%-6% of total gene copies and *nosZ* at 0.1%-0.5% of total gene copies (Henry, 2006). There may be less denitrifiers present in bioretention soils because overall they are much more oxygenized due to high soil porosity, decreasing the likelihood of anoxic zones.

Human error during DNA extraction or qPCR procedure could have caused low DNA concentrations in the samples that registered below the limit of detection. Samples from sections B2 and C2 in April were below the limit of detection for both *nirK*, *nosZ*, and 16s rDNA. Sample B1 was also below the limit of detection for *nosZ* and 16s rDNA, and had the lowest number of gene copies for *nirK* other than the sections below the limit of detection. These data are different from Section A, which had relatively high gene copies in the top sections. It is also different from fecal indicator bacteria concentrations, which generally decreased with depth. The observation of no 16s rDNA in these samples indicates that errors likely occurred during analyses.

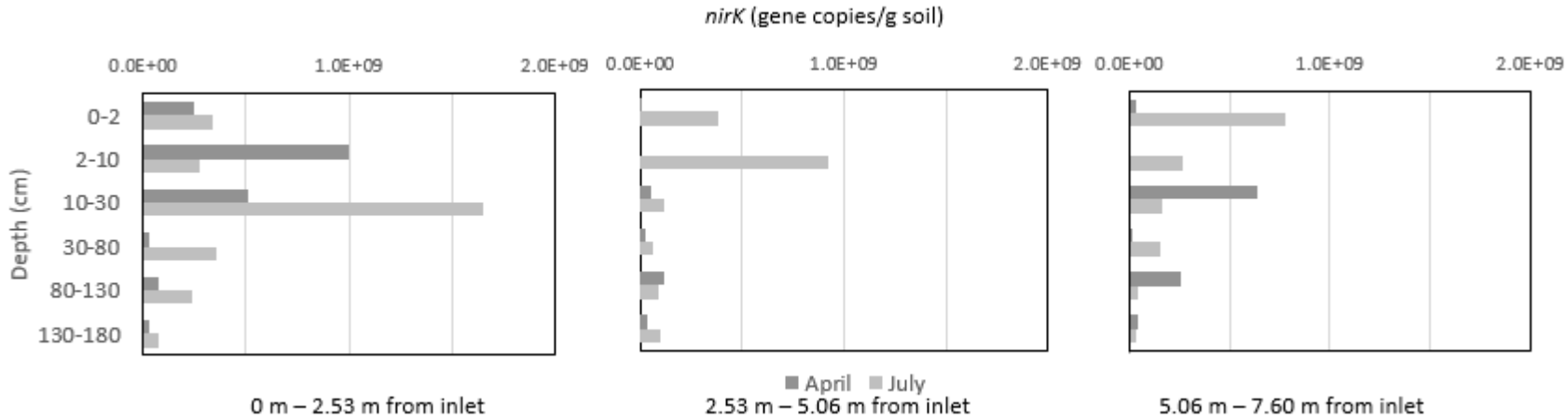

Figure 5.10. Gene copies of *nirK* per gram of soil present in the BRC at varying distances from the inlet and target depths. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m.

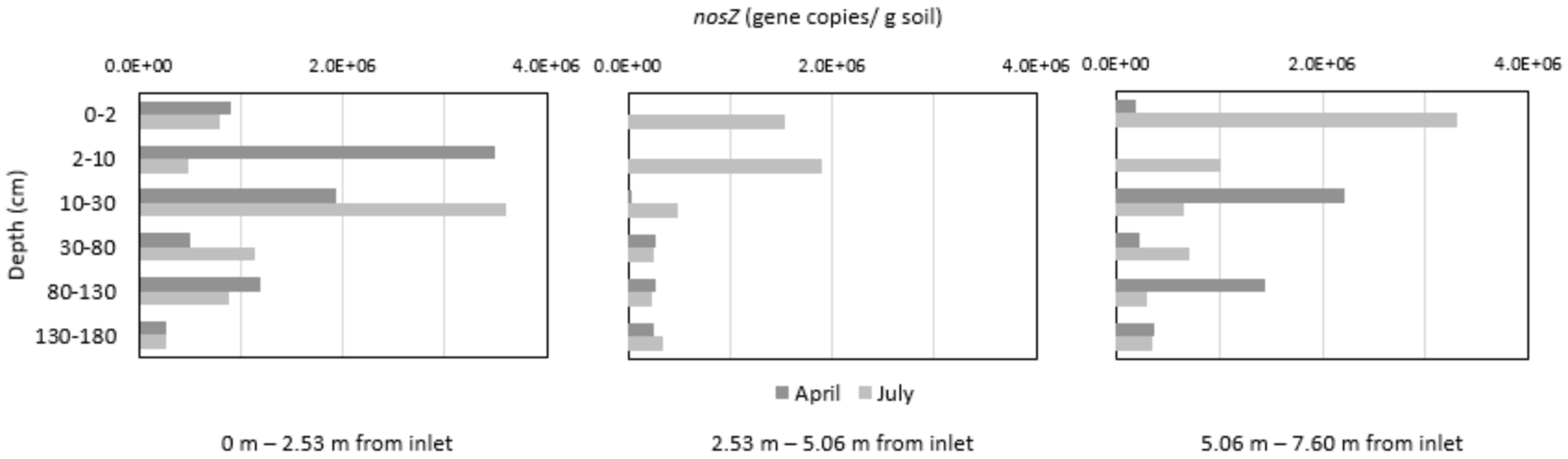

Figure 5.11. Gene copies of *nosZ* per gram of soil present in BRC at varying distances from inlet and target depths. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m.

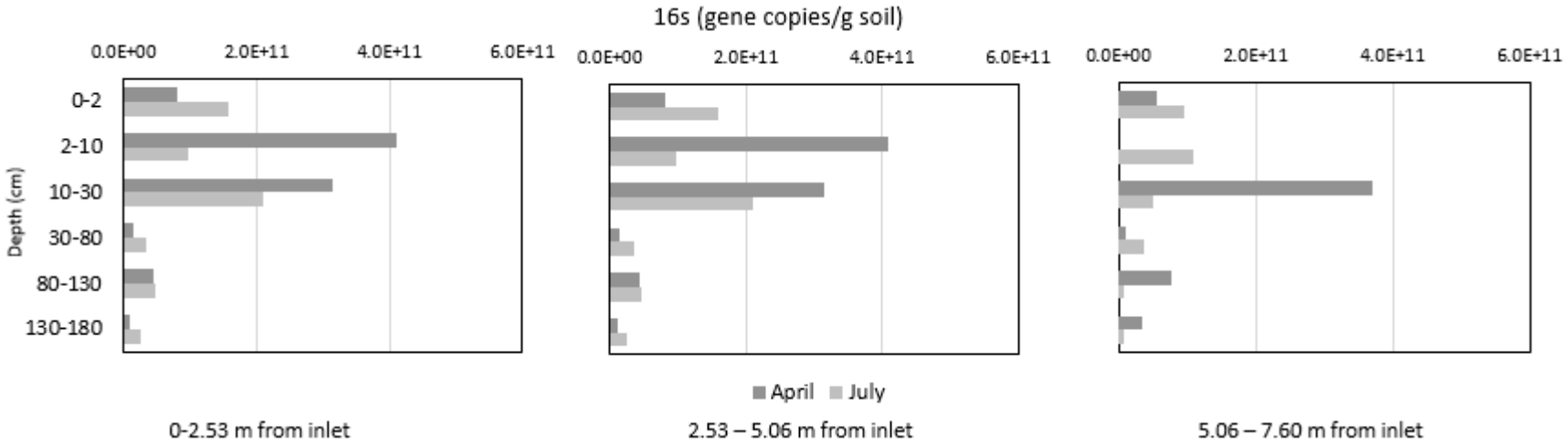

Figure 5.12. Gene copies of 16S rDNA per gram of soil present in BRC at varying distances from inlet and target depths. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m.

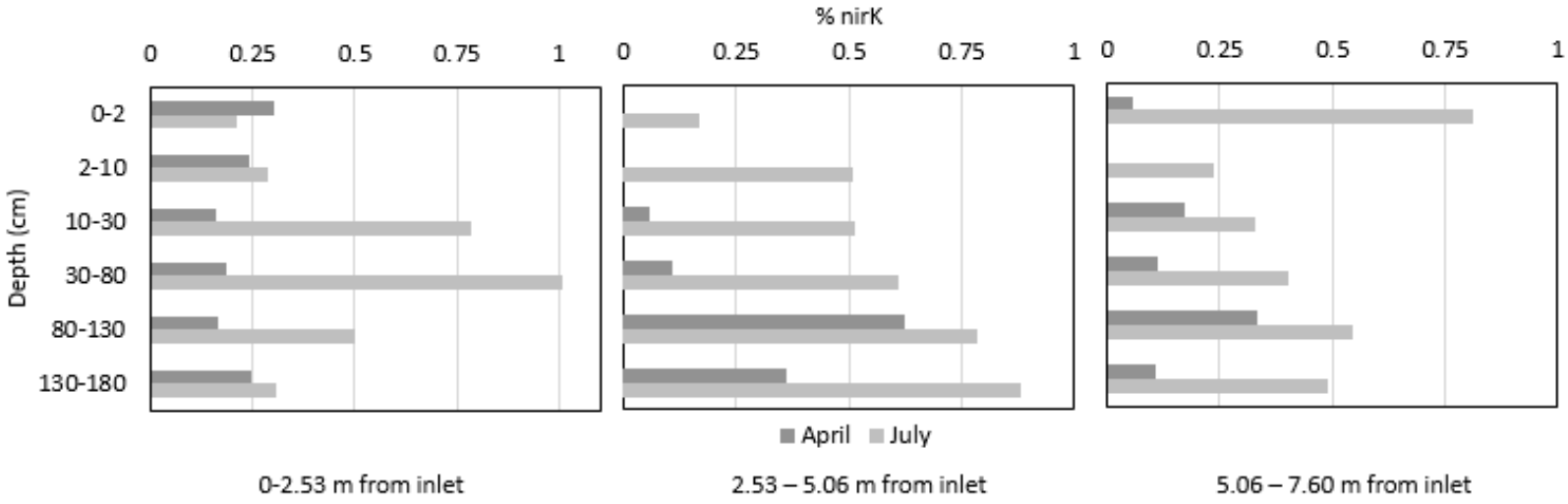

Figure 5.13. *NirK* genes present in BRC as a percentage of total 16s rDNA genes. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m.

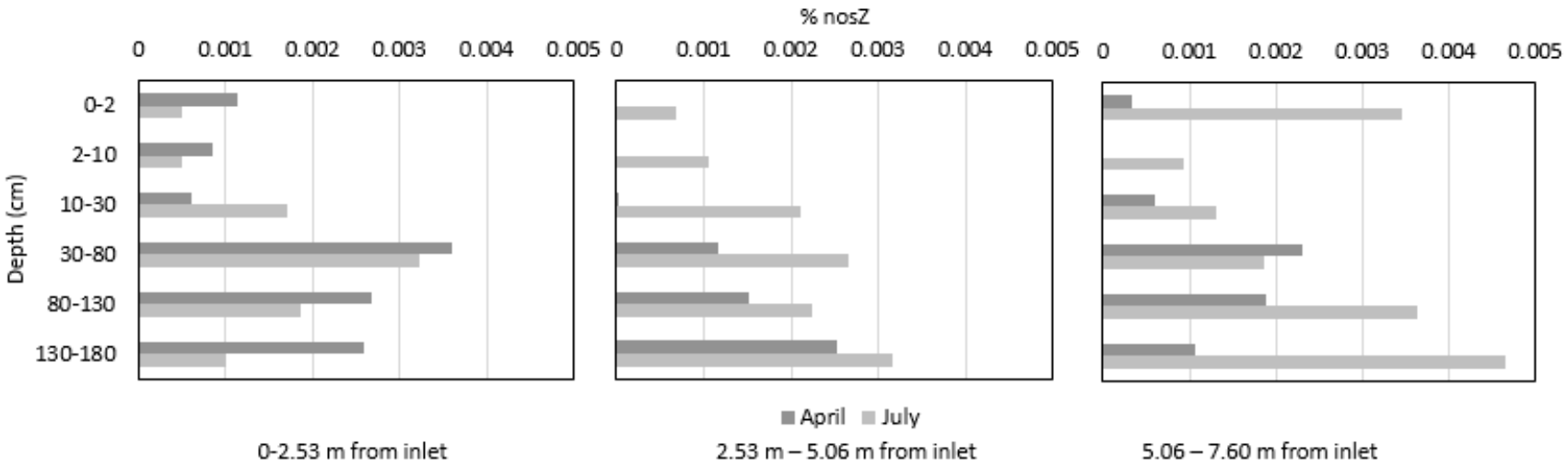

Figure 5.14. *NosZ* present in BRC as a percentage of 16s rDNA. Section A corresponds with 0-2.53 m from inlet, section B corresponds with 2.53-5.06 m, and section C corresponds with 5.06-7.60 m

5.2 Precipitation

Monitoring of the BRC inflow and outflow began on December 14th, 2013 and ended on June 4th, 2014. During this time, samples from 23 runoff events were collected for analysis with ten of those events producing outflow, as indicated in Table 5.1. Storms 8 and 9 consisted of runoff from a large snowfall event, and were therefore removed from calculations of parameters such as storm length, intensity, duration of preceding dry weather, and precipitation depth. Storms lasted for an average of 7.0 hours, with an average intensity of 2.0 mm/hr. Storms produced an average of 12.1 cm of rainfall.

During the previous study effort, the BRC was monitored from November 25th, 2007 to March 20th, 2008. Three of 21 storms sampled for analysis produced outflow. Of the sampled storms, the average storm duration was 2.6 hours, with an average intensity of 16.2 mm/hr and average precipitation depth of 0.5 cm; thus the storms during the current study were less intense but of longer duration and depth. A Mann-Whitney test confirmed that the current study period had significantly longer storms ($p = 0.0068$) with greater total precipitation ($p = 0.0162$) than the previous study. There was no significant difference in average storm intensity. A comparison of rainfall totals over the entire 2007-2008 study period, the 2013-2014 study period, and the 30-year average (1981-2010) for Blacksburg, Virginia in Figure 5.15 shows that the 2007-2008 study period was drier than average and the 2013-2014 study period was wetter than average. This difference in precipitation between the two studies was likely the reason more outflow events occurred for the current study. Additionally, as the BRC media has aged, macropores could have formed, leading to preferential flow pathways within the BRC.

As with the 2007-2008 study, most of the storms during this study period took place during the winter, which in the mid-Atlantic region is characterized by frontal systems that produce storms of longer duration and lower intensity. Summer storms are characterized by convective systems, which have lower duration and higher intensity. Figure 5.16 indicates that the earlier storm events had longer durations with lower precipitation totals. This pattern begins to shift around storm 14, which took place on March 25th, 2014. Figure 5.17 shows a similar plot from the 2007-2008 study, with a similar shift taking place between February and March.

Hydrologic and pollutant data for each storm are contained in **Error! Reference source not found.** and **Error! Reference source not found.**.

Table 5.1. Summary of storms during 2013-2014 study period. A check mark indicates a sample taken at the inflow or outflow of the BRC.

Storm	Date	Inflow	Outflow
1	12/14/2013	✓	
2	12/17/2013	✓	
3	12/23/2013	✓	✓
4	1/10/2014	✓	
5	1/11/2014	✓	✓
6	2/5/2014	✓	
7	2/15/2014	✓	
8	2/18/2014	✓	
9	2/19/2014	✓	✓
10	2/21/2014	✓	✓
11	3/3/2014	✓	✓
12	3/7/2014	✓	
13	3/17/2014	✓	
14	3/25/2014	✓	
15	4/3/2014	✓	
16	4/4/2014	✓	
17	4/7/2014	✓	✓
18	4/15/2014	✓	
19	4/29/2014	✓	✓
20	5/10/2014	✓	
21	5/15/2014	✓	✓
22	6/4/2014 AM	✓	✓
23	6/4/2014 PM	✓	✓

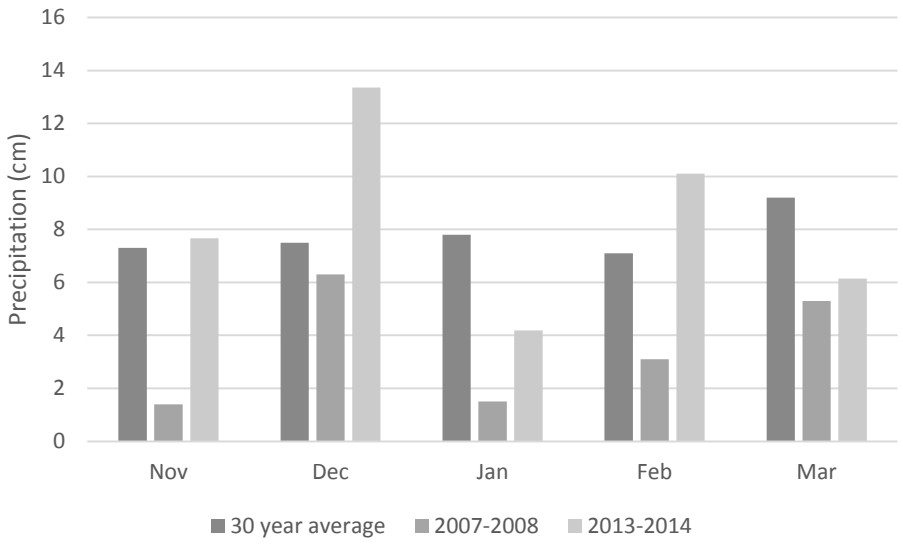

Figure 5.15. Monthly precipitation comparison of 2007-2008 study period, partial 2013-2014 study period, and 30 year precipitation (1981-2010) average of Blacksburg, VA.

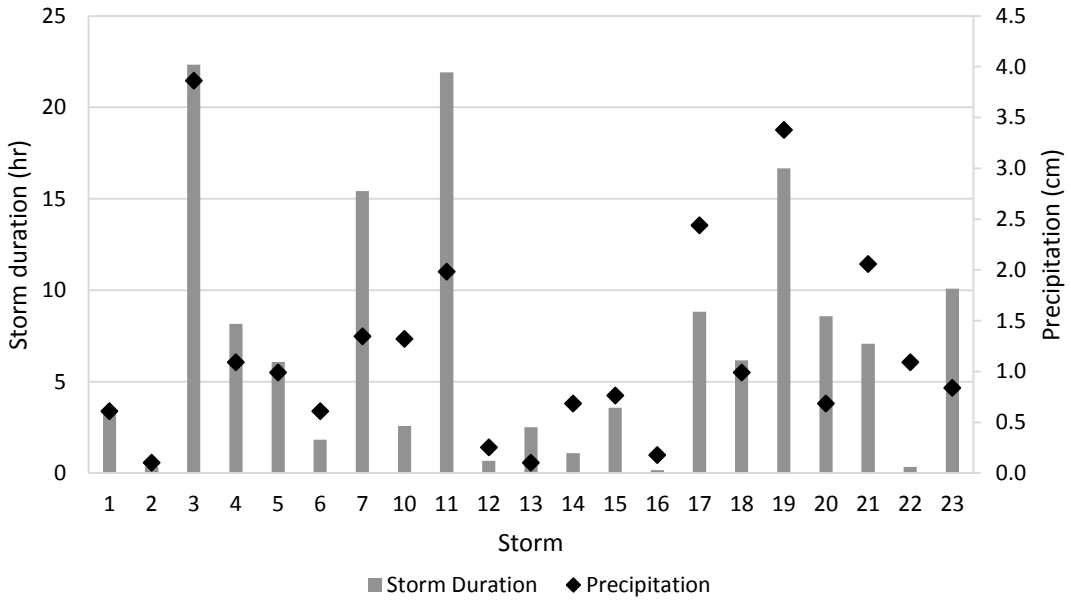

Figure 5.16. Summary of storm duration and precipitation depth during 2013-2014 BRC study period.

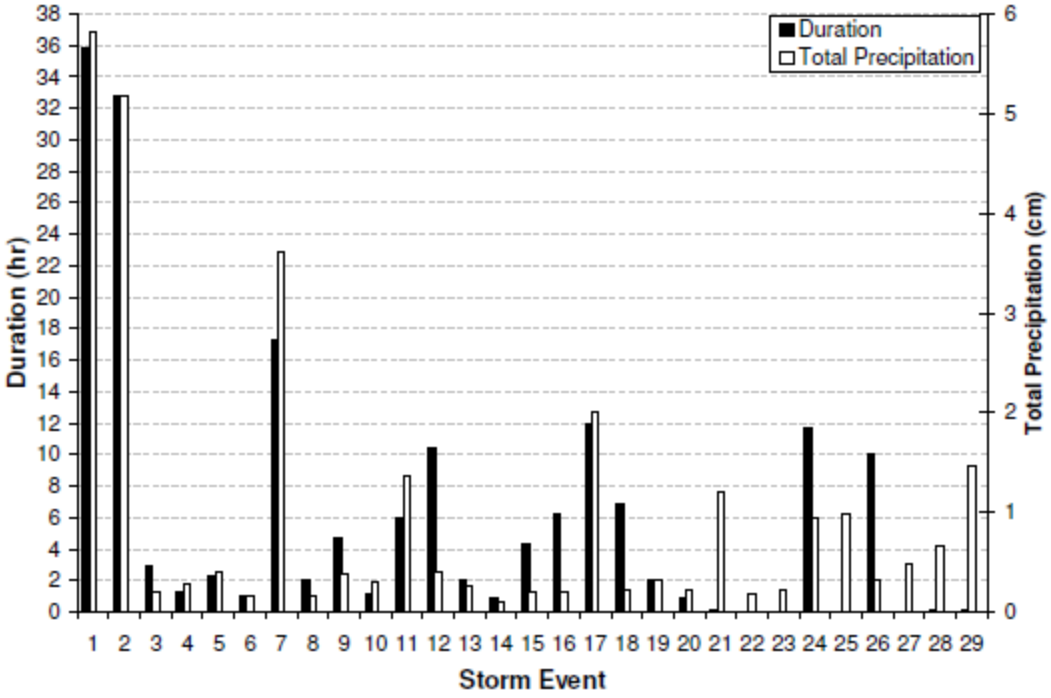

Figure 5.17. Summary of storm duration and precipitation during 2007-2008 study period. Storms 1-4, 7, 9, 24, and 26 were not used in data analysis (DeBusk, 2008).

Several storms produced outliers for multiple hydrologic parameters, as shown in Table 5.2. Outliers were defined as values that exceeded the median by 1.5 times the interquartile range (the difference between the 75th and 25th percentile), the same definition used in DeBusk, 2008. Storm 3 stood out as being an outlier in duration, total precipitation, effluent volume, and effluent peak flow. Storms 21 and 22 were also outliers in maximum and average intensity, respectively, effluent volume, and peak flow volume. All of the outliers were in the high range of values except for the outlier for influent peak flow (storm 7), which had a much lower value than the rest of the peak flow data. As outflow occurred during 14% and 43% of the storm events in the 2007-2008 study and this study, respectively, it is clear that outflow only occurs under certain hydrologic conditions. Although there was no set threshold for precipitation, intensity, or antecedent precipitation data, all storms producing outflow had an average intensity higher than approximately 1 mm/hr.

Table 5.2. Storms producing outliers during 2013-2014 sampling period.

Parameter	Storms with outliers
Duration	3, 11
Average intensity	14, 16, 22
Maximum intensity	21
Total precipitation	3, 19
Effluent volume	3, 17, 21, 22
Influent peak flow	7
Effluent peak flow	3, 5, 21, 22

Figure 5.18 shows how water moved though the BRC during storm 17 on April 7th, 2014. During this storm it took approximately 3 hours for influent runoff to reach the uphill piezometer (2.5 m from the inlet), and another 45 minutes for runoff to reach the downhill piezometer (5 m from the inlet). Peak inflow occurred about 1.5 hours before peak outflow. Water levels reached approximately 65 cm above the uphill piezometer and 60 cm above the downhill piezometer. Outflow began when water pooled approximately 50 cm above the uphill piezometer and ceased when the water level had resided to approximately 40 cm. All of these values are slightly higher than those observed by DeBusk (2008). As discussed by DeBusk, the fact that outflow ceases before all of the water has drained off the uphill piezometer indicates that it is likely leaving the BRC through the sides of the media.

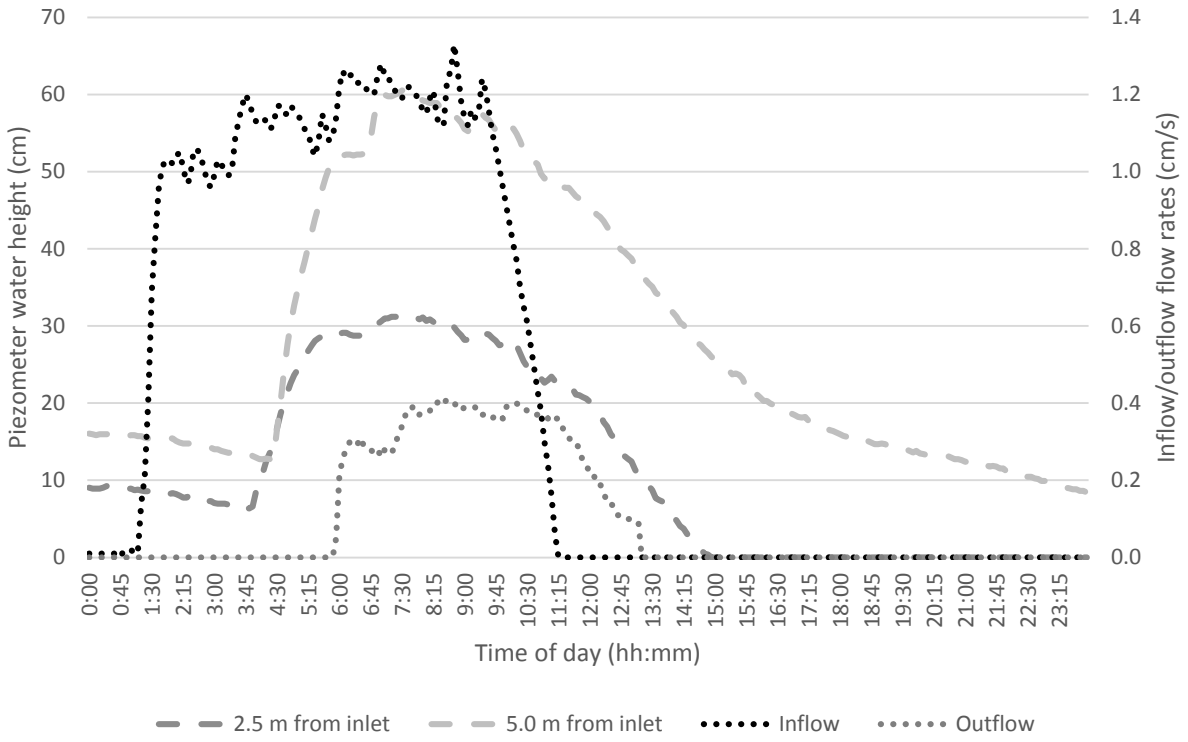

Figure 5.18. Water-level data for storm 17 (April 7th, 2014) as recorded by HOBO data loggers and ISCO stormwater samplers. The left axis corresponds with piezometer distance from inlet (2.5 m or 5.0 m) and indicates water height above the piezometers, which were located at the bottom of the BRC (1.8 m deep). The right axis corresponds with the flow observed at the inflow and outflow ISCO and data labeled "inflow" and "outflow".

5.2.1 Hydrology

5.2.2 Peak Flow Rate

Peak inflow rates for the current BRC study period ranged from 3 L/s (storm 7) to 16 L/s (storm 22), with a mean peak inflow rate of 11.3 L/s and a median peak inflow rate of 11.8 L/s. Peak outflow rates for storms with outflow ranged from 0.5 L/s (storm 23) to 12.2 L/s (storm 21), with a mean of 3.9 L/s and a median of 2.5 L/s. Figure 5.19 shows inflow and outflow peak flow rates for storms generating outflow.

The median peak flow rate reduction for the entire study period was 100%. For storms generating outflow, the mean and median peak flow rate reductions were 72.6% and 81.4%, respectively. Storm 21 had the lowest peak flow rate reduction at 19.2%. This storm was characterized by a typical average intensity (2.9 mm/hr), the highest maximum intensity (48.8 mm/hr), and average storm duration (425 min). This percent reduction may indicate that at the highest intensity, runoff did not infiltrate the BRC and flowed straight to the outlet, as was also observed by DeBusk (2008). A nonparametric Spearman's ρ correlation indicates that peak flow reduction is negatively correlated with maximum intensity ($\rho = -0.54$, $p = 0.012$) and total precipitation ($\rho = -0.73$, $p = 0.0002$) (as maximum intensity and total precipitation increase, peak flow rate reduction decreases). It is positively correlated with volume reduction ($\rho = 0.9880$, $p = 0.0001$).

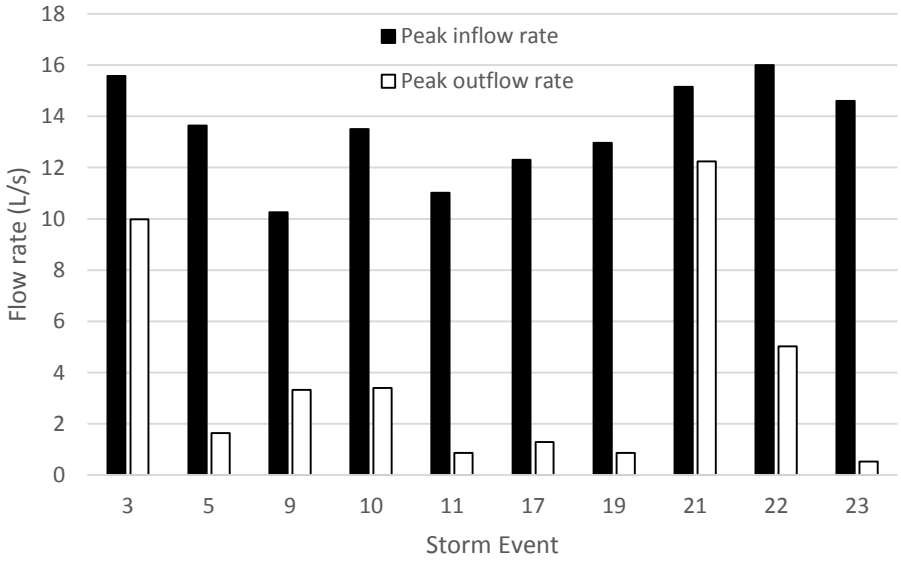

Figure 5.19. Peak inflow and outflow rates for storms producing outflow in the BRC from 2013-2014.

The previous study effort observed peak inflows ranging from less than 1 L/s to 13 L/s, with a mean of 1.5 L/s and a median of less than 1 L/s. Peak outflows for storms that generated outflow ranged from 0.01 L/s to 0.2 L/s. Median peak flow rate reduction was 100% for all

storms, and 99.3% for storms generating outflow. Figure 5.20 shows a quantile plot for peak flow rate reduction for storms producing outflow. A Mann-Whitney test indicated that there was no significant difference between percent peak flow rate reduction at construction and presently when only storms generating outflow were considered ($p = 0.091$). When all storms from both study periods are considered, peak flow rate reduction was significantly lower in the current study ($p = 0.019$). However, peak inflow and outflow rates were also significantly higher ($p < 0.0001$ and $p = 0.013$, respectively), so a lower reduction was expected.

Other studies have observed peak flow reductions ranging from 14% (Passeport et al., 2009) to 84% (Hatt et al., 2009); therefore, the Blacksburg BRC functions within the range of expected peak flow reduction when only storms generating outflow are considered. When all storms are considered, it performs well above this range, which is probably due to the loss of runoff through the sides of the BRC.

Passeport et al. (2009) observed grassed BRCs in North Carolina for peak flow reductions. Generally the BRC did reduce peak flows, which was attributed to the volume and time needed to saturate the BRC after a dry period. During a few storms, peak flow rate increases were observed from the inlet to outlet, which were attributed to short antecedent dry periods where the BRC remained saturated and large storms that falsely showed a decreased influent peak flow due to the weir causing back-up. Li et al. (2009) compiled data from studies on BRCs of varying sizes in College Park, MD, Silver Spring, MD, Louisburg, NC, and Greensboro, NC and used linear regression analyses to determine correlations between peak flow reduction and rainfall depth, duration, intensity, temperature, and antecedent dry weather periods. They observed that peak flow reduction may be a function of precipitation depth and average intensity, similar to the results observed at the Blacksburg BRC (although in Blacksburg peak flow reduction correlated with maximum intensity). Hatt et al. (2009) monitored BRCs in Australia and also observed negative correlations between peak flow rate reduction and inflow volume. Based on all of these studies, BRC design should focus on .

It is expected that a mature BRC would reduce peak flow more efficiently than a newly constructed BRC due to mature vegetation that promotes infiltration. Although the Blacksburg BRC was mowed before winter, stalks remaining after mowing were about 5 cm to 10 cm tall, and likely provided more resistance to flow than the young vegetation and mulch in the earlier

study. However, as the drought conditions during the first study likely contributed to the very high peak flow reductions, it is difficult to directly compare the two studies.

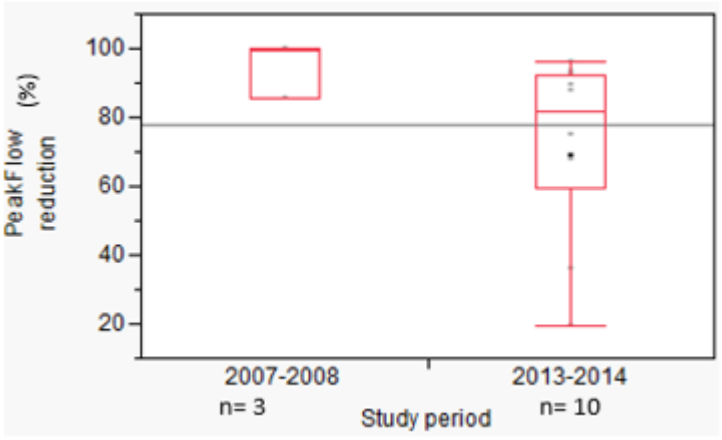

Figure 5.20. Quantile box plot comparing peak flow rate reduction in storms producing outflow in 2007-2008 and 2013-2014.

5.2.3 Flow Volume

Of the 23 storms that produced inflow to the BRC, 10 generated outflow. The BRC has a total design capacity of 25,500 L (including pore space and surface ponding volume), which was exceeded in 20 of the storm events. Inflow volumes ranged from 15,000 L (storm 2) to 570,000 L (storm 3), with a mean of 181,000 L and a median of 111,000 L. Outflow volumes from storms generating outflow ranged from 630 L (storm 23) to 149,000 L (storm 3), with a mean of 39,100 L and a median of 14,200 L. Figure 5.21 shows the inflow and outflow volumes for each storm event (the outflow volume of storm 23 was 630 L, and therefore is not visible). Figure 5.22 shows the stormwater volume reductions due to the BRC.

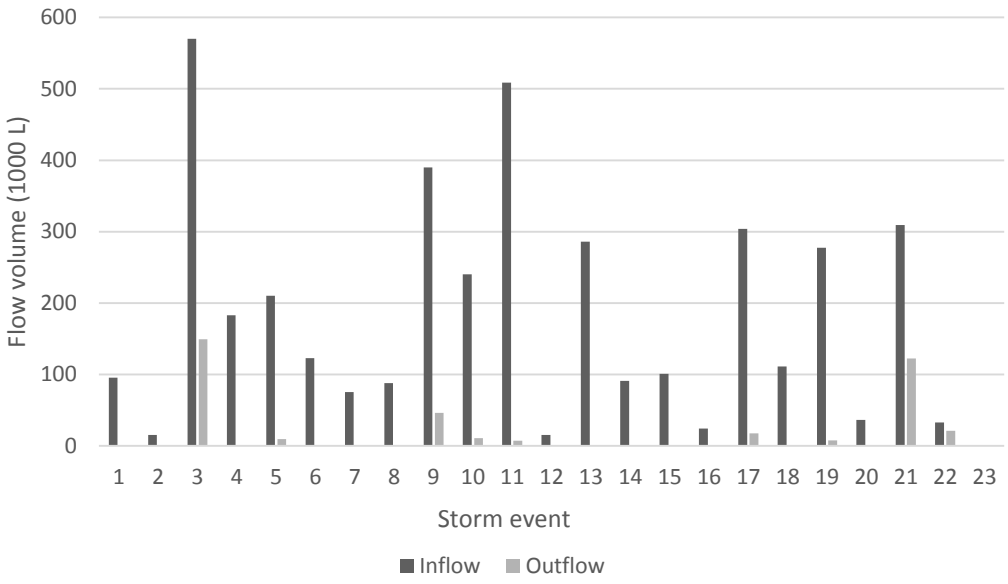

Figure 5.21. Inflow and outflow volumes of 2013-2014 BRC storm events.

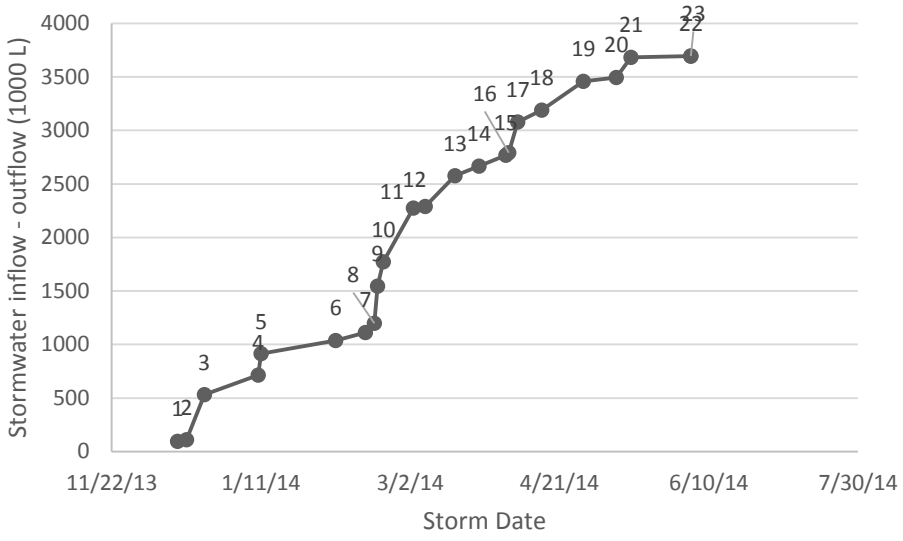

Figure 5.22. Cumulative stormwater reduction plot for BRC. Point labels indicate storm number.

Over the entire study period, 4,163,00 L entered the BRC and 391,400 L exited the BRC. The cumulative stormwater runoff volume reduction by the BRC was 91%. The median volume reduction based on all storms was 100%. For storms generating outflow, the mean volume reduction was 95% and the median volume reduction was 84%. Storm 22 had the lowest volume reduction percentage at 37%. This storm was also characterized by the highest average intensity of 33 mm/hr, suggesting the BRC stormwater volume reduction performance is dependent on the storm intensity; therefore, the ability of BRCs to reduce runoff will likely vary with climate and season. A nonparametric Spearman's ρ correlation ($\alpha = 0.05$) indicates that percent volume reduction is negatively correlated with the maximum intensity of a storm event ($\rho = -0.53$, $p = 0.013$) and total precipitation ($\rho = -0.73$, $p = 0.001$) (as maximum intensity and total precipitation increase, percent volume reduction decreases). These results are expected, because the BRC has a set volume capacity and may not be able to hold all of the precipitation from a large storm. Precipitation occurring during high intensity storms would infiltrate through the BRC media

quickly, so it also makes sense that these storms would be more likely to generate outflow quickly.

Ermilio and Traver (2006) also observed a strong negative correlation between influent runoff volume (which is a function of precipitation depth) and overall reduction while collecting data from a BRC over several years. Li et al (2009), as discussed with peak flow reduction, also analyzed volume reduction using regression analyses based on studies in Maryland and North Carolina. The analyses indicated that percent volume reduction may be a function of rainfall depth. As observed at the Blacksburg BRC, antecedent dry weather, storm duration, and average rainfall intensity appeared to have no correlation with volume and peak flow reduction. Li et al. (2009) also developed boundaries for cell storage depth (the maximum precipitation depth a BRC can hold before generating outflow) and cell storage intensity (the maximum intensity a BRC can handle before generating outflow). Data from every site show that the BRCs can hold more precipitation as storm duration increases, but all had different specific thresholds. Therefore, BRC design should continue to focus on using regional climate data with an emphasis on precipitation depth as a first design consideration and intensity as a second consideration for flow volume reduction.

In ten of the 13 storms that did not generate outflow, the total inflow volume exceeded the BRC's capacity of 25,500 L by 700 L to 260,000 L. Although plant uptake may contribute to increased water storage, it seems more likely that water was lost via seepage through the sides of the BRC (DeBusk, 2008). Because of adjacent utility lines, the BRC was constructed as a cube; the bottom of the cell is flat and the sides are nearly vertical. Therefore, the clay lining is present only on the bottom of the cell and water stored in the BRC can infiltrate the surrounding soils through the sides. A cell constructed with all sides lined would ensure no water seepage.

The previous study observed inflow volumes ranging from less than 1 L to 9,700 L with a mean inflow volume of 1,743 L and a median inflow of 800 L. Outflow volumes ranged from less than 1 L to 468 L for the three storms with outflow. The cumulative water balance for the initial study included 36,600 L entering the BRC and 492 L exiting the BRC, indicating a cumulative volume reduction of 98.7%. The median volume reduction for all storms was 100%, and the median volume reduction for storms producing outflow was 98.7%. A Mann-Whitney test indicated that there was not a significant difference between the volume reduction for storms generating outflow in the previous study and the current study, although the sample sizes are

small for this comparison, especially since only three storms produced outflow in 2007-2008. When all storms are considered, the runoff volume reduction was significantly lower seven years after BRC construction ($p = 0.019$). However, given the differences in precipitation and the fact that inflow and outflow volume were significantly lower in the prior study ($p < 0.0001$ and $p = 0.013$, respectively), the comparison is not necessarily meaningful.

Figure 5.23 shows a quantile box plot comparing volume reduction of storms that generated outflow during the previous and current studies.

The Blacksburg BRC remains effective at reducing stormwater runoff volume. Other studies (taking place over four months to two years) observed flow reductions ranging from 7% to 54% (Hunt et al., 2006; Hatt et al., 2009) and little decrease in BRC infiltration (Le Coustumer, 2009). Bioretention is largely accepted as an effective BMP to reduce stormwater volume, and this study does not indicate that performance of the Blacksburg BRC has significantly declined, especially considering the differences in precipitation during the two study periods. Further monitoring to compare flow reduction to storm events more similar to the 2007-2008 study would help determine a more accurate change in BRC performance; however, given the difficulty of predicting dry vs wet years, implementing such a study would be difficult.

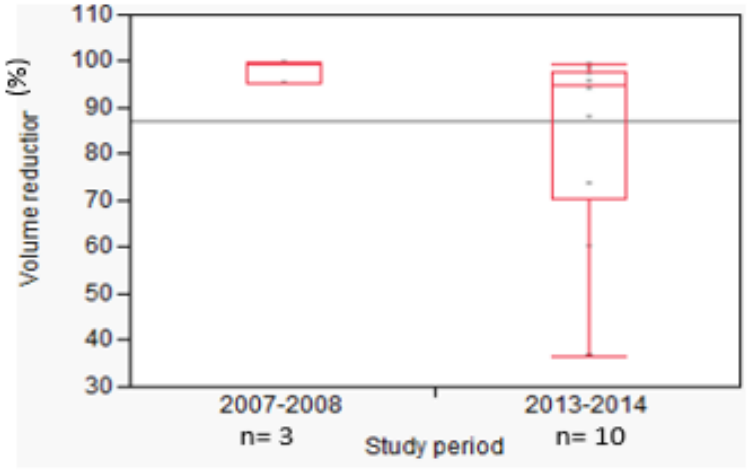

Figure 5.23. Quantile box plot comparing volume reduction from storms generating outflow in 2007-2008 and 2013-2014.

5.2.4 Hydrology Summary

Seven years post-construction, the BRC is still effective at reducing flow volume and peak flow rates. A majority (57%) of storms did not generate outflow. The BRC reduced the cumulative flow volume by 90.6%, and for storms that produce outflow the median volume reduction is 83.9%. Although this is lower than volume reductions observed from 2007-2008, this present study period comprised a period of higher than average precipitation for the region, while the 2007-2008 study occurred during a drought period. High volume reductions may also be attributed to water loss via seepage through the sides of the cell.

Peak flow rate reduction from 2013-2014 remains high as well, with median reduction at 100% for all storms and 81.4% for storms with outflow. This is lower than average peak flow rate reduction for 2007-2008, but the smaller sample size and drought period likely contributed to high reductions during the initial study.

Comparing both study periods using a Mann-Whitney test reveals a significant difference in both volume reduction and peak flow reduction when every storm (including those with no outflow) is included. When only storms generating outflow are considered, there is no significant difference in either volume or peak outflow rate reductions, although the sample size is particularly small.

This study observed that total precipitation depth and maximum intensity are negatively correlated with volume and peak flow reduction. Data from storm 22 indicate that very high average intensity may influence volume reduction as well, although more data are needed to make a conclusion. Similar results have been observed across the east coast (Li et al., 2009; Passeport et al., 2009) and Australia (Hatt et al., 2009). All of the studies indicated a correlation with total precipitation depth, suggesting that regardless of media type, vegetation, and any other environmental factors, BRC design should first be based on the precipitation amount that a municipality or other group aims to reduce.

5.3 Impacts on Water Quality

5.3.1 Total suspended sediment

TSS inflow concentrations for the current BRC study period ranged from 1.6 mg/L (storm 8) to 296 mg/L (storm 15) with a mean concentration of 69.0 mg/L and a median concentration of 46.7 mg/L. TSS outflow concentrations ranged from 0 mg/L (storms 5 and 9) to 23.8 mg/L (storm 3), with a mean of 7.1 mg/L and a median of 4.25 mg/L. TSS influent concentrations were significantly greater than effluent concentrations assessed using a Mann Whitney test ($p = 0.004$).

TSS inflow mass loads for the current BRC study period ranged from 140 g (storm 8) to 29800 g (storm 15) with a mean TSS load of 9570 g and a median TSS load of 5590 g. TSS loads for storms with outflow ranged from 0 g (storms 5 and 9) to 3560 g (storm 3), with a mean of 488 g and a median of 37 g. Figure 5.24 shows influent and effluent loads for each storm event. Figure 5.25 shows the accumulation of sediment within the BRC due to storm runoff over this study. Influent TSS mass loads were significantly greater that effluent loads for all storms ($p < 0.0001$) and storms generating outflow ($p = 0.002$) when analyzed using a Mann Whitney test.

The cumulative TSS reduction by mass for the entire study period was 98%. The median TSS reduction for the entire study period was 100%. For storms generating outflow, the median and mean TSS reductions were 100% and 96%, respectively. Storm 3 had the lowest TSS reduction at 70%. Storm 3 was an outlier for storm duration, total precipitation, effluent volume, and effluent peak flow. A nonparametric Spearman's ρ correlation indicates that TSS reduction is negatively correlated with maximum intensity ($\rho = -0.49$, $p = 0.025$), total precipitation ($\rho = -0.75$, $p < 0.0001$), inflow volume ($\rho = -0.47$, $p = 0.024$), outflow volume ($\rho = -0.82$, $p < 0.0001$), peak inflow rate ($\rho = -0.68$, $p = 0.0004$), and peak outflow rate ($\rho = -0.84$, $p < 0.0001$). In storms producing outflow, only peak outflow rate is correlated with TSS reduction ($\rho = -0.69$, $p = 0.028$). Therefore, high precipitation amounts have a great influence on TSS reduction because they are more likely to generate outflow, reducing TSS removal. In storms that do produce outflow, high peak outflow rates are negatively correlated with TSS reduction. This inverse relationship may be due to more intense storms transporting higher sediment loads and more

turbulent flow throughout the BRC that has enough energy to carry sediment, or overflow that exits the cell untreated.

Immediately post-construction, TSS inflow mass loads ranged from 0.03 g to 3,867 g, and TSS outflow mass loads ranged from 0.0 g to 15.2 g. Mean and median inflow loads were 500 g and 68.5 g, and mean outflow loads were 0.8 g. The cumulative reduction during this period was 99.8%. Inflow TSS loads were significantly greater in the 2013-2014 study ($p = < 0.0001$). However, there was no significant difference in outflow loads or TSS reduction for the two studies.

These data suggest that even after seven years, the Blacksburg BRC reliably reduces TSS concentrations to a significant degree. This continued performance may be due to the maturity of the cell; the vegetation has grown enough to slow runoff and to prevent media clogging through its root system as Emerson and Traver (2008) observed while studying a six-year-old BRC for two years to assess infiltration rates in a mature BRC. Li and Davis (2008b) determined in a field study that most incoming fine particles settle within the first 20 cm, so as long as a BRC is not clogged it should still remove TSS through settling. Davis (2007) observed poor TSS removal on two side-by-side BRCs that were monitored from 3 months post construction to approximately 1.5 years post construction. The author attributed initial increases in effluent TSS concentration to fines in the media mix washing out after construction, but one of the BRCs also experienced increases in TSS concentration late in the study, while the other did not. The two cells were identical other than a 0.3 m anaerobic zone (which also made this cell deeper) installed in the cell that exhibited better TSS removal. The Blacksburg BRC media contained washed sand, which likely contributed the >99% TSS reduction post-construction. Hunt et al. (2006) also observed mass TSS export of 170% in a BRC less than one year old, but Hatt et al. (2009) observed mass removals over 80% on more mature BRCs. Based on these studies, it seems that using a washed media, such as the Blacksburg BRC did, will reduce initial TSS effluent concentrations, and over time BRC TSS removal for cells using unwashed media may improve as long as clogging does not occur.

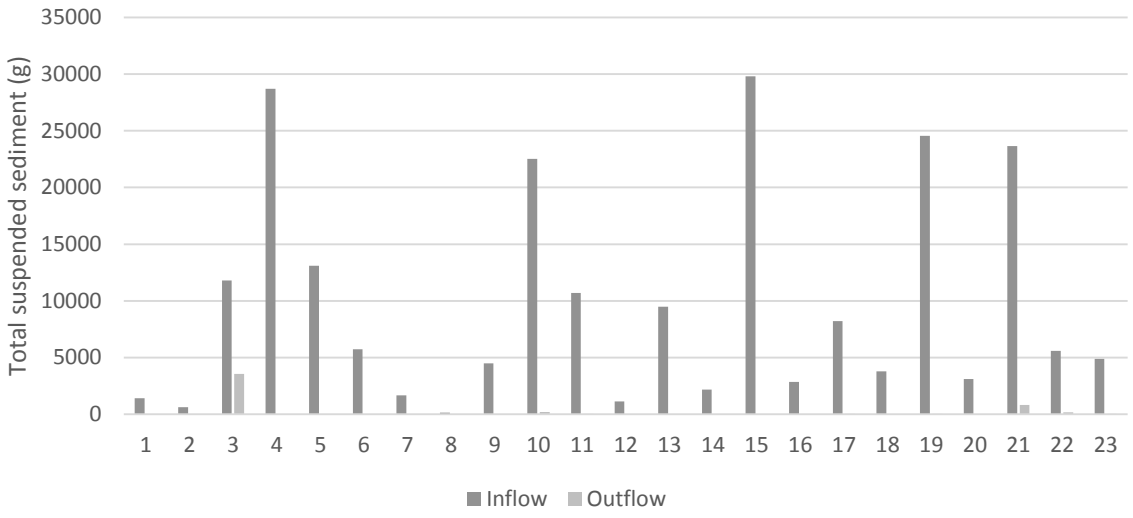

Figure 5.24. Inflow and outflow sediment load for each storm event.

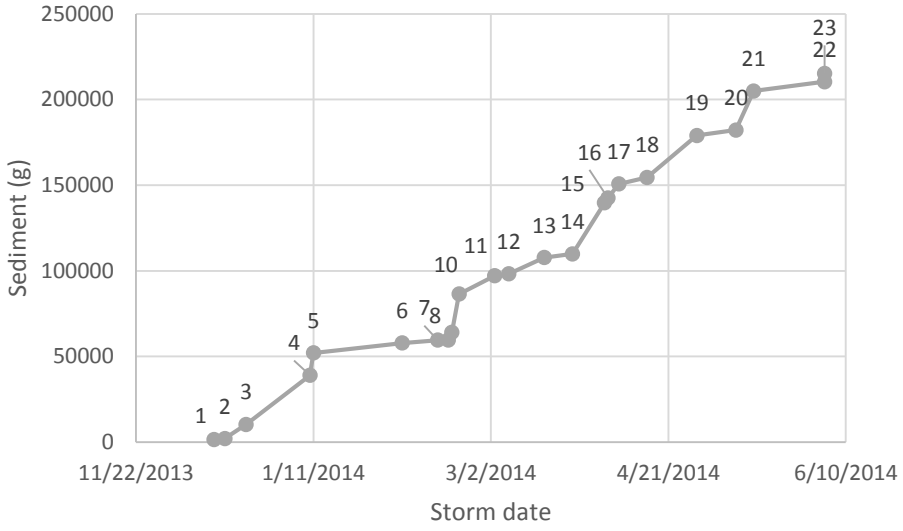

Figure 5.25. Cumulative sediment plot for BRC.

5.3.2 Nitrogen

Due to concerns related to laboratory QA/QC checks, storms 12, 21, and 22 were removed from TN performance analyses. TN inflow concentrations for the current BRC study period ranged from 0.27 mg/L (storm 3) to 4.07 mg/L (storm 15) with a mean concentration of 0.96 mg/L and a median concentration of 0.75 mg/L. TN outflow concentrations for storms with outflow ranged from 0.29 mg/L (storms 17) to 1.30 mg/L (storm 10), with a mean of 0.78 mg/L and a median of 0.73 mg/L. There is no significant difference in inflow and outflow TN concentrations for storms generating outflow.

While there is no significant difference between TN concentrations for the inflow and outflow, there was a significant reduction in TN mass loads. TN inflow mass loads ranged from 13 g (storm 2) to 410 g (storm 15) with a mean TN load of 143 g and a median TN load of 103 g. TN loads for storms with outflow ranged from 0.3 g (storm 23) to 188 g (storm 3), with a mean of 33 g and a median of 6.5 g. The influent TN mass load was significantly greater than the effluent mass load for all storms ($p < 0.0001$) and storms generating outflow ($p = 0.016$). Figure 5.26 shows the TN mass loads for each storm event. Figure 5.27 shows how TN from the storm events accumulated within the BRC.

The cumulative TN mass reduction for the entire study period was 91%. The median TN reduction for the entire study period was 100%. For storms generating outflow, reductions ranged from -22% to 99%. The mean and median TN reductions were 80% and 96%, respectively. Storm 3 had the lowest TN reduction at -22%, meaning that more TN left the BRC as effluent than entered during this storm event. As discussed earlier, storm 3 was an outlier for storm duration, total precipitation, effluent volume, and effluent peak flow. A nonparametric Spearman's ρ correlation indicates that TN reduction is negatively correlated with storm duration ($\rho = -0.53$, $p = 0.023$), total precipitation ($\rho = -0.71$, $p = 0.001$), inflow volume ($\rho = -0.72$, $p = 0.0004$), outflow volume ($\rho = -0.99$, $p < 0.0001$), peak inflow rate ($\rho = -0.53$, $p = 0.016$), and peak outflow rate ($\rho = -0.995$, $p < 0.0001$). In storms producing outflow, TN reduction is correlated with outflow volume ($\rho = -0.81$, $p = 0.015$) and peak outflow rate ($\rho = -0.93$, $p = 0.0007$).

During the 2007-2008 study, influent TN concentrations ranged from below detection to 7.2 mg/L, with mean and median values of 2.7 mg/L and 2.2 mg/L, respectively. Outflow

concentrations ranged from below detection to 5.8 mg/L, with mean and median of 3.8 mg/L and 5.3 mg/L, respectively. TN inflow loads ranged from 0.0 g to 40 g with a mean of 4.4 g and median of 1.3 g. Outflow loads ranged from 0 g to 0.13 g, with a mean of 0.01 g. The cumulative reduction was 99.7%. Total nitrogen loads were significantly greater in the current study for both inflow ($p < 0.0001$) and outflow ($p = 0.036$), due to the large increase in runoff volume. Comparing TN reduction of storms generating outflow, there was no significant difference between current reductions and reductions immediately post construction. When all storms are considered, TN removal was significantly greater in 2007-2008 ($p = 0.049$). However, when storm 3 of the current study was removed (the TN reduction of -22% during storm 3 represented the only export of nitrogen), there was no significant difference in TN reduction percentage.

Cumulative TN load reduction for this study was higher than most field studies, which observed TN reductions ranging from -7% (Hatt et al., 2009) to 54% (Passeport et al., 2009) in cells aged 1 to 3 years. Hatt et al. (2009) observed three BRCs different media and varying levels of organic matter (two BRCs contained no OM, one contained 10% compost and 10% hardwood mulch by volume), but all constructed in parallel and receiving the same stormwater influent. No significant difference in nitrogen removal was observed among the three BRCs, and there was no significant difference in influent and effluent NO_x concentrations. Since NO_x is soluble and does not sorb to soil, the authors suggested that nitrogen removal is mostly influenced by plant species present and denitrification occurring in the cell, and these are important in overall BRC design. Passeport et al. (2009) studied two BRCs in North Carolina that contained 0.18% humic matter, but one contained clay overlaying loam while the other contained loam overlaying clay. The BRC with clay as the bottom layer removed nitrogen species better (data showed both removed TN equally well), likely due to the IWS layer staying saturated due to reduced hydraulic conductivity from the clay.

Considering that the Blacksburg media has experienced a 94% reduction in nitrogen content, it seems that nitrogen accumulation within the cell is negligible. Also, unlike the 2007-2008 study, effluent TN concentrations are lower than influent TN concentrations. This may be a result of the BRC aging; there is no longer any excess TN from BRC media (i.e. polymers on the washed sand) that can be exported in outflow and the vegetation and microbial communities are mature enough to impact TN. Soil media results indicate that microorganisms with the *nirk*

and *nosZ* genes are present in the bottom 30 cm of the BRC for denitrification to occur, and in a higher relative percentage than at the top layer of the BRC, indicating conditions are likely favorable for denitrifiers to survive, and to contribute to nitrogen removal within the BRC.

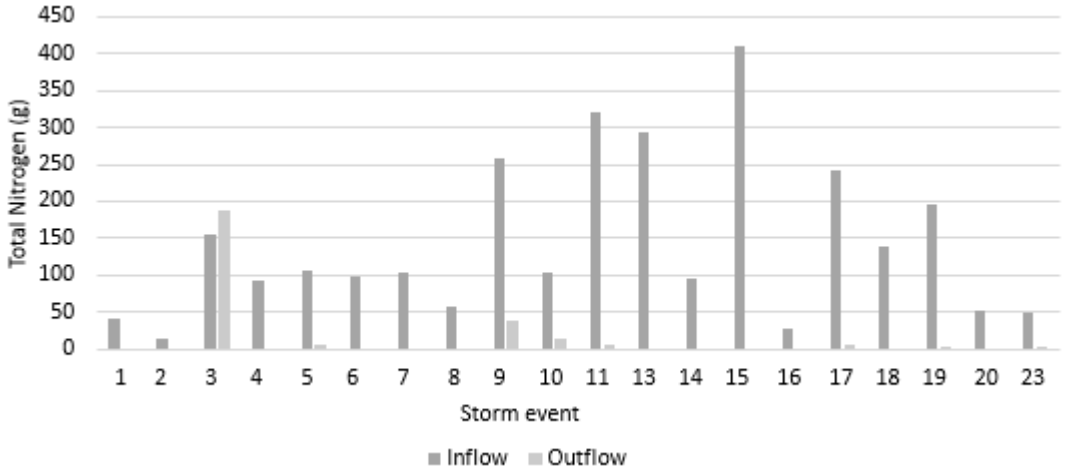

Figure 5.26. Influent and effluent total nitrogen mass loads.

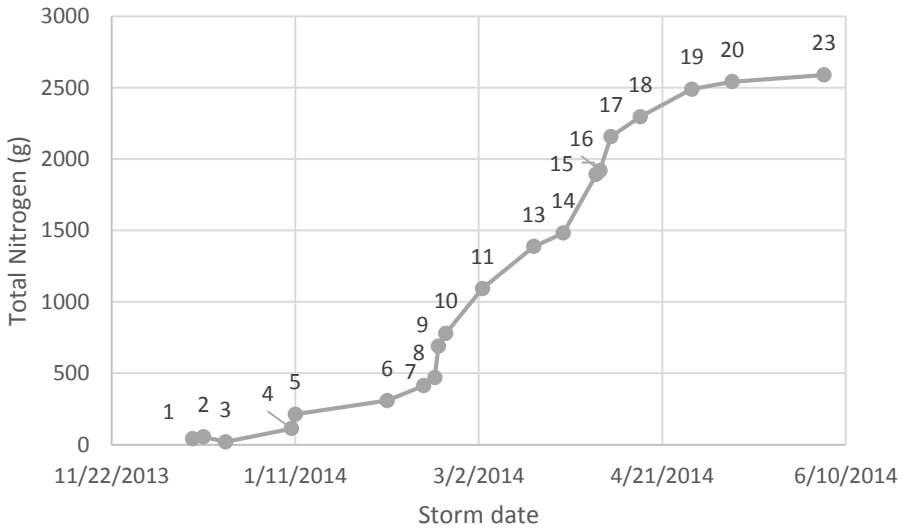

Figure 5.27. Nitrogen accumulation within BRC during study period.

5.3.3 Phosphorus

TP inflow concentrations for the current BRC study period ranged from 0.01 mg/L (storm 6) to 0.38 mg/L (storm 15) with a mean concentration of 0.08 mg/L and a median concentration of 0.04 mg/L. TP outflow concentrations for storms with outflow ranged from 0.02 mg/L (storms 17) to 0.32 mg/L (storm 3), with a mean of 0.09 mg/L and a median of 0.05 mg/L. Inflow and outflow TP concentrations were not significantly different.

TP inflow mass loads ranged from 0.3 g (storm 6) to 87 g (storm 11) with a mean TP load of 14.2 g and a median TP load of 5.5 g. TP outflow loads for storms with outflow ranged from 0.0 g (storm 23) to 48 g (storm 3), with a mean of 6.6 g and a median of 0.5 g. Figure 5.28 shows the phosphorus mass loads for each storm event. Figure 5.29 shows the accumulation of phosphorus due to stormwater runoff during this study. Overall the mass of TP entering the BRC was significantly greater than TP exiting the cell ($p = 0.0005$). When only storms generating outflow are considered, influent mass loads were not significantly different than effluent loads.

The cumulative TP reduction for the entire study period was 81%. The median TP reduction for the entire study period was 100%. For storms generating outflow, the mean and median TP reductions were 59% and 95%, respectively. Storm 3 had the lowest TP reduction at -180%, meaning that more TP left the BRC as effluent than entered during this storm event. As discussed earlier, storm 3 was an outlier for storm duration, total precipitation, effluent volume, and effluent peak flow, and possible untreated overflow likely contributed to high pollutant loads. This is similar to TN results, suggesting that media hold nutrients until a large storm flushes them from the cell. These results are comparable to studies that suggest that all pollutant removal is most closely negatively correlated outflow volume (Hatt et al., 2009; Li et al., 2009). A nonparametric Spearman's ρ correlation indicates that TP reduction is negatively correlated with storm duration ($\rho = -0.52$, $p = 0.027$), total precipitation ($\rho = -0.70$, $p = 0.001$), inflow volume ($\rho = -0.67$, $p = 0.001$), outflow volume ($\rho = -0.98$, $p < 0.0001$), peak inflow rate ($\rho = -0.59$, $p = 0.006$), and peak outflow rate ($\rho = -0.99$, $p < 0.0001$). In storms producing outflow, TP reduction is correlated with outflow volume ($\rho = -0.79$, $p = 0.021$) and peak outflow rate ($\rho = -0.92$, $p = 0.001$).

The 2007-2008 study observed influent TP concentrations ranging from 0.1 mg/L to 5.0 mg/L with mean and median concentrations of 2.7 mg/L and 2.2 mg/L, respectively. Effluent concentrations ranged from 0.1 mg/L to 2.3 mg/L with mean and medians of 3.8 mg/L and 5.3 mg/L. Cumulative reduction was 99.6%. The inflow mass load of TP during the 2013-2014 study was significantly greater ($p < 0.0001$), but the outflow mass load was not significantly different. TP removal was significantly lower ($p = 0.049$) for the two studies unless storm 3, which had a removal rate of -180, was removed from the statistical analysis.

Other field studies reported phosphorus reduction ranging from -398% to 86% (Hatt et al., 2009), with most reductions ranging from 60% to 86%. Hatt et al. (2009) attributed high phosphorus effluent loads to the media used, which contained dissolved forms of phosphorus, and the fact that Australian plants are adapted to low P environments. The high phosphorus reduction seen in the Blacksburg BRC can be attributed to the flow reductions. Studies attributed reductions in TP loads to vegetation (Lucas and Greenway, 2008), microbial uptake (Henderson, 2009), and clays mixed with a sandy media (Lucas and Greenway, 2011). As with TN concentrations, the observed TP concentrations were reduced from inflow to outflow in the current study, but not in the study immediately post-construction. Given that phosphorus was only exported during one storm event, it seems that the mature cell is able to utilize most of the TP from stormwater and likely exports TP after accumulation over a few months. A study over several years is needed fully assess how TP is transported through the BRC.

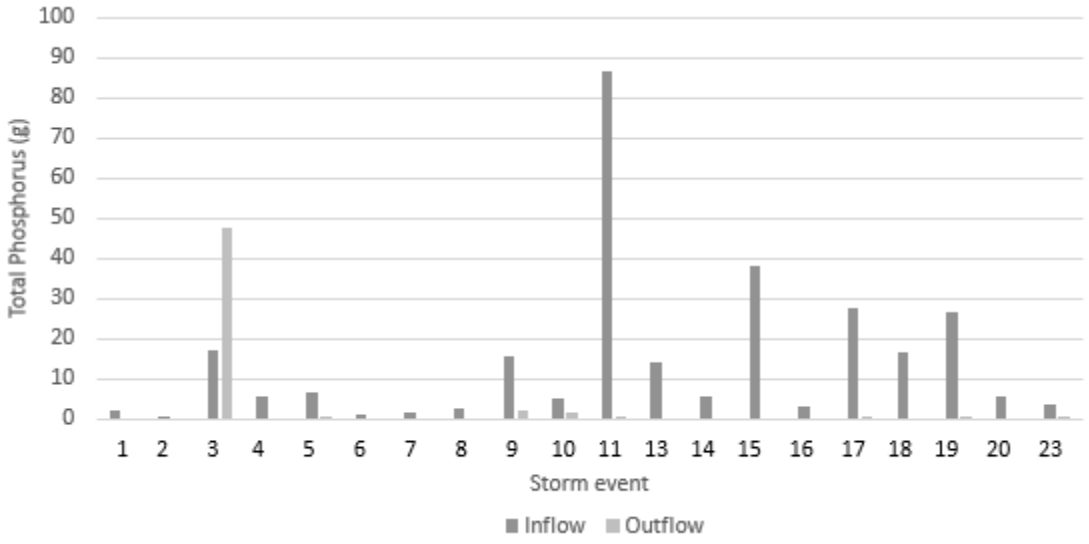

Figure 5.28. Influent and effluent total phosphorus mass loads.

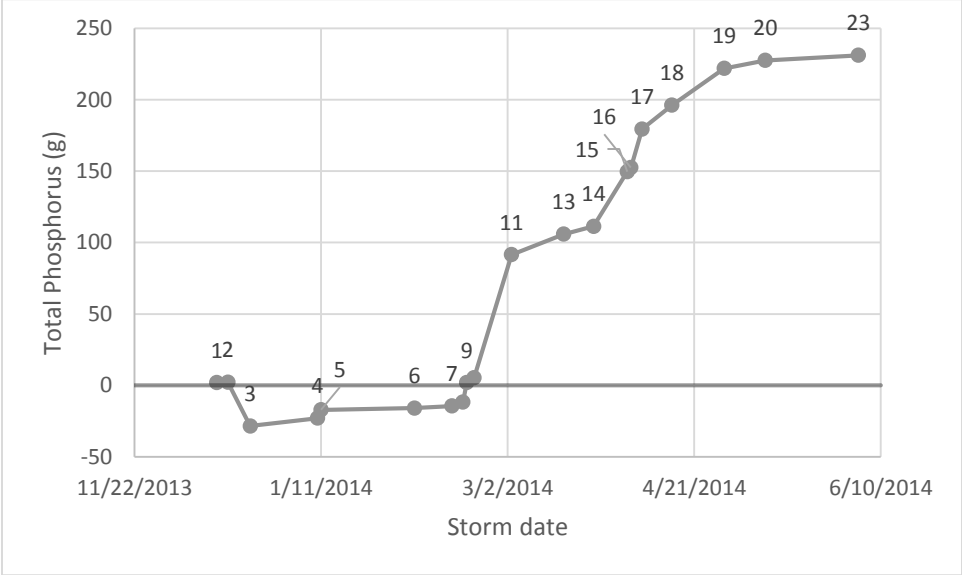

Figure 5.29. Accumulation of total phosphorus in BRC due to stormwater runoff.

5.3.4 Total coliforms

Total coliform inflow concentrations for the current BRC study period ranged from $4.68x10^2$ MPN/100 mL (storm 13) to above the limit of detection of $3.15x10^5$ MPN/100 mL (storms 20, 21, 23) with a mean concentration of $4.83x10^4$ MPN/100 mL and a median concentration of $3.53x10^3$ MPN/100 mL. Values above the limit of detection for all FIB were reported as the limit of detection, and therefore statistical values are likely lower than actual values. Coliform concentrations for storms with outflow ranged from $7.47x10^2$ MPN/100 mL (storm 11) to $1.68x10^5$ MPN/100 mL (storm 22), with a mean of $3.74x10^4$ MPN/100 mL and a median of $2.70x10^3$ MPN/100 mL. There was no significant difference between inflow and outflow total coliform concentration in storms generating outflow.

Coliform inflow mass loads ranged from $9.6x10^7$ MPN (storm 12) to over $9.7x10^{11}$ MPN (storm 21) with a mean load of $6.7x10^{10}$ MPN and a median load of $1.0x10^{10}$ MPN. Coliform loads for storms with outflow ranged from $5.3x10^7$ MPN (storm 11) to $3.7x10^{10}$ MPN (storm 21), with a mean of $7.9x10^9$ MPN and a median of $5.8x10^8$ MPN. Total coliforms entering the BRC were significantly higher than total coliforms exiting the BRC for all storms ($p < 0.0001$) and for storms generating outflow ($p = 0.002$). These values were not log transformed for any FIB due to concern with total load rather than concentration. Figure 5.30 contains the influent and effluent total coliforms for each storm event. Figure 5.31 shows the accumulation of total coliforms within the BRC from 2013-2014. This figure does not take into account any growth or decay that the populations may experience, however.

The cumulative coliform reduction for the entire study period was 95%. The median coliform reduction for the entire study period was 100%. For storms generating outflow, the mean and median coliform reductions were 74% and 98%, respectively. Storm 22 had the lowest coliform reduction at -92%. Storm 22 was an outlier for average intensity, effluent volume, and effluent peak flow. A nonparametric Spearman's ρ correlation indicates that coliform reduction is negatively correlated with maximum intensity ($\rho = -0.53$, $p = 0.013$), total precipitation ($\rho = -0.72$, $p = 0.0003$), inflow volume ($\rho = -0.56$, $p = 0.005$), outflow volume ($\rho = -0.98$, $p < 0.0001$), peak inflow rate ($\rho = -0.65$, $p = 0.0007$), and peak outflow rate ($\rho = -0.99$, $p < 0.0001$). In storms producing outflow, coliform reduction is correlated with outflow volume ($\rho = -0.82$, $p = 0.004$) and peak outflow rate ($\rho = -0.87$, $p = 0.001$). Therefore, the large effluent

volume and high peak flow resulted in poor coliform reduction, possibly due to overflow occurring. Leaving dead vegetation in a BRC is one way to increase flow resistance, which promotes infiltration and makes overflow less likely.

It is worth nothing that most FIB studies focus on concentration reduction rather than total load reduction. For this study, the mean and median total coliform concentration reductions were 65% and 100%, respectively. For storms generating outflow, the median concentration reduction was 51%. Passeport et al. (2009) and Hathaway et al. (2009) both observed high fecal coliform concentration reductions (85% - 95%), and this study has comparable total coliform reduction. When performance evaluations are based on concentration reductions for individual storms, results indicate that BRCs are not effective at reducing total coliforms; however, analyses based on FIB load indicate that BRCs are effective. Since the TMDL studies are based on total load rather than concentration, load reduction efficiency is more relevant for water quality management, especially for fecal indicator bacteria, where toxicity is not a concern as it is for metals and organics.

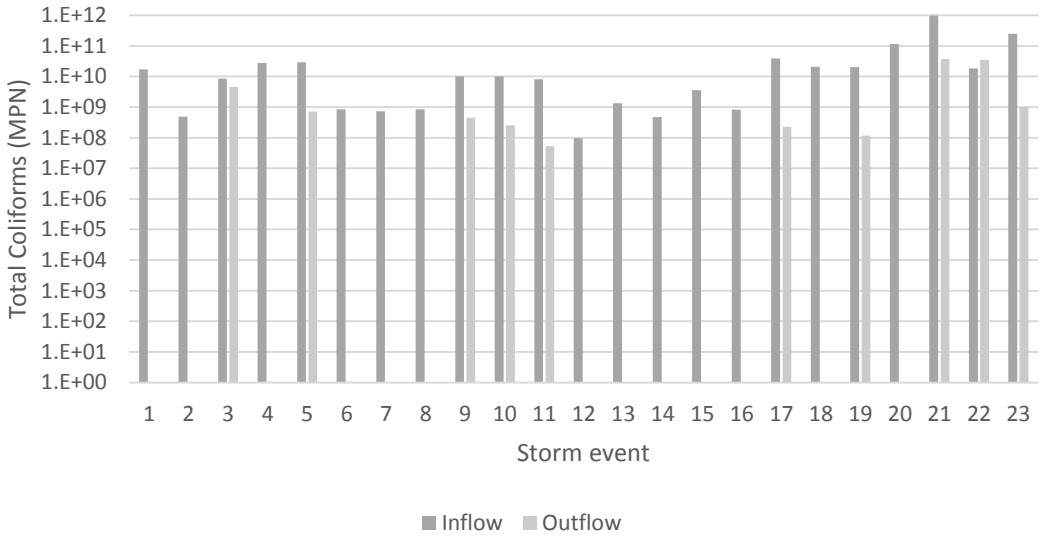

Figure 5.30. Influent and effluent total coliform MPN.

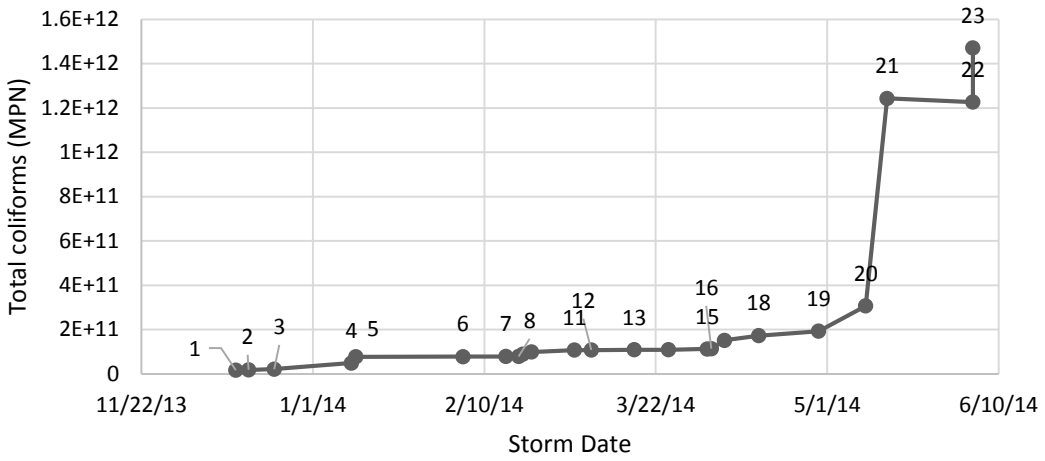

Figure 5.31. Total coliform content during 2013-2014 study period based on mass influent and effluent of viable organisms.

5.3.5 Escherichia coli (*E. coli*)

E. coli inflow concentrations for the current BRC study period ranged from 0 MPN/100 mL (storms 1, 2, 7, 8, and 12) to 1.92×10^3 MPN/100 mL (storm 21) with a mean concentration of 1.58×10^2 MPN/100 mL and a median concentration of 5.2 MPN/100 mL. *E. coli* concentrations for storms with outflow ranged from 3.1 MPN/100 mL (storms 17 and 19) to 4.70×10^2 MPN/100 mL (storm 3), with a mean of 75.1 MPN/100 mL and a median of 26.2 MPN/100 mL. For storms with outflow, there was no significant difference between influent and effluent *E. coli* concentrations ($p = 0.084$).

E. coli inflow mass loads ranged from 0 MPN (storms 1, 2, 7, 8, and 12) to 5.9×10^9 MPN (storm 21) with a mean load of 5.7×10^8 MPN and a median load of 1.1×10^7 MPN. *E. coli* effluent loads for storms with outflow ranged from 1.2×10^5 MPN (storm 23) to 7.0×10^8 MPN (storm 3), with a mean of 7.6×10^7 MPN and a median of 5.2×10^6 MPN. Figure 5.32 shows the total influent and effluent *E. coli* for each storm. Figure 5.33 shows the accumulation of *E. coli* within the BRC during the current study period, although it does not account for growth and decay of the bacteria population. For storms with outflow, influent mass loads were significantly higher than effluent mass loads ($p = 0.004$).

The cumulative *E.coli* reduction for the entire study period was 94%. The median *E. coli* reduction for the entire study period was 100%. For storms generating outflow, the mean and median *E. coli* reductions were 76% and 91%, respectively. Storm 9 had the lowest *E. coli* reduction at -42%, which was likely due to the large effluent volume and high peak flow rate of the storm. A nonparametric Spearman's ρ correlation indicates that *E. coli* reduction was negatively correlated with maximum intensity ($\rho = -0.46$, $p = 0.036$), total precipitation ($\rho = -0.68$, $p = 0.0007$), inflow volume ($\rho = -0.58$, $p = 0.004$), outflow volume ($\rho = -0.96$, $p < 0.0001$), peak inflow rate ($\rho = -0.61$, $p = 0.002$), and peak outflow rate ($\rho = -0.95$, $p < 0.0001$). In storms producing outflow, *E. coli* reduction did not correlate with any other observations.

The high *E. coli* reduction is similar to reductions observed in column studies by Bright et al. (2010) and Bratieres et al. (2008) and a field study by Hathaway et al. (2009), although these other observations were based on concentration, not "mass"/total, reductions. Hathaway observed that a shallow BRC performed poorly with regard to *E. coli* concentration reduction compared to a deeper BRC in Wilmington, NC, which was attributed to the shallow BRC staying wet due to a smaller bowl volume and larger contributing runoff area. The Blacksburg BRC reduced mean and median *E. coli* concentrations by -18% and 100%, respectively, on a storm-by-storm basis. For storms generating outflow, median *E. coli* concentration reduction was 58%. These reductions are all low because several storms had very low influent and effluent concentrations that resulted in high percent export. Once again, load reductions are preferable for assessing BRC performance.

Figure 5.34 shows the inflow and outflow concentrations of *E. coli* from the study period and compares the values to the Virginia Water Quality Standards. The *E. coli* standards state that the geometric mean of a water body with four weekly samples per month shall not exceed 126 MPN/100 mL and a single sample shall not exceed 235 MPN/100 mL (SWCB, 2011). During this study, three inflow concentrations (13% of inflow samples) and one outflow concentration (10% of outflow samples) exceeded the single sample standard, indicating that *E. coli* is a concern in urban stormwater and bioretention can be an effective way to reduce *E. coli* before storm runoff enters waterways.

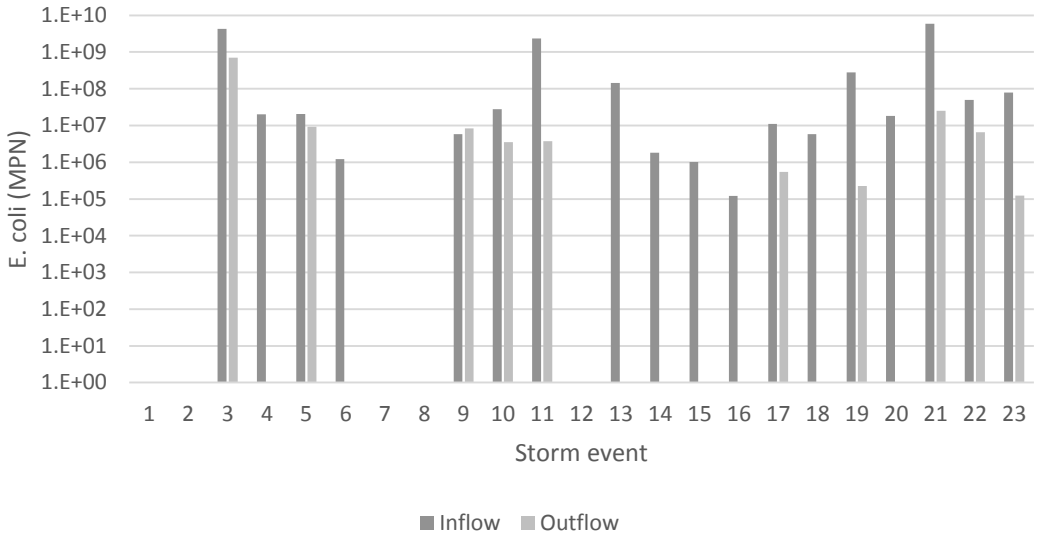

Figure 5.32. Influent and effluent *E. coli* MPN.

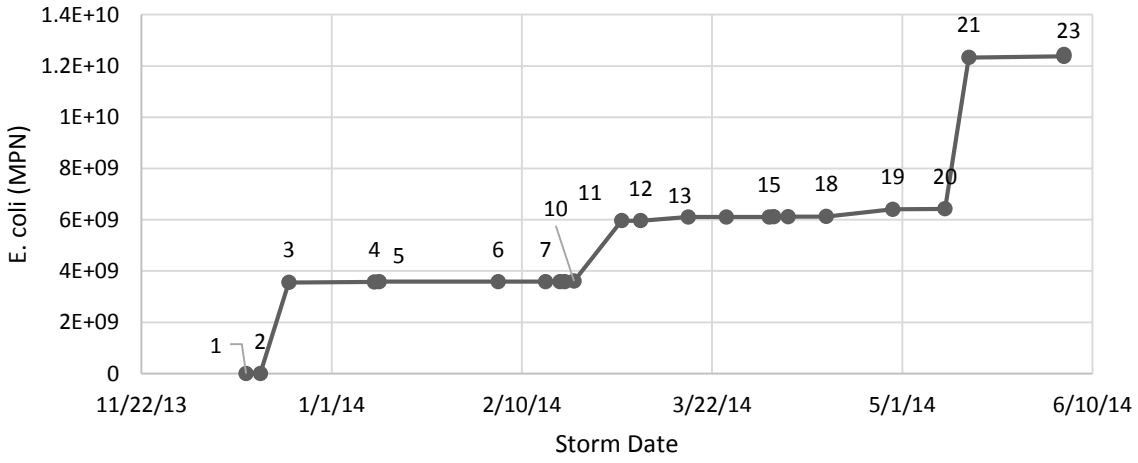

Figure 5.33. *E. coli* content during 2013-2014 study period based on mass influent and effluent of viable bacteria.

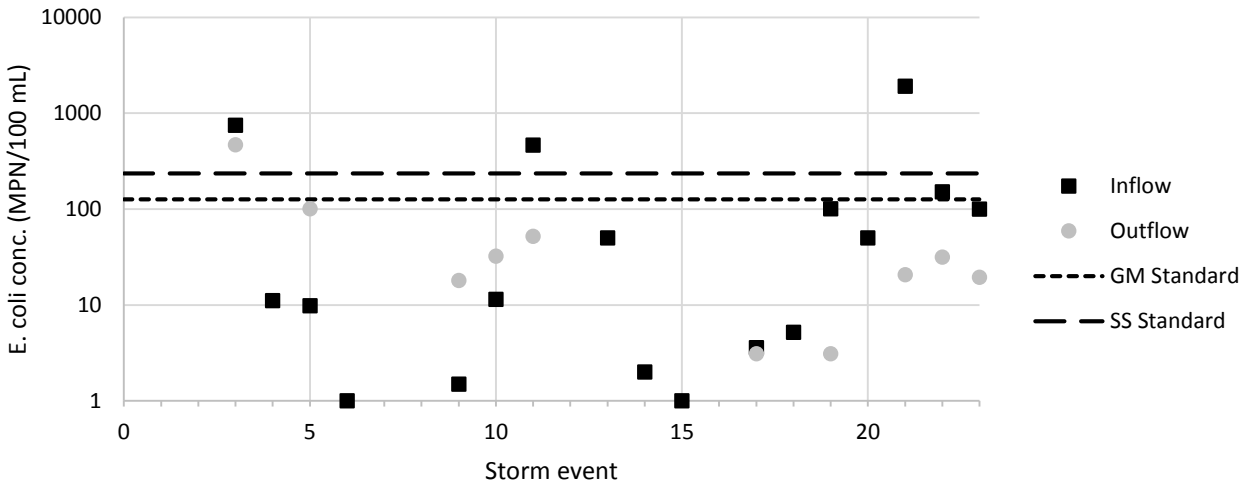

Figure 5.34. Inflow and outflow *E. coli* concentrations of storm events compared to Virginia Water Quality Standards for geometric mean (GM) standards and single sample (SS) standards.

5.3.6 Enterococci

Enterococci inflow concentrations for the current BRC study period ranged from 23.9 MPN/100 mL (storm 14) to 5.72×10^4 MPN/100 mL (storm 23) with a mean concentration of 2.88×10^3 MPN/100 mL and a median concentration of 1.52×10^2 MPN/100 mL. Enterococci concentrations for storms with outflow ranged from 19.3 MPN/100 mL (storm 17) to 1.74×10^3 MPN/100 mL (storm 3), with a mean of 3.45×10^2 MPN/100 mL and a median of 1.26×10^2 MPN/100 mL. There was no significant difference between influent and effluent enterococci concentrations for storms generating outflow.

Enterococci inflow mass loads ranged from 7.5×10^6 MPN (storm 12) to 4.5×10^{10} MPN (storm 23) with a mean load of 2.5×10^9 MPN and a median load of 2.0×10^8 MPN. Enterococci loads for storms with outflow ranged from 1.9×10^6 MPN (storm 23) to 2.6×10^9 MPN (storm 3), with a mean of 3.3×10^8 MPN and a median of 1.26×10^7 MPN. Influent enterococci MPN values were significantly higher than effluent enterococci MPN ($p = 0.002$). Figure 5.35 shows the total enterococci numbers for the inflow and outflow of each storm. Figure 5.36 shows the accumulation of enterococci within the BRC over the study period, although it does not take bacterial growth and decay into consideration.

The cumulative enterococci reduction for the entire study period was 94%. The median enterococci reduction for the entire study period was 100%. For storms generating outflow, the mean and median coliform reductions were 89% and 97%, respectively. As with other pollutants, the lowest enterococci removal rate of 55% was associated with storm 3. A nonparametric Spearman's ρ correlation indicated that enterococci reduction was negatively correlated with maximum intensity ($\rho = -0.54$, $p = 0.011$), total precipitation ($\rho = -0.74$, $p = 0.0001$), inflow volume ($\rho = -0.62$, $p = 0.002$), outflow volume ($\rho = -0.98$, $p < 0.0001$), peak inflow rate ($\rho = -0.64$, $p = 0.001$), and peak outflow rate ($\rho = -0.98$, $p < 0.0001$). In storms producing outflow, enterococci reduction was correlated with outflow volume ($\rho = -0.79$, $p = 0.006$).

Most published studies focused on enterococci concentration reduction rather than total load reduction, rendering direct comparison difficult. The mean and median enterococci concentration reduction values were 66% and 100%, respectively. In storms generating outflow, the median concentration reduction was 24%. These values were within the range Hathaway et al. (2009) observed in Wilmington, NC. As with *E. coli* in Hathaway et al.'s study, enterococci concentration reduction was lower in a shallow BRC, probably due to the wetter conditions in the small cell. However, concentration reductions are not the most reliable way to measure BRC performance if the influent concentrations are already below a target concentration.

Figure 5.37 shows the influent and effluent enterococci concentrations as they compare to the Virginia Water Quality Standards. For enterococci in freshwater, the geometric mean standard is 35 MPN/100 mL and the single sample standard is 104 MPN/100 mL (SWCB, 2011). Only two influent (9.5%) and 1 effluent (10%) storm events had concentrations below 35 MPN/100 mL. Eleven influent (48%) and four effluent (40%) storm events had concentrations at or below 104 MPN/100 mL, the single sample standard, indicating that the majority of the water leaving the BRC does not meet Virginia surface water quality regulations.

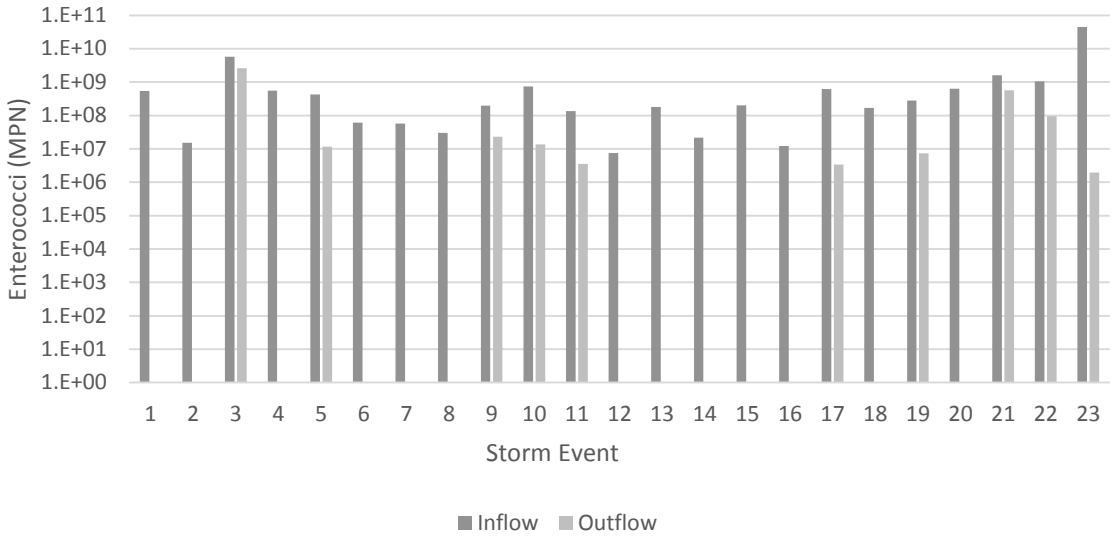

Figure 5.35. Influent and effluent enterococci loads.

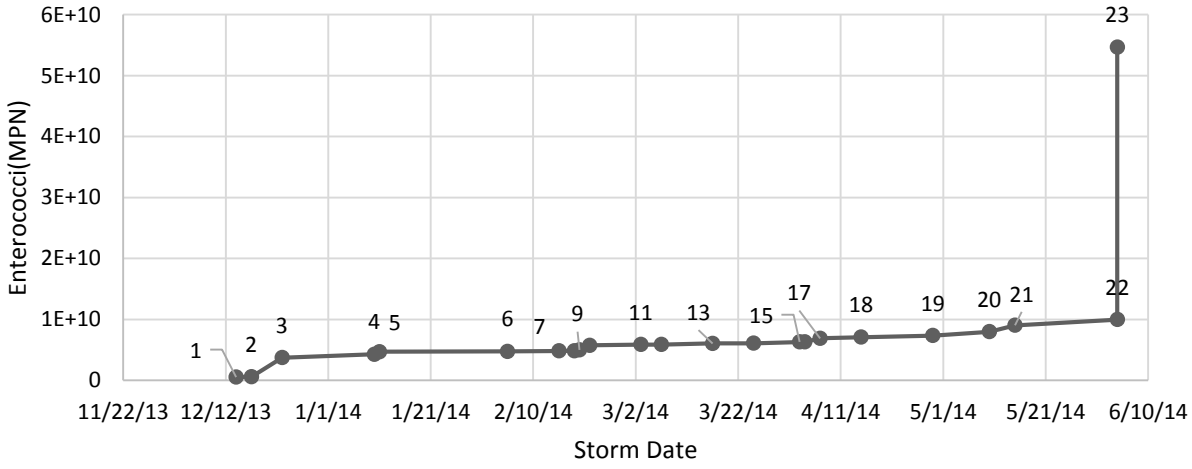

Figure 5.36. Enterococci content over 2013-2014 BRC study period based on mass influent and effluent of viable organisms.

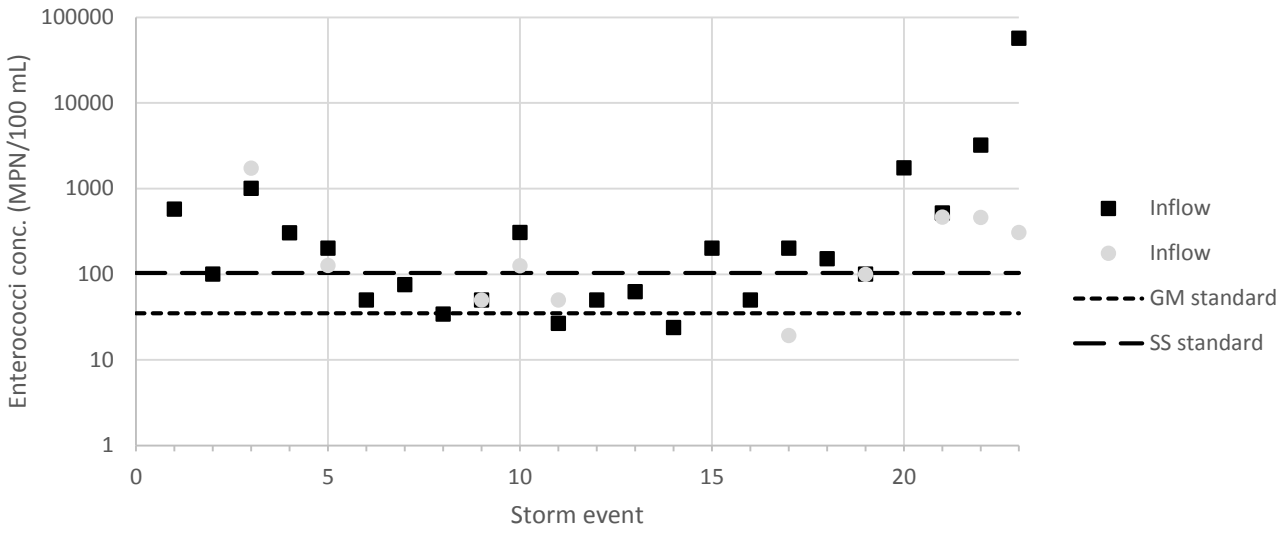

Figure 5.37. Influent and effluent enterococci concentrations from storm events compared to Virginia Water Quality geometric mean (GM) standard and single sample (SS) standard.

5.3.7 Water Quality Summary

The large hydrologic reductions in the BRC resulted in large mass reductions for all pollutants. In keeping with previous studies with observed sediment reductions ranging from 22 to 93%, the BRC was most effective in terms of TSS removal, with load reductions for individual storm events ranging from 70% to 100%, with a cumulative reduction of 98% for the study period.

While cumulative reductions over the course of the study period were high for all nutrients and bacteria (>80%), there was substantially more variability between storms, and export of nutrients and bacteria (e.g. greater quantities in the outflow) was observed for the storm 3, which could statistically be designated an outlier in terms of storm duration, total precipitation, effluent volume, and effluent peak flow. The observed variability between storms is in keeping with previous studies as well, which suggest that the removal of nutrients and bacteria is complex and less predictable.

Analysis of potential correlations between pollutant load removal and recorded climatic parameters does seem to suggest factors that could be somewhat predicative of expected performance for a single given storm. A summary of percent reductions and correlations is included in Table 5.3. All pollutant load reductions were negatively correlated with total precipitation, influent and effluent volume load, and influent and effluent peak flow rate. TSS, total coliform, *E. coli*, and enterococci reductions were also negatively correlated with maximum intensity. TN and total coliform reductions were negatively correlated with storm duration. However, when only storms with outflow were considered, most reduction rates were only correlated with effluent volume or peak flow rate, or in the cases of volume, peak flow, and *E. coli*, no correlations. Therefore, it seems that once a storm reaches the point of generating outflow, the volume of runoff, rather than the characteristics of the storm, is most critical in determining effluent pollutant concentrations.

All cumulative reductions were lower than those observed in the 2007-2008 study; however, it is important to remember that the previous study was conducted under drought conditions, and only three storms generated outflow. Differences in performance were significant for TN and TP, but not for TSS. Bacteria were not measured in the previous study.

Table 5.3. Negative correlations associated with hydrologic and pollutant % reductions in the Blacksburg BRC. Bolded items indicate a correlation for all storm events and events generating outflow. "PF" refers to peak flow rate. "V" refers to volume. "In" and "out" refer to inflow and outflow, respectively.

% Reduction	Negative correlations for all storms
Peak flow	maximum intensity, total precipitation
Volume	maximum intensity, total precipitation
TSS	maximum intensity, total precipitation, Vin, Vout, PFin, **PFout**
TN	storm duration, total precipitation, Vin, **Vout**, PF in, **PF out**
TP	maximum intensity, total precipitation, Vin**, Vout**, PF in, **PF out**
total coliforms	storm duration, total precipitation, Vin, **Vout**, PF in, **PF out**
E. coli	maximum intensity, total precipitation, Vin, Vout, PF in, PF out
enterococci	maximum intensity, total precipitation, Vin, **Vout**, PF in, PF out

6. Conclusions

Bioretention cells are designed to treat stormwater runoff from impervious areas by introducing influent runoff at the top of a box filled with selected media. It is filtered by flowing through the media to a subsurface drainage system, which eventually leads to streams and waterways in the area. This study documented that most of the treatment occurs in the top layers. In the top few centimeters of media, influent gravel, clay, and silt are deposited, TN, TP, and TC accumulate, vegetation grows, and both fecal indicator bacteria and environmental bacteria live. Due to the accumulation of sediment and nutrients at the top of the media, many are concerned that BRCs have a short design life, and media should be changed regularly to prevent clogging and nutrient exportation. This study indicates that performance of a BRC after seven years of use is not significantly different than performance immediately post-construction. However, this BRC may be unique in that it much of the water seems to leave the BRC through the sides, potentially allowing untreated stormwater to enter the environment, albeit in a pattern that mimics more natural hydrology.

Comparing current performance of the Blacksburg BRC to other research, there are several aspects that promoted long-term pollutant removal, and several that did not. The media was comprised of 88% sand, 8% clay/silt, and 4% leaf litter by volume. The high sand content caused the BRC to maintain larger pore space, so TSS are not as likely to clog the BRC as finer media. Although research indicates that media with high sand content may not be as effective at removing nutrients, less overall maintenance and slightly reduced performance may make more sense for long-term water treatment (Hatt et al., 2006; Le Coustumer et al., 2009). Li et al., (2008b) observed that TSS only settles within the top 20 cm of the BRC media, and if clogging does occur only the top media needs to be replaced.

Conversely, this media provided little carbon for denitrifying bacteria and plant use. The fact that maintenance workers at the BRC mowed the cell each year and removed leaf litter lowered the carbon content of the BRC further. This removal of vegetation should not be a standard maintenance practice, and is an example of why communication between landscapers and those responsible for stormwater maintenance is important. The lack of carbon, organic matter, and oxides and hydroxides also means there is little potential for nitrogen and phosphorus to sorb to, which may explain why media experienced high reductions of TN and TP (94% and

74%, respectively) in the media over the last seven years. The cell also may have started out with unnaturally high TN content due to polymers used to wash the sand.

Perhaps the most important factor in the large removal reductions of this BRC is that runoff most likely seeps through the BRC walls into the surrounding soil. In 10 of 13 storms not producing outflow, the bowl volume was exceeded. Many studies, including this one, have documented a negative correlation between total precipitation (which is directly related to influent flow volume) and percent hydrologic and pollutant reductions (Hatt et al., 2009; Li et al., 2009; Emilio and Traver, 2006). Although the seepage in the Blacksburg BRC reduces effluent volume from the BRC, which keeps TSS, nutrient, and bacteria loads to the stormwater system low, it is difficult to determine what pollutants are entering groundwater. Therefore, the BRC is meeting hydrologic goals by promoting infiltration and likely groundwater recharge, but is likely not meeting pollutant reduction goals. This is not an ideal scenario in Blacksburg, which has karst geologic features, or areas that have naturally high groundwater levels, such as coastal regions. In these areas, environmental treatment of pollutants after stormwater leaves the BRC would also be minimal due to low contact time with natural soils. Piezometers could be installed to track water seepage in the future.

FIB loads were also reduced due to high volume reduction and potentially the depth of the cell. Hathaway et al. (2009) observed export of bacteria in a shallow BRC that received high flow volumes and stayed saturated frequently, unlike the Blacksburg BRC. This study also indicated that the Blacksburg BRC can effectively reduce *E. coli* concentrations to meet Virginia water quality standards; however, it could not reduce enterococci concentrations to meet fresh water standards in Virginia.

Analysis of denitrifying bacteria indicate that while the IWS layer at the bottom of the BRC has fewer bacteria, the overall percentage of denitrifiers within the population is higher; conditions in the IWS may be suitable for their survival and for some denitrification to occur. However, the low carbon content and lack of total saturation of the IWS layer present a less than ideal environment for optimal denitrification. Chen et al. (2013) suggests a focus on long periods of saturation, ideal media pH, and sufficient carbon content for denitrification. The Blacksburg BRC did not meet these requirements for saturation or carbon content, and pH data were not collected. Isotopes and tracers are two methods that could be used to quantify actual denitrification, as discussed by Groffman et al. (2006).

Volume, peak flow, TSS, TN, and TP reductions were all lower in 2013-2014 than they were immediately after construction. However, it was difficult to directly compare the two studies because the initial study occurred during a drought period and the 2013-2014 study took place during a time period that received more precipitation than average. Overall, the BRC still functioned effectively after seven years, cumulatively reducing 91% of volume, 98% of TSS, 91% of TN, 81% of TP, 95% of total coliforms, 94% of *E. coli*, and 94% of enterococci. Median peak flow rate reduction was 100%.

From a regulatory standpoint, BRCs may not be able to reduce volume enough to meet predevelopment hydrologic conditions, especially for extreme storm events. For example, storm 22 in this study was characterized by the highest average intensity and had the lowest volume reduction at 37%. Recent stormwater guidelines in Washington DC require that a retention site retain a 1.2 inch rainfall event occurring over 24 hours with a 72-hour antecedent dry condition (DDOE, 2013). Depending on the maximum intensity of a rainfall event, this may be impossible. Li et al. (2009) observed a rainfall storage depth (i.e. the depth of rainfall over the contributing impervious area) and rainfall storage intensity before which outflow occurred for six BRCs across NC and Maryland. The rainfall storage depths ranged from 0.06 cm to 0.46 cm, and the rainfall storage intensities ranged from 0.007 cm/h to 0.08 cm/h. The most recent study of the Blacksburg BRC indicates a rainfall storage depth of 0.3 cm and a rainfall storage intensity of 0.08 cm/hr, both of which are comparable to Li et al.'s (2008) results. It is important to understand the limitations of bioretention and other LID technologies before assuming they will solve all stormwater management problems.

Based on the data at the Blacksburg BRC and previous studies, BRCs design should focus on treating storm events of a certain depth, because this is the most important predictor of effluent volume and ultimately effluent pollutant loads. Media should contain enough sand content to prevent clogging, yet enough organic matter, carbon, and oxides to promote denitrification and sorption of nutrients and metals to the media. Based on data from this study, a volumetric sand content of 88% is high enough that no clogging occurs, but 8% clay and 4% leaf litter was too low for nutrient sorption and denitrification. Therefore, based on this study and prior studies, a sand content between 60-70%, clay (including oxides) content of 20%, and organic matter content of 10-20% may make a more ideal media mixture. If media does become clogged, only the top 20 cm – 30 cm near the inlet should be removed for maintenance. If

denitrification is a priority, media of an optimal pH and carbon content should be included, both of which encourage microbial denitrification. Although prior design objectives have focused on including an IWS layer to promote denitrification, the data observed at the Blacksburg BRC indicate that denitrification may primarily occur in the top layers of the media, when it is inundated for short periods of time during storm events. If this is the case, it may make more sense to leave the IWS out of the design and focus on surface media conditions for denitrification. The pH levels can also affect the bioavailability of nitrogen and phosphorus in the soil. Media should not contain excess, soluble phosphorus that will leach into the environment or not be used by plants. Routine maintenance should include additions of mulch or other organic matter to promote vegetation growth and nutrient sorption. Unless clogging is an issue or stormwater influents have significantly high nutrient inputs, this study suggests that mature BRCs may not need media replacement to maintain pollutant removal over long time periods.

7. Future Research

This study highlighted the changes that have taken place in a BRC over a seven-year period, including addition of particles, changes in nutrient content, potential changes in microbial community, and cumulative reduction rates of common pollutants of concern. However, there are few field studies with which these can be directly compared. Not only are most previous BRC studies short-term, but they focus on single sample or mean concentration removals rather than total mass removal. This is important, because an examination of concentration may only suggest that certain pollutants are being exported, while a consideration of volume (on mass balance) would indicate that there is a reduction in the quantity entering the environment.

Future research should investigate the accumulation of TN, TP, and TC within BRC media in order to improve maintenance guidelines. Similarly, few studies have directly researched microbial communities within BRCs in order to determine how microbial ecology affects nutrient removal and/or processing. Studying the presence of denitrifying bacteria in mature BRCs may indicate how BRCs utilize nitrogen and what combination of vegetation, media, and inundation is best to promote denitrification. It is also important to understand how fecal indicator bacteria behave within a BRC, and if they can colonize and be exported from a BRC. While this study examined all of these aspects, it was over a short time period. Ideally, samples could be taken between storm events to assess both short-term and long-term changes in media and microbial colonization.

With increasing concerns for climate change, it is also important to understand how climate, bioretention media, and vegetation all interact for effective stormwater management. While different bioretention media have been studied on the laboratory scale, there have been few field studies focused on mature cells to assess performance. The current study only applies to one BRC in Virginia, and even a seemingly slightly differently designed BRC in another climatological region may behave differently. Research on how to design BRCs for heavy storm events (i.e. long duration and/or high intensity) may be important in the future as extreme storm events become more common.

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Appendix A. Original planting list and layout.

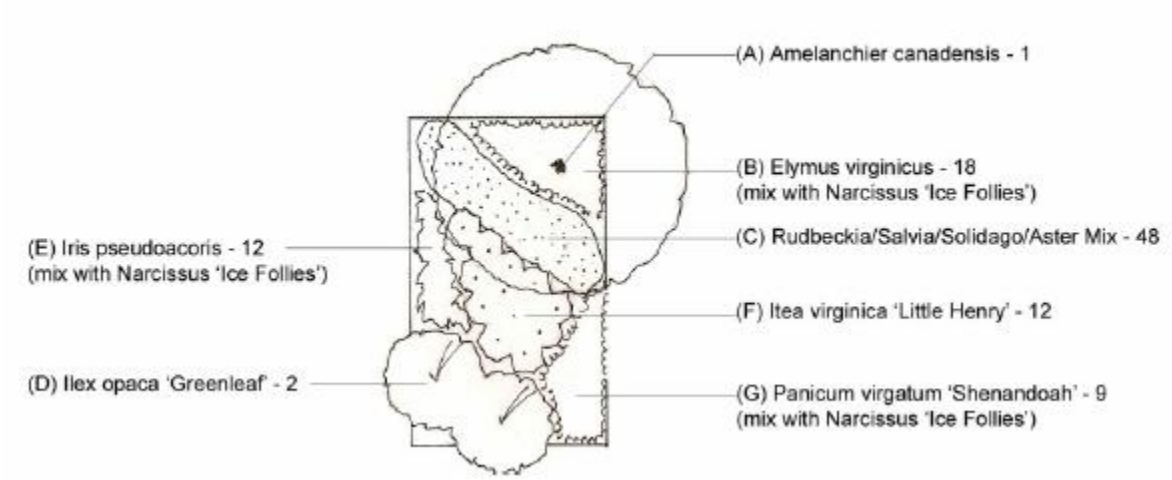

Table A. 1. Planting list and layout for bioretention cell (DeBusk, 2008).

Appendix B. Particle size distribution data

Table B. 1. Particle size distribution based on distance from inlet and depth

Distance from inlet (m)	Depth (cm)	% Clay	% Silt	% Sand	% Gravel
A (0-2.53)	0-2	1.2	8.4	77.7	12.7
	2-10	1.5	2.6	88.4	7.5
	10-30	0.4	0.6	90.3	8.8
	30-80	0.4	0.5	95.0	4.1
	80-130	0.3	0.6	95.9	3.2
	130-180	0.2	0.2	92.5	7.1
B (2.53 - 5.06)	0-2	1.5	1.3	86.1	11.2
	2-10	0.9	0.9	89.9	8.4
	10-30	0.4	0.5	92.9	6.2
	30-80	0.4	0.5	94.4	4.7
	80-130	0.3	0.3	93.1	6.3
	130-180	0.2	0.4	93.9	5.5
C (5.06-7.60)	0-2	1.0	2.9	86.0	10.2
	2-10	0.7	2.4	90.7	6.2
	10-30	0.2	0.2	94.0	5.6
	30-80	0.4	0.5	94.2	5.0
	80-130	0.4	0.6	93.0	6.0
	130-180	0.2	0.4	92.0	7.4

Appendix C. Nutrient content of bioretention media

Table C. 1. Total nitrogen and total phosphorus content of BRC during 2007-2008 study.

Source	TN (kg)	TP (kg)
Sand	165.65	1.00
Mulch 1	11.99	0.51
Mulch 2	4.28	0.18
Leaf Compost	62.32	4.08
Top Soil	5.39	1.81
Potting soil	0.26	0.05
Total	249.89	7.63

Table C. 2. Total nitrogen, total phosphorus, and total carbon content of BRC in 2013.

Distance from inlet	Depth (cm)	TN (kg)	TP (kg)	TC (kg)
A (0-2.53m)	0-2	0.05	0.01	1.92
	2-10	0.75	0.06	30.45
	10-30	1.18	0.06	53.42
	30-80	0.65	0.18	17.42
	80-130	0.68	0.18	10.39
	130-180	0.73	0.18	12.15
B (2.53-5.06m)	0-2	0.24	0.02	4.85
	2-10	0.47	0.04	10.43
	10-30	1.27	0.06	31.77
	30-80	1.75	0.18	39.14
	80-130	1.21	0.18	19.07
	130-180	1.11	0.18	22.13
C (5.06-7.6m)	0-2	0.19	0.01	4.09
	2-10	1.29	0.05	31.53
	10-30	0.83	0.06	22.97
	30-80	1.44	0.18	30.64
	80-130	0.77	0.18	12.77
	130-180	1.14	0.18	20.18
Total		15.74	1.95	375.33

Appendix D. Fecal indicator bacteria in BRC

Table D. 1. Fecal indicator bacteria in BRC in July 2013 and April 2014

Section	Depth (cm)	Total coliforms (MPN/dry g soil)		E. coli (MPN/dry g soil)		Enterococci (MPN/dry g soil)	
		April	July	April	July	April	July
	0-2	4000	4000	0	42.8	1258.9	109.4
	2-10	4000	4000	0	59.2	1556.7	81.2
	10-30	869.7	4000	0	5.8	10.1	4.7
	30-80	15.8	4000	1.1	29.7	0.5	28.8
	80-130	27.7	4000	0	9.0	0	6.2
A	130-180	19.0	4000	0	5.6	1.7	19.0
	0-2	4000	4000	0	429.8	1567.2	275.5
	2-10	4000	4000	0	17.8	1181.3	57.2
	10-30	1.1	4000	0	7.9	3.3	4.8
	30-80	2.1	2503.4	0	2.1	0	4.2
	80-130	4.4	4000	0	0	1.1	6.0
B	130-180	4.3	4000	0	3.2	0.5	30.8
	0-2	4000	4000	4.8	670.4	1578.9	79.8
	2-10	4000	4000	0	282.2	1139.9	32.2
	10-30	83.2	4000	0	7.9	2.3	1.1
	30-80	0	2532.9	0	2.1	1.0	3.2
	80-130	42.4	4000	0	18.4	2.2	4.9
C	130-180	74.3	602.8	0	2.1	1.6	0.5

Appendix E. Denitrifying genes in BRC

Table E. 1. *NirK*, *nirS*, Eubacteria, and Fungi copies per gram of soil.

Section	Depth (cm)	*nirK* (copies/g soil)		*nosZ* (copies/g soil)		Eubacteria (copies/g soil)		Fungi (copies/g soil)	
		April	July	April	July	April	July	April	July
A	0-2	2.43E+08	3.34E+08	9.07E+05	7.87E+05	8.01E+10	1.59E+11	4.05E+08	1.05E+09
	2-10	9.97E+08	2.76E+08	3.49E+06	4.90E+05	4.10E+11	9.62E+10	7.69E+08	5.86E+08
	10-30	5.07E+08	1.66E+09	1.94E+06	3.61E+06	3.15E+11	2.11E+11	3.89E+08	4.19E+08
	30-80	2.62E+07	3.53E+08	5.06E+05	1.13E+06	1.40E+10	3.50E+10	1.01E+07	1.65E+08
	80-130	7.40E+07	2.37E+08	1.18E+06	8.74E+05	4.42E+10	4.72E+10	5.05E+07	8.97E+07
	130-180	2.55E+07	7.87E+07	2.69E+05	2.61E+05	1.04E+10	2.57E+10	1.31E+07	1.72E+07
B	0-2	1.07E+05	3.85E+08		1.54E+06		2.26E+11		7.02E+08
	2-10		9.26E+08		1.91E+06		1.82E+11		4.21E+08
	10-30	5.63E+07	1.20E+08	2.87E+04	4.93E+05	9.90E+10	2.33E+10	1.62E+08	3.85E+07
	30-80	2.44E+07	5.81E+07	2.62E+05	2.52E+05	2.24E+10	9.51E+09	3.33E+07	2.10E+07
	80-130	1.15E+08	8.53E+07	2.76E+05	2.43E+05	1.83E+10	1.09E+10	2.29E+07	8.23E+07
	130-180	3.66E+07	9.51E+07	2.56E+05	3.40E+05	1.02E+10	1.08E+10	9.18E+06	1.75E+07
C	0-2	3.10E+07	7.79E+08	1.89E+05	3.31E+06	5.59E+10	9.57E+10	1.70E+08	1.31E+09
	2-10		2.63E+08		1.02E+06		1.10E+11		3.73E+08
	10-30	6.39E+08	1.63E+08	2.21E+06	6.51E+05	3.70E+11	4.96E+10	4.59E+08	2.36E+08
	30-80	1.12E+07	1.54E+08	2.30E+05	7.13E+05	9.93E+09	3.83E+10	1.36E+07	1.12E+08
	80-130	2.54E+08	4.48E+07	1.44E+06	3.00E+05	7.63E+10	8.23E+09	8.43E+07	2.13E+07
	130-180	3.70E+07	3.66E+07	3.70E+05	3.50E+05	3.47E+10	7.51E+09	3.47E+07	1.45E+07

Appendix F. Storm data

Table F. 1. Precipitation data of 2007-2008 storms.

Date	Storm	Duration (min)	Precip (cm)	Average Intensity (mm/hr)
11/26/2007	5	144	0.4	1.7
12/3/2007	6	66	0.2	1.6
12/21/2007	8	120	0.2	0.9
12/23/2007	10	60	0.3	3.1
12/28/2007	11	360	1.0	1.6
12/30/2007	12	618	0.5	0.4
1/5/2008	13	120	0.3	1.4
1/8/2008	14	48	0.1	1.8
1/10/2008	15	54	0.2	2.2
1/11/2008	16	264	0.2	0.5
2/1/2008	17	720	2.1	1.7
2/4/2008	18	420	0.2	0.3
2/6/2008	19	120	0.3	1.6
2/6/2008	20	60	0.2	2.2
2/13/2008	21	9	1.2	80.0
2/18/2008	22	9	0.2	12.0
2/23/2008	23	4.8	0.2	28.8
3/7/2008	25	9	1.0	66.7
3/14/2008	27	4.8	0.5	62.5
3/15/2008	28	18	0.7	22.0
3/19/2008	29	18	1.5	48.3

Table F. 2. Precipitation data of storms during 2013-2014 study period.

Date	Storm	Storm Length (min)	Avg intensity (mm/hr)	Max intensity (mm/hr)	Duration of preceding dry weather (hours)	Total Precipitation (cm)
12/14/2013	1	200	1.8	6.1	48	0.6
12/17/2013	2	20	3.0	3.0	76	0.1
12/23/2013	3	1340	1.7	24.4	98	3.9
1/10/2014	4	490	1.3	10.1	185	1.1
1/11/2014	5	365	1.6	18.3	15	1.0
2/5/2014	6	110	3.3	27.4	54	0.6
2/15/2014	7	925	0.9	12.2	220	1.3
2/18/2014	8					
2/19/2014	9					
2/21/2014	10	155	5.1	21.3	30	1.3
3/3/2014	11	1315	0.9	6.1	232.5	2.0
3/7/2014	12	40	3.8	6.1	92	0.3
3/17/2014	13	150	0.4	3.0	96.5	0.1
3/25/2014	14	65	6.3	12.2	160	0.7
4/3/2014	15	215	2.1	21.3	122	0.8
4/4/2014	16	10	10.7	15.2	20	0.2
4/7/2014	17	530	2.8	6.1	52	2.4
4/15/2014	18	370	1.6	12.2	192	1.0
4/29/2014	19	1000	2.0	42.7	67	3.4
5/10/2014	20	515	0.8	15.2	261	0.7
5/15/2014	21	425	2.9	48.8	110	2.1
6/4/2014 AM	22	20	32.8	33.5	144	1.1
6/4/2014 PM	23	605	0.8	18.3	4.5	0.8

Appendix G. Hydrologic data

Table G. 1. Volume and peak flow data observed in 2007-2008 study.

Date	Storm	Volume			Peak Flow		
		In (L)	Out (L)	Reduction (%)	In (L^3/s)	Out (L^3/s)	Reduction (%)
11/26/2007	5	467.8	0	100	0.7	0	100
12/3/2007	6	24.7	0	100	0.1	0	100
12/21/2007	8	512.1	0	100	0.1	0	100
12/23/2007	10	1725.8	0	100	1.5	0	100
12/28/2007	11	9782.6	468	95	1.2	0.17	86
12/30/2007	12	1420.3	0	100	1.7	0	100
1/5/2008	13	0.4	0	100	0.0	0	100
1/8/2008	14	87.2	0	100	0.2	0	100
1/10/2008	15	153.7	0	100	0.1	0	100
1/11/2008	16	800	0	100	0.6	0	100
2/1/2008	17	5548.5	22.8	100	1.2	0.008	99
2/4/2008	18	233.9	0	100	0.3	0	100
2/6/2008	19	858.9	0	100	1.2	0	100
2/6/2008	20	1633.5	0	100	2.8	0	100
2/13/2008	21	2537	0	100	1.8	0	100
2/18/2008	22	318.6	0	100	0.2	0	100
2/23/2008	23	132.3	0	100	0.3	0	100
3/7/2008	25	545.5	0	100	0.2	0	100
3/14/2008	27	3577.1	0	100	2.4	0	100
3/15/2008	28	995.9	0	100	0.5	0	100
3/19/2008	29	5240	0.81	100	12.6	0.011	100

Table G. 2. Volume and peak flow data observed in 2013-2014 study.

Date	Storm	Volume			Peak Flow		
		In (L)	Out (L)	Reduction (%)	In (L^3/s)	Out (L^3/s)	Reduction (%)
12/14/2013	1	95200	0	100	11.0	0.0	100
12/17/2013	2	15000	0	100	8.1	0.0	100
12/23/2013	3	570100	149400	74	15.6	10.0	36
1/10/2014	4	182600	0	100	12.6	0.0	100
1/11/2014	5	210100	9130	96	13.6	1.6	88
2/5/2014	6	122600	0	100	12.0	0.0	100
2/15/2014	7	75100	0	100	3.0	0.0	100
2/18/2014	8	87600	0	100	5.4	0.0	100
2/19/2014	9	390100	46200	88	10.3	3.3	68
2/21/2014	10	240200	10800	96	13.5	3.4	75
3/3/2014	11	508900	7160	99	11.0	0.9	92
3/7/2014	12	15000	0	100	6.2	0.0	100
3/17/2014	13	285900	0	100	9.7	0.0	100
3/25/2014	14	91000	0	100	8.9	0.0	100
4/3/2014	15	100800	0	100	13.2	0.0	100
4/4/2014	16	24100	0	100	11.0	0.0	100
4/7/2014	17	303900	17600	94	12.3	1.3	90
4/15/2014	18	111000	0	100	11.8	0.0	100
4/29/2014	19	277300	7330	97	13.0	0.9	93
5/10/2014	20	36200	0	100	11.1	0.0	100
5/15/2014	21	309100	122400	60	15.2	12.2	19
6/4/2014 AM	22	32700	20770	36	16.0	5.0	69
6/4/2014 PM	23	78100	629	99	14.6	0.5	96

Appendix H. Total suspended solids, total nitrogen, and total phosphorus storm data

Table H. 1. Mass loads and reductions of TSS, TN, and TP over 2007-2008 study.

Date	Storm	Total Suspended Solids			Total Nitrogen			Total Phosphorus		
		In (g)	Out (g)	Reduction (%)	In (g)	Out (g)	Reduction (%)	In (g)	Out (g)	Reduction (%)
11/26/2007	5	77.7	0	100	1.6	0	100	0.11	0	100
12/3/2007	6	4.2	0	100	0.2	0	100	0.01	0	100
12/21/2007	8	10.2	0	100	0.9	0	100	0.09	0	100
12/23/2007	10	165.7	0	100	2.4	0	100	0.44	0	100
12/28/2007	11	430.9	15.2	96.5	2.5	0.12	95.1	1.34	0.086	93.6
12/30/2007	12	39.1	0	100	1.6	0	100	0.20	0	100
1/5/2008	13	0.0	0	100	0.0	0	100	0.00	0	100
1/8/2008	14	17.0	0	100	0.2	0	100	0.04	0	100
1/10/2008	15	16.3	0	100	0.2	0	100	0.04	0	100
1/11/2008	16	47.2	0	100	1.3	0	100	0.16	0	100
2/1/2008	17	1242.9	0.2	100.0	40.0	0.13	99.7	2.41	0.003	99.9
2/4/2008	18	68.5	0	100	0.8	0	100	0.21	0	100
2/6/2008	19	186.6	0	100	5.5	0	100	4.26	0	100
2/6/2008	20	875.6	0	100	5.4	0	100	7.78	0	100
2/13/2008	21	3867.4	0	100	12.9	0	100	1.79	0	100
2/18/2008	22	31.2	0	100	1.0	0	100	0.13	0	100
2/23/2008	23	34.3	0	100	0.3	0	100	0.08	0	100
3/7/2008	25	44.8	0	100	0.8	0	100	0.15	0	100
3/14/2008	27	1168.5	0	100	10.7	0	100	1.90	0	100
3/15/2008	28	122.4	0	100	1.3	0	100	0.34	0	100
3/19/2008	29	2058.5	0.71	100.0	3.7	0.004	99.9	1.78	0.0006	100.0

Table H. 2. Mass loads and reductions of TSS, TN, and TP in 2013-2014.

Date	Storm	Total Suspended Solids			Total Nitrogen			Total Phosphorus		
		In (g)	Out (g)	Reduction (%)	In (g)	Out (g)	Reduction (%)	In (g)	Out (g)	Reduction (%)
12/14/2013	1	1428	0	100	40.9	0.0	100	1.9	0	100
12/17/2013	2	620	0	100	13.2	0.0	100	0.3	0	100
12/23/2013	3	11801	3555.7	70	153.9	188.2	-22	17.1	47.8	-180
1/10/2014	4	28686	0	100	93.1	0.0	100	5.5	0	100
1/11/2014	5	13089	0	100	105.1	5.9	94	6.3	0.5	93
2/5/2014	6	5725	0	100	96.9	0.0	100	1.2	0	100
2/15/2014	7	1682	0	100	103.6	0.0	100	1.5	0	100
2/18/2014	8	140	0	100	57.8	0.0	100	2.6	0	100
2/19/2014	9	4486	0	100	257.5	37.4	85	15.6	1.8	88
2/21/2014	10	22531	219.2	99	103.3	14.0	86	4.8	1.4	71
3/3/2014	11	10687	37.9	100	320.6	7.0	98	86.5	0.3	100
3/7/2014	12	1133	0	100		0.0	100		0	100
3/17/2014	13	9492	0	100	294.5	0.0	100	14.3	0	100
3/25/2014	14	2184	0	100	94.6	0.0	100	5.5	0	100
4/3/2014	15	29796	0	100	410.3	0.0	100	38.3	0	100
4/4/2014	16	2853	0	100	27.2	0.0	100	2.9	0	100
4/7/2014	17	8205	37.0	100	243.1	5.1	98	27.4	0.4	99
4/15/2014	18	3796	0	100	139.9	0.0	100	16.7	0	100
4/29/2014	19	24541	23.5	100	195.5	2.8	99	26.3	0.5	98
5/10/2014	20	3124	0	100	51.8	0.0	100	5.6	0	100
5/15/2014	21	23646	832.3	96						
6/4/2014 AM	22	5592	178.6	97						
6/4/2014 PM	23	4889	0.6	100	48.4	0.3	99	3.5	0.0	99

Appendix I. Fecal indicator bacteria storm load and reduction data

Table I. 1. Total loads and reductions of total coliforms, *E. coli*, and enterococci over 2013-2014 study.

Date	Storm	Total Coliforms			*E. coli*			Enterococci		
		In (MPN)	Out (MPN)	Reduction (%)	In (MPN)	Out (MPN)	Reduction (%)	In (MPN)	Out (MPN)	Reduction (%)
12/14/2013	1	1.71E+10	0	100	0	0	100	5.47E+08	0	100
12/17/2013	2	4.81E+08	0	100	0	0	100	1.51E+07	0	100
12/23/2013	3	8.40E+09	4.53E+09	46	4.25E+09	7.02E+08	83	5.76E+09	2.60E+09	55
1/10/2014	4	2.73E+10	0	100	2.03E+07	0	100	5.59E+08	0	100
1/11/2014	5	2.89E+10	707593260	98	2.06E+07	9.21E+06	55	4.23E+08	1.16E+07	97
2/5/2014	6	8.49E+08	0	100	1.23E+06	0	100	6.15E+07	0	100
2/15/2014	7	7.29E+08	0	100	0	0	100	5.68E+07	0	100
2/18/2014	8	8.47E+08	0	100	0	0	100	3.01E+07	0	100
2/19/2014	9	1.01E+10	455208600	96	5.85E+06	8.32E+06	-42	1.95E+08	2.31E+07	88
2/21/2014	10	1.01E+10	2.56E+08	97	2.76E+07	3.50E+06	87	7.41E+08	1.36E+07	98
3/3/2014	11	8.12E+09	5.34E+07	99	2.36E+09	3.72E+06	100	1.36E+08	3.58E+06	97
3/7/2014	12	9.55E+07	0	100	0	0	100	7.50E+06	0	100
3/17/2014	13	1.34E+09	0	100	1.44E+08	0	100	1.79E+08	0	100
3/25/2014	14	4.69E+08	0	100	1.82E+06	0	100	2.17E+07	0	100
4/3/2014	15	3.56E+09	0	100	1.01E+06	0	100	2.03E+08	0	100
4/4/2014	16	8.27E+08	0	100	1.21E+05	0	100	1.21E+07	0	100
4/7/2014	17	3.86E+10	2.26E+08	99	1.09E+07	5.46E+05	95	6.14E+08	3.40E+06	99
4/15/2014	18	2.07E+10	0	100	5.77E+06	0	100	1.68E+08	0	100
4/29/2014	19	2.04E+10	1.18E+08	99	2.80E+08	2.27E+05	100	2.80E+08	7.34E+06	97
5/10/2014	20	1.14E+11	0	100	1.82E+07	0	100	6.35E+08	0	100
5/15/2014	21	9.74E+11	3.71E+10	96	5.93E+09	2.53E+07	100	1.61E+09	5.70E+08	65
6/4/2014 AM	22	1.82E+10	3.49E+10	-92	4.95E+07	6.58E+06	87	1.06E+09	9.60E+07	91

Date	Storm	Total Coliforms			*E. coli*			Enterococci		
		In (MPN)	Out (MPN)	Reduction (%)	In (MPN)	Out (MPN)	Reduction (%)	In (MPN)	Out (MPN)	Reduction (%)
6/4/2014 PM	23	2.46E+11	9.95E+08	100	7.84E+07	1.23E+05	100	4.47E+10	1.93E+06	100