ABSTRACT

Title of Document:	MULTI-CRITERIA VEGETATION SELECTION FOR MARYLAND BIORETENTION, WITH NITROGEN FOCUS
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Stormwater is a leading source of nutrient pollution in natural waters. Bioretention cells can mitigate stormwater pollution. This study examines the role of vegetation in bioretention. In a bioretention field study; of *Eutrochium dubium, Solidago rugosa*, and *Erigeron* sp.; *E. dubium* had the thickest root and tallest aboveground biomass. The root length of the three species averaged 29.1 cm. A greenhouse bioretention mesocosm study examined three plant species: *Eutrochium dubium*, *Iris versicolor,* and *Juncus effusus.* Only *J. effusus* created significant nitrate (NO_3^-) removal from synthetic stormwater influent, 0.21 mg to 0.066 mg NO_3^--N L^{-1}, only in low-density plantings. However, all planted treatments prevented nitrogen export vis-à-vis the unplanted treatment in two storms. *J. effusus* had the greatest average biomass growth of the three species, 29-fold vis-à-vis 1.3- and 2.7-fold. *J. effusus* is the most highly recommended plant for Maryland bioretention in this study. *E. dubium* is cautiously recommended.

MULTI-CRITERIA VEGETATION SELECTION FOR MARYLAND
BIORETENTION, WITH NITROGEN FOCUS

By

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Chapter One: Introduction and Objectives

1.1 Bioretention

Non-point pollutant sources, such as stormwater runoff, are leading sources of impairment in natural bodies of water in the United States (US EPA 2004). Low Impact Development (LID) techniques can reduce and treat stormwater runoff at its source. Bioretention is a LID practice that consists of a depressed area, filled with engineered media, covered with mulch, and planted (Figure 1.1).
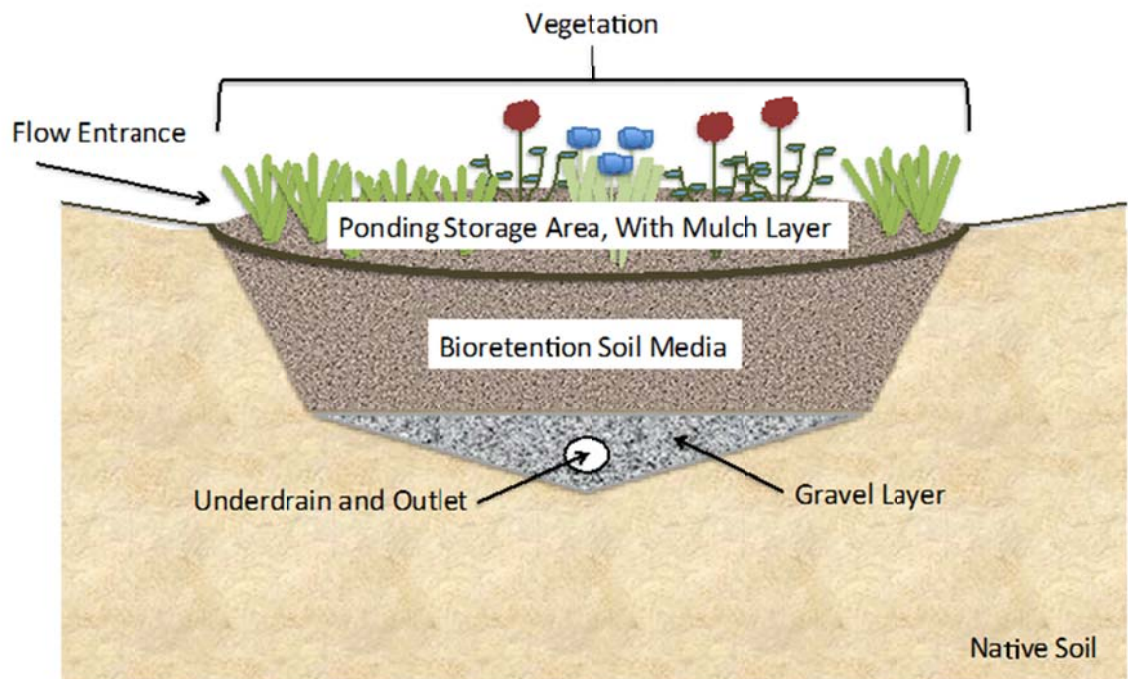
Figure 1.1   Cross-section of a typical bioretention cell

The bioretention cell is situated so that stormwater is directed into it and infiltrates the media. Depending on the size of the storm, the cell will either partially or entirely manage the runoff volume (Davis et al. 2012). Water quality is also typically improved. Laboratory and field bioretention studies have shown high levels of suspended solids and heavy metals retention (Hunt et al. 2006; Sun and Davis 2007; Li and Davis 2008). Variable removal of phosphorus (P) and nitrogen (N) species has been found, depending upon the particular experimental setup or cell and the species considered (e.g. Davis et al. 2001, 2003, 2009; Dietz 2007; Hunt et al. 2006; Hunt et al. 2012).

In Maryland, the U.S. Environmental Protection Agency's Chesapeake Bay Total Maximum Daily Load (TMDL) for N, P, and sediment have brought increased attention to reducing the nonpoint contributions of stormwater runoff to these three constituents. Bioretention cells represent a promising stormwater control measure with the ability to address the TMDL requirements.

1.2 Vegetation in Bioretention

One aspect of bioretention that has not been thoroughly studied is the role of the vegetation. The existing literature is dominated by studies from outside the United States, in areas that have different climates than Maryland, such as western Australia. Bioretention design guidance generally specifies the use of native species since they are well adapted to local conditions. A direct translation of vegetation results to other locations can be difficult because native species may not have a large geographic range, so the other locations may be outside of the range where the species in question can grow.

Therefore, this project has several objectives:

1. Identify vegetation species that generally grow well in bioretention facilities in Maryland and are aesthetically pleasing.

2. Characterize the overall vegetation community, and measure the root characteristics and aboveground height of successful plant species in an established bioretention cell.

3. Quantify N and P uptake of successful bioretention plants identified in objectives 1 and 2, in bioretention conditions. Determine if single-species plantings have different uptake than mixed-species plantings. Additionally, quantify the amount of biomass change and overall aesthetics for these plants over the course of a year, when grown in bioretention conditions.

   a. Hypotheses:

      i. N [nitrate (NO_3^-) and ammonium (NH_4^+)] and P uptake will change over the course of the year, with vegetation growth and bloom times. Higher nutrient uptake will occur during vegetation growth and bloom times, e.g. as illustrated in the hypothetical diagram of Figure 1.2.
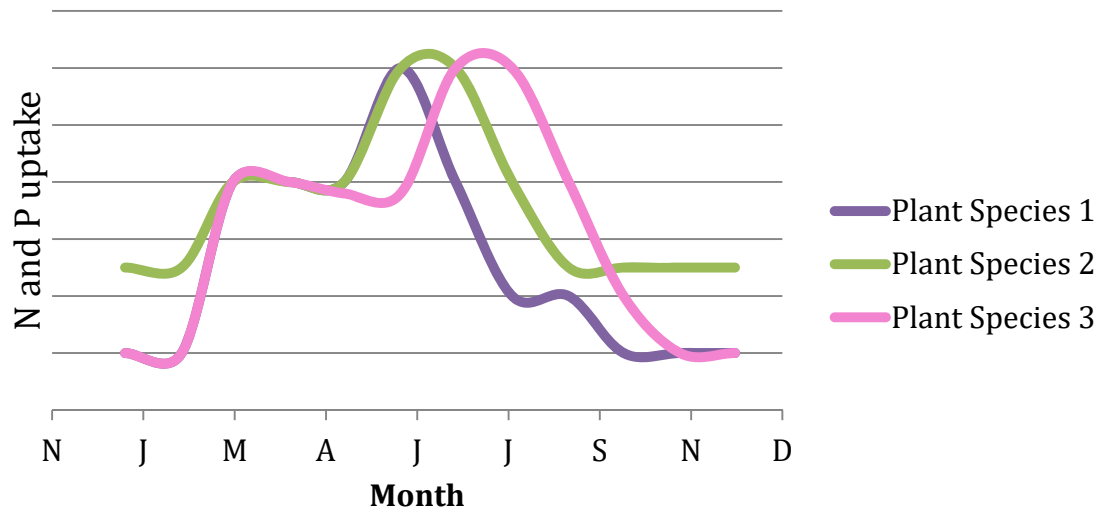
Figure 1.2 Expected changes in N and P uptake of three example plants with differing growth and bloom times. Uptake is expected to be highest during blooming periods (which correspond to the peaks) and active growth periods (in the Spring and early Summer). Plant species 2 remains green year-round and as such is expected to have higher nutrient uptake during the winter months than species 1 or 3, whose aboveground biomass dies back in the winter.

The resulting information on the temporal spread of nutrient uptake over a full year by Maryland bioretention vegetation would provide valuable information for planting and maintenance recommendations.

ii. N and P uptake will be consistently highest in a mixed planting, because it will include multiple plant species with differing growth and bloom times, which will maximize uptake at all times of the year. Existing mesocosm studies described in the literature generally compare mesocosms with a single species in each mesocosm, which is not a realistic representation of most existing bioretention cells, which contain multiple species. Some studies, such as Henderson et al. (2007) and Lucas and Greenway (2008), used mixed-species mesocosms in comparison with a soil-only control, but they lack

comparisons with single-species mesocosms. Read et al. (2008) notes this lack of in-depth information about mixed-species vegetation in bioretention and encourages further study on the topic. It is further important because most bioretention cells in the field are planted with several different plant species.

iii. More densely planted treatments will remove more NO_3^- and NH_4^+ from the influent than less densely planted treatments of the same plant species.

iv. Multiple species will have differential growth and aesthetics. This will lead to differences among the species in the quantity of N incorporated into the plant biomass over the course of the year. Different aesthetics between the plant species will lead to different recommendations for each species on use for aesthetic considerations in bioretention.

Methods to address these objectives:

In order to address these objectives, a variety of approaches will be necessary, from office to lab to field work. The method for addressing each objective is outlined below.

1. Speak with bioretention design and maintenance professionals, consult design manual plant lists, and conduct in-person surveys of bioretention cells in Prince George's County, Montgomery County, and Washington DC. Assemble information on recommended species.

2. Destructively survey an existing, well-established bioretention cell to determine plant community makeup, survivorship of planted species, and root and aboveground height characteristics.

3. Over the course of a year, perform a greenhouse bioretention mesocosm study of three native Maryland plant species that have been proven to grow in field bioretention.

    i. Three species with different peak bloom times will be selected for experimental testing, including one species that remains green year-round, while the other species' aboveground growth dies back during the winter. Different bloom and growth times are expected to correspond to different peak nutrient uptake times (e.g. Figure 1.2).

    ii. The selected species will be planted in both mixed-plant-species mesocosms and mesocosms with only one plant species, to examine competition and synergistic effects on nutrient uptake.

    iii. Additionally, one plant species will be planted at different densities in different treatments, to quantify vegetation density effects on nutrient uptake.

    iv. Plant survivorship, growth, and aesthetics will be tracked over the year. To examine growth, the dry mass and percent N of above- and belowground representative samples of each plant species will be found at both the beginning and end of the experiment.

    v. The treatments will all be watered with synthetic stormwater. Influent and effluent samples will be collected at select dates throughout the year. The samples will be analyzed for N species: total N, total

dissolved N, NO_3^-, nitrite (NO_2^-), and NH_4^+, and for total phosphorus (TP). Nutrient results for planted treatments will be compared to an unplanted control to determine which treatments are the most effective at nutrient removal at different times of year.

vi. Media samples will be taken at both the beginning and end of the experiment and analyzed for N content, to help determine the role of the media in N uptake.

Such a study, focusing on vegetation species suitable for use in Maryland bioretention cells, has not been performed to the author's knowledge. The scientific data produced by such a study could inform vegetation selection for the most reliable and effective nutrient management in bioretention, as well as aesthetics.

Chapter Two: Literature Review

2.1 Stormwater Characteristics

Nitrogen

N enters stormwater from fertilizers, animal waste, septic and sewage leaks, plant matter, and atmospheric deposition (Collins et al. 2010; Davis and McCuen 2005). These sources may exist on pervious or impervious surfaces, and combine with stormwater during or following a precipitation event. Stormwater N concentrations in urban areas are not consistent, but tend to be greater than stormwater N concentrations in undisturbed natural areas (Dodd

et al., 1992; Groffman et al., 2004; Line et al., 2002).

N is typically present in several forms in stormwater, including organic N (several forms), NO_2^-, NO_3^-, and NH_4^+. Since the NH_4^+ pKa is 9.3 (Stumm and Morgan 1981) and stormwater pH is typically below a pH of 9.3 (Kayhanian et al. 2012; Pitt et al. 1995), NH_4^+ is the dominant form in stormwater rather than NH_3. Additionally, NH_3 is a gas, unlike NH_4^+. A nationwide study of stormwater composition (The National Stormwater Quality Database 2015) found the average concentrations of different types of N presented in Table 2.1. Because it is a nationwide study with data from varying land uses, variability is high, as demonstrated by the large standard deviation values compared with the average concentrations in Table 2.1.

Table 2.1 Average N concentrations in stormwater (The National Stormwater Quality Database 2015)

N Form	Average Concentration (mg N L^{-1})	Standard Deviation (mg N L^{-1})
NO_3^--N	0.966	1.35
NO_2^--N	0.172	0.373
NH_4^+-N	0.772	1.16
Organic N	2.61	2.76
Total N (TN)	2.92	3.65

An international study of highway runoff only (Kayhanian et al. 2012) found the

N concentrations shown in Table 2.2.

Table 2.2 Average N concentrations in highway runoff, values assumed as N (Kayhanian et al. 2012)

N Form	Average Concentration (mg L^{-1})
NO_3^--N	1.74
NO_2^--N	0.375
NH_4^+-N	1.36
Organic N	2.16

The average concentrations found in the National Stormwater Quality Database

(2015, Table 2.1) are higher in NO_3^- and NO_2^- than the highway runoff numbers

(Kayhanian et al. 2012, Table 2.2): 0.966 mg NO_3^--N versus 1.74 mg NO_3^-N, and 0.172

mg NH_4^+-N L^{-1} versus 0.375 mg NH_4^+-N L^{-1}. This may be because the large amount of

impervious surfaces in highway systems results in a high percentage of deposited

pollutants entering runoff, as opposed to other land uses that have more natural areas to

interact with and detain pollutants, instead of allowing them to be carried by runoff. The

majority of N entering urban streams has been attributed to such impervious surface

runoff (Metropolitan Washington Council of Governments, 1983).

Phosphorus

  As with N, P enters stormwater from several sources: fertilizer, plant matter, and

animal waste (Davis and McCuen 2005). P in stormwater may be either particulate or

dissolved. P in stormwater also exists in either inorganic (as various orthophosphate

compounds of formula $H_XPO_4^{X-3}$) (Stumm and Morgan 1981) or organic forms.

  P concentrations from the sources cited above for N are presented in Tables 2.3

and 2.4.

Table 2.3   Average P concentrations in stormwater (The National Stormwater Quality
Database 2015)

P Form	Average Concentration (mg P L^{-1})	Standard Deviation (mg P L^{-1})
Dissolved P	0.223	0.452
Orthophosphate	0.222	0.364
TP	0.401	0.685

Table 2.4   Average P concentrations in highway runoff, as found in Kayhanian et al.
2012.

P Form	Average Concentration (mg P L^{-1})
Orthophosphate	0.183
TP	0.475

Other Pollutants

Stormwater can contain many other pollutants, including hydrocarbons, metals, suspended solids, and microbes. Typical levels of these pollutants and their removal rates in bioretention have been discussed in a number of papers, including but not limited to Davis et al. (2009), LeFevre et al. (2015), and Roy-Poirier et al. (2010).

<u>*2.2 Plant Biology Related to Bioretention Performance*</u>

N Uptake by Vegetation

N is essential for plant growth (Fageria et al. 2006). N is a component of amino acids and therefore proteins (Shuman 2000), as well as many other organic compounds (Fageria et al., 2006). Plants are 2–5% N by dry weight (Shuman 2000).

NO_3^- and NH_4^+ are the two major forms of N taken up by plants (Pilbeam and Kirkby 1992; Shuman 2000; Morot-Gaudry and Lea 2001). Some plant species can take up organic N compounds, while other species cannot (Chapin et al. 1993; Jones and Darrah, 1993; Raab et al. 1999). Most plants can take up both NO_3^- and NH_4^+, however, NO_3^- is favored by most plants, perhaps because it is generally the most readily available form of N in most soils (Pilbeam and Kirkby 1992). Additionally, except for plants adapted to acid soils, most plants grow better when supplied with NO_3^- instead of NH_4^+ (Pilbeam and Kirby 1992).

However, plant species that prefer NO_3^- have shown the ability, in controlled laboratory experiments with constant nutrient levels, to take up both NO_3^- and NH_4^+ simultaneously. Those plants supplied with both NO_3^- and NH_4^+ increased their growth rate as compared to those plants supplied with only NO_3^- (Cox and Reisnauer 1973;

Lewis and Chadwick 1983). This could be useful in bioretention, where multiple N sources are typically present in incoming stormwater and additional plant growth and biomass could lead to increased transpiration and pollutant uptake.

The levels of NO_3^- and NH_4^+ uptake also influence the pH of the surrounding soil. The uptake of a NO_3^- ion is typically paired with either the release of a hydroxide ion from the root or the uptake of a monovalent cation, to maintain charge balance (Pilbeam and Kirby 1992). Similarly, the uptake of a NH_4^+ ion is typically paired with either the release of an H^+ from the root or the uptake of a monovalent anion (Pilbeam and Kirby 1992).

Available NO_3^- can greatly influence plant growth. In general, if there are no other restrictions for plant growth, increasing the available N will increase leaf area until the leaf reaches full size (Hageman and Below 1990). This is of particular note in bioretention where vegetation will be provided with large quantities of typically N-rich stormwater, which could create more plant growth than is typical for the plant outside of bioretention conditions.

P Content of Vegetation

Plant tissue percent P can vary widely depending on species, but 0.2–0.5% P by dry weight are typical values (Shuman 2000; Fitter and Hay 2002), an order of magnitude less than the N content. Plants can concentrate P with respect to the soil concentration, with concentrations in plant xylem sap being 100 to 1,000 times the P concentrations found in the soil (Shuman 2000). P is crucial for many energy transfer processes in plants, as it is necessary for the production of adenosine triphosphate (ATP) (Shuman 2000), which serves as an energy carrier.

2.3 Stormwater Benefits of Vegetation in Bioretention

Pollutant Removal

N Removal

Several studies, primarily consisting of mesocosm studies, from a variety of geographic locations have examined N removal in bioretention in relation to the vegetation present.

Zhang et al. (2011) found that all four tested native species of vegetation in the Mediterranean-like climate of Western Australia significantly increased NO_x ($= NO_3^- +$ NO_2^-), NH_4^+, total dissolved N, and total N (TN) removal in greenhouse mesocosms vis-à-vis non-planted controls, as long as a saturated zone was present in the media. A slight difference in NH_4^+ removal capacity was noted between the species grown with saturated zones, but for all other N species the removal capacities of the different plant species were not, on average, differentiable. The tested species included a *Juncus* (reed) species, *Baumea* (sedge) species, and *Melaleuca* (myrtle family) species. No native *Baumea* or *Melaleuca* species occur in the United States, but there are *Juncus* species native to the United States.

In a different mesocosm study on the east coast of Australia in Brisbane, Henderson et al. (2007) found that a mix of five species of vegetation in mesocosms removed substantially more TN than non-vegetated mesocosms. The vegetation included two shrub/tree species, a groundcover, a lily, and a grass. The vegetation examined were Australian species that do not have similar American counterparts. Eighteen months later

in the same mesocosms Lucas and Greenway (2008) found that TN retention was still enhanced by the presence of vegetation.

Beyond the simple presence of vegetation, the selection of vegetation type can sometimes have a large impact on N removal effectiveness, again based primarily on mesocosm/column studies. As mentioned above, Zhang et al. (2011) found slight differences among their tested plant species in NH_4^+ removal capacity. Read et al. (2008) found variation among 20 plant species native to southeast Australia in effluent concentrations of TN, dissolved organic N (DON), NO_x, and NH_4^+ in a mesocosm column study. Some plant species created significant removal of these four N species vis-à-vis the influent. None of the species created significant removal of particulate organic N. Both monocots and dicots, which are different plant categories distinguished by the number of initial seed leaves of the species among other traits, were tested. Some of the genera tested, such as *Poa, Juncus,* and *Carex*, have American species, but many do not. The *Carex* and *Juncus* species in this study created statistically significant NO_x and NH_4^+ removal, and the *Carex* and one of the *Juncus* species removed a significant amount of DON.

Similarly, in Cape Town, South Africa, Milandri et al. (2012) found that some of the nine tested plant species performed much better than others in NO_3^- removal in mesocosms. All species were native to South Africa except for one species native to east Africa. One plant species they tested did not, however, remove more NO_3^- than the control, again indicating the potential importance of vegetation selection in nutrient removal.

In contrast, as noted above Zhang et al. (2011) found no difference in NO_x, total dissolved N, and TN removal among their tested plant species. However, it is possible that all of these species are similar enough in N removal capacity that their differences were not measureable, whereas Read et al. (2008) and Milandri et al. (2012) may have chosen species with more inherent variation in uptake ability.

Additionally, a field study in North Carolina by Passeport et al. (2009) found comparable removal of TN between turfgrass (Bermuda sod)-only bioretention and conventional bioretention with trees, shrubs, etc. However, these grass-only cells also had a specialized Stalite (a rotary kiln expanded slate lightweight aggregate) fill media and an internal storage zone, which may have increased the N removal efficiency of the cell enough to diminish the impact of vegetation selection.

With regard to specific traits that make plants more effective in bioretention, plants with more extensive root systems seem to be the most effective, though so far this has been found through correlation and not direct causation. For example, in a study in Texas, Barrett et al. (2013) showed that Big Muhly grass (*Muhlenbergia lindheimeri*), a large bunch grass with a root depth of ~460 mm in the mesocosms, removed significantly more NO_x than Buffalograss 609, a turf grass whose roots were only found in the top ~100 mm of the media. Similarly, Bratieres et al. (2008) found that the strongest performer in NO_x and TN removal in an Australian column study was *Carex*, which has a dense root architecture and many fine root hairs. Other species (all Australian) tested by Bratieres et al. under the same conditions exported NO_x, showing the importance of vegetation selection.

In short, these papers generally argue for a definite link between bioretention N removal performance and plant selection. This aligns with the importance of biological pathways in N processing, and preliminarily argues for a deeply and extensively rooted plant palette.

P Removal

Henderson et al. (2007) and Lucas and Greenway (2008) found that TP retention in bioretention mesocosms was enhanced by the presence of vegetation (a mix of multiple species of Australian vegetation), with TP retention by bare media eventually becoming exhausted.

P removal has more physically and chemically dependent pathways than N removal, which relies more heavily on biological pathways. This may contribute to the impact of vegetation type on P removal in bioretention being less distinct than for N. In Texas, Barrett et al. (2013) found that vegetation improved TP removal but the vegetation type was not important. In Australia, Bratieres et al. (2008) found somewhat higher TP removal with certain species than others, but noted that removal was generally high (77–95%) in all mesocosms, including those that were not vegetated (which had an 81% TP removal rate). Read et al. (2008) found in another Australian mesocosm study that only one of twenty plant species tested removed significantly more TP than the soil-only control; in contrast, all but one tested species removed more total dissolved P than the soil-only control. For phosphate, Milandri et al. (2012) found that significant removal depended upon input concentration. For an input concentration of 1.47 mg PO_4(-III)-P L^{-1}, only one of the tested plant species removed significantly more PO_4(-III) than the soil-only control. For an input concentration of 2.62 mg PO_4(-III)-P L^{-1}, four out of the nine

tested plant species tested removed significantly more $PO_4(-III)$ than the soil-only control.

While mesocosm studies typically test non-turfgrass vegetation, in a North Carolina field study Passeport et al. (2009) had good success with TP removal rates in grass-only bioretention, with comparable removal rates to conventional bioretention with trees, shrubs, etc., in the same manner as TN as noted above. However, again the specialized Stalite fill media and an internal storage zone may increase the P removal efficiency of the cell enough to overshadow the impact of vegetation selection.

Again, the majority of these studies occurred outside of the United States, with many species that do not have American counterparts of the same genus.

Hydrocarbon Removal

In general, vegetated soils remove more total petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAH) than unvegetated soils (United States Environmental Protection Agency, 2000). In bioretention specifically, both column and field studies have found consistent removal of greater than 96% removal of oil and grease (Hsieh and Davis 2005). In column studies without vegetation, Hong et al. (2006) found that the mulch layer was important for removing hydrocarbon contaminants (naphthalene, toluene, and dissolved motor oil) and facilitating microbial degradation of those contaminants by harboring an appropriate microbial population. In Maryland field bioretention, PAH event mean concentration reductions of 31–99% were documented (DiBlasi et al. 2009). Sorption and particulate capture are documented mechanisms for this reduction (DiBlasi et al. 2009); the contribution of vegetation to the process was not documented in this study, but has the potential to aid in the process.

LeFevre et al. (2011) specifically examined the role of vegetation in the removal of naphthalene in bioretention columns in Minnesota. Three setups were used: a legume (Purple Prairie Clover, *Dalea purpureum*), a grass (Blue-Joint Grass, *Calamagrostis canadensis*), and an unplanted control. Both plants are native to Minnesota, are recommended for stormwater use, and have a deep root structure. The planted columns removed 93% of the naphthalene vis-à-vis 78% for the unplanted columns, suggesting that vegetation played an important role in removal. Naphthalene tracing showed that adsorption to the media was the dominant removal mechanism, removing 56–73% of the added naphthalene. Mineralization also played a role (12–18% of removal, with no significant difference between setups), but plant uptake did factor in, accounting for 2.5–23% of naphthalene removal. The grass took up significantly more naphthalene than the clover, with 2.5% uptake for clover and 23% uptake for grass. The vegetative biomass of the grass was more than two times greater than the clover, but regardless of the biomass difference the traced carbon (C) concentration in the grass tissue was about three times greater than in the clover tissue. The difference in incorporation into plant biomass is likely attributed to several factors, including the extensive root structure of the grass. Interestingly, for both plant species the majority (88 to 92%) of the traced naphthalene-C was found in aboveground biomass rather than belowground biomass (i.e., roots), which indicates that naphthalene or its subsequent degraded products may be transported from the roots to the shoots of both plants. Further research is needed to determine the form of the C in the plant biomass and its role in the bioretention system after plant death and decay. Beyond uptake, both plant species helped prevent naphthalene-C leaching (7% leaching for vegetated columns) in comparison to the unplanted column (22% leaching),

and plant growth encouraged bacterial activity which can lead to enhanced naphthalene degradation. The grass columns had significantly more naphthalene dioxygenase functional genes (which indicate the presence of microorganisms with the ability to degrade naphthalene) present than the clover or unplanted columns.

In a field study by the same authors (LeFevre et al. 2012), greater numbers of two bacterial genes that code for proteins that aid in hydrocarbon breakdown were found in Minnesota bioretention field sites with deeply-rooted vegetation than those sites with grass only or mulch only (non-vegetated). This argues that more complex vegetation better supports a bacterial population that can break down hydrocarbons, leading to increased removal efficiencies.

Metals Removal

In an eight-month study of metal uptake in Australia, vegetation was found to be a significant factor in the levels of iron, aluminum, and chromium removal from stormwater in bioretention mesocosms (Feng et al. 2012). Four of the five tested species performed similarly but one species, *Melaleuca ericifolia*, had lower removal of those three metals. On average, all five species performed similarly, however, in the removal of lead, copper, and zinc. Metal uptake varied with time for all species, indicating changes in conditions as plants grow and develop and media conditions evolve. The plant species were Australian natives and were selected from Read et al. (2008)'s plant palette. *Carex* was the only tested genus with an American species.

In a bioretention pot study, Sun and Davis (2007) found that zinc, copper, lead, and cadmium in the influent were subsequently found as 88–97% held in soil media, 2–11.6% not captured by the media, and 0.5–3.3% accumulated in plant tissue of the

grasses grown in the pots (*Panicum virgatum*, Kentucky-31, and *Bromus ciliates*). This suggests that the media is the largest factor in metals removal in bioretention, however hyperaccumulating plants may generate a higher level of metal retention than the plants used in Sun and Davis (2007).

Total Suspended Solids (TSS) Removal

Vegetation helps slow water flow in a bioretention cell and can physically filter solids from runoff (see Slowing Water Flow and Encouraging Infiltration sections in section 2.4 below). Many pollutants, including several metals and P, are associated with particulates (Sansalone and Buchberger 1997; Fritioff and Greger 2003), so decreased TSS levels in effluent can also decrease levels of other target pollutants. However, no specific guidance exists on the best types of vegetation for this purpose, or at what density vegetation should be planted to best remove suspended solids (Hunt et al. 2012). The media is highly effective at suspended solids removal and vegetation type or even presence is likely not important.

Microbial Community

Vegetation also has the potential to enhance bacterial presence in the media, which can contribute to nutrient removal if the correct bacteria are present. For example, Chen et al. (2013) examined bacterial populations in a bioretention cell in Kansas to investigate the potential for biological transformation of N in the soil media. Media cores taken in a densely vegetated area of a bioretention cell had bacterial 16S rDNA concentrations an order of magnitude higher than sparsely vegetated areas, suggesting higher bacterial abundance in vegetated areas. Along the same lines, but examining vegetation type in addition to presence/absence, LeFevre et al. (2012) found that field

bioretention sites in Minnesota with deeply-rooted plants contained significantly more bacterial 16S rRNA copies than sites planted with turfgrass only or containing only mulch. This suggests that increased complexity of plant palette leads to increased bacterial presence in bioretention, which can affect nutrient transformations. Further study is needed to better understand microbial action in bioretention (Li and Davis 2009).

2.4 The Role of Vegetation in Bioretention Hydrology

Slowing Water Flow

Vegetation can slow the rate of water flow across the surface of a bioretention cell (Davis and McCuen 2005; Hsieh and Davis 2005; Davis et al. 2009). Denser vegetation would presumably slow water flow more than minimal vegetation. For example, in a study of the nearshore area of a lake (Petticrew and Kalff 1992) plant surface area accounted for the majority of the variance in flow reduction, after the effect of changing water depth was removed. In bioretention, this slowing helps to increase TSS and particulate removal rates (Hunt et al. 2012).

Encouraging Infiltration

From an infiltration perspective, Le Coustoumer et al. (2012) showed (in a bioretention mesocosm study in Australia) that thicker roots encourage infiltration. For example, the no-vegetation treatment declined from 199 mm hr^{-1} infiltration to 53 mm hr^{-1} infiltration 56 weeks later. The planted native Australian species also all declined in infiltration over the course of the experiment except for the thickly rooted *Melaleuca ericafolia*, which actually increased infiltration rate from 155 mm hr^{-1} to 295 mm hr^{-1} 56

weeks later. Infiltration is crucial to maintaining media permeability and preventing clogging. Similarly, Feng et al. (2012) also found that over eight months of a bioretention column study, hydraulic conductivity decreased for all columns except for the columns planted with *M. ericifolia*. Again, *M. ericifolia* had thicker roots than all of the other vegetation species used in the study.

These results would generally argue for larger plants than turfgrass, unless the grass has an exceptional root structure. In keeping with this philosophy, Hatt et al. (2009) found a correlation between vigorous vegetation growth in a field bioretention site and significant increases in infiltration, in Victoria and Queensland, Australia. Skorobogatov et al. (2013) discuss the infiltration benefits of more complex/woody vegetation over grass in bioretention settings.

Transpiration

Once the water has infiltrated into the media, transpiration of water by vegetation also helps to maximize the amount of stormwater treated by the cell. In a green roof system, Voyde et al. (2010) found that small, succulent plants transpired about a third of the total water that left the system via evapotranspiration. However, different plant species can transpire at very different rates (e.g. Farrell et al. 2013). Larger vegetation, such as trees and shrubs, generally has the ability to transpire more water than smaller vegetation (Center for Watershed Protection 2012). Hunt et al. (2012) recommend bioretention vegetation with extensive roots in order to maximize water movement out of the cell through transpiration.

Media Shading and Thermal Attenuation

Vegetation will shade the bioretention media surface, possibly leading to thermal attenuation of the stormwater. For this reason, vegetation that produces a near 100% canopy cover is recommended for bioretention by Hunt et al. (2012). However, shading can encourage pathogen survival, if pathogens are captured by the bioretention media.

2.5 Non-Stormwater Benefits of Vegetation in Bioretention

Aesthetics

From an aesthetics perspective, the Maryland Stormwater Design Manual (CWP and MDE 2000) states that "Aesthetics and visual characteristics should be a prime consideration" for stormwater best management practices. Also, the 2007 Prince George's County Bioretention Manual (PGCo 2007) mentions that designers can increase "real estate values up to 20 percent by using aesthetically pleasing landscaping" which argues for more diverse, visually pleasing bioretention landscaping rather than turf-grass-only bioretention.

A variety of plant species can also increase the resilience of the plant community. The Prince George's County Manual (PGCo 2007) states: "A minimum of three species of trees and three species of shrubs should be selected to ensure diversity. This will protect the system against collapse from insect and disease infestations and can ensure a more constant rate of evapotranspiration and nutrient and pollutant uptake throughout the growing season." A variety of plants will also attract a variety of pollinators, providing habitat for animals (see the following section).

A very important component of aesthetics is plant survivorship. While some plants may provide attractive aesthetic benefits while dead or dormant, typically green plants and flowers are desired. Bioretention plants need to be able to withstand both inundation with water during storms and drought between storms, because properly functioning bioretention media has a rapid infiltration rate and water will move fairly quickly out of the plants' root zone; the recommended rate by Davis and McCuen (2005) is 3.81 to 10.2 cm hr^{-1}. Muerdter et al. (2015, Chapter 3) found that the root zone of a bioretention cell in Silver Spring, Maryland extended about 30 cm below the surface of the cell. To predict the rate of movement through the media from the infiltration rate, the pore space volume is accounted for by dividing the infiltration rate by 0.4. Therefore, at the recommended Davis and McCuen (2005) rates, in the Silver Spring cell water should infiltrate through the root zone between 1.2 and 3.1 hours after introduction of the water to the cell surface. However, the measured infiltration rate in Muerdter et al. (2015) was 34 cm hr^{-1}, in which case the water moves through the root zone in 21 minutes. Therefore, it is important that bioretention plants can withstand periods of drought, because available water will quickly move through their root zone.

Many plants cannot tolerate these conditions and will die in bioretention, as occurred in Li et al. (2011). Native plants are often used in bioretention because of their adaption to the local climate and contribution to the local ecosystem. Plants that can tolerate extremes in moisture and also match the desired aesthetic of the cell and surrounding area are recommended when maximization of aesthetic value is of concern. Ideally these plants will also serve pollutant removal functions.

Animal Habitat

   Kazemi et al. (2009a, 2009b) found a statistically significant difference in invertebrate biodiversity between bioretention basins and lawn-type greenspace, with an average of 22 invertebrate species in bioretention basins vis-à-vis five species in lawn-type greenspace. In their study, the highest biodiversity was found in sites with a greater depth of leaf/plant litter, the highest number of plant taxa, and a higher amount of mid-stratum (i.e., not trees or groundcover) vegetation. Bioretention cells with complex and varied vegetation therefore have the potential to provide more invertebrate habitat than cells with only one low-growing plant species.

Chapter Three: Field Studies

3.1 Montgomery County Bioretention Survey

In the fall of 2013, the author rode along with Mary Travaglini, a Planning Specialist with Montgomery County, to several bioretention sites to examine existing vegetation and learn which plants have grown well and which plants have died in bioretention. The results are summarized in Tables 3.1, 3.2, and 3.3.

Table 3.1   Plants that have grown well in bioretention facilities in Montgomery County, Maryland and are aesthetically pleasing.

Plant	Notes
Amelanchier sp.	Can tolerate being in the basin of a bioretention cell
Asclepias incarnata (swamp milkweed)	Can handle wet conditions
Baptisia sp.	
Beautyberry	
Calimagrostis sp.	• Does well in inlets especially • Not native
Carex spp. (sedge species)	Can tolerate sunny conditions
Chasmanthium (river oats)	• Does better in shade than sun • Can be aggressive • Seeds stay on in the winter for winter interest
Clethra alnifolia (summersweet)	Can handle wet conditions
Cornus sp.	Variegated versions add interest
Eutrochium sp. (Joe-Pye Weed)	Can handle wet conditions
Hibiscus moscheutos (swamp rose mallow)	
Iris versicolor	• Widely used in Montgomery County • Tolerates inlets well

Plant	Notes
Itea virginica (Virginia sweetspire)	Can handle wet conditions
Juncus effusus (common rush)	- Can have a messy look - Good for wet and dry areas - They do get complaints about their looks sometimes and therefore trim them - In winter and spring it is the only green thing (besides hollies), but then it gets messy as the warm weather continues - Works well in inlets
Lindera benzoin (spicebush)	Red berries add interest
Lobelia spp. (cardinal flower)	
Swamp magnolia	
Panicum virgatum (switchgrass)	- Widely used in Montgomery County - Dense - Bloom: Mid-summer - Fruit/seed period: Summer to Fall - Pros: - Very successful in bioretention in survivability (takes drought, water, salt, heat, shade, etc.) and looks. - Deeply rooted - Provides winter interest - Good for slopes and preventing erosion - Doesn't mind organic debris input into the cell - Not favored by deer for herbivory - Cons: - Can be a little tall for some sightlines (dwarf species are still about 4 feet tall, which can be too tall for driveways. Montgomery County has also received complaints about *Panicums* blocking vision for older people) - Aesthetically does not appeal to everyone
Rudbeckia hirta (black eyed Susan)	Blooms generate nice seed heads in winter

Plant	Notes
Rudbeckia fulgida (orange coneflower)	For moist side slopes
Solidago sp. (goldenrod)	
Spartina sp.	Very tall
Sporobolus heterolepis (prarie dropseed)	
Viburnum dentatum (arrowwood viburnum)	Larger shrubs, may need to keep trimmed

Table 3.2   Plants that have grown well on the drier slopes of bioretention facilities in Montgomery County, Maryland

Plant	Notes
Aster spp.	Use for upper dry border only, they do not handle wet conditions.
Dwarf holly	
Echinacea	
Liatrus sp. 'blazing star'	Features tall spikes of flowers
Morella sp. (bayberry)	• Salt-tolerant • Leggy, tall shrub
Phlox sp.	
Schizachyrium scoparium (little bluestem)	
Yarrow	
Witch hazel	

Table 3.3   Plants that have not grown well in bioretention facilities in Montgomery County, Maryland: plants to avoid

Plant	Notes
Ilex verticillata, Winterberry	
Ilex glabra 'shamrock'	Short lifespan
Onoclea sensibilis (sensitive fern)	Some individual plants grow, others do not.

3.2 Silver Spring Field Study

In the fall of 2013, an opportunity arose to destructively evaluate the condition of the vegetation at a successful 7-year-old bioretention cell in Silver Spring, Maryland. Species survival, growth, and condition were evaluated, with a goal of informing both bioretention design and maintenance practices.

3.3 Silver Spring Objectives

The study had three objectives:

1) Determine the community makeup of the vegetative population of a seven-year-old bioretention cell that had not been maintained for 11 months.

2) Determine the root structure and aboveground height of the three dominant plant species in the cell, and determine any differences in spatial distribution close to the cell inlet vis-à-vis further from the cell inlet.

3) Examine spatial variation in vegetation, media infiltration rate, and media P content in the cell.

3.4 Silver Spring Methodology

Site

The bioretention cell was installed in March 2006 and has been described previously, e.g., in Li and Davis (2009). The cell is located in located in Silver Spring, Montgomery County, Maryland (39.025638, -77.041988) (Figure 3.1a).

(a)

(b)

(c)

Figure 3.1 The study bioretention cell located in Silver Spring, Montgomery County, Maryland; **(a)** August 2006, five months after installation; **(b)** June 2009, with prominent species delineated; **(c)** October 2013, at the time of this study.

The cell surface area is approximately 102 m^2, representing 2% of the cell's 0.45 ha drainage area. The drainage area is 90% impervious surfaces: asphalt parking lots and driveways next to a health service facility complex.

The installed media depth was 0.9 m. As of December 2005, the installed media texture distribution consisted of 54% sand, 26% silt, and 20% clay, forming a sandy clay loam with a pH of 7.7 and 12.2% organic matter (Li and Davis 2009). Below the media is a sand layer underlain by a gravel layer. 15-centimeter perforated PVC pipes in the gravel layer drain the cell to an existing stormwater network. The existing soil below the gravel layer was not analyzed but is expected to have high fines content and low permeability (Davis et al. 2012).

The facility has been successful and effective during its lifespan. It was shown in Li and Davis (2009) to produce significant reductions in runoff concentrations of TSS,

TN, NO$_3^-$, NO$_3^-$, TP, chloride, *E. coli*, chromium, copper, lead, and zinc. Successful hydrologic management was also noted in 2008–2010 (Davis et al. 2012, Olszewski and Davis 2013).

Routine maintenance of the site was last performed on November 8 and 9, 2012, 11 months before the plant survey conducted in this study, and included weeding of all non-planted species and mulch replenishment. No maintenance was performed after that date because the site was scheduled to be demolished. Some non-scheduled vegetation cutting appears to have occurred since the last maintenance date, but it was not clear when that happened. Between installation in 2006 and the final maintenance in 2012, maintenance records and communications with those in charge of the site indicate that non-planted species were removed at least once per calendar year during the facility's lifespan. Figure 3.1b, taken in 2009, helps to illustrate that the prescribed maintenance regime took place.

Plant Survivorship and Coverage Survey

A list of the original species planted in the cell and their approximate locations was obtained. In October 2013, as part of this study, the cell was surveyed and a list of the plants present in the cell was created. By comparison with the information on initial plantings, each plant species was noted as either an original planting or as a volunteer species that subsequently established itself without human intervention.

The percent cover of the cell by each plant species was estimated by eye (per Grieg-Smith 1983; Hill et al. 2005). A measurement of <1% cover in the field was counted as 0.2% during analysis. Using this procedure, the summed percent cover for the entire cell was 105.6%. To obtain a standard percentage, the field-measured percent

cover of each species was normalized by 1.056. The sums of percent cover by planted and volunteer species were then calculated.

Plant Height and Root Survey

Of the species found onsite in October 2013, three species were chosen for examination of root structure. Two of the species were originally planted at the site, *Eupatorium dubium* (Joe Pye weed) and *Solidago rugosa* (wrinkleleaf goldenrod). The third species was a volunteer, an *Erigeron* species with white flowers (Figure 3.2); instances of this species were sampled for above- and belowground characteristics.

Figure 3.2 *Erigeron* species found in the Silver Spring bioretention site in October 2013, with pencil and gloved finger for scale.

The species were selected primarily by percent cover. *E. dubium* and *S. rugosa* had the highest percent cover of the planted species. The volunteer species with the highest percent cover, *Polygonum* sp., is a genus that contains species listed as noxious weeds and therefore should not be encouraged in bioretention. Therefore, the volunteer species with the second highest percent cover, *Erigeron* sp., was chosen for further measurements.

To determine if any difference existed between vegetation close to the single runoff inlet and vegetation further away from the inlet, three instances of each species were selected from the half of the cell nearest to the inlet (<8.85 m from inlet along center line) and three were selected from the half of the cell furthest from the inlet (>8.85 m). The instances ranged from a single plant to several plants, but each instance was physically grouped together and not located directly next to any other instances of the species.

Each instance was examined by a method based on the "simple spade" methods described in Böhm (1979), which references Görbing (1930) and Pittman (1962). Each instance was carefully dug up with hand-held shovels, with as much root structure as possible preserved intact. Soil was cleaned off of the roots either by hand or by gentle shaking.

The height of the tallest point of each instance, from soil level, was measured with a tape measure. The longest root was straightened as much as possible and its length measured. The diameter of the thickest root of each instance was also measured and recorded.

Data for each combination of plant species and measurement were analyzed independently. First, the data for the three inlet instances was compared to the data for the three outlet instances. Because of the small sample size and lack of normality of some of the data, non-parametric statistical methods were used. The small sample size results in a lack of sufficient power for a Wilcoxian Rank-Sum test. Instead, a generalized linear model is used. Statistical significance was accepted at $p < 0.05$ when p is rounded to the nearest hundredth. If inlet and outlet plants were not significantly different, the mean for

all six plants of the same species was calculated for plant height, length of longest root, and thickest root measurement. A Kruskal-Wallis test, followed by Dunn's test if significant difference was found in the Kruskal-Wallis test, was used to test for significant difference between the means of each species for each characteristic, with statistical significance accepted at $p < 0.05$ when p is rounded to the nearest hundredth.

Quadrats for Infiltration and Media Sampling

Nine 1 m by 1 m quadrats were established for infiltration testing and media sampling (Figure 3.3). To accomplish this, a tape measure was stretched from the cell inlet to the far edge of the cell, forming a "centerline". The center of the first line of three quadrats was placed at 5 m from the inlet, with quadrat 2 on the centerline and quadrats 1 and 3 on the axis perpendicular to the centerline. Quadrats were separated by 1 m distance between quadrat edges.
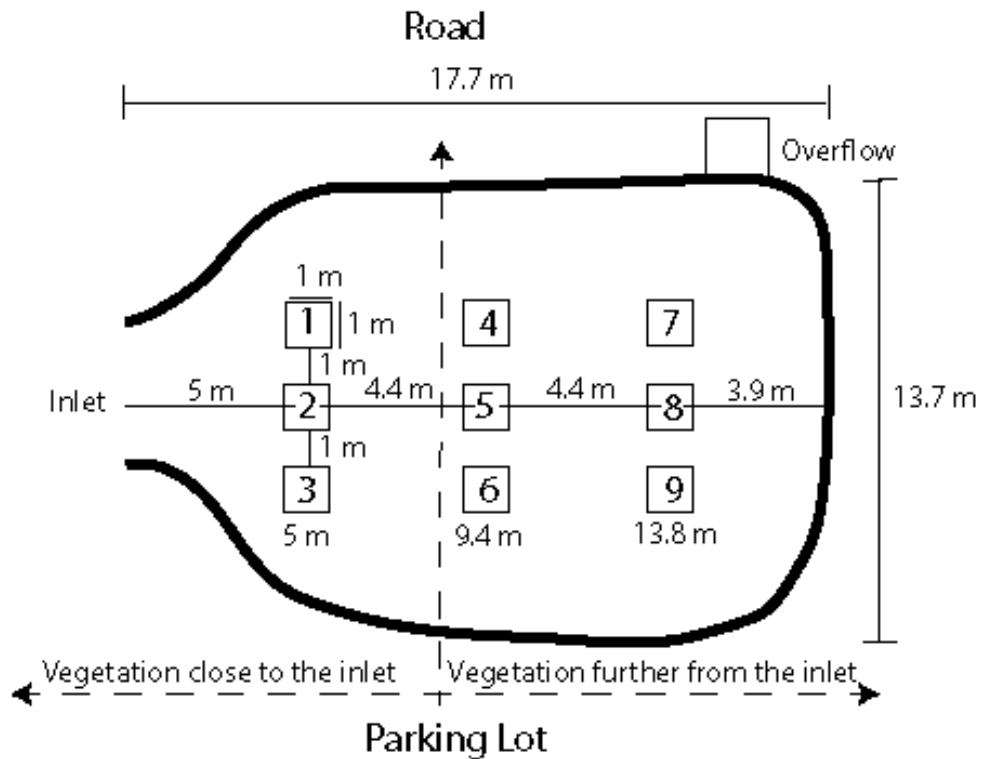
Figure 3.3 The quadrat layout established for media sampling and infiltration testing at the bioretention site.

The location on the centerline of the remaining two lines of three quadrats (quadrats 4-6 and quadrats 7-9) were established by dividing the remaining length of the centerline into equal distances, so that the distance between centers of quadrats measured along the centerline was 4.4 m.

Once the edges of all quadrats were established, the vegetation around each quadrat was trimmed to allow for easy access to each quadrat.

Infiltration Testing

Infiltration testing was performed using a single-ring infiltrometer constructed of 11.4 cm (4.50 in.) outer diameter, 9.50 cm (3.75 in.) inner diameter PVC pipe. The pipe was pounded into the media near (within a few centimeters of) the midpoint of the inlet side of the quadrat so that 15 centimeters of pipe was below grade. 1.24 L of water, representing 17.4 cm of water in the pipe, was poured into the pipe. The time between the water being poured into the pipe and the time when no water was visible above the media was measured and recorded. Each quadrat was sampled once. More samples would have been ideal but were not logistically possible in this study. Infiltration testing for quadrats 1 through 5 was conducted four days before infiltration testing for quadrats 6 through 9. Besides the 1.24 L of water, no additional water is added. Rain occurred on the seventh through fourth days prior to the first infiltration test (total of more than 9.7 cm), and on the day after the first infiltration test and three days prior to the second infiltration test, (less than 0.3 cm). Therefore these measurements represent the infiltration rate of field-moist soil, not saturated hydraulic conductivity measurements.

In order to investigate a possible correlation with location in the cell, infiltration rate values measured in the nine quadrats when grouped by distance from the inlet. Differences between the groups were established using a Kruskal-Wallis test. Significance was declared at $p < 0.05$ when p is rounded to the nearest hundredth. The Kruskal-Wallis test was followed by Dunn's test when significant differences were found.

3.5 Silver Spring Results and Discussion

The cell was heavily vegetated, as demonstrated by the 105.6 field-measured percent vegetative cover, indicating overlap between species of varying heights. A few small patches of uncovered mulch, however, were apparent in the half of the quadrat nearest to the inlet.

Plant height ranged from just above 0 to about 1.5 m, excepting the three shrubs on the edges of the cell, which were taller than 1.5 m. All of the plants present appeared to generally be in good condition. Some evidence of herbivory was noted on the leaves of several species, but not to the extent where plant growth appeared to be severely affected.

Plant Survivorship and Coverage

Table 3.4 lists the plants present at the time of the survey. The top six plants in terms of surface area coverage were (from largest to smallest coverage): *Eupatorium dubium, Polygonum* sp. (smartweed), *Erigeron* sp., *Ampelopsis brevipedunculata*, stiltgrass, and *Solidago rugosa.* Of these six, only *Eupatorium dubium* and *Solidago rugosa* were originally planted, the other four were volunteers.

After 11 months since last maintenance, volunteer plants constituted more than half of the vegetation in the cell: coverage by planted species was 48.7% (five species) and coverage by volunteer species was 51.3% (22 species). This distribution illustrates that the vegetative makeup of a bioretention cell can change drastically in a relatively short amount of time due to colonization by volunteers. The impact of this change in the vegetative community upon bioretention performance depends on the species makeup and the evaluation criteria selected. Given time to establish a root structure, depending

upon the species, the volunteer plants may provide similar, better, or worse function in terms of water uptake, maintenance of media permeability, and other vegetation functions.

Many of the species initially planted in the cell did not survive; Table 3.5 lists these plants. Given the failure of many of the planted species to survive and the large percentage of volunteer species, *E. dubium*'s dominance at 43% of the cell's cover is noteworthy. Three of the species that did not survive: *Ilex glabra, Ilex verticillata,* and *Onoclea sensibilis* have all been noted as also not performing well in other Montgomery County bioretention cells (Mary Travaglini, personal communication, fall 2013). The lack of survivorship of the species in Table 3.5 indicates that designers should use caution when specifying these plants for bioretention in a climate similar to the U.S. mid-Atlantic. However, *Panicum virgatum*, though it did not persist in this facility, is widely used in Montgomery County bioretention, has been successful in establishment of other bioretention cells, and has low maintenance requirements (Mary Travaglini, personal communication, fall 2013 and winter 2014).

Table 3.4 All plant species present in the Silver Spring bioretention cell at the time of the survey, October 2013.

Planted Species Present at Time of Survey		
Scientific name	**Common name**	**Percent Cover of the Cell in October 2013**
Eupatorium dubium	Joe Pye weed	43
Solidago rugosa	Wrinkleleaf goldenrod	4
Cephalanthus occidentalis	Buttonbush	2
Lobelia siphilitica	Great blue lobelia	0.2
Cornus amomum	Silky dogwood	0.2
Volunteer Species Present at Time of Survey		
Scientific name	**Common name**	**Percent Cover of the Cell in October 2013**
Polygonum sp.	Smartweed	24
Erigeron sp.	Fleabane	9.5
Ampelopsis brevipedunculata	Porcelain berry	8.5
Microstegium vimineum	Stiltgrass	4.7
Erechtites hieracifolia	Fireweed	0.95
Viola sp.	Violet	0.95
Rubus sp.	Berry	0.2
Solanum sp.		0.2
Taraxacum sp.	Dandelion species	0.2
	Unknown with small pink/white flower	0.2
	Unknown with very lobbed leaves	0.2
	Unknown	0.2
	Unknown with prickly leaves	0.2
	Turf grass	0.2
	Clover with yellow flowers	0.2
	Creeping herb with small white flower	0.2
	Chives	0.2
	Herb with opposite leaves	0.2
	Strawberry	0.2
Robinia pseudoacacia	Black locust	0.2
Euonymus fortuneii		0.2
Setaria lutescens		0.2

Table 3.5   All planted species that were not present in the Silver Spring bioretention cell at the time of the survey, October 2013.

Planted Species, Non-Survivors	
Scientific name	**Common name**
Symphyotrichum novae-angliae	New England aster
Baptisia australis	False indigo
Ilex glabra	Inkberry
Ilex verticillata	Winterberry
Lobelia cardinalis	Cardinal flower
Monarda fistulosa	Wild bergamot
Onoclea sensibilis	Sensitive fern
Panicum virgatum	Switchgrass
Physostegia virginiana	Obedient plant
Viburnum dentatum	Southern arrowwood

Additionally, historical records show that 63 of the initial 470 herbaceous plants had to be replaced after dying in the first year after planting. Interestingly, these 63 plants included 10 *E. dubium*s, which represents a failure rate of 20% of the original 50 planted *E. dubium*s. Ten *Symphyotrichum novae-angliae*s and 20 *Panicum virgatum*s were also replaced at the same time, along with three *Ilex verticillata*. For comparison, Figures 3.1a and 3.1b show the cell in 2006 and 2009, respectively, and an October 2013 photograph of the site taken at the time of the study, is given in Figure 3.1c. In Figure 3.1b, planted species (the switchgrass, lobelia, and false indigo) can be seen that were no longer present in 2013, suggesting that the maintenance regime was successfully preserving some planted species that either subsequently died of their own accord or could not compete with weeds after maintenance stopped.

In terms of volunteer species, *Polygonum* sp. was the most successful in establishing maximum coverage. Multiple *Polygonum* spp. are listed as noxious weeds in

several U.S. states (USDA NRCS 2015a). Two of the other volunteer species, *Erechtites hieracifolia* and *Ampelopsis brevipedunculata* were noted as common bioretention weeds in Montgomery County (Mary Travaglini, personal communication, fall 2013).

Plant Height and Root Survey

When considering the results of the plant height and root survey, it is important to distinguish that the *E. dubium* and *S. rugosa* populations are based on individuals planted seven years prior to the study, whereas the *Erigeron* sp. presumably had only had 11 months to establish. Therefore while the data for the three species are presented together, this difference in establishment time must be considered when comparing traits between the three species.

When plant instances in the half of the cell closest to the inlet vis-à-vis the half farther from the inlet are compared, no significant difference was found for any of the three examined species in terms of plant height, length of longest root, or thickest root measurement. This is an interesting result, because if the inlet area received a significantly greater amount of water and nutrients than the outlet area, then the plants near the inlet could be larger than the plants further from the inlet. This was not observed, suggesting that a great enough difference in water and nutrients to cause differential growth was not present. Therefore, for each of the three species, all data from each species (n=6) were analyzed together through a Kruskal-Wallis test followed by Dunn's test.

Average tallest aboveground height and average longest root length of each species in the Silver Spring bioretention site are presented in Figure 3.4a. *E. dubium* was on average significantly taller (88.7 ±10.2 cm) than the *Erigeron sp.* (56.7 ± 20.3 cm) and

S. rugosa (56.0 ±19.9 cm) (Figure 3.4a). The *Erigeron* sp. may not have had enough establishment time to reach its full potential height. Height should be considered when designing for areas where plant height may be of concern, e.g., where plants may limit sightlines near roads and driveways. At this particular site sightlines are not a concern and therefore taller plants; unless they have fewer and/or smaller leaves, stems, and vertical growth; will provide more biomass, which may lead to increased water and pollutant uptake.

The average height of 88.7 cm is typical for *E. dubium* plants grown outside of bioretention, which range from 40 to 120 cm generally and sometimes to 170 cm (Lamont 2006). The *S. rugosa,* at an average of 56 cm, is shorter than typically found outside of bioretention, where it ranges from 61 to 152 cm (Lady Bird Johnson Wildflower Center, 2014). This lack of height may mean that bioretention conditions are not ideal for this species. However, further data not collected as part of this study, such as plant width and leaf size, would further inform this statement. *Erigeron* species have a wide range of heights, from 2 to 90 cm (Nesom 2006), so lacking a species identification a definitive comparison cannot be made with the height in this bioretention site.

No statistically significant difference was found among the three species in the length of their longest root (Figure 3.4a), with an average of 29.1 cm for all three species. Again, *Erigeron* sp. had much less time to establish a root network than the other two species, yet it was able to produce a longest root that was not significantly different than the other two originally planted species, in only 11 months. Additionally, this lack of difference occurred even though the tallest aboveground biomass of *E. dubium* was significantly taller than that of the other two species. Root length varies inherently

between plant species, but also depends on a variety of soil characteristics including soil texture, structure, bulk density, and water content (Pagès 2002). However, given that bioretention sites typically use the same media throughout the site, large differences in root structure due to varying soil structure are not expected. Plant roots tend to grow toward areas of high water and nutrient concentration (Huang and Eissenstat 2000). It is therefore reasonable to assume that the three plants studied in this cell are all able to obtain enough water and nutrients in the first roughly 30 cm of media to sustain themselves.

(a)

(b)
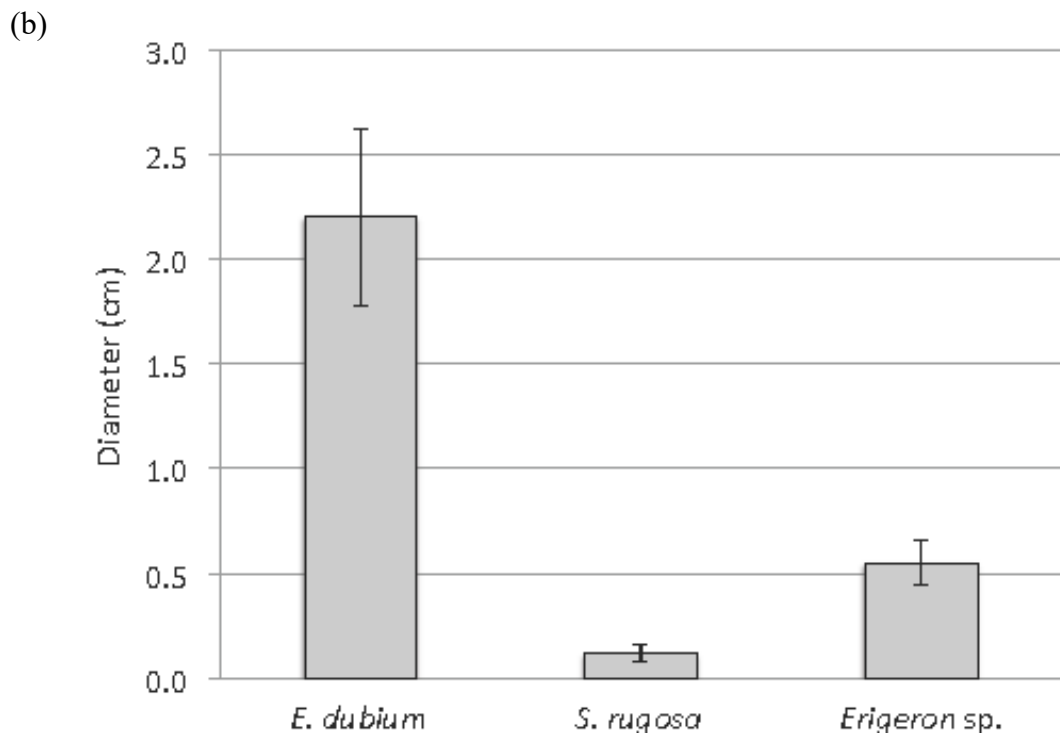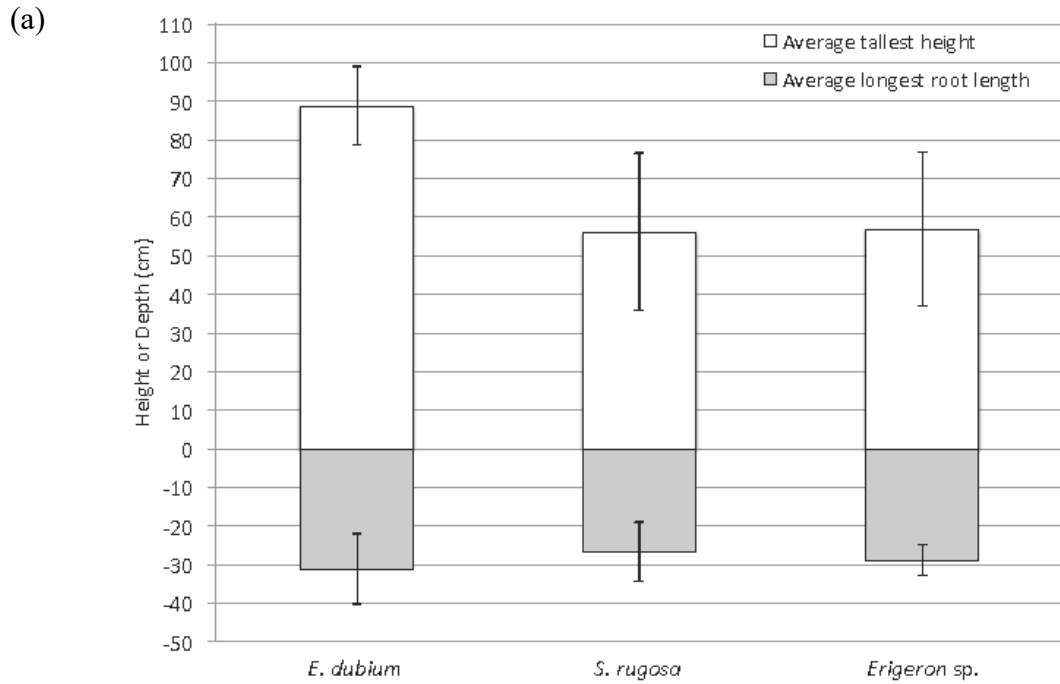
Figure 3.4 **(a)** Average tallest aboveground height and average longest root length of each species. Error bars indicate +/- one standard deviation. **(b)** Average diameter of the thickest root for each species. Error bars indicate +/- one standard deviation.

Regardless of root length, the root network of *E. dubium* can be seen in Figure 3.5 to be more extensive than that of the other two species, which should support high infiltration. On the east coast of the United States, on average between 50–60% of root biomass is found in the upper 30 cm of soil (Jackson et al. 1996). Therefore unless long, fine roots were not successfully extracted from the soil in this study and therefore were not measured, the plants in this study appear to be somewhat less deeply rooted than average natural plant communities in the area.

Figure 3.5 Photos of representative plant instances sampled for root characteristics at the Silver Spring site, categorized by species (scale varies among pictures).

Root growth was not constrained by media depth. The installed media depth was 90 cm (Li and Davis, 2009), and the longest root measured (from one of the *E. dubium* replicates) was 41 cm. This has implications for evapotranspiration and stormwater management performance. Plants can only transpire the water that their roots have access

to, so any water below the root zone will not leave the cell through plant transpiration. In order to maximize the rooting profile in bioretention, the use of drought-resistant plants should be considered. To withstand drought, such plants have deep root systems with extensive branching (Kramer 1983). However, for bioretention use these plants must also be able to withstand inundation.

The diameter of the thickest roots of *E. dubium*, at an average of 2.2 cm, was significantly greater than either of the other two species (Figure 3.4b). The *Erigeron* sp. in turn has significantly wider thickest roots than *S. rugosa*, despite having much less time to establish itself. Le Coustoumer et al. (2012) showed that larger-rooted plants were able to maintain bioretention media permeability over time. Nonetheless, it should be noted that the majority of the *E. dubium* roots were much thinner than the thickest root, as can be seen in Figure 3.5.

In addition to roots, *S. rugosa* had suckers that also penetrated the media near the surface more extensively than the roots alone would, thereby presumably increasing the plant's contribution to media permeability.

Other Vegetation Results

All plants that were dug up had holes in their leaves due to herbivory. Two of the *Erigeron* sp. also had orange eggs on their leaves. During the course of the study, three different invertebrate species were observed in the cell, and others likely could be found through focused study. Therefore, insect habitat provided by the bioretention vegetation in this study agrees with findings of a statistically significant difference in invertebrate biodiversity between bioretention facilities and lawn-type greenspace (Kazemi et al., 2009b; 2011). Depending upon type, invertebrates can contribute to organic matter

decomposition, serve as a food source for animals higher in the food chain, and pollinate a variety of plant species (Samways, 1995). Additionally, one instance of animal droppings was also found in the cell in this study, indicating usage of the cell by larger animals.

In terms of aesthetics, *E. dubium*, *S. rugosa*, and *Erigeron sp.* all produce pleasant flowers that were still present in October. These aesthetics are an important consideration for community acceptance of the bioretention cell.

A few other species beyond the three main species were also examined: *Ampelopsis brevipendunculata, Microstegium vimineum, Erechtites hieracifolia,* and an unknown violet species. While not replicated at a sufficient level to warrant statistical comparison with the three main species, photos of these species are presented in Figure 3.6 as reference. All three of the known species in Figure 3.6 do not have extensive root systems, but the root system in the violet is the most extensive of the four and may warrant further investigation into the use of perennial violets in bioretention. This is especially true as, assuming all species not originally planted were removed with the last maintenance occurrence, the violets had 11 months to become established. With additional time their root structure may become even more extensive, potentially helping to increase infiltration.

Ampelopsis brevipedunculata

Erechtites hieracifolia

Microstegium vimineum

Two specimens of unknown violet species
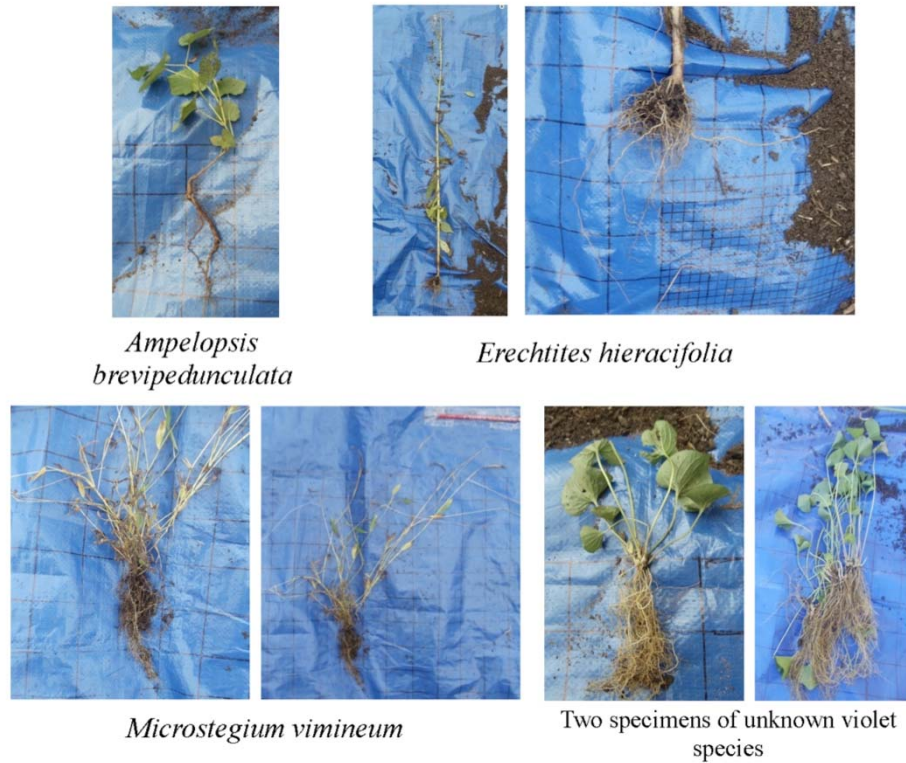
Figure 3.6 Other species at the Silver Spring bioretention cell whose root structure was examined but not quantified.

Infiltration Testing

The infiltration rates in the nine quadrats ranged from 21 to 54 cm per hour. The average for the entire cell was 34 ± 11 cm hr^{-1}. Infiltration rate in bioretention cells is considered an important metric to evaluate the cell's performance (Asleson et al. 2009, Paus et al. 2014). The infiltration rates observed in this study are well above the infiltration rates recommended by Davis and McCuen (2005).

The average value for each group of quadrats are given in Figure 3.7. Asleson et al. (2009) and Paus et al. (2014) have shown that infiltration rates, which they measured by saturated hydraulic conductivity, vary spatially within a single bioretention cell, although not always in a consistent pattern. Although the data are limited, when infiltration rates of the quadrats in the Silver Spring cell were grouped based on distance

to the inlet (Figure 3.7), it was observed that average infiltration rates decreased with increasing distance from the inlet; nonetheless, only the difference between the average infiltration rate of the quadrats closest (quadrats 1–3; 46.3±10.5 cm hr^{-1}) and furthest from the inlet (quadrats 7–9; 25.2±6.6 cm hr^{-1}) was statistically significant ($p = 0.01$).
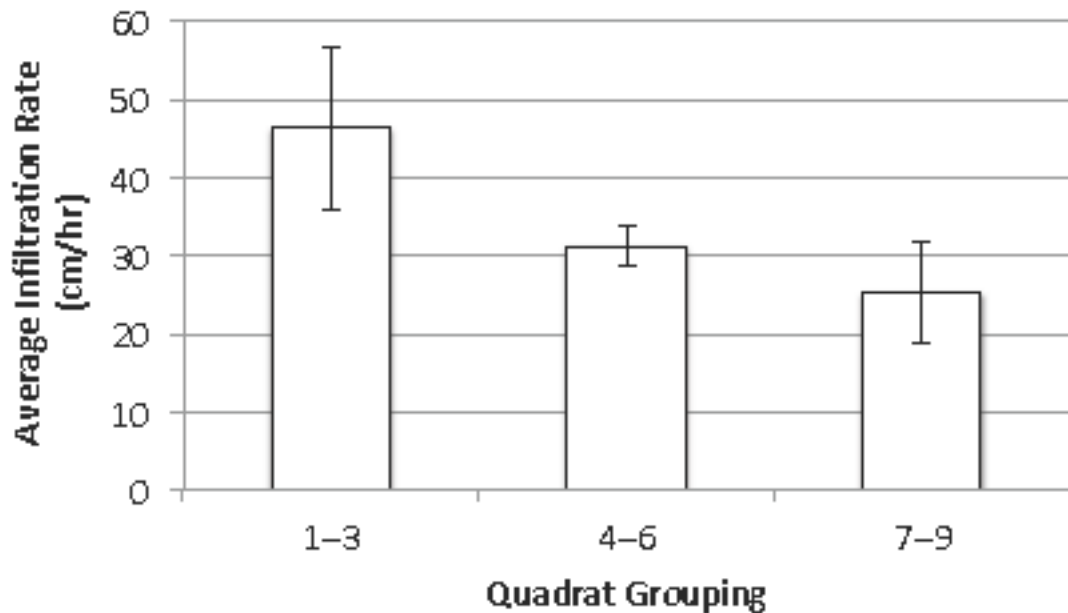
Figure 3.7 Average infiltration rate for quadrats (grouped by distance from the inlet) in the Silver Spring site

The impact of root structure on infiltration rates, at least for the three species measured in this study, is not expected to contribute to infiltration differences among quadrats since no difference was observed spatially in the vegetation characteristics. However, vegetation characteristic measurements were not correlated with quadrat location. Therefore, no claim can be made regarding the exact vegetation composition of each quadrat. It is possible that different species composition between quadrats resulted in differing root characteristics that may contribute to the difference in observed

infiltration rates. However, overall, the reason for the observed infiltration trend is not clear and with the given data it cannot be correlated to other observations made for this cell.

Design Recommendations

Design recommendations are offered based on data collected during this study. The cell was heavily vegetated, which in general enhances the benefits that vegetation provides. However, many of the originally-planted species had not survived. More data from additional cells is necessary to draw wide-reaching conclusions, but based upon this study, the species listed in Table 3.5 should be cautiously considered for use in bioretention in the mid-Atlantic region. Conversely, the most successful planted species, in terms of coverage, was by far *E. dubium*; the high percentage of *E. dubium* coverage constitutes a strong recommendation for using this species in bioretention in the mid-Atlantic, especially considering that the other survivors trailed behind markedly in coverage. *S. rugosa* was the only other herbaceous species remaining of the original plantings that maintained a cover greater than 1%. As noted in Table 3.4, scattered blue lobelia was also found, and the two shrubs persisted.

Volunteer plants constituted more than half of the cell vegetation coverage after less than a year without maintenance. Many of these, such as *Ampelopsis brevipedunculata,* are commonly found weed species in Maryland. While the presence of a natural area next to the cell may contribute more weed seeds than a cell in a highly urban setting would receive, this finding does suggest the importance of maintenance if a long-lasting vegetative makeup with any close resemblance the original planting is desired. It also indicates the importance of specifying successful species like *E. dubium*,

which in this case persisted for approximately six years with a maintenance regime while other planted species did not, and then maintained a high percent cover for 11 months without a maintenance regime. Previous studies have noted the influx of weed species in bioretention if maintenance is not performed: near Atlanta, Georgia in Hunt et al. (2012), and in Texas in Li et al. (2011). Further studies of a variety of cells, especially studies that provide controlled experimental manipulation, would provide useful data toward determining the average weed invasion into bioretention cells.

It is also worth considering if a plant palette consisting largely or entirely of volunteer species could provide aesthetic and nutrient benefits while also greatly reducing maintenance cost and effort. The benefits will of course depend on the geographic area and exact volunteer species that thrive there. Noxious weeds should also be avoided. Further work comparing cells with unmaintained vegetation vis-à-vis cells with maintained vegetation in both aesthetic and nutrient removal performance have the potential to produce results that could greatly change cell maintenance regimes. The success of *Erigeron* sp. in establishing similar root depth as the two planted species in this study is an example of the potential of volunteer species to quickly establish notable root depth, though not an extensive root network.

Additionally, no statistical difference was found between the root length of *E. dubium* and *S. rugosa*, despite their aboveground heights being statistically different. This is an important consideration for design: for these three species at least, root depth cannot be judged by the aboveground height of the plant.

In this study, the benefits of the roots in aeration of the media and introduction of channels for water was limited to only approximately the top ~30 cm, though as noted

above it is possible that fine roots were not removed from the media for measurement, and may penetrate deeper. It should be noted that root depth can be highly species- and location-dependent, for example Barrett et al. (2013) found that Big Muhly grass (*Muhlenbergia lindheimeri*) grew roots to a depth of ~46 cm in bioretention mesocosms, and Buffalograss 609, a turfgrass, had roots only in the top ~10 cm of the media in bioretention mesocosms.

Of the three species examined in this study, *E. dubium* roots were the most dense and extensive; this species also had the largest average measurement of the thickest root of the three species. In previous bioretention studies, thick roots have been shown to encourage infiltration. As stated previously, *Erigeron* sp. had much less time to establish itself than the other two species, so with additional time its root thickness may change.

E. dubium was also significantly taller than the other two intensively-studied species. Aboveground width, leaf size, and other factors can also contribute to the amount of aboveground biomass, but these measurements were not made. In general, maximizing biomass production is desirable, as more biomass can potentially lead to additional pollutant removal and water uptake. If sightlines are a consideration, however, then shorter species such as *S. rugosa* may be more desirable, but the more minimal vegetative coverage of the cell by *S. rugosa* vis-à-vis *E. dubium* may be a tradeoff in such a case.

Overall, based on this study's findings, due to its survivorship, cell coverage, and its extensive root network, *E. dubium* is the most highly recommended species for bioretention use of those species examined in this study. Further quantitative study of other cells in the mid-Atlantic region is needed to confirm this finding, but based on the

results of both this study and observation of multiple cells in Maryland, *E. dubium* appears to grow successfully in bioretention.

Chapter Four: Greenhouse Study Methodology

4.1 Plant Selection

Commonly specified Maryland bioretention plant species that also survive well in bioretention conditions were identified via design manuals (CWP and MDE 2000, PGCo 2007); personal conversation and field inspection of bioretention sites with Mary Travaglini of Montgomery County, Maryland (personal communication, Fall 2013); and through a field study (Muerdter et al. 2015, Chapter 3). *Eutrochium* (Joe Pye) species, *Iris versicolor* (blue flag Iris), and *Juncus effusus* (common rush) have all been widely used in the state and have been successful at establishment and persistence. *Eutrochium dubium* was by far the most successful (in percent of cell area covered) planted species in the Silver Spring, Maryland bioretention field study in 2013 (Muerdter et al. 2015, Chapter 3). *Juncus effusus* provides greenery during the winter months when other plants have lost their leaves, and it tolerates bioretention inlets well (Mary Travaglini, personal communication, fall 2013). *Iris versicolor* has also been successful in inlets and bioretention in general (Mary Travaglini, personal communication, fall 2013) and is included in the bioretention plant lists for both the Maryland Stormwater Design Manual (2000) and the Prince George's County Bioretention Manual (2007). In addition to past success in bioretention, seasonal uptake and comparison of uptake during bloom period vis-à-vis non-bloom periods were also of interest in this study. Therefore, these three species were selected for use in this study on the basis of their differing bloom times as well: the Iris in early summer (PGCo 2007), *J. effusus* in May through August (USDA

NRCS 2015b), and *E. dubium* in July through September (Missouri Botanical Garden 2015). Additionally, the ability of *Juncus effusus* to remain green during the winter months was of interest, for comparison with the two other species that do not.

4.2 Study Setup

A greenhouse section in the University of Maryland Research Greenhouse Complex was used for this study. The section receives ambient light through glass on two side walls and through the roof. The roof is occasionally shaded with retractable, semi-light-permeable fabric to maintain desired temperatures. Shading is automatically controlled through the automated greenhouse temperature regulation system. One wall of the section is covered in black plastic to prevent light contamination from a neighboring experiment that uses artificial light at night, and one wall is the brick wall of the greenhouse headhouse. The overall effect is similar to outdoor, ambient lighting.

A temperature regime (Appendix A) was derived from *weather.com* data of average daily high, low, and mean temperatures in College Park, Maryland. Weather.com uses data from the National Climatic Data Center (NCDC) for its average temperature information, from the NCDC station within about 30 miles of the given zip code and at a similar elevation. The exact station used is not specified. A weekly average high, low, and mean were found from these data. These weekly averages were used as the temperature regime in this study. The low temperature is used from 1 a.m. to 5 a.m., the mean temperature for 5 a.m. to 1 p.m., the high temperature for 1 p.m. to 5 p.m., and the mean temperature again from 5 p.m. to 1 a.m. Throughout the year the low temperature ranged from -3.9 °C to 20.6 °C for the minimum temperature, 0 to 26.1 °C for the mean temperature, and 5.6 to 31.7 °C for the maximum temperature. The greenhouse climate

control system matches the desired temperatures as closely as possible, though negative

(°C) temperatures were not possible.

13.2 L HDPE plastic buckets were used to form the mesocosms. The buckets are

27.31 cm tall, with a top outside diameter of 30.16 cm and bottom outside diameter of

26.35 cm. A 0.635 cm diameter hole was drilled in the bottom of each bucket, as close to

the edge of the bucket as possible. All buckets were washed with Alconox and tap water,

rinsed with deionized (DI) water, and allowed to air dry before use.

Materials to create a bioretention mesocosm were obtained: pool sand, gravel,

hardwood shredded mulch, and Maryland SHA 920 bioretention media. Media

specifications are given in Table 4.1.

Table 4.1   Specifications of the bioretention media used in the greenhouse mesocosm
experiment

Component	Parts out of 10, by volume
Coarse sand: washed silica or crushed glass the conforms to ASTM Fine Aggregate C-33	5
Base soil: 1 to 10 % organic matter by weight 50 to 85% sand by weight 5 to 45% silt by weight 5 to 10% clay by weight	3
Fine bark: Bark of hardwood trees, milled and screened to a uniform particle size of 2 inches or less and then composted and aged for six months or longer.	2

Pool filter sand was packed by Southern Products & Silica Co. from Hoffman,

North Carolina. The sand was washed with deionized (DI) water until the runoff ran

clear, which typically required four changes of water. The gravel, mulch, and media were

all donated by Stancills, Inc. in Perryville, Maryland, who is listed in the Maryland SHA

Base Soil Suppliers Database. The mulch was hardwood shredded mulch sieved with a

1.27 cm screen. Any gravel pieces larger than 1.27 cm in diameter were removed, to

correspond with the bucket dimensions. The remaining gravel, 1.27 cm or less in

diameter, was washed in the same manner as the pool sand. Both media and mulch were

sorted before use in the study, so that particles larger than 3.81 cm in length and/or 0.635

cm in width were removed, to correspond with the bucket dimensions.

Each bucket was assembled as follows: A single layer of 31.6 cm^2 of washed

gravel was placed over the hole in the bottom of the bucket so that it formed a roughly

triangular shape, with a height of 6.35 cm between the hole at the edge of the bucket and

the center of the bucket (Figure 4.1).

Figure 4.1   Gravel in the bottom of the bucket

This gravel was covered with a layer of washed, moist pool sand to 2.54 cm

above the bottom of the bucket (Figure 4.2).

Figure 4.2 Washed pool sand was added to 2.54 cm above the bottom of the bucket

Media was added on top of the pool sand to 21.08 cm above the bottom of the bucket. 1.27 cm of mulch was added on top of the media, so that the top of the mulch was 22.35 cm above the bottom of the bucket. A cross section of the full arrangement is given in Figure 4.3.
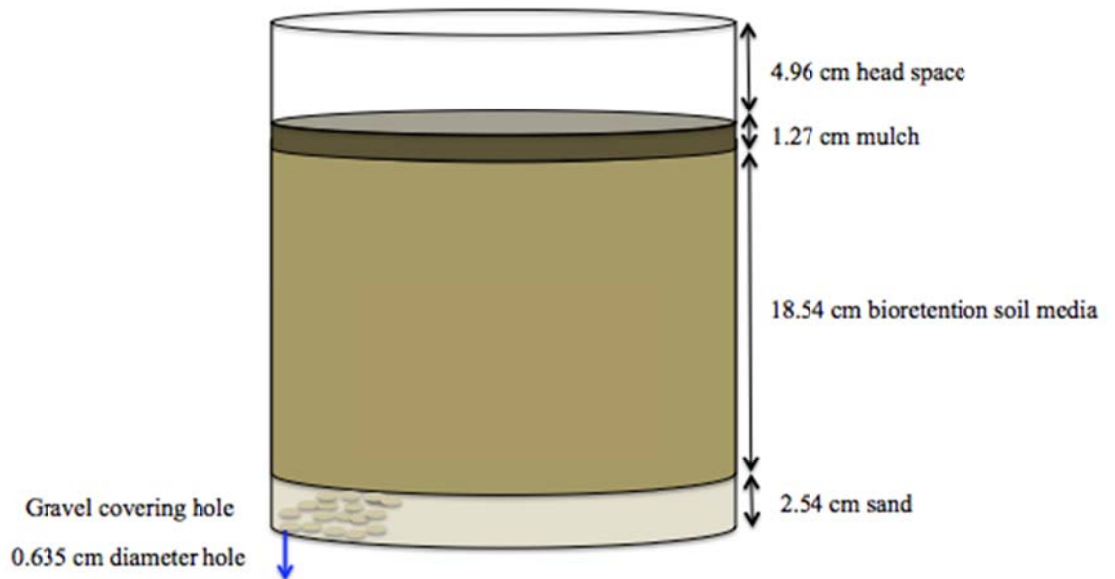
Figure 4.3 Cross section of an assembled mesocosm, before planting

4.3 Applied Water

Freshly assembled buckets produced very murky effluent when DI water was run through them. Because clearer water is needed for analysis of N species, assembled buckets were flushed with DI water and then synthetic stormwater (Table 4.2) before being planted with vegetation. Each assembled bucket was flushed with 224 cm (140 L) of DI water to produce sufficiently clear effluent, followed by 26.9 cm (16.8 L) of stormwater before plants were planted in week one of the experiment. Stormwater chemical composition is detailed in Table 4.2.

Table 4.2 Composition of synthetic stormwater used as influent. Bold components were added beginning in week 15.

Chemical	Concentration (mg L^{-1} unless noted)
Sodium nitrate	0.56 as N
Glycine	0.5 as N
Ammonium chloride	0.3 as N
Sodium dihydrogen phosphate	0.17 as P
Sodium chloride	0.01 M
Potassium chloride	**5.6 as K**
Magnesium chloride and magnesium sulfate	**0.78 as Mg**
Magnesium sulfate and zinc sulfate	**0.68 as S**
Sodium borate	**0.045 as B**
Zinc sulfate	**0.028 as Zn**

Phosphate concentration was designed to be similar to the concentration found in typical stormwater (see Tables 2.3 and 2.4). Sodium chloride concentration was determined based on Corsi et al. (2010), who used urban stream chloride concentrations to study road salt in urban runoff. Corsi et al. (2010) found that, in urban streams in the Washington, DC area, the US EPA chronic chlorine (Cl) water quality criterion of 230 mg L^{-1} was never exceeded in May–October; and was exceeded in less than 20% of the

sampled sites in Washington, DC, in November–April. 230 mg Cl L^{-1} is equivalent to 0.0065 mol Cl L^{-1}. The 0.01 mol NaCl L^{-1} used in this study was equivalent to 0.0061 mol Cl L^{-1}, slightly less than the EPA chronic Cl water quality criterion that was found to be rarely exceeded in Corsi et al. (2010).

As a result of the media testing following *E. dubium* deaths, supplemental chemicals for plant health, indicated in bold in Table 4.2, were added to the synthetic stormwater beginning in week 15. Table 4.3 gives the results of the media testing that prompted the addition of the chemicals in bold. The concentrations for supplemental chemicals were designed to apply the mass of each chemical required to make up the deficit between the tested concentration and an optimal concentration, in three months of stormwater applications.

Table 4.3 Results of media testing in week 11 of the experiment showing plant nutrient deficiencies. Three media samples were taken from each mesocosm at 10 cm below the media's surface. The samples for each treatment were combined for nutrient analysis. Nutrient levels were determined by Mehlich-3 extraction. Bold nutrients were added to the synthetic stormwater beginning in week 15 (see Table 4.2).

Nutrient	Joe-Pye Treatment Concentration (ppm)	Joe-Pye Treatment Rating for Plant Growth	All-plants Treatment Concentration (ppm)	All-plants Treatment Rating for Plant Growth
Phosphorus (P)	24	Low	19	Low
Potassium (K)	**29**	**Very Low**	**19**	**Very Low**
Calcium (Ca)	484	Medium	401	Medium
Magnesium (Mg)	**54**	**Medium**	**46**	**Medium**
Sulfur (S)	**4**	**Very Low**	**3**	**Very Low**
Boron (B)	**0.3**	**Very Low**	**0.2**	**Very Low**
Copper (Cu)	1.5	Medium	1.0	Medium
Iron (Fe)	321	Very High	252	Very High
Manganese (Mn)	34	Optimum	22	Optimum
Zinc (Zn)	**2.3**	**Medium**	**1.1**	**Low**
Sodium (Na)	243	Very High	210	Very High

Thus, the TN concentration in the stormwater is 1.36 mg L^{-1} and the concentration of P is 0.17 mg L^{-1}. Stormwater was mixed using DI water and the above chemicals in a BRUTE 44 gallon trash can, washed with Alconox and tap water, and then rinsed with DI water before use. DI water is measured by volume and chemicals are measured by mass. At the beginning of the experiment, dry chemicals were slowly added into the DI water as a Talboys Laboratory Stirrer, model number 103, from Troemner LLC, homogenized the mixture. Beginning in week 11 of the experiment, stock solutions of 250 or 500 mL per 120 L of stormwater were made with the dry chemicals and DI water. These stock solutions were added to the DI water in the container, in place of dry chemicals, to make the synthetic stormwater for all 0.69 cm or greater storms. From week 37 through the end of the experiment, stock was used for all storms.

4.4 Treatments

After flushing, mesocosms were planted with vegetation in week one of the study. Vegetation was obtained through as plugs from Signature Horticultural Services, Inc., in Freeland, Maryland, approximately 50 miles northeast of the University of Maryland. Six types of vegetation buckets and one no-vegetation control were assembled, with varying species and plant densities, as shown in Figure 4.4.
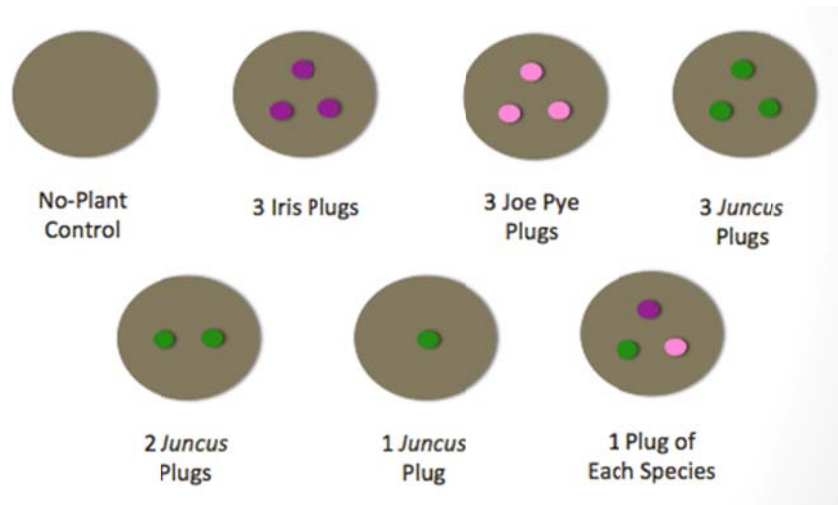
Figure 4.4 Mesocosm setup

Each of the seven types of mesocosms was replicated four times, for a total of 28 mesocosms. The mesocosms are arranged in four rows of seven, on 77.5 cm-high tables, with each row having one of each type of mesocosm. Mesocosm arrangement within each row is such that adjacent rows have a different order of the seven types of mesocosms. Mesocosms are propped up above table level to allow for a funnel to be placed directly below the drainage hole. Funnels were obtained from Parco Scientific Company in Westland, Michigan, and were washed with Alconox and tap water and rinsed with DI water before use.

4.5 Planting

To prepare for planting, the mulch in the area of the pot where the plug was to be planted was scraped to the side. A plastic spade was used to make a hole about as deep as the plug (6.35 cm) and about twice as wide (~8.89 cm) (Figure 4.5). Before planting in a mesocosm, each plug had as much soil as possible gently removed from the roots by gloved hand. Then the plug was rinsed in two sequential buckets of DI water and

massaged by hand to remove as much of the soil as possible while leaving the roots intact (Figure 4.6).

Figure 4.5 A pot being planted

Figure 4.6 A plug before and after removal of soil

The plug was placed in the hole so that the top of the roots was slightly above the top of the hole, to allow for the height of the mulch. Then the hole was filled in with the media that was previously removed, and the mulch was replaced above the media, to surround the plant.

Figure 4.7 The overall setup, after the pots were planted

4.6 Water Application

Immediately after planting, each bucket was watered with 1.60 cm of tap water, as it likely would be in the field during a bioretention installation. Each mesocosm was then watered daily until two weeks after planting, again with 1.60 cm of tap water each day, to allow for plant establishment, except for two "storms" with stormwater during this period, on which days only stormwater was applied. This initial establishment watering regime of watering once per day for 14 days is indicated as common in the Maryland Stormwater Design Manual (2000). Two applications of 2.40 cm tap water per mesocosm were then applied during the nine days following the initial establishment period. Subsequently, except for an establishment period after plant deaths that necessitated replanting, only stormwater was applied, in amounts and frequency to mimic natural Maryland precipitation: generally twice a week. The frequency of a given type of storm is selected to represent the average distribution of different sizes of storms in Maryland, as found by Kreeb and McCuen (2003).

The stormwater watering schedule is given in Table 4.4. Both the rainfall depths simulated and the actual depths of water applied to the mesocosms are given. To convert from rainfall depth to depth of water applied, scaling factors are used to represent the typical amount of water that would enter a bioretention cell for a storm of that depth. This calculation is given in Equation 4.1:

$$0.9 \; x \; 20 \; x \; rainfall \; depth \; in \; cm \qquad\qquad (4.1)$$

$$= \; stormwater \; depth \; applied \; to \; a \; single \; mesocosm$$

The depth of rainfall is multiplied by 0.9 to find the runoff volume, and by 20 to represent the typical design in which a bioretention cell receives runoff from an area 20 times the size of the cell.

Table 4.4 Watering, sampling, and measurement regime

Week of the Experiment	Calendar Week	Rainfall Depth Simulated (in cm)	Depth of Synthetic Stormwater Applied (in cm)	Sampling Regime	P or Other Measurements (other measurements are typically for selected mesocosms, not all mesocosms)
1	7/13/2014	0.28	5.04	Composite	P, conductivity
2	7/20/2014	1.38	24.8	Discrete	
3	7/27/2014	None	None		
4	8/3/2014	1.38	24.8	Discrete	
5	8/10/2014	0.09	1.62		
		0.34	6.12		
6	8/17/2014	0.09	1.62		
		0.69	12.4	Discrete	
7	8/24/2014	0.18	3.24		Infiltration rate
		0.69	12.4		
8	8/31/2014	0.18	3.24		
		0.34	6.12		Effluent volume
9	9/7/2014	1.03	18.5	Discrete	
		2.76	49.7		
10	9/14/2014	0.18	3.24		

Week of the Experiment	Calendar Week	Rainfall Depth Simulated (in cm)	Depth of Synthetic Stormwater Applied (in cm)	Sampling Regime	P or Other Measurements (other measurements are typically for selected mesocosms, not all mesocosms)
		1.38	24.8		
11	9/21/2014	0.34	6.12		
		1.03	18.5		
12	9/28/2014	1.03	18.5		
		0.18	3.24		pH of influent and effluent
13	10/5/2014	0.34	6.12		
		0.18	3.24	Composite	Effluent rate
14	10/12/2014	0.18	3.24		
		0.09	1.62		
		2.76	49.7		Infiltration rate
15	10/19/2014	0.34	6.12		
		2.76	49.7		
16	10/26/2014	1.03	18.5		pH
		0.18	3.24	Composite	
17	11/2/2014	0.18	3.24		
18	11/9/2014	0.34	6.12		
		1.03	18.5		
		0.34	6.12		
19	11/16/2014	0.18	3.24		
		1.03	18.5		Infiltration rate
20	11/23/2014	0.18	3.24		
		0.34	6.12		
21	11/30/2014	1.38	24.8		
		0.34	6.12	Composite	pH
22	12/7/2014	0.18	3.24		
		0.34	6.12		
23	12/14/2014	1.38	24.8		
24	12/21/2014	1.03	18.5		
25	12/28/2014	1.38	24.8		
26	1/4/2015	2.76	49.7		
		0.18	3.24		
		0.69	12.4		
27	1/11/2015	0.69	12.4	Discrete	P, conductivity
		0.18	3.24		
		2.76	49.7		
28	1/18/2015	1.03	18.5		
		2.76	49.7		

Week of the Experiment	Calendar Week	Rainfall Depth Simulated (in cm)	Depth of Synthetic Stormwater Applied (in cm)	Sampling Regime	P or Other Measurements (other measurements are typically for selected mesocosms, not all mesocosms)
29	1/25/2015	0.34	6.12		Infiltration rate
		1.38	24.8		
30	2/1/2015	0.18	3.24		pH
		0.18	3.24		
		1.03	18.5		Effluent rate
31	2/8/2015	1.38	24.8		
		0.69	12.4	Discrete	
32	2/15/15	0.18	3.24		
		2.76	49.7		
33	2/22/15	1.03	18.5		pH
		1.38	24.8		Effluent rate
34	3/1/15	0.18	3.24		Infiltration rate
		2.76	49.7	Composite	
35	3/8/15	0.18	3.24		
		0.34	6.12		
36	3/15/15	2.76	49.7		
37	3/22/15	1.38	24.8		
		1.03	18.5		Effluent rate
		0.18	3.24		Infiltration rate
38	3/29/15	0.18	3.24		
		1.03	18.5	Composite	P, conductivity
39	4/5/15	0.69	12.4		pH
		2.76	49.7		
40	4/12/15	0.18	3.24		
		1.03	18.5		
		1.38	24.8		
41	4/19/15	0.18	3.24		
		2.76	49.7		
42	4/26/15	0.18	3.24		
		1.38	24.8	Composite	P, conductivity (for 22/32 samples)
		0.18	3.24		Effluent rate
43	5/3/15	0.18	3.24		Infiltration rate
		1.38	24.8		
		1.38	24.8		pH
		0.18	3.24		
44	5/10/15	0.34	6.12		
		0.34	6.12		

Week of the Experiment	Calendar Week	Rainfall Depth Simulated (in cm)	Depth of Synthetic Stormwater Applied (in cm)	Sampling Regime	P or Other Measurements (other measurements are typically for selected mesocosms, not all mesocosms)
45	5/17/15	0.34	6.12		
46	5/24/15	0.34	6.12		
47	5/31/15	0.69	12.4	Composite	
		1.38	24.8		
48	6/7/15	2.76	49.7		
		0.09	1.62		
49	6/14/15	0.09	1.62		
		0.18	3.24		pH
		1.03	18.5		
50	6/21/15	0.18	3.24		Infiltration rate
		1.38	24.8		Effluent rate
51	6/28/15	2.76	49.7		
		1.38	24.8		
52	7/5/15	1.03	18.5	Discrete	
		1.38	24.8		
53	7/12/15	0.34	6.12		Effluent rate
Total		**92.6**	**1,670**		

Note that watering with tap water, which occurred in weeks one through four of the study and in weeks 16 through 18 following *E. dubium* replacement, is not included in Table 4.4. All applications of tap water were 1.0 L, equivalent to a 0.09 cm storm, except for two applications of 1.4 L, equivalent to a 0.13 cm storm, in weeks three and four. The sum of tap water was applied to each pot during these two periods was 2.42 cm simulated rainfall depth and 43.6 cm applied depth.

The total simulated rainfall depth delivered during this study is 92.6 cm, or 1,670 cm of applied depth. The average amount of precipitation for one year (which is approximately the time period of this study) from Beltsville, Maryland, which is the closest US National Oceanic and Atmospheric Administration (NOAA) weather station

to the study location, between 2000 and 2014, is 109.9 cm (US NOAA National Centers for Environmental Information 2015). Therefore this regime represents about 84% of average precipitation for the area.

4.7 Mesocosm Drainage

Beginning about a month into the study, one mesocosm (the fourth replicate of the three-*Juncus* treatment) began to drain noticeably slower than the other mesocosms. Within two more weeks, two additional mesocosms (both one-*Juncus* replicates) were also draining slowly. By week 13 there were eight slow-draining mesocosms from five different treatments. Water had to be reserved during storms for later application because these mesocosms were draining too slowly to apply the fully storm amount on the day of the storm. In week 14 the fourth three-*Juncus* treatment replicate was disassembled because it was so far behind in water to be applied. It was replaced with a new all-plants mesocosm, which was assembled and washed with tap and then stormwater and then planted, in the same manner as the other mesocosms at the beginning of the experiment. In week 29 of the experiment, 15 of the mesocosms, representing all treatments, were draining slow enough so as to need water reserved that could not be applied on the day of a storm. Therefore in week 29, an acid bath-cleaned 1000 uL pipette tip was inserted into the hole at the bottom of each mesocosm as far as possible (about 4.5 cm) and then removed, three times. A new, clean pipette tip was used for each mesocosm. This procedure resulted in marked improved drainage rates for all of the slow mesocosms, so that water no longer needed to be reserved for any mesocosms. To maintain these drainage rates, the poking procedure was repeated approximately every two weeks for the remainder of the experiment. This succeeded in maintaining drainage rates.

4.8 Sampling Storms

Fifteen total storms were sampled, eight as composite sampling and seven as discrete sampling. Influent samples were collected for each batch of stormwater applied to the mesocosms during a given storm. Effluent samples were collected either as composite samples or discrete samples. For all sample types, the funnels under the mesocosms were removed and cleaned with Alconox and tap water, rinsed with DI water, and then replaced before sample collection. For a composite sample, the effluent buckets were cleaned with Alconox and tap water, rinsed with DI water, and allowed to air dry prior to one bucket being placed under each mesocosm during the sampling storm. Once all effluent has drained through the mesocosms, the effluent was stirred with a clean rubber spatula, and then a 125 mL sample bottle was submerged into the effluent until the bottle is full, minus head space for expansion during freezing. For a discrete sample, a sample bottle was placed directly below the funnel while a batch of the stormwater was applied to the top of the mesocosm. The sample bottle was held until place until it is full, minus head space for freezing. Usually multiple samples were taken per storm during discrete sampling. For all sampling regimes, collected samples were kept in a cooler with an ice pack until they were able to be transported to a freezer, which occurred either during or directly after the storm. Samples were then kept frozen until analysis.

4.9 Other Measurements

As noted in Table 4.4, on an approximately monthly basis other measurements were taken, typically on one row of mesocosms, i.e., one mesocosm of each treatment, on a given date:

1. Solution pH was measured for the influent and the first ~70 mL of effluent from each of the mesocosms from one row of mesocosms (i.e., seven samples), collected in a 125 mL sample bottle. pH of the samples was measured using a Mettler Toledo MA235 pH/ion analyzer, as soon as possible after sampling.

2. One measurement of effluent volume after all water had drained through the mesocosms was made in September 2014. Subsequently, effluent flow rate was measured, i.e., how much time elapsed between the time of application of the first application of stormwater for the day and the time when 2.54 cm of effluent was present in the effluent collection bucket. Some sampling storms also included measurement of the volume of effluent between sample collections.

3. Infiltration rate was also measured, as the time elapsed between the start of application of the first batch of stormwater for the day to the time when the water had infiltrated below the mulch surface.

4. Pictures of each mesocosm were taken with a scaled grid background of 5 cm x 5 cm per square (Figure 4.8) on approximately monthly basis, to allow for monitoring of aboveground biomass growth.

Figure 4.8   A mesocosm, with the scaled grid background (one square = 5 cm x 5 cm)

4.10 Weeding

Any unplanted species that began growing in the mesocosms were removed as soon as they were seen.

4.11 Sample Analysis

Sample analysis consisted of determining the TN, total dissolved N, NH_4^+-N, NO_3^--N, and NO_2^--N levels for effluent and influent samples collected during either composite or discrete samplings storms. TP and conductivity were also determined for the samples noted in the watering regime in Table 4.4, above. Table 4.5 shows the methods used for analysis.

Table 4.5 Sample analysis methods for N and P concentrations in influent and effluent samples

Parameter	Method Used	Detection Range
TN	Either, or both for verification: • Bachmann and Canfield (1996), except HCl is used instead of sulfuric acid prior to reading. Also, to remove particulate matter: for all unfiltered samples the samples were centrifuged at 3,000 rpm for eight minutes and then decanted directly before reading on the spectrophotometer, except select 7/23/14 and 10/31/14 samples which were either not centrifuged or centrifuged at a lower speed. • Shimadzu TOC-L Total Organic Carbon Analyzer with Shimadzu TNM-L TN measuring unit	0.05–2.0 mg L^{-1}
Total dissolved N	After filtration of sample with 0.22 μm filter: Either, or both for verification: • Bachmann and Canfield (1996), except HCl is used instead of sulfuric acid prior to reading. Also, to remove particulate matter: for all unfiltered samples the samples were centrifuged at 3,000 rpm for eight minutes and then decanted directly before reading	0.05–2.0 mg L^{-1}

Parameter	Method Used	Detection Range
	on the spectrophotometer, except select 7/23/14 and 10/31/14 samples which were either not centrifuged or centrifuged at a lower speed. • Shimadzu TOC-L Total Organic Carbon Analyzer with Shimadzu TNM-L TN measuring unit	
NH_4^+-N	Standard Method 4500-NH_3 F (Eaton et al. 1995)	0.1–2.0 mg L^{-1}
NO_3^--N	Standard Method 4500-NO_3^- B. (Eaton et al. 1995)	0.1–2.0 mg L^{-1}
NO_2^--N	Standard Method 4500-NO_2^- B. (Eaton et al. 1995)	0.02–0.3 mg L^{-1}
TP	Standard Method 4500-P E. (Eaton et al. 1995), ascorbic acid method	0.1–0.4 mg L^{-1}

DON was calculated as dissolved TN – (NO_3^- + NO_2^- + NH_4^+). For storms in which NO_2^- was not measured, DON is presented as DON*, and was calculated as dissolved TN – (NO_3^- + NH_4^+). Particulate organic N was calculated from TN – total dissolved N. If a concentration was read or calculated as negative, it was corrected to zero before being used in calculation or plotted. If a concentration was between zero and the lowest non-zero standard that can be differentiated from zero, then it was recorded as half of the concentration of the lowest non-zero standard that can be differentiated from zero.

Concentrations were statistically compared for significant differences. Unless otherwise noted, an approximative k-sample permutation test was performed using the coin package in R, with the discrete method used to find the p values for each treatment. Differences were deemed significant at $p \le 0.05$.

4.12 Plant and Media Testing and Measurements

To establish a fuller picture of the N and P budget of the system, plant biomass and percent N content was measured at both the beginning and end of the experiment. At the beginning of the experiment, six extra plugs of each species of plant were cut into above- and belowground portions after being washed in the same manner as the plugs that were planted (Figure 4.9).

Figure 4.9 Joe Pye plugs washed and cut into above- and portions, in preparation for drying

These above- and belowground portions were dried at 80° C until a constant mass was obtained and recorded, per Mills et al. (1996). Three aboveground samples and three belowground samples for each of the three plant species, from a range of dry masses, were sent to the University of Delaware Soil Testing Program, where their percent TN was determined by combustion.

At the end of the experiment, one plant from three different one-*Juncus* treatment

replicates, one plant from three different Iris treatment replicates, one plant from three different Joe-Pye treatment replicates, one Iris from three different all-plants treatment replicates and one *Juncus* from each of the same three all-plants treatment replicates, had their roots washed in the same manner as the plugs that were originally planted. They then were cut into above- and belowground portions as the plugs were, dried at 80° C, had their masses recorded, and were sent to the University of Delaware Soil Testing Program in the same manner as the plugs at the beginning of the experiment. For both *Juncus* and Iris, for each species the three replicates from the all-plants treatment were compared with the three replicates from the mono-plant species mesocosms, using a t-test. Both the root masses and shoot masses were compared, in a separate t-test, to determine if growing with different plant species in the same mesocosm significantly affected biomass production.

Media percent N was also tested at the beginning and end of the experiment. At the beginning of the experiment, composite samples of the bioretention media both before the washing with DI and stormwater and after washing were taken. The after-washing sample was a composite sample consisting of small amounts of media collected from planting depth from all but one mesocosm during planting, from between the top of the media and the bottom of the holes dug for planting (at about 6.35 cm below the top of the mulch). At the end of the experiment, media samples were collected from the same depth range from each of the following treatments: one-*Juncus*, three-*Juncus*, no-plants, and Iris. Samples from replicates of one treatment were combined into a single composite sample. Both the plant and media samples were sent to the University of Delaware Soil Testing Program, and percent N was found through combustion of the dried samples.

Throughout the experiment, at each sampling storm, dead plant biomass that had separated from the plants was collected, with the biomass from each mesocosm kept separate. The biomass was dried at 80° C and weighed. The mass collected gives an idea of the amount of plant biomass returned to the system, which represents a return of the N and P to the system that would otherwise be sequestered in the living plant biomass.

Chapter Five: Greenhouse Study Results and Discussion

The greenhouse study ran for 53 weeks. Several measurements were taken periodically throughout the year to assess the system: pH of influent and effluent, infiltration rate, effluent discharge rate, and mass of dead biomass. Additionally, plant survivorship, aesthetics, and health were assessed throughout the experiment through visual observation. Biomass growth over the course of the study was determined by cleaning and drying representative specimens of each species at both the beginning and end of the study, separated into roots and shoots. Dry mass and percent N for each root or shoot sample was determined, so that the change in N content and biomass could be assessed.

Additionally, several influent and effluent samples from time points throughout the study were analyzed for nutrient concentrations.

5.1 pH

The pH of the influent and effluent from seven selected mesocosms, from one row of mesocosms, was measured 10 times throughout the course of the study. Five of those occasions were measurements of the same row (row three), two of the occasions were measurements of row one, and the pH of row two and row four mesocosms were each measured once. These data are given in Table 5.1a. Additionally, the pH of the full volume of a storm's effluent was also measured for row three in December 2014 (Table 5.1b).

Table 5.1a pH data from the first 0.1 cm of effluent (=75 mL), or 75 mL influent. n = 1 for each datum. All mean values and standard deviation ranges are geometric.

	Date ->	10/4/14	10/28/14	1/5/15	2/2/15	2/3/15	2/24/15	4/7/15	5/5/15	6/18/15	All Sample Dates
	Mesocosm Row ->	**3**	**1**	**2**	**3**	**3**	**4**	**3**	**1**	**3**	**1–4**
	Influent	6.33	6.53	6.05	5.92	6.21	6.21	5.88	5.63	6.38	6.04
	No-plants	6.6	6.32	6.45	6.06	6.07	6.04	5.65	5.95	6.26	6.07
	All-plants	6.41	6.45	6.41	5.98	5.94	6.15	5.94	5.43	5.84	5.94
	All-plants 5	n/a	n/a	n/a	n/a	n/a	6.41	n/a	n/a	n/a	6.41
Sample Source	Iris	6.57	6.25	6.42	6.04	6.09	6.34	5.74	5.73	5.64	5.98
	Joe-Pye	6.35	6.39	6.51	6.06	6.18	6.14	5.96	5.8	6.35	6.14
	Three-*Juncus*	6.36	6.37	6.45	6.07	5.85	n/a	6.37	5.56	5.79	5.98
	Two-*Juncus*	6.66	6.39	5.97	5.96	6.05	6.09	5.64	5.85	5.68	5.94
	One-*Juncus*	6.53	6.39	6.45	5.96	6.06	6.55	5.87	5.46	5.96	5.99
	Effluent Mean	6.48	6.36	6.34	6.02	6.02	6.21	5.83	5.64	5.87	6.01
	Effluent Mean ± Standard Deviation	6.37–6.63	6.30–6.43	6.14–6.73	5.97–6.07	5.92–6.16	6.07–6.42	5.67–6.09	5.48–5.91	5.68–6.19	5.76–6.65

Table 5.1b Composite pH measurements of the full volume of effluent, measured the day after a 1.03 cm simulated storm = 18.54 cm applied storm. Numbers in sample column designate the mesocosm row from which effluent was sampled. Mean value and standard deviation range are geometric.

12/5/14 Storm	
Sample Source	**pH**
Influent	6.32
No-plants 3	6.61
All-plants 3	6.67
Iris 3	6.66
Joe-Pye 3	6.37
Three-*Juncus* 3	6.68
Two-*Juncus* 3	6.66
One-*Juncus* 3	6.46
Effluent mean	6.57
Effluent mean ± standard deviation	6.45–6.74

The influent pH of the 75-mL samples varies over the course of the experiment with no clear temporal trend, from a minimum of 5.63 to a maximum of 6.53 (Table 5.1a). In general, the average pH of the effluent fell over the course of the study, from an effluent pH average of 6.50 on October 4, 2014, to an effluent pH average of 5.93 on June 18, 2015, with the influent pH values for these two days being similar: 6.33 on October 4, 2014 and 6.38 on June 18, 2015. This indicates that some mechanism that over time resulted in a lowered pH of the stormwater that has passed through the mesocosms. Because the no-plants treatment effluent pH varied between being higher and lower than the planted treatments effluent pH average throughout the course of the experiment, the mechanism cannot be clearly attributed to plant growth and was therefore likely a media effect. One hypothesis is that the respiration of the microbial community in the media increased over time, producing CO_2 to react with the water and form hydrogen ions, lowering the pH.

Over the course of the experiment, the pH of the 75-mL influent samples averaged 6.13, with a standard deviation of 0.282. Kayhanian et al. (2012) found highway runoff pH values ranging from 6.4 to 7.7, and the National Stormwater Quality Database (2015) has an average stormwater pH of 7.31, with a standard deviation of 0.82. Therefore, the influent pH in this study was slightly lower than typical stormwater pH found in the field.

The pH of all of the first 0.1 cm (representing 75 mL of volume) of all effluent samples averaged 6.01 (Table 5.1a). Therefore, despite the temporal trend discussed above, overall during the course of the experiment the average pH of the influent and effluent were similar: 6.04 for the influent and 6.01 for the effluent from all treatments.

The three plant species used in the study are adaptable to a variety of pH conditions. *Juncus effusus* can tolerate a pH of 5.5 to 8.8 (USDA NRCS 2015b), *Eutrochium*s and *Iris versicolor* are also adaptable to a range of pH from acidic to basic (Mount Cuba Center 2015, Ohio Northern University Medicinal Herb Gardens 2015),

5.2 Infiltration Rate

In addition to pH, the rate of infiltration of stormwater when applied to the top of the media was also periodically measured. The time of infiltration (i.e., from the time the water was applied to the pot to the time no water was visible above the media surface) for 3.2 cm of influent (simulating a 0.18 cm storm) was measured for row three mesocosms on five occasions throughout the study, allowing for analysis of change over time. Rate measurements were also taken for all of the other mesocosms during the study, but never from more than one mesocosm in a treatment at once, so n =1 per treatment at all points,

and comparison of different mesocosms within the same treatment is not feasible because both the date and mesocosm change between data points. Therefore, only the data from row 3 mesocosms, which have the most dates with recorded data, are presented. Because n=1, no standard deviation is reported.

Any rates slower than 7.78 cm hr^{-1} were removed from the data set because, based on observation, these rates resulted from clogged mesocosms. This assumption is supported by the fact that no rates slower than 7.78 cm hr^{-1} were found after regular poking of mesocosm effluent holes was started in late January 2015. The data are presented in Figure 5.1.
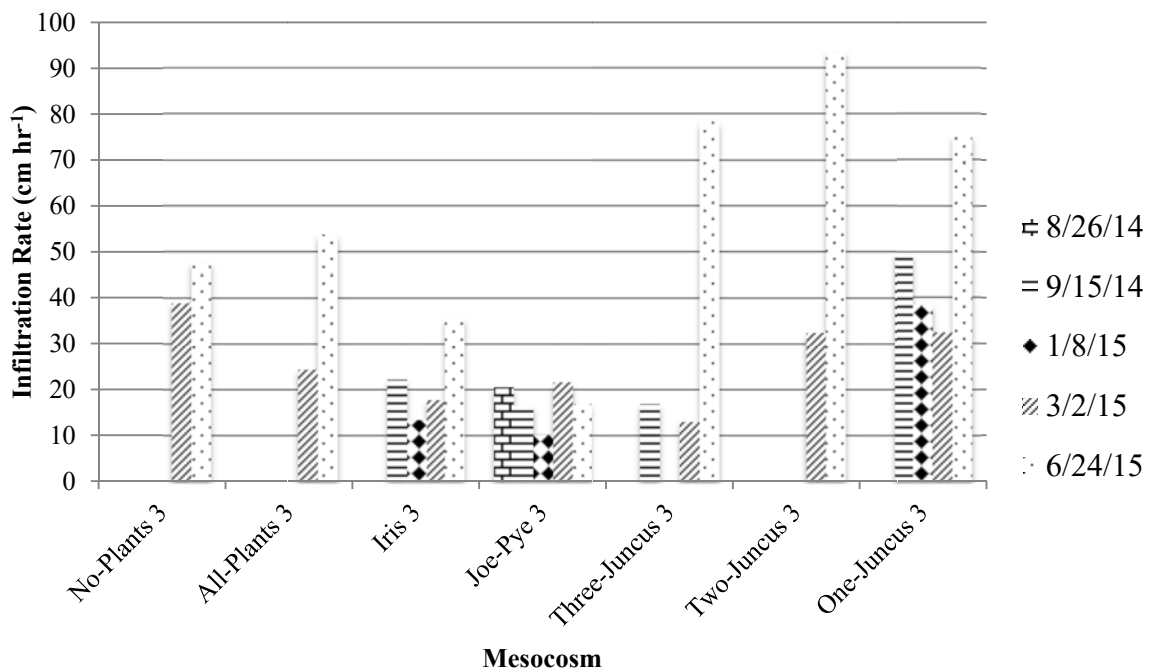
Figure 5.1 The infiltration rate measurements for five dates for the row three mesocosms. The rates were found by dividing the amount of applied water (3.24 cm) by the time in hours between water application and when the water was no longer visible on the media/mulch surface.

The measured infiltration rates range from 10 to 93 cm hr^{-1}. Infiltration rates in field bioretention have been measured between 3 and 72 cm hr^{-1} (Asleson 2009), which are consistent with all of the mesocosms in Figure 5.1 except for the three-*Juncus* mesocosms in June 2015, which had rates of 78.3 cm hr^{-1}, vis-à-vis 93.4 cm hr^{-1} for two-*Juncus* and 75.2 cm hr^{-1} for one-*Juncus*. These *Juncus* rates are much higher than the recommended basic range for infiltration rates in bioretention by Davis and MeCuen (2005): 9.53 to 25.5 cm hr^{-1}. Over the course of the study, the most noticeable differences between dates occur between March 2015 and June 2015. An increase in infiltration rate is seen for all of the mesocosms except for a 4.7 cm hr^{-1} decrease for Joe-Pye 3. Those mesocosms with *Juncus* plants see the greatest increase: more than 6-fold for three-*Juncus*, 2.9-fold for two-*Juncus*, 2.3-fold for one-*Juncus*, and 2.2-fold for all-plants 3. The Iris 3 mesocosm increases two-fold. The no-plants mesocosm increases 1.2-fold, about the same increase as the Joe-Pye 3 mesocosm decreases: 0.78-fold. While the Joe-Pye and no-plants changes are not of a large enough magnitude to be unquestionably above experimental variation, the Iris mesocosms and mesocosms with *Juncus* show substantial increases that are likely well above any produced by experimental variation. Therefore, the Iris and *Juncus* appear to have a seasonal effect on infiltration rate. Since the mulch and media are the same for all mesocosms, the effect is presumably from either the effect of the plants upon the media or mulch or from the plants themselves, most likely due to root growth creating channels for faster water infiltration. This result corresponds to previous studies discussed in Chapter Two, which have found that plant root growth in bioretention, especially of thick-rooted plant species, can help maintain or improve infiltration rates (Feng et al. 2012, Hatt et al. 2009, Le Coustoumer et al. 2012).

Root biomass in the three- and two-*Juncus* treatments was not compared to the one-*Juncus* treatment, but the biggest increase in infiltration rate between March and June 2015 was for the three-*Juncus* treatment, possibly because three plants produced more root biomass during this period than two plants or one plant.

Infiltration rates were measured for 3.24 cm applied stormwater storms, which simulate 0.18 cm of precipitation. Effluent rate was measured for only one 3.24 cm applied stormwater storm, the 10/11/14 storm. Only two mesocosms were not clogged for the 10/11/14 storm: Iris 3 and One-*Juncus* 3. The closest storm for which infiltration was measured was 9/15/14. On 9/15/14 the infiltration rate of Iris 3 was 22.2 cm hr^{-1}; on 10/11/14 the effluent rate of Iris 3 was 8.64 cm hr^{-1}. On 9/15/14 the infiltration rate of One-*Juncus* 3 was 50.0 cm hr^{-1}; on 10/11/14 the effluent rate of One-*Juncus* 3 was 10.7 cm hr^{-1}. Therefore for both the Iris 3 and One-*Juncus* 3 mesocosms, the infiltration rate is more than double the effluent rate. Stormwater more quickly entered the mesocosm mulch and media than it left the mesocosm. This is expected, because the effluent rate accounted for travel time throughout the whole mesocosm, whereas the influent rate was only for movement of water into the top of the mulch and media.

5.3 Effluent Rate

The elapsed time between influent introduction to the top of the mesocosm and collection of 2.54 cm of effluent was measured throughout the course of the study, most frequently for row three. Row three effluent rate data are presented in Table 5.2. Note that the elapsed time was measured for the first application of stormwater on each day, so for all storms of simulated 0.34 cm or greater (i.e., all but the 10/11/14 storm), the data below represent the same amount of applied influent during the data collection, i.e., the

same head.

Table 5.2 Effluent flow rate data from row three mesocosms: elapsed time in h:mm:ss from influent introduction to the top of the mesocosm, to the collection of 2.54 cm of effluent in the effluent bucket below the mesocosm. Shaded cells indicate clogged mesocosms (defined as > 25 minutes elapsed time). Data from 2/5/15 and 3/26/15 were recorded to the nearest minute, not to the second.

Date	10/11/14	12/18/14	2/5/15	3/26/15	6/27/15	7/13/15
Simulated Storm (cm)	0.18	0.34	1.03	1.03	1.38	0.34
Applied Depth (cm)	3.2	6.1	18.5	18.5	24.8	6.1
Mesocosm	**Average Elapsed Time (h:mm:ss)**					
No-plants 3	2:10:20	3:20:42	0:05:00	0:06:00	0:06:11	0:06:10
All-plants 3	2:28:15	3:39:55	0:08:00	0:06:00	0:06:33	0:07:45
Iris 3	0:22:30	0:21:45	0:12:00	0:17:00	0:13:33	0:12:15
Joe-Pye 3	0:31:45	0:42:44	0:13:00	0:15:00	0:14:27	0:13:20
Three-*Juncus* 3	0:33:30	0:26:52	0:11:00	0:18:00	0:07:57	0:11:40
Two-*Juncus* 3	2:18:45	1:18:13	0:09:00	0:12:00	0:09:10	0:11:10
One-*Juncus* 3	0:18:15	0:13:12	0:10:00	0:11:00	0:16:55	0:17:50

As described in the Methodology chapter, slow-draining mesocosms were an issue in weeks 4 through 29 of the study. In week 29 (the week of January 25, 2015), a regular regime of inserting a pipette tip into the effluent hole of each pot to clear it was instigated, and drainage rates improved markedly and remained that way for the remainder of the experiment. This change can clearly be seen in Table 5.2. For example, the all-plants 3 mesocosm changes from taking more than 3.5 hours to produce 2.54 cm of effluent on December 18, 2014, to only eight minutes on February 5, 2015. The same mesocosm then remains in the range of 6 to 7.75 minutes drainage time for the remainder of the study, indicating that the effluent-hole-clearing method worked to maintain good drainage.

The infiltration rates for the different *Juncus* mesocosms are similar in February 2015, ranging from 9 to 11 minutes. Close to two months later, on March 26, 2015, the three-*Juncus* 3 mesocosm has slowed by seven minutes, the two-*Juncus* mesocosm by three minutes, and the one-*Juncus* mesocosm by one minute, so that one-*Juncus* 3 was the fastest draining of the three *Juncus* mesocosms, and three-*Juncus* 3 the slowest. However, this ranking had reversed by the June and July data points, with one-*Juncus* 3 being the slowest draining of the three *Juncus* mesocosms. In June, one-*Juncus* 3 is 2.13 times the rate of three-*Juncus* 3, and 1.85 times the rate of two-*Juncus* 3. In July the difference is slightly less, with one-*Juncus* 3 being 1.53 times the rate of three-*Juncus* 3 and 1.59 times the rate of two-*Juncus* 3. This has potentially important consequences, as it affects the contact time the stormwater has with the media and plants. A slower effluent rate, such as one-*Juncus* exhibits, results in more contact time for the stormwater with the media and plants. This slower effluent rate, vis-à-vis the higher density *Juncus* mesocosms, is presumed to be due to less root growth with one plant compared to multiple plants. Interestingly, the difference between the two-*Juncus* and three-*Juncus* mesocosm rates is minimal, despite the presence of an extra plant in the three-*Juncus* mesocosm. It appears that the third *Juncus* has less of an effect on rate than the second *Juncus*.

Generally only one row of mesocosms was measured for a given storm. However at the end of the study, the effluent rate for all mesocosms was measured on the same day. The results are presented in Table 5.3.

Table 5.3 Average time from influent introduction to the top of the mesocosm to the time when 2.54 cm of effluent was collected in the effluent bucket, for each treatment on July 13, 2015

Treatment	Average elapsed time for 2.54 cm of effluent (h:mm:ss)	Standard Deviation (h:mm:ss)	n
No-plants	0:10:01	0:09:28	4
All-plants	0:09:26	0:04:45	4
Iris	0:09:34	0:04:17	4
Joe-Pye	0:09:12	0:04:02	4
Three-*Juncus*	0:11:18	0:00:33	3
Two-*Juncus*	0:10:48	0:05:20	4
One-*Juncus*	0:18:01	0:07:18	4

The fastest rate was found in the Joe-Pye mesocosms, with an average of 9 minutes and 12 seconds. As in the measurements of row three only, when all mesocosms are measured the one-*Juncus* treatment is still clearly the slowest. The effluent rate for one-*Juncus* is the slowest of any of the mesocosms, 80% slower than the no-plants control, 67% slower than the two-*Juncus* treatment, and 59% slower than the three-*Juncus* treatment. A slower effluent rate means the synthetic stormwater has more contact time with the media and plant roots. Plants can adjust their N uptake rate but only within a certain range (e.g., Cárdenas-Navarro 1998), so more contact time with the synthetic stormwater should allow for more uptake of N by the plants.

However, the replicates within a single treatment vary: the standard deviation is about 40 to 50% of the average for all treatments except no-plants where the standard deviation is 95% of the average, and three-*Juncus*, where the standard deviation is 5% of the average. Therefore, the most densely planted treatment is the most consistent in effluent rate among replicates, and the unplanted treatment is the most variable. Due to the high standard deviations, an approximative k-sample permutation test finds no significant differences at an alpha of 0.05; the p-value for the one-*Juncus* treatment is

0.08. Therefore, the slowness of the one-*Juncus* treatment vis-à-vis the other treatments is not quite statistically significant. But, it is still a result worth noting for its impact on stormwater contact time with the media and plant roots.

5.4 Plant Survivorship

All of the *Juncus* except for one plug in the all-plants 3 pot survived and grew well. The all-plants 3 *Juncus* turned completely brown and generated no new aboveground growth. All of the Iris survived throughout the course of the study and grew well.

The Joe Pye plants in both the all-species and Joe-Pye mesocosms were beginning to struggle in August 2014 (two months into the experiment), and had declined significantly by September (Figure 5.2). The Joe Pye leaves displayed chlorosis, which occurs when insignificant chlorophyll is produced and causes the leaves to lose their green color. The leaves thus turned yellow and then brown and dry.

This was an unexpected result, given the success of Joe Pye in the Silver Spring study conducted in fall of 2013 (Chapter 3), when it dominated the vegetation coverage in the cell. Multiple factors could contribute to the failure of the Joe Pyes in this study. First, the Joe Pyes have a different leaf structure than the other two species, which may contribute to a different reaction to drought stress. Sack et al. (2003) found that different leaf shapes produced different hydraulic conductivities between plant species. Secondly, the Silver Spring site was a full-scale bioretention cell, whereas the mesocosms in this experiment are much smaller and therefore have less water-holding capacity. The mesocosms are thus more prone to drying out between storm events than the Silver Spring cell, which means the Joe Pyes in this study were likely subject to more drought

stress than the Silver Spring Joe Pyes. Thirdly, the mesocosms in this study were watered with synthetic stormwater that only contained N, P, and sodium chloride for the first 15 weeks of the experiment. Following media testing, it was determined that the media was not providing sufficient levels of several essential plant nutrients. This could have contributed to the stress that the Joe Pyes were experiencing during this period. Finally, during the few insect infestations during the study, which were quickly controlled, the vast majority of the insects were found on the Joe Pye plants and not the Iris or *Juncus* plants, which may have added some additional stress to the Joe Pye plants. Finally, even though Joe Pye did very well in percent cover in a field cell seven years after planting, in the first year after planting in that cell 20% of the planted Joe Pyes had to be replaced due to plant failure, as noted in Chapter three. Therefore, even in the field Joe Pye had less than perfect survivorship.

August 22, 2014	September 8, 2014	October 31, 2014 (New Plants)
December 5, 2014	January 14, 2015	February 22, 2015
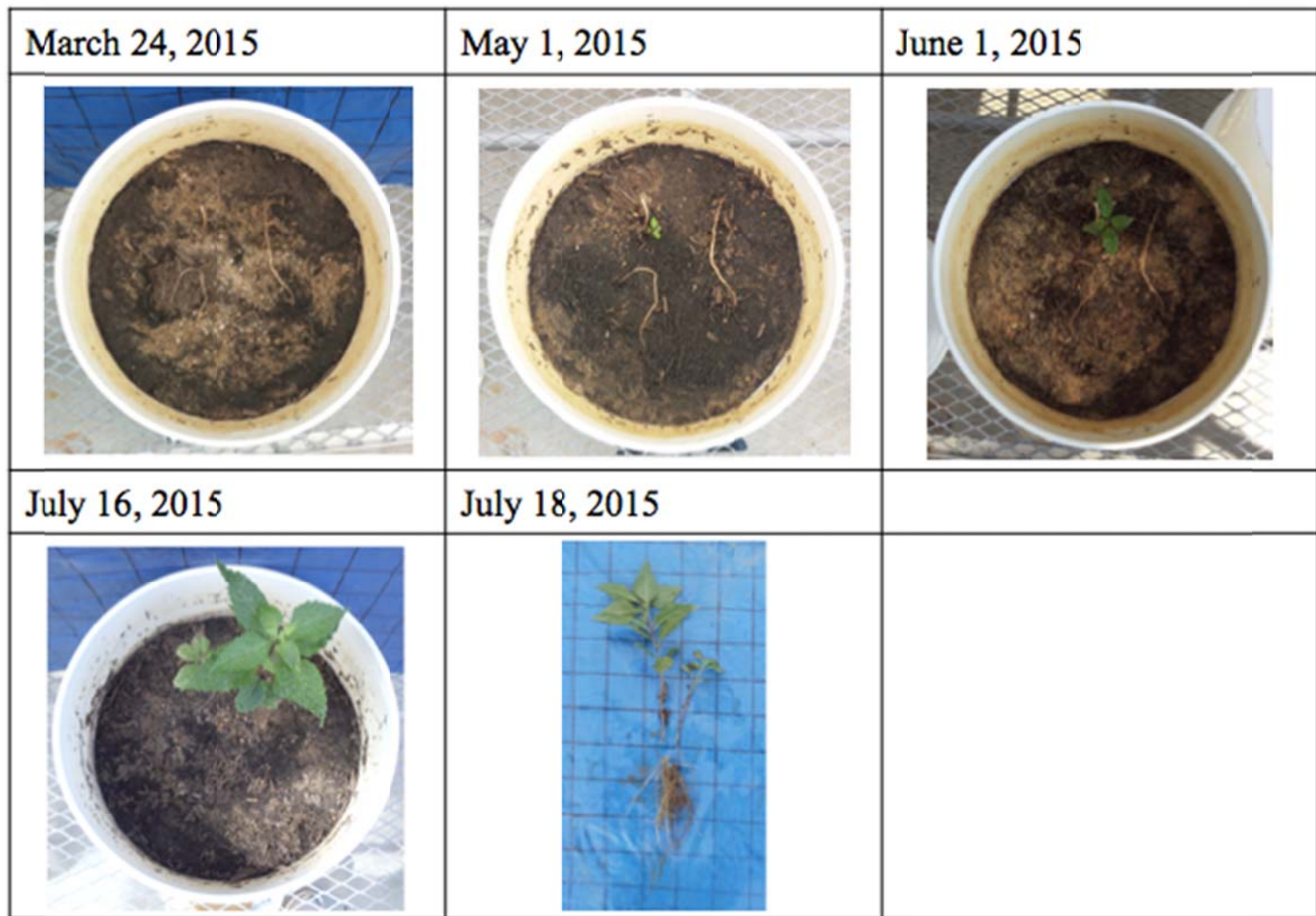
Figure 5.2 Plant growth over time for a representative Joe-Pye mesocosm (row 4), top view except for July 18, 2015. Squares on blue background are 5 cm x 5 cm.

In the summer and fall of 2014 the Joe Pyes were the focus of white fly and aphid infestations; the insects almost wholly ignored the Iris and *Juncus*. Insects were removed by Aria- Enstar ll and Botanigard 22-WP treatment, one treatment each.

As described previously, in week 16 the Joe Pyes in the mesocosms were removed and replaced with new plugs. Those plants did well until they died back for the winter. However, only slightly less than half of the replanted Joe Pyes sprouted again in the Spring. The ones that did sprout did well, but the lack of more than half of the Joe Pyes to come back after the winter raises doubts about their hardiness in bioretention.

5.5 Plant Biomass Accumulation

As part of the establishment of a N mass balance for the mesocosms, and to document plant growth, representative plant samples of each species were collected and dried at 80° C until a constant mass was obtained, at both the beginning and end of the experiment. These dry mass results are given in Table 5.4, with full data given in Appendix B.

No significant difference was found in either the shoot or root biomass from the *Juncus* samples from the all-plants treatment vis-à-vis the one-*Juncus* treatment, both collected at the end of the study (as established by t-tests with alpha = 0.05, calculations not shown). Similarly, no significant difference was found in either the shoot or root biomass from the Iris samples from the all-plants treatment vis-à-vis the Iris treatment, both collected at the end of the study (as established by t-tests with alpha = 0.05, calculations not shown). Therefore, all of the data for each species were analyzed together and summarized in Table 5.4.

Table 5.4 Dry plant biomass sample measurements: Summary. Full data are given in Appendix B.

Plant Species	Average Root Beginning of Study Mass ± Standard Deviation (g)	Average Root End of Study Mass ± Standard Deviation (g)	Average Change in Root Biomass (g)	Average Times Increase in Root Biomass	Average Shoot Beginning of Study Mass ± Standard Deviation (g)	Average Shoot End of Study Mass ± Standard Deviation (g)	Average Shoot Change in Biomass (g)	Average Times Increase in Shoot Biomass
Joe Pye	1.5 ± 0.79	1.6 ± 0.97	**+0.1 (in 38 weeks)**	**0.067**	0.46 ± 0.13	2.9 ± 0.97	**+2.4 (in 38 weeks)**	**5.2**
Juncus	0.61 ± 0.28	20 ± 8.4	**+19 (in 54 weeks)**	**31**	1.3 ± 0.38	38 ± 8.4	**+37 (in 54 weeks)**	**28**
Iris	1.1 ± 0.33	4.8 ± 2.3	**+3.7 (in 54 weeks)**	**3.4**	1.3 ± 0.37	4.1 ± 2.3	**+2.8 (in 54 weeks)**	**2.2**

The Joe-Pye data are not a perfect comparison to the other two species, because the Joe Pyes were replanted in week 16 of the experiment. The plugs were obtained from the same source both before week one and for week 16, and appeared of similar size and quality, so the data from the Joe Pye plant samples at the beginning of the study are assumed to also represent the Joe Pyes that were planted in week 16. The data from the beginning-of-study Joe Pye samples were therefore compared to the end-of-experiment Joe Pye samples, with the note that only 38 weeks' worth of growth is included, vis-à-vis 54 weeks of growth for the other two species.

The difference in biomass added by each species (Table 5.4, with full data given in Appendix B) is large. The average Joe Pye only added an additional 0.14 g root mass (a 0.067-fold increase) + 2.40 g shoot mass (a 5.2-fold increase) = 2.54 g total mass over 38 weeks. In comparison, the average Iris added 3.72 g root mass (a 3.4-fold increase) + 2.84 g shoot mass (a 2.2-fold increase) = 6.56 g total mass over 54 weeks. The average *Juncus*, in comparison, added 18.8 g root mass (a 31-fold increase) + 37.0 g shoot mass (a 28-fold increase) = 55.8 g total mass over 54 weeks, almost 10 times as much as the average Iris plant and more than 20 times as much as the average Joe Pye plant. *Juncus* remained green all year round and may have been able to contribute some additional biomass during the winter; this was not measured. However, importantly it did not loose biomass during the winter, as both the Joe Pye and Iris plants did when their aboveground biomass died back. Such a difference in magnitude of added biomass can be important when trying to maximize N uptake by the vegetation. As long as the percent N in the different plant species' biomass is similar (see the Change in Plant and Media N Levels

During the Study section below for data), then having more biomass results in more uptake of N in order to build that biomass.

Pictures of typical plants from each species at both the beginning and end of the study are given in Figure 5.3, as a visual presentation of these biomass changes.

a.

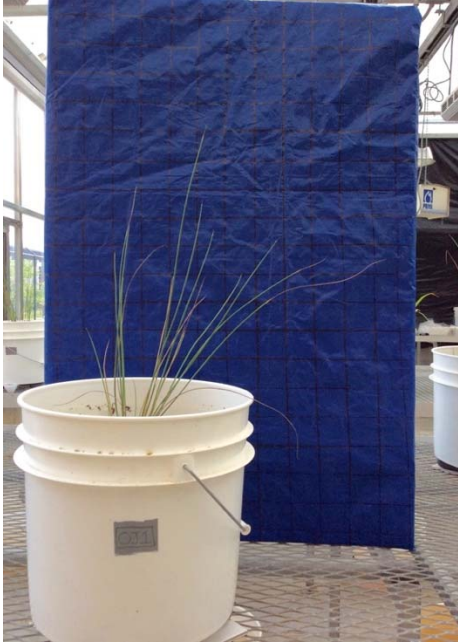| Beginning of Study	End of Study
8/18/14	7/16/15
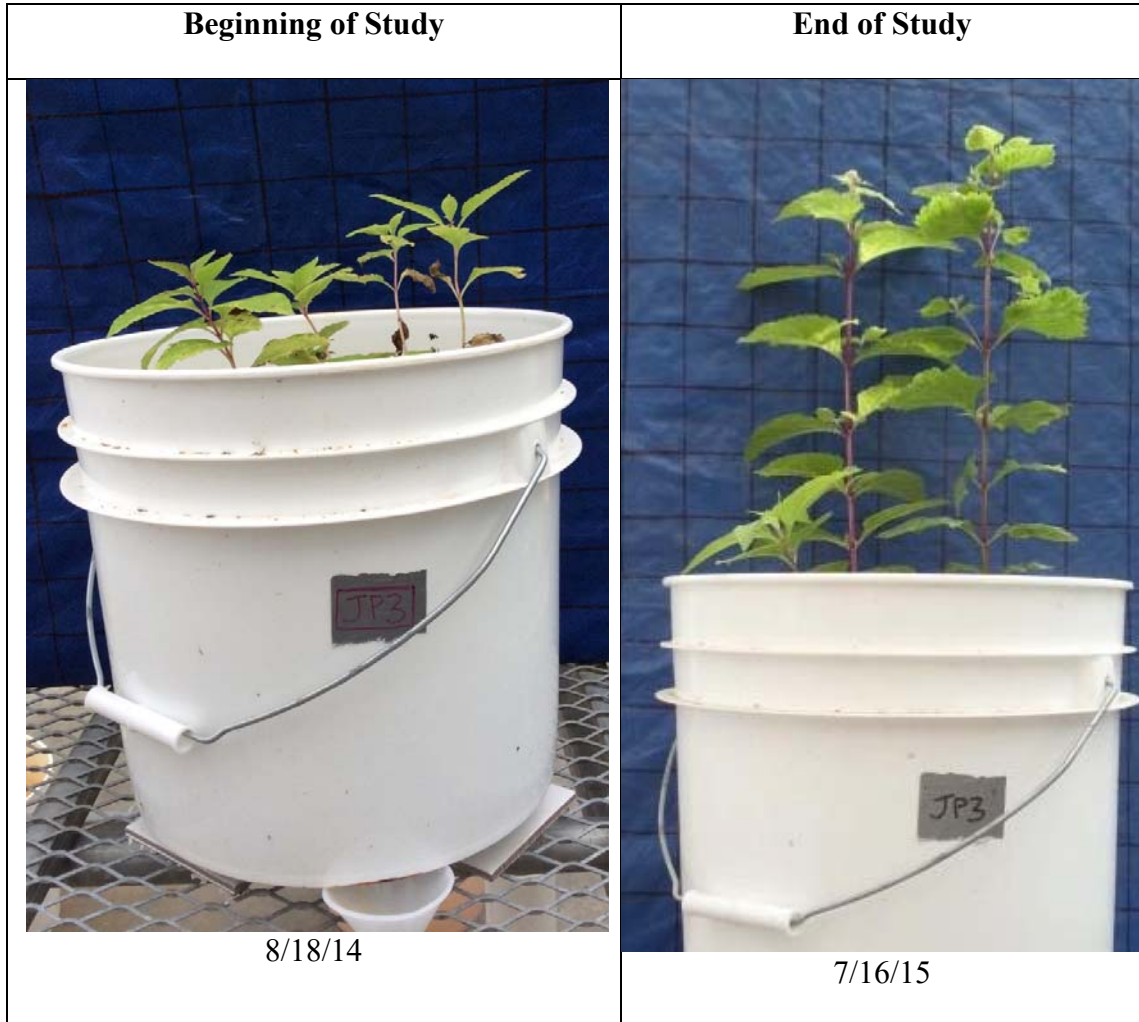
Beginning of Study	End of Study
7/15/14	7/18/15

b.

Beginning of Study	End of Study
8/18/14	7/16/15
7/14/14	7/18/15

c.

Beginning of Study	End of Study
8/18/14	7/16/15
7/14/14	7/18/15

Figure 5.3 Typical specimens of each species at the beginning and end of the study: a: Joe Pye, b: *Juncus*, c. Iris. The squares on the blue background are 5 cm x 5 cm. The full ruler length is equivalent to ~the height of six blue squares.

5.6 Dead Shoot Biomass

Dead shoot biomass that had disconnected from the plant (in order to ensure that all biomass collected is truly dead) was collected from each mesocosm at each sampling, placed in a paper bag, and dried at 80°C until a consistent mass was obtained. Masses less than 0.01 g (less than the detection limit of the balance) were recorded as 0.005 g. The amount of dead biomass generated provides information about the amount of N and P returned to the system. However, there are two sources of error: 1. The dead biomass is only collected if it has disconnected from the plant. It is possible for dead biomass that is connected to the plant to decompose while still connected to the plant, and contribute nutrients to the system. 2. Because of varying times between sampling, sometimes several weeks, it is possible for dead biomass to decay between sampling periods and therefore not be collected and weighed. This is especially true of thinner Joe Pye leaves.

Cumulative average dead shoot biomass data per mesocosm are presented in Figure 5.4.

Using an approximative k-sample permutation test, the only treatment that is significantly (i.e., $p<0.05$) different than the others is the one-*Juncus* treatment. The Iris treatment is almost significantly different, with a $p = 0.06$. Though not quite significant, the Iris treatment did generate the most dead biomass on average and likely drove the similarly high dead biomass average for the all-plants treatment. As expected, the thin-leaved Joe Pyes did not contribute as much dead biomass. The Joe-Pye treatment, three-*Juncus* treatment, and two-*Juncus* treatments all had similar amounts of dead biomass
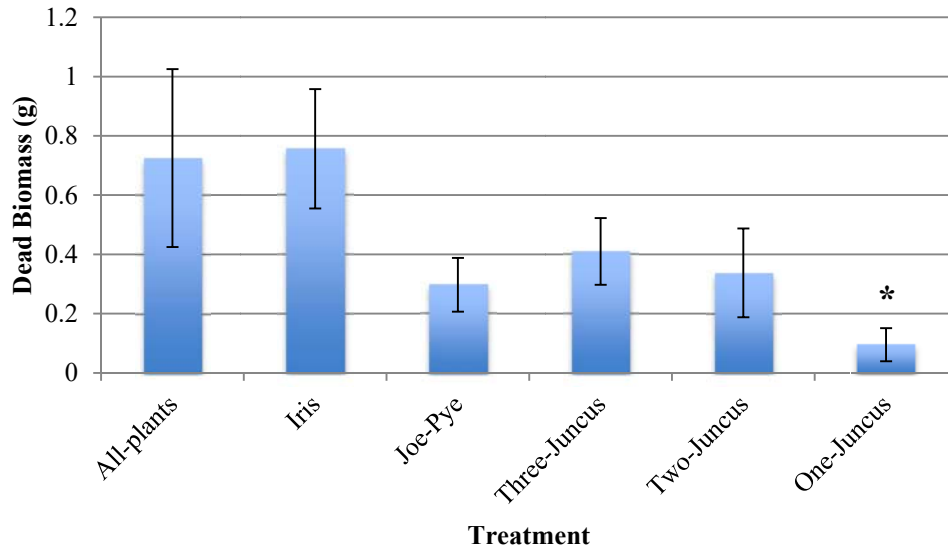
Figure 5.4 Average cumulative mass of dried, dead shoot biomass per mesocosm for the entire study. Error bars are +/- one standard deviation. Statistically significant differences are indicated with *.

produced. The one-*Juncus* treatment was the clear choice, however, for minimizing dead biomass production and therefore minimizing return of nutrients in dead biomass to the system.

However, the relative accumulation of live biomass (a gain in nutrient storage capacity) should be weighed against the dead biomass production (a return of nutrients to the media and potentially the effluent). These ratios are presented in Table 5.5.

Table 5.5 Ratio of dry shoot live biomass produced during the study to dry shoot dead biomass. Note: the all-plant and Joe-Pye ratios were calculated by assuming three plants per mesocosm, which was not the case consistently throughout the experiment due to plant death and some Joe Pye plants not resprouting in Spring 2015.

Treatment	Ratio of Living Shoot Biomass (g) : Dead Shoot Biomass (g)
All-plants	58
Iris	11
Joe-Pye	24
Three-*Juncus*	270
Two-*Juncus*	220
One-*Juncus*	390

Table 5.5 clearly shows that the one-*Juncus* treatment produces the most mass of live shoot biomass per gram of dead shoot biomass produced: 390 g g^{-1} vis-à-vis a low of 11 g g^{-1} for Iris. All of the treatments with a *Juncus* plant are much better than the other treatments at maximizing production of live biomass for each gram of dead biomass produced, because of the ability of *Juncus* to accumulate a large amount of shoot biomass over a year as compared with the two other plant species.

5.7   Nutrient Removal from Synthetic Runoff

Influent Concentrations

For the N species that were added to the synthetic stormwater, the measured influent concentration of each species was on average less than the expected concentration (Table 5.6).

Table 5.6 The expected concentrations of N species in the synthetic stormwater, and the average measured concentration of each species.

N Species	Expected Concentration (mg N L^{-1})	Average Measured Concentration ± Standard Deviation (mg N L^{-1})	Average Measured Concentration (mg N L^{-1})/ Expected Concentration (mg N L^{-1})	n
NO$_3^-$-N	0.56	0.44 ± 0.26	0.79	5
NH$_4^+$-N	0.3	0.29 ± 0.10	0.97	5
DON-N	0.5	0.25 ± 0.25	0.50	3
Particulate Organic N-N	0	0.024 ± 0.033	undefined	2
NO$_2^-$-N	0	0.01 ± 0	undefined	4
Total N	1.4	0.77 ± 0.12	0.55	3

All of the measured concentrations were within 50% of the expected concentrations, for expected concentrations greater than zero. The NO$_3^-$ and NH$_4^+$ measured concentrations were within 30% of the expected concentrations. The average DON measurement was 50% of expected and had high variability, with the standard deviation being greater than the average. The total N had less variability but was still only 55% of the expected concentration on average. The measurements that were expected to be zero, particulate organic N and NO$_2^-$, met the expectation with low measured values. Only measured concentrations are presented in the figures below.

NO$_3^-$

NO$_3^-$ data for all five storm/storm sections were analyzed (Figure 5.5). NO$_3^-$ removal in bioretention occurs through denitrification or vegetative uptake (reviewed in Hunt et al. 2012). Denitrification requires anaerobic conditions.

Significant differences among treatments are not seen until June 1, 2015. However, the overall magnitude of removal changes between the earlier storms. In the

7/16/14 and 10/31/14 storms, the difference between the measured influent and the effluent NO_3^- concentrations ranges from 42% removal to 54% export (calculations not shown), with none of the differences among treatments being significant. Because the no-plants control is not significantly different than the planted treatments in both storms, the presence of vegetation did not impart a significant impact on NO_3^- removal, even after more than three months of growth for the 10/31/14 storm. And since there is no significant NO_3^- removal, denitrification of NO_3^- also does not appear to be occurring at a significant level, despite 12 of the 26 sampled mesocosms in the 10/31/14 storm being slow-draining (in this case, taking greater than an hour and 15 minutes to fully drain), which has the potential to create anoxic conditions for denitrification. As an anion with limited media affinity (Davis and McCuen 2005), if denitrification or vegetative uptake are not present then NO_3^- can wash through the media without significant removal, as appears to be occurring in these two storms.

7/16/14
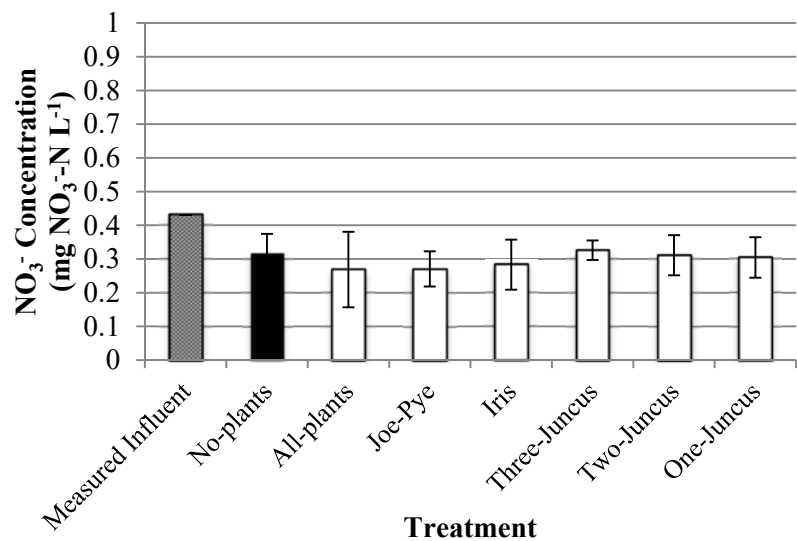
7/23/14 Second (Left) and Fourth (Right) Flushes
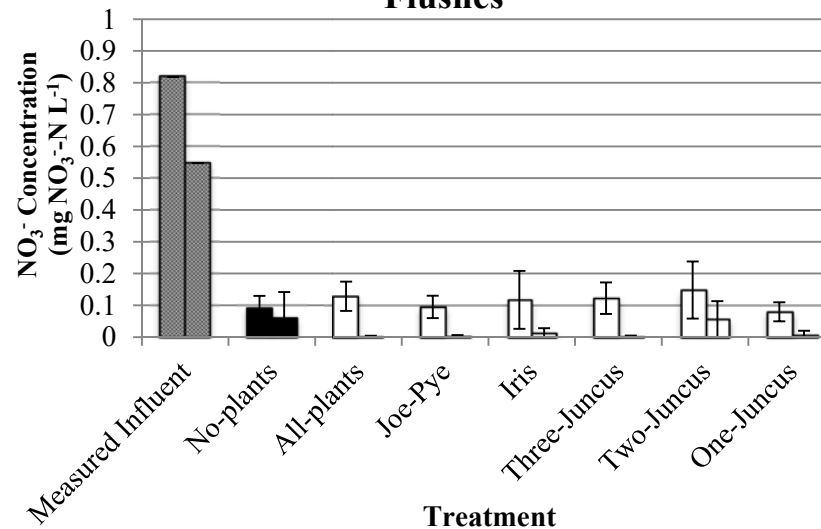
10/31/14
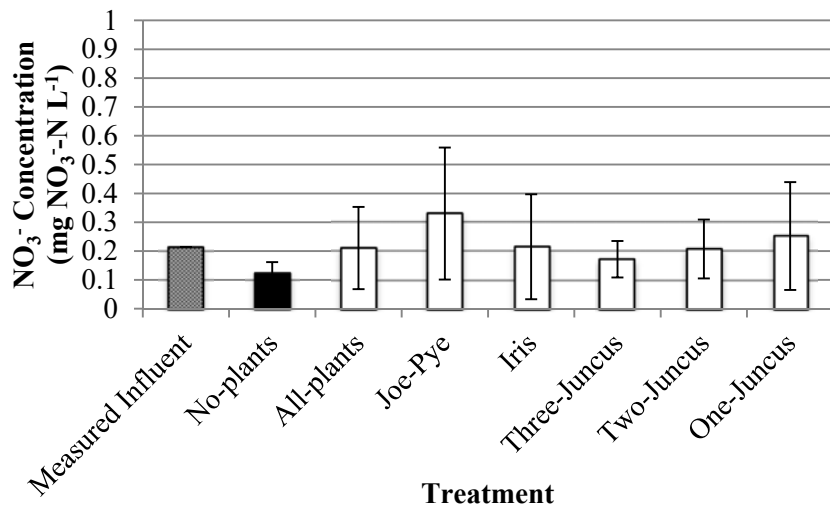
6/1/15
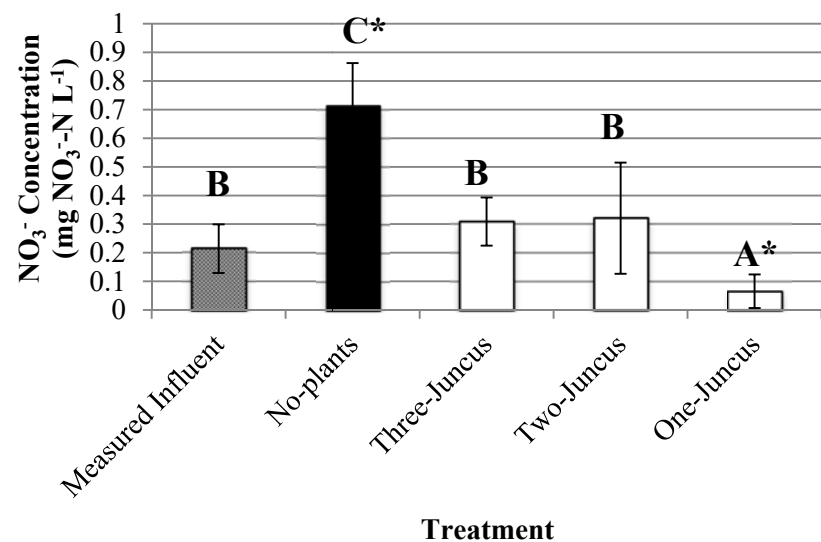
Figure 5.5 NO_3^- concentrations from synthetic stormwater influent and the effluent from seven treatments of bioretention mesocosms with different vegetation. Each graph represents a simulated storm. The 7/16/14 storm is a composite sample of a simulated 0.27 cm storm, or 4.9 cm applied water. The 7/23/14 storm is a simulated 1.4 cm storm, or 25 cm applied water. The second flush was sampled after 6.2 cm of water was applied; the fourth flush was sampled after 19 cm of water was applied. The 10/31/14 storm is a composite sample of a simulated 0.18 cm storm, or 3.2 cm of applied water. The 6/1/15 storm is a composite sample of a simulated 0.69 cm storm, or 12 cm of applied water. The * denotes a significant difference between that treatment and the measured influent. Differences among treatments and/or the influent are denoted with letters, if found. n=1–2 for influent, n=2–4 for effluent, usually n=4. Error bars are ± one standard deviation.

Both flushes of the 7/23/14 storm also do not have statistically significant NO_3^- removal (i.e., a statistically significant difference between influent and effluent NO_3^- concentrations), however looking at the graph for the storm (Figure 5.5), the large difference between the influent concentration and all of the effluent concentrations is noticeable. The removal ranges from 82–100%. With both the no-plants and planted treatments in this 18% range, there is no significant vegetative uptake. This is not unrealistic at this early stage of the study, when the plants were small. There were no slow-draining mesocosms at this date, so denitrification should not have been occurring. Therefore, the cause for the high NO_3^- removal rates in this storm is unclear. At 25 cm of applied stormwater, with discrete samples taken after 6.3 cm (second flush) and 19 cm (fourth flush) of applied stormwater, this is a much larger storm than the 7/16/14 (5.0 cm of applied stormwater) and 10/31/14 (3.24 cm applied stormwater) storms. The 7/16/14 and 10/31/14 storms were also composite samples, rather than the 7/23/14 discrete samples.

Significant differences between influent and effluent concentrations are finally found in the 6/1/15 storm. For the first time, the no-plants control is significantly exporting NO_3^-, at 0.71 mg N L^{-1} vis-à-vis the influent concentration of 0.21 mg N L^{-1}. Previous studies in bioretention mesocosms and in the field (Davis et al. 2001, Davis et al. 2006, Dietz and Clausen 2006, Hsieh et al. 2007, Hunt et al. 2006, Line and Hunt 2009) have found a NO_3^- washout, i.e., a higher concentration of NO_3^- in the effluent than the influent. This washout is attributed to aerobic nitrification that takes place between storms. Given aerobic conditions and the correct microbial community, organic N can be converted to NH_4^+, and NH_4^+ can be converted to NO_3^-, which then washes out in the

effluent when new stormwater is applied. In this study, the influent has N at all of these three steps: organic N, NH_4^+, and NO_3^-. Organic N is also provided by the media, which is specified to have a minimum of 1.5% organic matter, as provided by the fine bark and soil components (Maryland State Highway Administration 2008). Therefore through a cascade effect, the effluent NO_3^- concentration can exceed the influent NO_3^- concentration, due to conversion of organic N and/or NH_4^+ to NO_3^-. Interestingly, however, this export is not seen earlier in the study, from any of the treatments including the no-plants control.

The no-plants treatment is the only treatment that created NO_3^- washout in the 6/1/15 storm. The two-*Juncus* and three-*Juncus* treatments did not create significant removal or export, as they are not statistically different than the measured influent. In this manner, having *Juncus* planted at a two- or three-*Juncus* density is better than having no plants, because NO_3^- export will be avoided. Presumably nitrification also occurs between storms in the *Juncus* treatments and the no-plants treatment, but in the *Juncus* treatment the produced NO_3^- is fully or largely taken up by the plants. However, *Juncus* at the two- and three-*Juncus* densities did not create NO_3^- removal.

As the first instance of a planted treatment causing NO_3^- reduction from the synthetic stormwater concentration, at 0.066 mg N L^{-1} the one-*Juncus* treatment NO_3^- concentration is significantly less than the NO_3^- concentration of the other two *Juncus* treatments (0.31 mg N L^{-1} for three-*Juncus*, 0.32 mg N L^{-1} for two-*Juncus*) the influent (0.21 mg N L^{-1}), and the non-planted treatment (0.71 mg N L^{-1}). This one-*Juncus* concentration represents a 69% removal vis-à-vis the measured influent.

Further data are needed to determine if the one-*Juncus* NO_3^- removal effect can be demonstrated to have occurred on more than a single date. However, based on the 6/1/15 results, *Juncus* plantings in bioretention at the one-*Juncus* treatment density are recommended for NO_3^- removal. This result is counter to the expected hypothesis that a higher density of plants would produce higher NO_3^- removal. A hypothesis to explain this finding is that the additional root structure of additional plants leads to water moving through the mesocosm at too fast of a rate, so the plant roots are not able to absorb as much NO_3^- as they could given more contact time. This hypothesis is supported by the effluent rate data collected on July 13, 2015, which are presented in Table 5.3 and paired with N removal data in Figure 5.6. In Figure 5.6, the two-*Juncus*, three-*Juncus*, and no-plants treatments' effluent rates are all within 1.5 minutes of each other, showing that the denser *Juncus* plantings have a similar contact time as the unplanted control. However, the NO_3^- concentration in the two planted treatments is less than half of that of the unplanted treatment, indicating that plants improve NO_3^- removal vis-à-vis unplanted treatments, even when contact time of the stormwater and the mesocosm is similar in the planted and unplanted treatments.
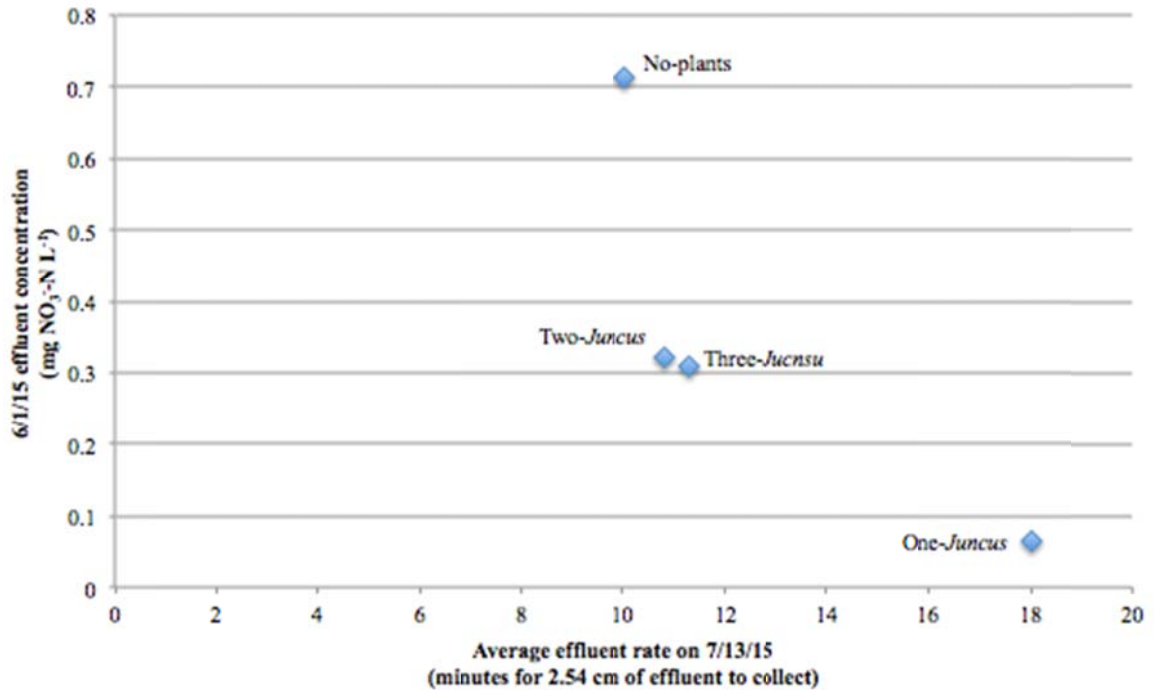
Figure 5.6 June 1, 2015 effluent NO_3^- concentrations (from Figure 5.5) versus July 13, 2015 average effluent rate (from Table 5.3), for four treatments.

Figure 5.6 also shows that the largest effluent rate, i.e. the slowest movement of stormwater through the mesocosm, occurs in the one-*Juncus* treatment, and leads to the lowest NO_3^- concentration in the effluent. A slower effluent rate should create more contact time with the media and plant roots, allowing more time for NO_3^- uptake by the plants and microbial community. Since mesocosm clogging was not observed after January 2015, denitrification is not presumed to play a role in the demonstrated one-*Juncus* NO_3^- removal. Barrett et al. (2013), Bratieres et al. (2008), Milandri et al. (2012), Read et al. (2008), Zhang et al. (2011) all also found increased NO_3^- or NO_x removal in some of their planted bioretention mesocosms vis-à-vis unplanted mesocosms.

NH$_4^+$

NH$_4^+$ concentrations were measured for all five storm/storm sections analyzed.

Results are presented in Figure 5.7 In all of the storms up to 6/1/15, NH$_4^+$ removal

occurred between the measured influent and the treatments, with the exception of the all-

plants treatment in 7/16/14 and the no-plants treatment in the fourth flush of 7/23/14,

which both have large standard errors (0.15 mg NH$_4^+$-N L^{-1} and 0.35 mg NH$_4^+$-N L^{-1},

respectively), ranging from 71–99%. The removal is not statistically significant for any of

these storms, however, because as in the NO$_3^-$ results it is difficult to register significance

with n=1 or 2 for the influent and n=3 or 4 for all of the other treatments. But all average

effluent concentrations, except for the two treatments and dates mentioned above with

large standard deviations, are below 0.1 mg NH$_4^+$-N L^{-1}. The average influent

concentration for all of the storms is 0.29 mg NH$_4^+$-N L^{-1}.

As with NO$_3^-$, it is only in the 6/1/15 storm, with well-developed vegetation, that

differences among treatments emerge. For the first time, removal in all treatments was

not found. However, as for the other dates, there are no statistically significantly

differences among treatments and/or the influent. But, the average effluent concentrations

become much higher than all of the previous storms, for all but the one-*Juncus* treatment.

The no-plants treatment concentration is 0.37 mg NH$_4^+$-N L^{-1}, the three-*Juncus* treatment

concentration is 0.20 mg NH$_4^+$-N L^{-1}, and the two-*Juncus* treatment is 0.21 mg NH$_4^+$-N

L^{-1}. The one-*Juncus* treatment is the only treatment that still provides removal, reducing

the influent 0.20 mg NH$_4^+$-N L^{-1} concentration to 0.092 mg NH$_4^+$-N L^{-1}, though again the

removal is not statistically significant. As with NO$_3^-$, the failure of the more densely

planted two- and three-*Juncus* treatments to create significant removal is again attributed

to the differing infiltration rates (see NO_3^- section above for full discussion). The ability of a planted treatment to provide more NH_4^+ removal than an unplanted-treatment was also found in Read et al. (2008) and Zhang et al. (2011).

The reason for the change from the no-plants, two-*Juncus*, and three-*Juncus* treatments providing 71–97% NH_4^+ removal (calculations not shown, again excepting the 7/23/14 no-plants fourth flush average which had a high standard deviation) in the July through October 2014 storms, to those treatments on average exporting NH_4^+ in the 6/1/15 storm, is unclear. Because the change appeared in both planted and un-planted treatments, the cause should be non-plant related, such as a change in the media or microbial community. Davis et al. (2001) attributed the NH_4^+ removal capacity most likely to the cation exchange capacity of the media, so perhaps a change has occurred in that capacity. Or, the microbial community has changed. If the change is non-plant related then it also should have occurred in the one-*Juncus* treatment, but that treatment appears able to counteract the effect somewhat and cause an overall NH_4^+ removal (though again, not statistically significant removal).
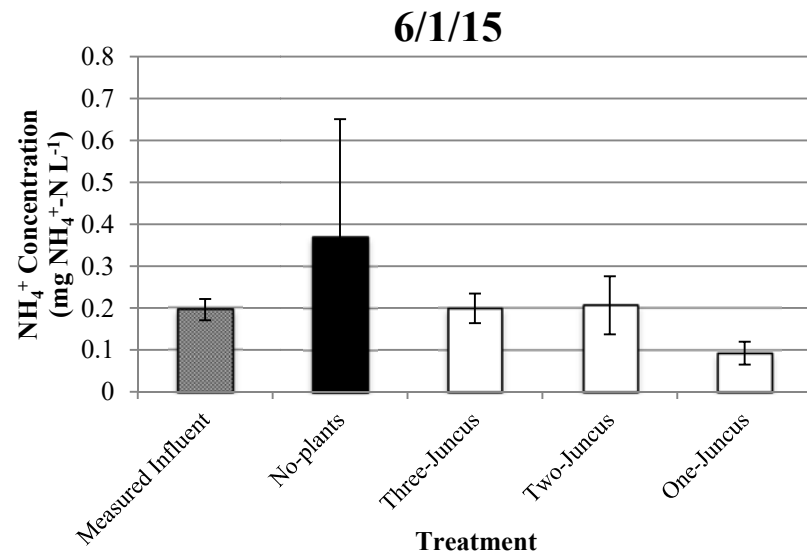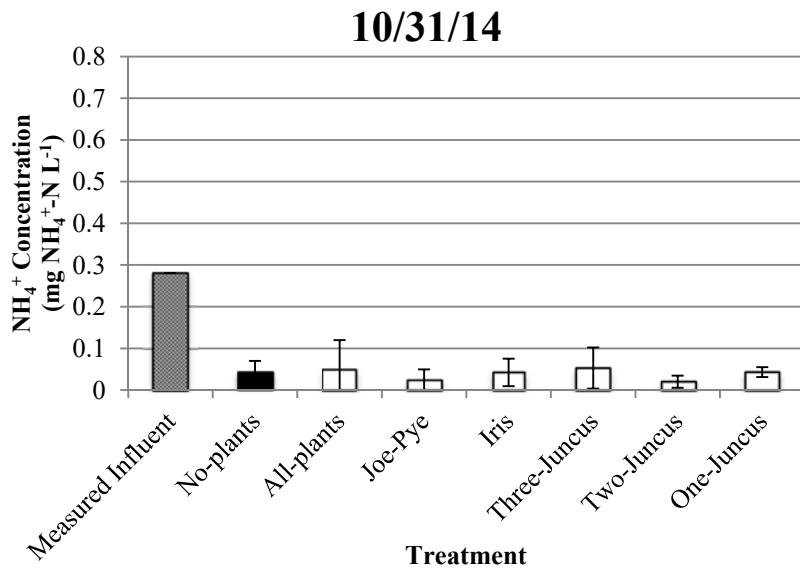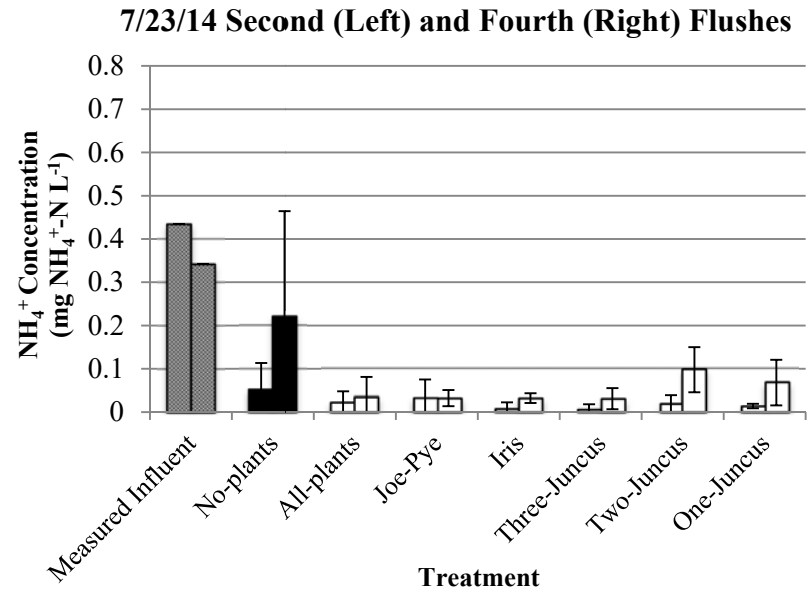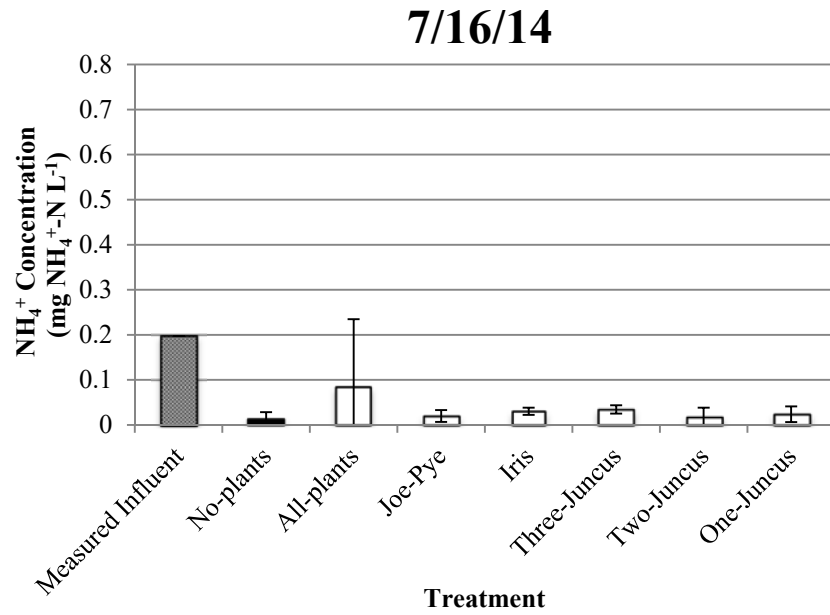
Figure 5.7 NH_4^+ concentrations from synthetic stormwater influent and the effluent from seven treatments of bioretention mesocosms with different vegetation. Each graph represents a simulated storm. The 7/16/14 storm is a composite sample of a simulated 0.27 cm storm, or 4.9 cm applied water. The 7/23/14 storm is a simulated 1.4 cm storm, or 25 cm applied water. The second flush was sampled after 6.2 cm of water was applied, and the fourth flush was sampled after 19 cm of water was applied. The 10/31/14 storm is a composite sample of a simulated 0.18 cm storm, or 3.2 cm of applied water. The 6/1/15 storm is a composite sample of a simulated 0.69 cm storm, or 12 cm of applied water. The * denotes a significant difference between that treatment and the measured influent. Differences among treatments and/or the influent are denoted with letters, if found. n=1–2 for influent, n=2–4 for effluent, usually n=4. Error bars are ± one standard deviation.

Organic N

DON data were found for both flushes of the 7/23/14 storm and for the 6/1/15 storm. Results are presented in Figure 5.8.

For both flushes of the 7/23/14 storm, effluent concentrations of DON average less than 0.2 mg DON-N L^{-1}. In the second flush, no effluent concentration is above 0.026 mg DON-N L^{-1}. Unexpectedly, given the theoretical DON influent concentration of 0.5 mg DON-N L^{-1}, no DON was found in the influent. The expected 0.50 mg DON-N L^{-1} was found in the fourth flush. DON removal for the treatments in the fourth flush ranged from 61–81%. However, the standard deviations are large, all at least 50% of their respective average concentration, so the actual removal may be considerably different than the 61–81%. This removal is primarily attributed to sorption processes with the organic material in the media and mulch. Davis et al. (2006) found that the majority of Total Kjeldahl Nitrogen (TKN, i.e.,NH_4^+ and organic N) removal in a bioretention box experiment occurred within the first few centimeters, and enhancement of the process by the media layer was suggested. For both flushes in the 7/23/14 storm in this study, no significant differences were found, despite the large removal percentages in the fourth flush.

By 6/1/15, with well-developed vegetation, a non-significant loss of removal capacity for both the no-plants control and all three *Juncus* treatments has developed. If previous removal was due to the organic material in the media, it is assumed that the loss of removal in the 6/1/15 storm is due to the breakdown and loss of capacity of this organic material. Poor DON removal was also found in a nine-year-old bioretention cell in Li and Davis (2014).

Particulate organic N (PON) data were found for the second flush of the 7/23/14 storm and the 6/1/15 storm. Results are presented in Figure 5.9. In the second flush of 7/23/14, export of PON is seen from all treatments, with the highest amount from the no-plants control. This storm was near the start of the study so particulate matter was likely still washing out from the media, despite extensive washing before vegetation planting. Since the plants were freshly planted and relatively small, they were not expected to contribute significantly to preventing particulate washout, but washout was highest for the no-plants control and lower for all planted treatments.

By 6/1/15, the amount of particulate washout was much reduced: under 0.5 mg N L^{-1} for all treatments, vis-à-vis a range of 0.46 to 1.6 mg N L^{-1} among the treatments in the 7/23/14 second flush. After more than 10 months, the majority of particulate matter from the media is expected to have washed out, so export is minimal. Plant contribution to particulate N washout is also expected to be minimal, because dead plant matter that had disconnected from the plant was removed before each sampling storm. However, dead or dying plant material still connected to the plant may have contributed small amounts of particulate organic matter. Li and Davis (2014) found that 79% of the PON captured in a field bioretention site was converted to DON and NO_3^-, contributing to leaching from the cell of both of those N forms. The amount of PON generated in this study was not measured, though none was added to the synthetic stormwater. It is likely that any PON produced did experience some mineralization, ammonification, and nitrification, and contribute to the DON and NO_3^- export from the mesocosms

7/23/14 Second (Left) and Fourth (Right) Flushes

6/1/15
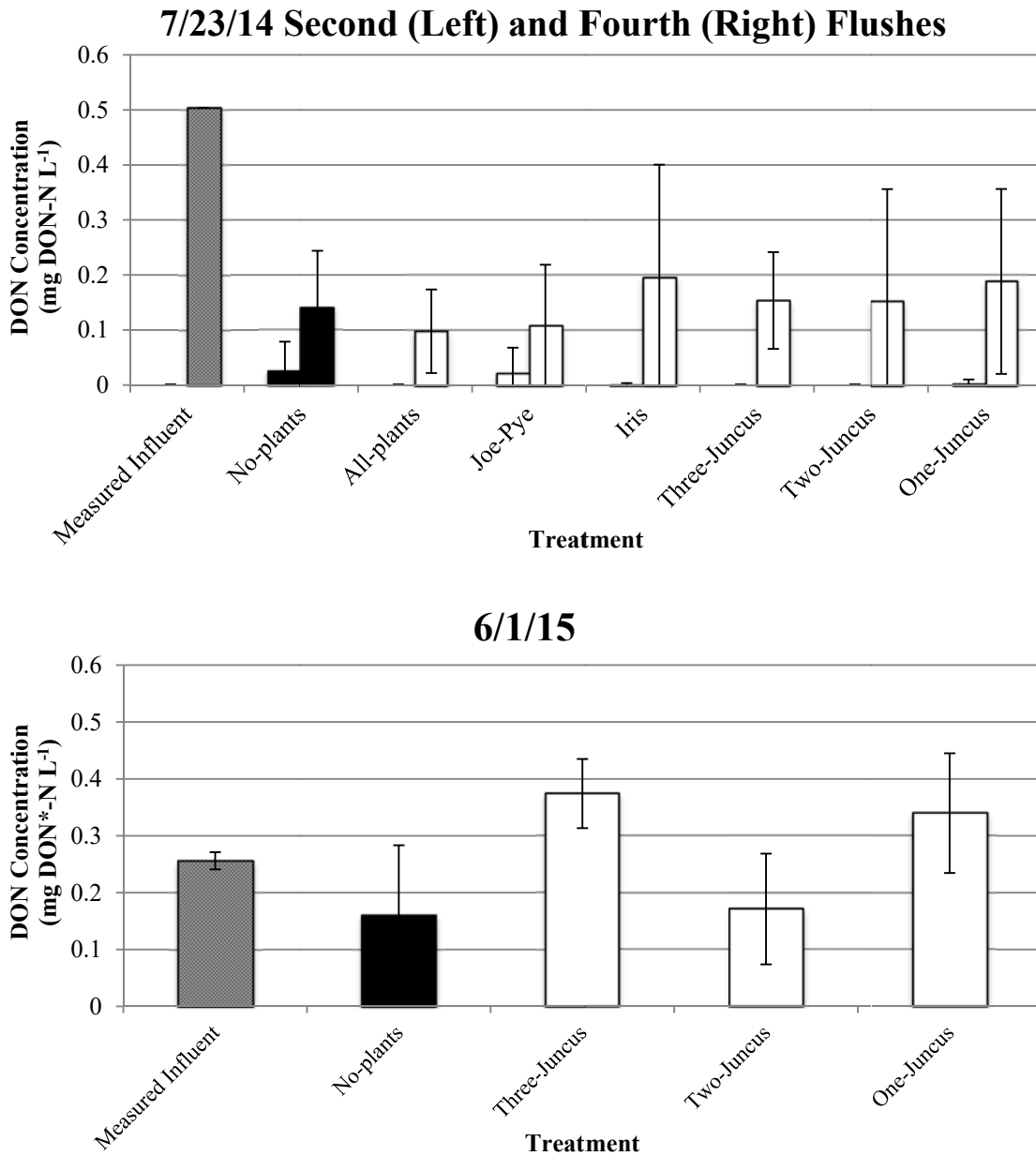
Figure 5.8 DON concentrations from synthetic stormwater influent and the effluent from seven treatments of bioretention mesocosms with different vegetation. Each graph represents a simulated storm. The 7/23/14 storm is a simulated 1.4 cm storm, or 25 cm applied water. The second flush was sampled after 6.2 cm of water was applied, and the fourth flush was sampled after 19 cm of water was applied. The 6/1/15 storm is a composite sample of a simulated 0.69 cm storm, or 12 cm of applied water. The * denotes a significant difference between that treatment and the measured influent. Differences among treatments and/or the influent are denoted with letters, if found. n=1–2 for influent, n=2–4 for effluent, usually n=4. Error bars are ± one standard deviation.

7/23/14 Second Flush
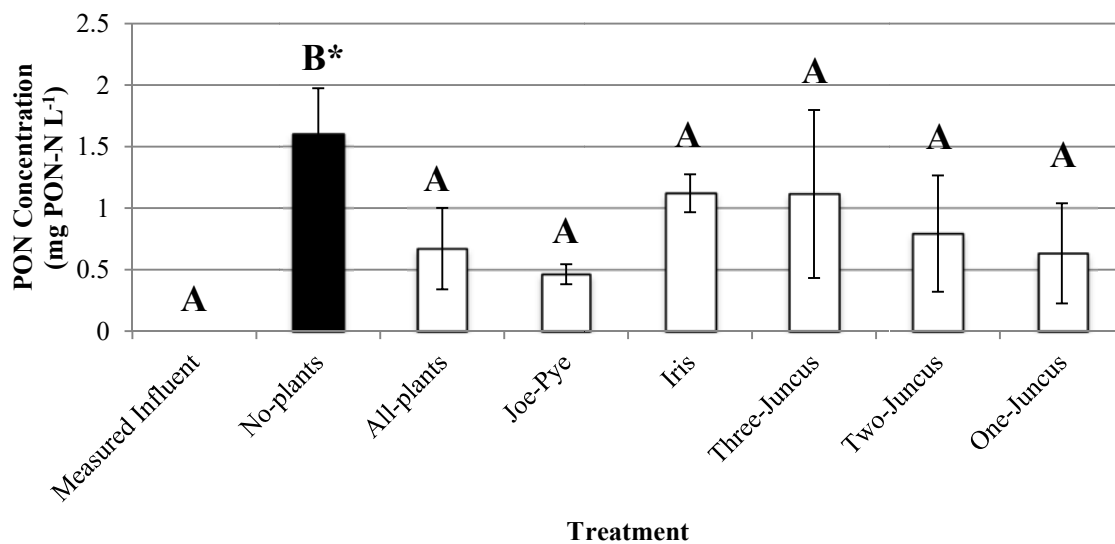
6/1/15

Figure 5.9 Particulate organic N concentrations from synthetic stormwater influent and the effluent from seven treatments of bioretention mesocosms with different vegetation. Each graph represents a simulated storm. The 7/23/14 storm is a simulated 1.4 cm storm, or 25 cm applied water. The second flush was sampled after 6.2 cm of water was applied. The 6/1/15 storm is a composite sample of a simulated 0.69 cm storm, or 12 cm of applied water. The * denotes a significant difference between that treatment and the measured influent. Differences among treatments and/or the influent are denoted with letters, if found. n=1–2 for influent, n=2–4 for effluent, usually n=4. Error bars are ± one standard deviation.

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NO_2^-

NO$_2^-$ data were found for 7/16/14, both the second and fourth flush of 7/23/14, and 10/31/14. Results are presented in Figure 5.10. No NO_2^- is introduced in the influent. NO_2^- is produced as an intermediate in the conversion of NH_4^+ to NO_3^-, but the NO_2^- to NO_3^- reaction is faster than the NH_4^+ to NO_2^- reaction, so as long as the correct bacterial populations are present then very low to no NO_2^- is expected in the system from these reactions (Rittmann and McCarty 2001). This appears to be the case in this study, because the effluent NO_2^- concentrations for all storms and treatments are minimal, never exceeding an average of 0.03 mg N L^{-1}.

7/16/14

7/23/14 Second (Left) and Fourth (Right) Flushes
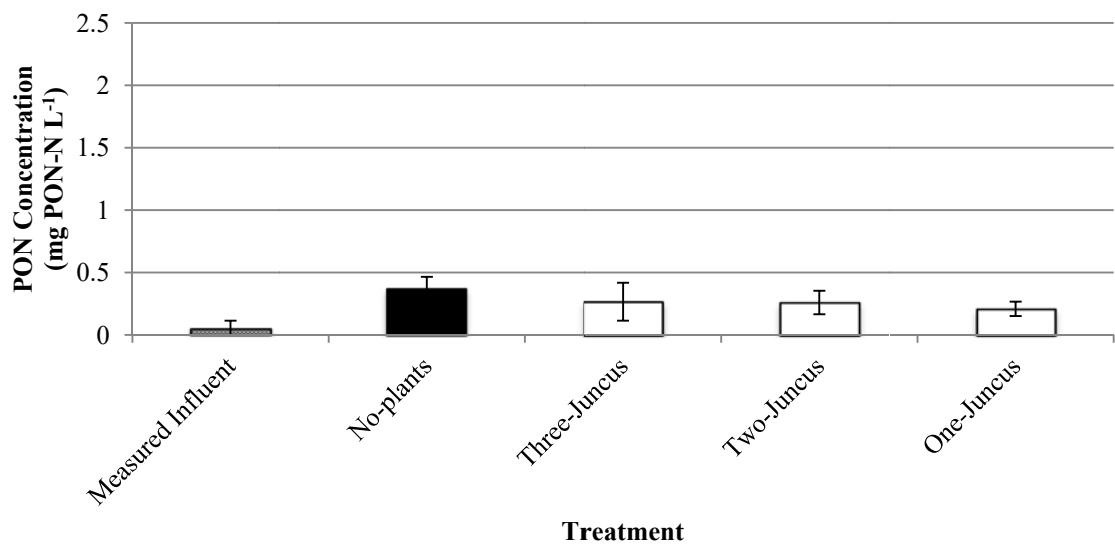
10/31/14
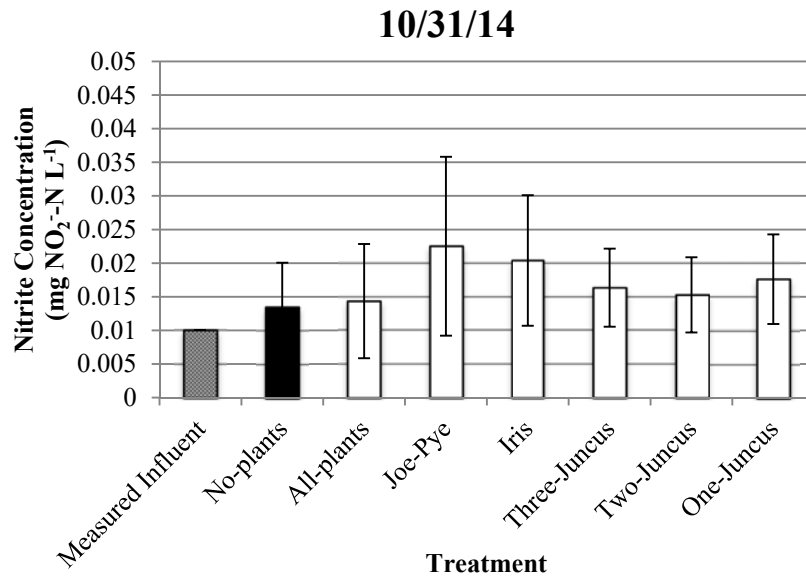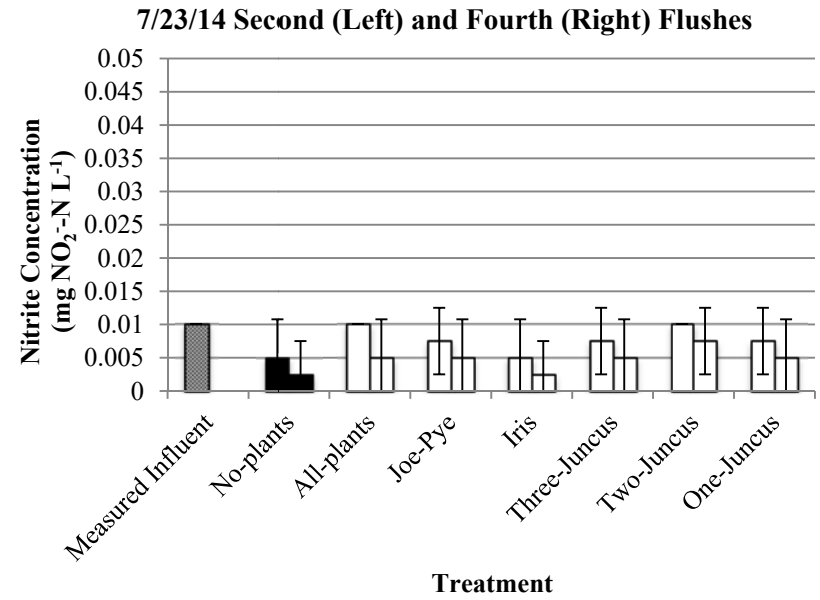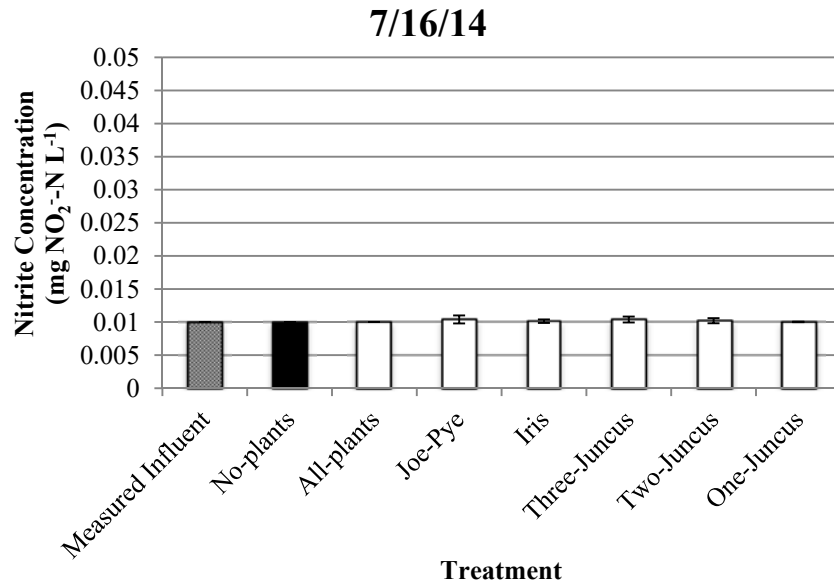
Figure 5.10 NO_2^- concentrations from synthetic stormwater influent and the effluent from seven treatments of bioretention mesocosms with different vegetation. Each graph represents a simulated storm. The 7/16/14 storm is a composite sample of a simulated 0.27 cm storm, or 4.9 cm applied water. The 7/23/14 storm is a simulated 1.4 cm storm, or 25 cm applied water. The second flush was sampled after 6.2 cm of water was applied, and the fourth flush was sampled after 19 cm of water was applied. The 10/31/14 storm is a composite sample of a simulated 0.18 cm storm, or 3.2 cm of applied water. The * denotes a significant difference between that treatment and the measured influent. Differences among treatments and/or the influent are denoted with letters, if found. n=1–2 for influent, n=2–4 for effluent, usually n=4. Error bars are ± one standard deviation.

TN

Total N data were obtained for 7/23/14 second flush, 10/31/14, and 6/1/15 (Figure 5.11). In the second flush of 7/23/14, the only significant difference between treatments and/or influent is that the no-plants treatment TN concentration is significantly higher than the influent and all other treatments. At 1.7 mg N L^{-1}, it is almost twice the concentration of the influent (0.91 mg N L^{-1}). All of the other treatments are not significantly different from the influent, ranging from a 44% removal vis-à-vis the influent in the Joe-Pye treatment to a 31% export in the Iris treatment. The results from this date and flush display a similar between-treatments trend as the PON results from this date and flush, suggesting that PON washout was the driving force behind the TN results.

In the 10/31/14 storm, the TN concentrations for all treatments decreased since 7/23/14. Like 7/23/14, only one treatment is significantly different than the influent, and that difference is TN export. In the 7/23/14 storm that treatment was the no-plants treatment; in the 10/31/14 storm it is the Joe-Pye treatment. This is likely due to the replanting of the Joe Pye plants that week prior to the storm, which may have dislodged media or plant particles, or microbes, which were washed out in the 10/31/14 storm. Organic N data for the 10/31/14 storm are not available, but the results above show no significant NO_3^- removal, along with non-significant NH_4^+ removal during the storm, for all treatments. Since there is no significant TN removal for the storm, it implies that the NH_4^+ removal is being compensated for by organic N export, resulting in no net removal of TN. In contrast to 7/23/14, in the 10/31/14 storm the no-plants treatment export is no longer significant.

By 6/1/15 there is again TN export, from all of the treatments except for one-*Juncus*. However, none of the exports are significant, even the export of 139% of the influent concentration, in the Joe-Pye treatment.

This storm includes data from the mesocosms that had fewer Joe Pye plants than before the winter, due to several of the Joe Pye plants not re-sprouting in Spring. The Joe-Pye treatment includes the three Joe-Pye mesocosms with only one Joe Pye plant, as opposed to the three plants in the mesocosms in the other two previous storms. The all-plants treatment includes the two all-plants mesocosms with an Iris plant and *Juncus* plant but no Joe Pye plant. The TN concentrations between the two storms for these two treatments increased for both treatments (1.7 times the 10/31/14 concentration for the all-plants treatment and 2.0 times the 10/31/14 concentration for the Joe-Pye treatment), but the TN concentration for all of the other treatments also increased during this time, from 1.5 to 3.9 times the respective 10/31/14 concentration. So, given that the increase in TN concentration during the time period for the all-plants and Joe-Pye treatments is within the range of the increase for the other treatments, it does not appear that the loss of some Joe Pye plants between the two dates had a large impact on TN removal effects.

Comparing the 6/1/15 TN data with data above for N species, it can be seen that the no-plants control export is primarily driven by NO_3^- export. By similar comparison, the three-*Juncus* export is driven primarily by DON export. Also, the TN concentrations of the *Juncus* treatments follows their densities: more dense treatments have higher export of TN, ranging from 1.1 mg N L^{-1} in the three-*Juncus* treatment to 0.96 mg N L^{-1} in the two-*Juncus* treatment and 0.71 mg N L^{-1} in the one-*Juncus* treatment.

7/23/14 Second Flush

10/31/14

6/1/15
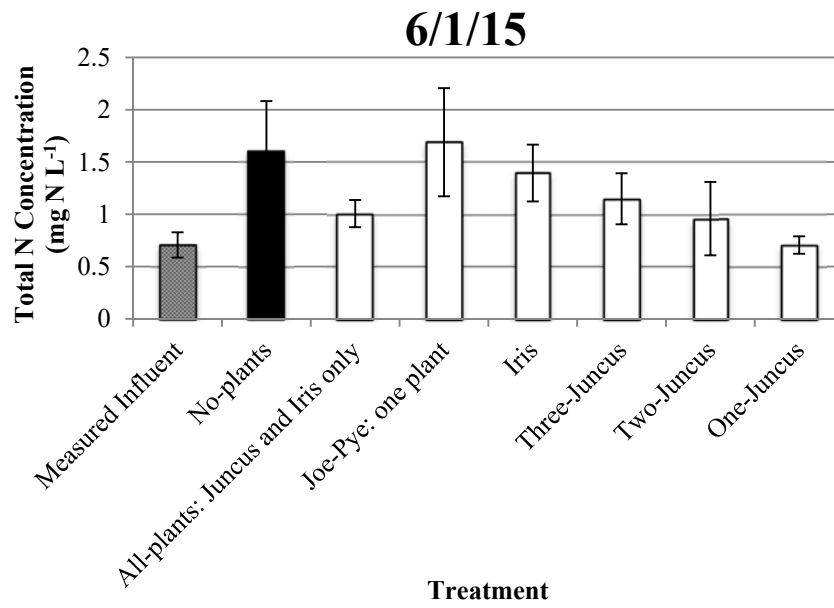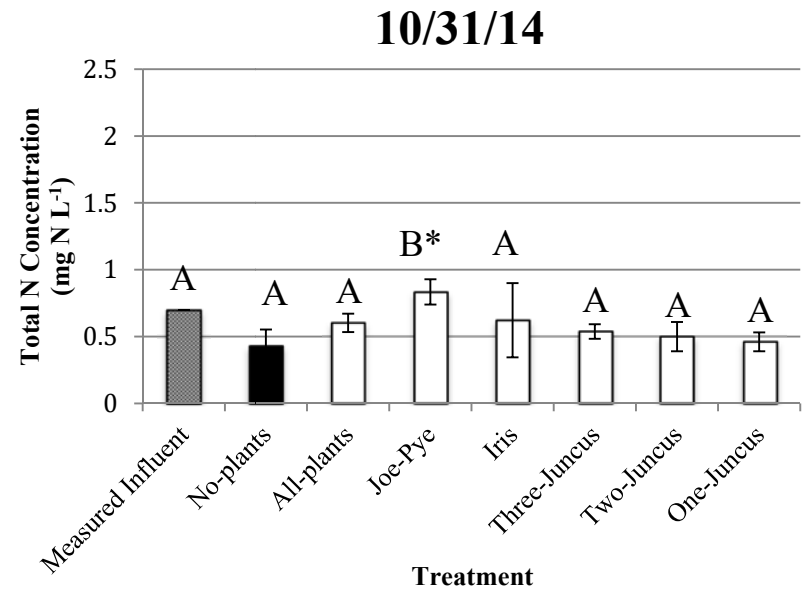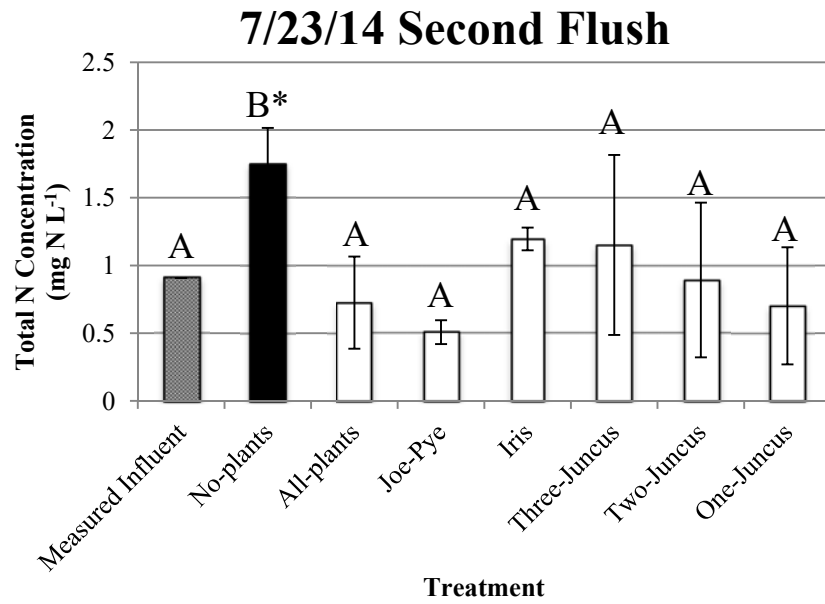
Figure 5.11 Total N concentrations from synthetic stormwater influent and the effluent from seven treatments of bioretention mesocosms with different vegetation. Each graph represents a simulated storm. The 7/23/14 storm is a simulated 1.4 cm storm, or 25 cm applied water. The second flush was sampled after 6.2 cm of water was applied. The 10/31/14 storm is a composite sample of a simulated 0.18 cm storm, or 3.2 cm of applied water. The 6/1/15 storm is a composite sample of a simulated 0.69 cm storm, or 12 cm of applied water. The * denotes a significant difference among that treatment and the measured influent. Differences among treatments and/or the influent are denoted with letters, if found. n=1–2 for influent, n=2–4 for effluent, usually n=4. Error bars are ± one standard deviation.

N Content in the Media and Plants

To account for N accumulation or removal from the mesocosm components, N analyses were conducted on media and plant samples at both the beginning and end of the experiment. At the beginning of the experiment, two composite media samples were taken: one from the eleven mesocosms planted on the first day of planting, and one from the 17 mesocosms planted on the second day of planting. Both samples included media from the unplanted controls as well. The two samples had N contents of 0.024% and 0.027%, for an average of 0.026%. Results from the end of the experiment, where media samples were segregated by treatment, are presented in Table 5.7.

Table 5.7 Media N content results

	Percent N				
Treatment	Beginning-of-Study Average (%)	Beginning-of-Study Standard Deviation (%)	End-of-Study Average (%)	End-of-Study Standard Deviation (%)	Times Change During the Study
No-plants			0.042	0.0036	**+1.6**
All-species			Not measured	Not measured	**Not measured**
Joe-Pye	From all mesocosms: 0.026	From all mesocosms: 0.0018	Not measured	Not measured	**Not measured**
Iris			0.044	0.0092	**+1.7**
Three-*Juncus*			0.047	0.016	**+1.8**
Two-*Juncus*			Not measured	Not measured	**Not measured**
One-*Juncus*			0.039	0.0028	**+1.5**

All of the measured mesocosms (four out of the seven treatments) increased the percent N in the media between the beginning and end of the experiment, between 1.5

and 1.8 times. The type of plant in the mesocosm did not appear to create a significant difference in N accumulation over the course of the study.

Plant percent N results are presented in Table 5.8. Full data are given in Appendix C. At the beginning of the experiment, *Juncus* shoots had the highest N content in their dry biomass, at 1.4%. Joe Pye roots had the least, at 0.68%. At the end of the study, Joe Pye shoots had the highest percent N in their dry biomass, at 2.1%. Iris roots from the all-plants treatment had the lowest percent N in their dry biomass, at 0.46%.

While statistically significant differences in biomass were not found for Iris roots or shoots nor *Juncus* roots or shoots biomass between the all-plants and mono-plant (i.e., one-*Juncus* and Iris) treatments at the end of the study (Table 5.4), statistically significant differences among the two treatments were found in root percent N for both species. *Juncus* root percent N averaged 0.52 in the one-*Juncus* treatment at the end of the study, vis-à-vis 0.64% in the all-plants treatment. Iris root percent N averaged 0.54% in the Iris treatment vis-à-vis 0.46% in the all-plants treatment. The differences in root conditions created between treatments evidently affected N allocation to the roots, while not significantly affecting biomass.

Statistically significant changes in percent N occurred between the beginning and end of the study. Both Joe Pye roots and shoots increased in their percent N: roots from 0.68 to 1.1%, and shoots from 1.1 to 2.1%. Iris shoots also increased, from 0.9 to 0.95%. *Juncus* shoots decreased, from 1.4 to 1.1%. Iris roots also decreased, from 0.81 to 0.54% in the Iris treatment, and 0.81 to 0.46% in the all-plants treatment.

Table 5.8 Summary of plant N results. Bold end-of-study data = significant difference between beginning- and end-of-study data. Significance determined using a t-test with alpha = 0.05. Full data are given in Appendix C.

Plant Species	Percent N			
	Root Beginning-of-Study Average ± Standard Deviation (%)	Root End-of-Study Average ± Standard Deviation (%)		Shoot Beginning-of-Study Average ± Standard Deviation (%)	Shoot End-of-Study Average ± Standard Deviation (%)
Joe Pye	0.68 ± 0.075	**1.1 ± 0.25**		1.1 ± 0.13	**2.1 ± 0.17**
Juncus	1.2 ± 0.48	One-*Juncus* treatment	0.52 ± 0.014	1.4 ± 0.55	**1.1 ± 0.30**
		All-plants treatment	0.64 ± 0.016		
Iris	0.81 ± 0.031	Iris treatment	**0.54 ± 0.047**	0.90 ± 0.17	**0.95 ± 0.14**
		All-plants treatment	**0.46 ± 0.015**		

N Mass in Mesocosm Components

      The N mass in the applied stormwater, effluent, plants, and media is calculated in this section. The change in N mass is calculated for the whole study.

Applied Stormwater

      The mass of total N applied in the stormwater to a single mesocosm during the full study, is calculated using the average measured influent TN concentration of 0.77 mg N L^{-1} (Table 5.6) in Equation 5.1, which finds that approximately 0.81 g N mesocosm^{-1} were applied.

$$(1{,}670 \; cm \; applied \; stormwater)\left(\frac{3.875 \; L}{6.12 \; cm \; applied \; stormwater \; x \; mesocosm}\right) \quad (5.1)$$

$$\left(\frac{0.77 \; mg \; N}{L}\right)\left(\frac{g \; N}{1{,}000 \; mg \; N}\right) = 0.81 \; g \; N \; mesocosm^{-1}$$

      The amount of stormwater applied during the year was less than the average precipitation for the region, as discussed in Section 4.6: a simulated 92.6 cm of precipitation were applied in this study, with average annual precipitation in the area being 109.9 cm. Additionally, 0.77 mg N L^{-1} is a low loading. As given in Section 2.1, a national database of stormwater averages 2.92 mg N L^{-1} (The National Stormwater Quality Database 2015), and Kayhanian et al. (2012) found an average highway runoff N loading of 5.64 mg N L^{-1}. Thus, due to the lower-than-average precipitation and N concentration, the total amount of N applied in the stormwater in this study is lower than would be found on average in Maryland.

<u>Effluent</u>

Concentrations of TN in the effluent ranged from below the influent concentration to higher than the influent concentration, depending on the storm. The range was from 56–227% of the influent concentration. Therefore, while variable, the range of effluent N concentration should fall between approximately 0.46 and 1.8 g N mesocosm^{-1}.

<u>Plants: Live Biomass</u>

Using the plant biomass data (from Table 5.4) and end-of-study plant percent N data (from Table 5.8), the amount of N accumulated in the biomass of an average plant of each species during the study was calculated using Equation 5.2; the results are presented in Table 5.9.

$$\left(\frac{End\ of\ Study\ \%\ N}{100}\right)\left(End\ of\ Study\ Biomass\ (g)\right) - \tag{5.2}$$

$$\left(\frac{Beginning\ of\ Study\ \%\ N}{100}\right)\left(Beginning\ of\ Study\ Biomass\ (g)\right)$$

$$= g\ N\ accumulated$$

Table 5.9 N mass accumulated in the biomass of a plant of each species during the course of the study. Joe Pye biomass is from 38 weeks of growth. Iris and *Juncus* biomass is from 54 weeks of growth. In columns six and seven, the "Fraction of stormwater N…" are found by dividing column four or five by 0.81 g N, the average N mass applied in stormwater to each mesocosm (Equation 5.1).

Plant Species	Treatment	Mass N accumulated in roots (g)	Mass N accumulated in shoots (g)	Mass N accumulated in one plant (roots and shoots) (g)	Fraction of applied stormwater N accumulated in shoots	Fraction of applied stormwater N accumulated in one plant (roots and shoots)
Joe Pye	Joe Pye	0.0074	0.056	0.063	**0.069**	**0.078**
Juncus	Average of one-*Juncus* and all-plants	0.11	0.42	0.53	**0.52**	**0.65**
	One-*Juncus*	0.10	0.43	0.53	**0.53**	**0.65**
	All-plants	0.12	0.41	0.53	**0.51**	**0.65**
Iris	Average of Iris and all-plants	0.015	0.040	0.055	**0.050**	**0.068**
	Iris	0.017	0.057	0.074	**0.070**	**0.091**
	All-plants	0.013	0.023	0.036	**0.028**	**0.044**

Table 5.9 clearly demonstrates the difference that biomass growth makes in N uptake. A single *Juncus* plant accumulated more than half (0.65 g N accumulated per g N applied in both the one-*Juncus* and all-plants treatments) of the applied N from the synthetic stormwater over the course of a year. In contrast, a single Joe Pye accumulated only about 8% (0.078 g N accumulated per g N applied), and a single Iris accumulated only about 7% (0.068 g N accumulated per g N applied) on average. However, the Joe Pye only had 38 weeks of growth vis-à-vis 54 weeks for the other two plant species.

The percent N values for the three species range from 0.46% N (Iris roots in the all-plants treatment) to 2.1% N (Joe Pye shoots), a 4.6-fold difference. In contrast, the differences between the change in biomass for roots and shoots (Table 5.4, with the Joe Pye having less time to accumulate biomass than the other two species) ranges from 0.1 g (Joe Pye roots) to 37 g (*Juncus* shoots), a 370-fold difference. Therefore the much larger amount of N accumulated in biomass by the *Juncus* vis-à-vis the other two species is primarily due to biomass accumulation rather than differences in percent N in the biomass. The biomass accumulation of the whole (roots and shoots) *Juncus* plant during the study was almost an order of magnitude more than the Iris, and more than an order of magnitude greater than the Joe Pye: 55.8 g biomass accumulated for one *Juncus* plant, 6.56 g accumulated for one Iris plant, and 2.54 g for one Joe Pye plant. The Joe Pye value represents only 38 weeks of growth vis-à-vis 54 weeks for *Juncus* and Iris, but considering its rate of growth, even given the additional 16 weeks, the Joe Pye plant is not expected to produce as much biomass as *Juncus*, and likely not even as much as Iris.

Plants: Dead Shoot Biomass

The average cumulative amount of dried dead shoot biomass for a single mesocosm was less than 1.0 g for all treatments (Figure 5.4). Average percent N for dried dead shoot biomass at the end of the study was 0.95–2.1% (Table 5.8). Therefore, the average amount of N in dried dead shoot biomass did not exceed 1.0 g x 0.0021 = 0.0021 g for any treatment. This is much less than any amount of N in the other components (Figure 5.13); it is 0.5% of the nearest category, the lowest end of the effluent g N (0.46 g N). Therefore, the contribution of N in dead biomass to the system is not included in Figure 5.13.

Media

Equation 5.2 was adapted for media use by replacing "Biomass" with "Media Mass." To find the media mass, a typical bulk density for dry sandy loam was used: 1.44 g cm^{-3} (Linsley 1975). It was assumed that the media was at field capacity [12% water (Linsley et al. 1975)] when it was sampled. 12% would increase the density to the media by 0.12 g cm^{-3}, so an approximate wet bulk density of 1.56 g cm^{-3} was used. From the known dimensions of the wet media in the mesocosms, the volume of media in each mesocosm was calculated as 9,387 cm^3. Therefore the mass of the media in each mesocosm is estimated as: (1.56 g cm^{-3})(9,387 cm^3) = 14,600 g. In Table 5.10, this mass is used along with N content data from Table 5.7 in order to find the mass of N in the media at both the beginning and end of the study, and the change during the study. N contents were determined from dried bioretention media.

Table 5.10 N mass accumulated in mesocosm media during the course of the study, for one mesocosm. Equation 5.2, with Mass of Media used in place of Biomass, is used to find the Mass of N Accumulated in Media. Percent N data are from Table 5.7.

| Treatment | Beginning-of-Study | | | End-of-Study | | | Difference |
	Percent N (%)	Mass of Media (g)	Mass of N in Media (g)	Percent N (%)	Mass of Media (g)	Mass of N in Media (g)	Mass of N Accumulated in Media (g)
No-plants	0.026	14,600	3.8	0.042	14,600	6.1	2.3
Iris	0.026	14,600	3.8	0.044	14,600	6.4	2.6
Three-*Juncus*	0.026	14,600	3.8	0.047	14,600	6.9	3.1
One-*Juncus*	0.026	14,600	3.8	0.039	14,600	5.7	1.9

Therefore, the average mass of N accumulated in the media during the study, for the measured treatments, is 2.5 g.

Comparison of N in Mesocosm Components

A summary of the N pools in the stormwater, effluent, plants, and media during the entire study is given in Figure 5.12.
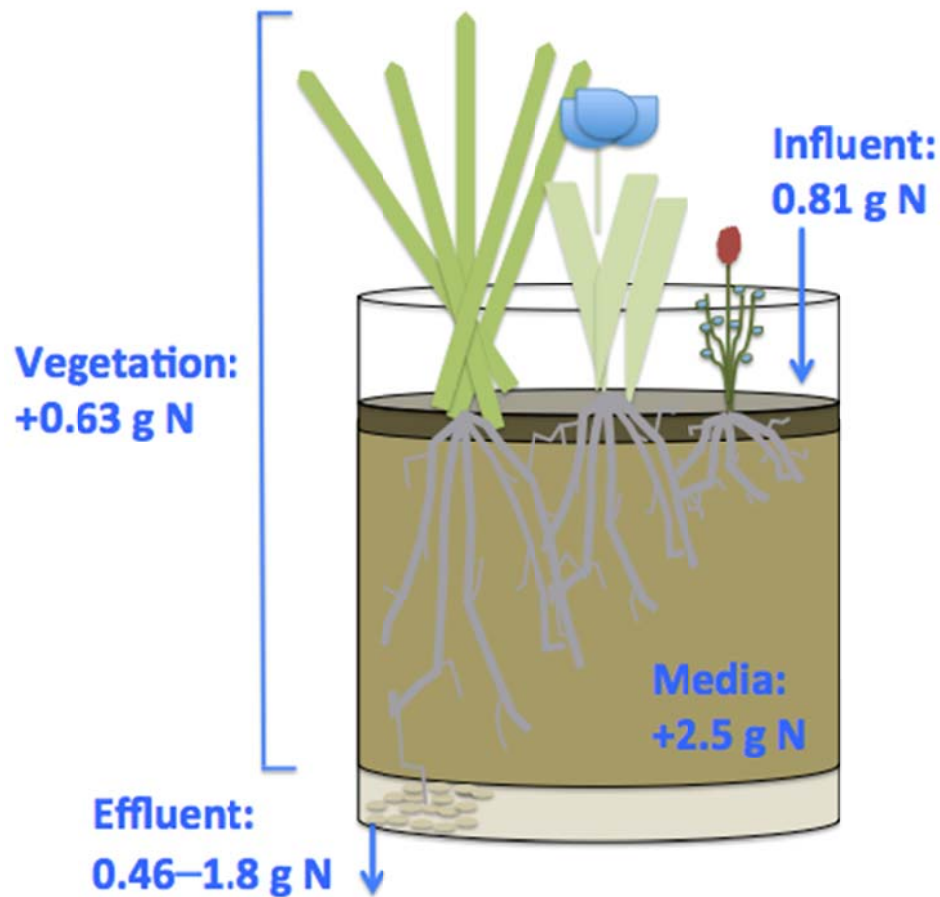
Figure 5.12 Average mass of N applied, accumulated in the plant(s), accumulated in the media, and exported in effluent during the course of the study, as g N. Data are taken from Tables 5.9 and 5.10 and from the Applied Stormwater and Effluent sections. The vegetation value is a sum of the average Iris and average *Juncus*, both from the all-plants treatment, plus a Joe Pye plant from the Joe-Pye treatment (with only 38 weeks of growth vis-à-vis 54 weeks for the other two plant species). Mass of N contributed by dead shoot biomass is not included due to its small magnitude (Plants: Dead Shoot Biomass section above).

Compared to the scale of N in the components (ranging from 0.46 to 2.5 g N), the

media mass is very large (14,600 g). Therefore, the media mass, which is estimated, has a

large influence on the media N mass estimate. The large influence of this estimated

media value may contribute to the imbalance between the sum of the uptake (into plants

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and into the media) and the effluent compared with the input value. Any error in the media mass will translate to the media N mass.

The estimated average amount of N accumulated in the media is 3.1 times the amount of N in the applied stormwater, and 4.0 times greater than the amount of N in plant biomass. The media N is also 1.4 times greater than the highest value in the effluent range. Therefore, the media dominates the N distribution among the system components. Despite this dominance, the vegetation biomass does contain 78% of the amount of N in the applied stormwater. So, plants do sequester N. However, the amount of N that plants accumulate over the study is only a quarter of the amount sequestered in the media, showing that, by mass of N, the media is the strongest force in N uptake in the system. For maximum N removal in bioretention, therefore, both media and vegetation should be carefully selected and optimized for N removal. However, based on the results of this study, a given change in media removal capacity will create a four-fold larger impact on overall N removal than the same amount of change from vegetation customization.

The wide range of effluent concentrations, ranging from N removal to N export, correlates with previous field work (Li and Davis 2014), which showed only a 9% net overall TN reduction over 16 storm events. N changed forms in Li and Davis (2014) and in this study, but the overall N removal was not consistently high.

The results of this study are based upon a drier than average year with lower than average N concentration, as mentioned previously. Therefore, the applied N mass is less than an average year in natural conditions, which also impacts the amount of N in the plants, media, and effluent.

N Mass in Mesocosm Components as a Function of Area and Time

The previous section described the mass of N in stormwater, effluent, plants, and media over the course of the study. In order to allow for easy comparison of these results with other studies, results are converted to mass-N area^{-1} time^{-1} (kg-N ha^{-1} year^{-1}), with area being the bioretention area. The study ran for one year, from July 2014 to July 2015, so the calculations for the full study time period represent one year. Additionally, the average surface area for a mesocosm was 624.3 cm^2. This information can be used to find the N mass in mesocosm components as a function of area and time.

Applied Stormwater

The applied stormwater during the study (i.e., over a year) was calculated as 0.81 g N mesocosm^{-1} year^{-1} in Equation 5.1. This mass is converted into kg-N ha^{-1} year^{-1} in Equation 5.3. The surface area (624.3 cm^2) is of the mesocosm, not of the drainage area. Therefore the resulting value is for bioretention area, not drainage area.

$$\left(\frac{0.81\ g\ N}{mesocosm \cdot year}\right)\left(\frac{mesocosm}{624.3\ cm^2\ surface\ area}\right)\left(\frac{10,000\ cm^2}{m^2}\right)\left(\frac{10,000\ m^2}{ha}\right)\left(\frac{kg}{1,000\ g}\right) \quad (5.3)$$

$$= 130\ kg \cdot N\ ha^{-1} year^{-1}$$

Effluent

The effluent in the study ranged from 0.46 to 1.8 g N mesocosm^{-1}, as established in the *N Mass in Stormwater, Effluent, Plants, and Media* section above. Using Equation 5.3 with 0.46 and 1.8 g N mesocosm^{-1} year^{-1}, the resulting range of N in the effluent is 73 to 290 kg-N ha^{-1} yr^{-1}.

<u>Plants</u>

The mass of N accumulated over a year in an average plant of each species, for a given treatment, is given in Table 5.9. These data are converted to kg-N ha^{-1} year^{-1} using Equation 5.4, with x being the shoot or root value from Table 5.9 and y being the typical number of plants per a given treatment (given in column three of Table 5.11). The results of Equation 5.2 are given in Table 5.11.

$$\left(\frac{x\ g\ N}{plant \cdot year}\right)\left(\frac{y\ plant(s)}{mesocosm}\right)\left(\frac{mesocosm}{624.3\ cm^2}\right)\left(\frac{10,000\ cm^2}{m^2}\right)\left(\frac{10,000\ m^2}{ha}\right)\left(\frac{kg\ N}{1000\ g\ N}\right) \quad (5.4)$$

$$= z\ kg \cdot N\ ha^{-1}year^{-1}$$

Table 5.11 Mass of N accumulated in the biomass of the plants of each species in a given treatment, during the course of the study (biomass for Joe Pye is from 38 weeks of growth, biomass is from 54 weeks of growth for *Juncus* and Iris). Plant biomass for treatments with three plants is estimated by multiplying the average for one plant by three.

Plant Species	Treatment	Number of Plants in the Given Treatment	Mass Accumulated in Total Plant Biomass in Given Treatment (kg-N ha^{-1} year^{-1})	Mass Accumulated in Shoot Plant Biomass in Given Treatment (kg-N ha^{-1} year^{-1})	Fraction of Shoot Plant Biomass (Total Plant Biomass)$^{-1}$
Joe Pye	Joe-Pye	1*	10	9.0	0.90
Juncus	One-*Juncus*	1	85	69	0.81
	All-plants	1	85	66	0.78
Iris	Iris	3	12	9.1	0.76
	All-plants	1	5.8	3.7	0.64

* One plant because only one of three plants survived in the mesocosms from which the Joe Pye plants were sampled

For comparison, a switchgrass (*Panicum virgatum*) study (Guretzky et al. 2011) with fertilization at 135 kg-N ha^{-1} yr^{-1}found N uptake into the switchgrass biomass at 99 kg-N ha^{-1} yr^{-1} when harvested after the frost. The one-*Juncus* and all-plants treatments provide comparable uptake to this study, with 85 kg-N ha^{-1} yr^{-1}, despite the time of year being different than the switchgrass study (July to July in this study, vis-à-vis harvested once a year in December or January in Guretzky et al. 2011).

Media

The mass of N accumulated in the media on a per mesocosm basis is presented in Table 5.12. These mass values in g N mesocosm^{-1} year^{-1} are converted using Equation 5.3, with the given g N, to kg-N ha^{-1} yr^{-1} in Table 5.12.

Table 5.12 Mass of N accumulated in the media in a given treatment, during the course of the study

Treatment	Beginning-of-Study Mass of N in Media (kg-N ha^{-1} yr^{-1})	End-of-Study Mass of N in Media (kg-N ha^{-1} yr^{-1})	Mass Accumulated in the Media (kg-N ha^{-1} yr^{-1})
No-plants		1040	400
Iris	640	1090	450
Three-*Juncus*		1150	510
One-*Juncus*		960	320

The average accumulation of N in the media, given the calculations for these four treatments, is 420 kg-N ha^{-1} yr^{-1}.

<u>Comparison of N in Mesocosm Components</u>

The ratios of the N mass in the applied stormwater and effluent, and accumulated

in the plants and media, are the same as in the *N Mass in Mesocosms Components* section

above. Only the units have changed. The average mass loadings per area and time are

given in Table 5.13.

Table 5.13   Average mass loadings of N applied, accumulated in the plant(s), accumulated in the media, and exported in effluent during the course of the study, as kg-N ha^{-1} yr^{-1}. Data are taken from Tables 5.11 and 5.12 and from the Applied Stormwater and Effluent sections. The vegetation value is a sum of the average Iris and average *Juncus*, both from the all-plants treatment, plus a Joe Pye plant from the Joe-Pye treatment (only 38 weeks of growth for Joe Pye plants vis-à-vis 54 weeks for the other two plant species). To find mass loading with ha as loading area (column three), the mass loading with ha as bioretention area (column two) is divided by 20 (20 is taken from Equation 4.1).

Mesocosm Component	Mass Loading (kg-N ha^{-1} yr^{-1}), with area based on area of bioretention area	Mass Loading (kg-N ha^{-1} yr^{-1}), with area based on area of loading area
Influent	130	6.50
Effluent	73–290	3.65–14.5
Vegetation	+101	+5.05
Media	+420	+21.0

When comparing values from Table 5.13 to other studies, the area (in ha) being

measured must be considered. Therefore, mass loadings are presented as both per ha of

bioretention area and per ha of loading area. Many other studies present the N loadings as

per loading area. For example, Beckert et al. (2011) found that an agricultural watershed

in the St. Martin River basin in eastern Maryland had an N loading of 20.4 kg-N ha^{-1} yr^{-1},

with area measured as watershed area. This loading is 3.1 times larger than the influent in

this study. This difference is expected due to fertilizer and animal waste input from

agricultural areas. This study represents stormwater runoff from areas that are not heavily agricultural.

The calculation of N distribution within the system shows that the majority of the N accumulated during the study was found in the media rather than the plants; with four times the N accumulated in the media as in the vegetation. However, an average of 78% of the N applied in the stormwater was sequestered in the vegetation biomass, so vegetation uptake does play an important role in N cycling. For all examined plants, more N was found in the shoots than the roots, suggesting that shoot harvesting may be feasible to remove N from the system permanently. Dead shoot biomass N accumulation was negligible. *Juncus* was the most successful plant at accumulating N in its live biomass, accumulating 65% of applied N vis-à-vis 8% for a Joe Pye and 7% for an Iris. These differences are primarily due to more biomass growth in the *Juncus* than the other two species.

Phosphorus

TP was measured for selected sampled storms: 7/16/14, the second flush of 1/14/15, and 4/2/15 (Figure 5.13).

No statistically significant removal was present for any of the treatments in the 7/16/14 storm. The influent TP concentration was 0.10 mg P L^{-1}, and effluent concentrations ranged from 0.20 to 0.72 mg P L^{-1}, with a wide range of standard deviations (from 0.063 to 1.1 times the average for the same treatment).

In the 1/14/15 storm, at least by the second flush, TP removal vis-à-vis the influent was noted for all treatments, with an influent concentration of 0.16 mg P L^{-1} and

effluent concentrations ranging from 0.034 to 0.068 mg P L^{-1}. No significant differences among the treatments were found. Similarly, on 4/2/15 the influent concentration was 0.17 mg P L^{-1} and the effluent concentrations ranged from 0.057 to 0.074 mg P L^{-1}. Again no significant differences among the treatments were found.

The 1/14/15 and 4/2/15 storms, with significant removal in both the planted and unplanted treatments and no statistically significant differences among treatments, suggest that the media is primarily responsible for the P removal and that any P uptake by the plants is not occurring at a significant level. Hunt et al. (2012) describes the primary P removal mechanisms in bioretention as particulate filtration for particulate-bound P, and chemical sorption for dissolved P. Because no particulate P was introduced in the influent in this study, chemical sorption should be the primary removal mechanism. The results also show that P removal can take time to develop. This may be due to washout of P from media organic matter breakdown (see Clark and Pitt 2009) counteracting any P removal by the media, despite pre-study washing of the media with DI and synthetic stormwater.

7/16/14

1/14/15 Second Flush

4/2/15
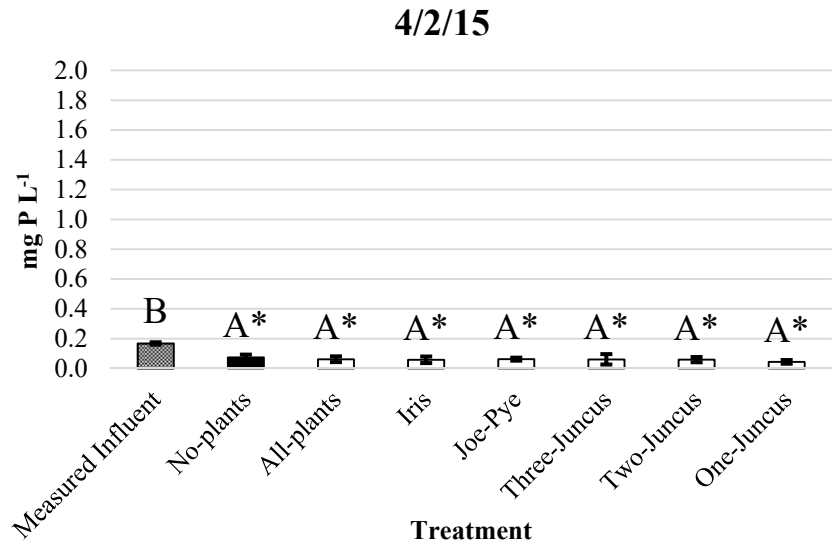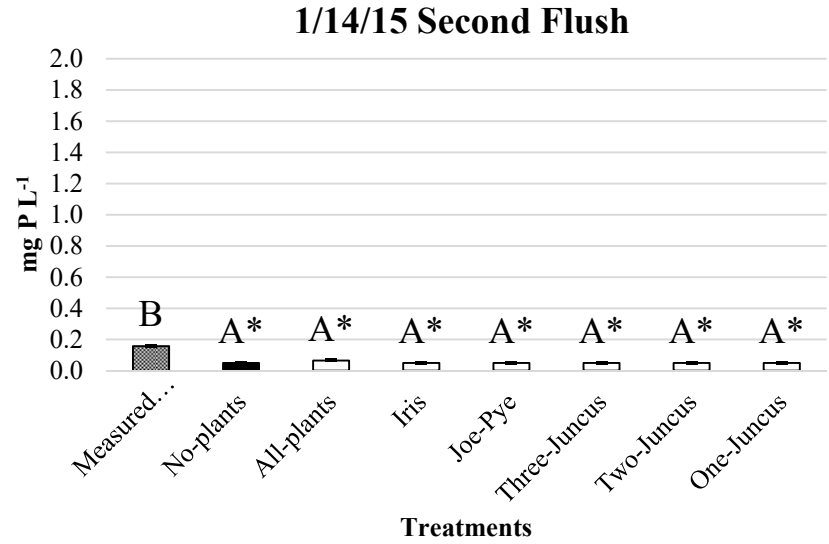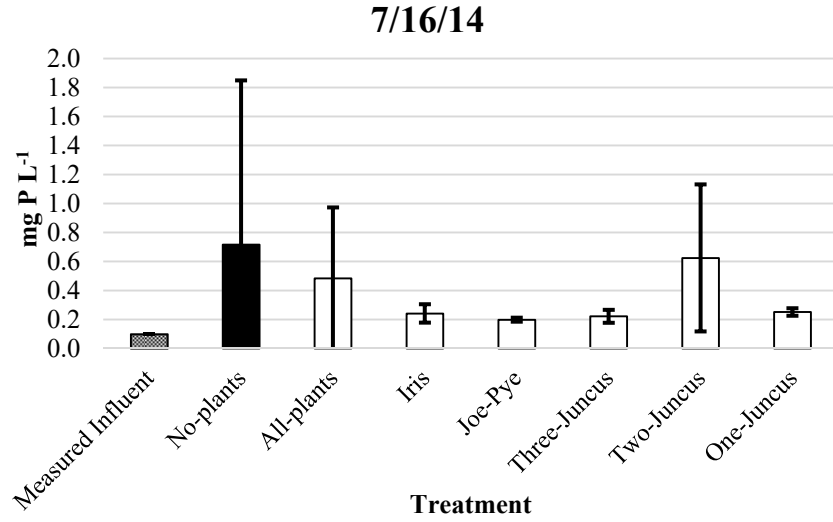
Figure 5.13 P concentrations from synthetic stormwater influent and the effluent from seven treatments of bioretention mesocosms with different vegetation. Each graph represents a simulated storm. The 7/16/14 storm is a composite sample of a simulated 0.27 cm storm, or 4.9 cm applied water. The 1/14/15 storm is a simulated 0.69 cm storm, or 12 cm of applied water. The second flush samples were collected as discrete samples after 6 cm of water had been applied. The 4/2/15 storm is a composite sample of a simulated 1.0 cm storm, or 19 cm of applied water. The * denotes a significant difference between that treatment and the measured influent. Differences among treatments and/or the influent are denoted with letters, if found. n=1–2 for influent, n=1–4 for effluent, usually n=4. Error bars are ± one standard deviation.

5.8 Aesthetics

During the spring and summer, Iris and *Juncus* provided pleasing greenery (Figure 5.14), as did the Joe Pyes, except for the chlorosis they developed during the first summer. In the fall the three species looked fairly green, but all displayed some brown leaves. As the weather became colder in the winter, the Iris leaves died back and became brown, giving them an untidy look. The Joe Pyes also died back, but in contrast to the Iris shriveled enough to be rather unnoticeable during the winter. The *Juncus* had the most pleasing winter aesthetic because it stays green throughout the winter, though it will retain brown shoots if they are developed over the summer (see Figure 5.14). Once the Iris plants started growing in the Spring they grew rapidly, as can be seen by the difference in less than three weeks in Figure 5.15.

All three of the species flowered at certain points throughout the experiment. Only one of the Iris plants flowered, in May 2015 (Figure 5.15). One of the Joe Pye plants, in the all species mesocosm in row two, flowered in late summer 2014 (Figure 5.16a). That individual plant subsequently died and was replaced with the other Joe Pyes in the replanting. Four out of nine Joe Pye plants that re-sprouted in Spring 2015 also put on buds in Summer 2015 (June and July), e.g., Figure 5.16b. None of the blooms were fully open by the time the study ended in mid-July. *Juncus* plants in five of the mesocosms bloomed in Spring 2015, with small blooms that started green in early May and dried to brown (Figure 5.17). The blooming plants were only in *Juncus*-only mesocosms: two one-*Juncus* mesocosms, one two-*Juncus* mesocosm, and two three-*Juncus* mesocosms. None of the *Juncus* in the all-plants mesocosms bloomed. The dried blooms were still on the plants at the end of the study in July 2015. Brown particles,

possibly seeds, were released from the blooms once they turned brown (Figure 5.17c).

	Joe Pye	*Juncus*	Iris
Fall: 11/18/14			
Winter: 1/20/15			

	Joe Pye	*Juncus*	Iris
Spring: 5/20/15			
Summer: 7/16/15			

Figure 5.14 Typical specimens from each species in each season

(a) May 1, 2015

(b) May 20, 2015

(c) May 27, 2015

(d) June 12, 2015

Figure 5.15   The Iris bloom in May and June 2015, in mesocosm Iris 2.

(a) September 12, 2014, all-plants 2 mesocosm
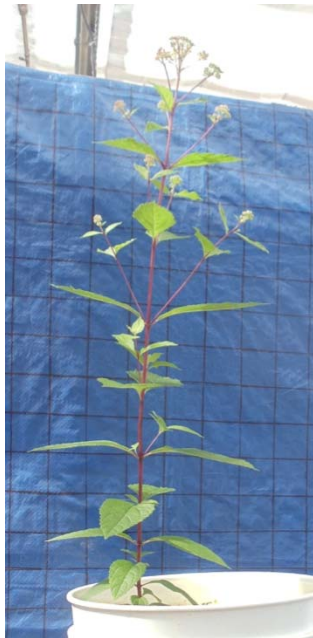
(b) July 16, 2015, Joe-Pye 2 mesocosm. The Joe Pye plant with the most buds in 2015.

Figure 5.16   Joe Pye blooms

(a) May 12, 2015: left: Three-*Juncus* 3 mesocosm, right: one-*Juncus* 1 mesocosm

(b) June 4, 2015: One-*Juncus* 1 mesocosm

(c) June 12, 2015: Small brown particles from blooms in one-*Juncus* 1 mesocosm

Figure 5.17 *Juncus* blooms

Chapter Six: Conclusions and Recommendations

This project consisted of field, greenhouse, and lab components. Multiple measures of vegetation success in bioretention were examined: survivorship, aesthetics, N uptake, and P uptake. The objectives of this project and their resulting conclusions based on the data are presented below.

Objective One

Identify vegetation species that generally grow well in bioretention facilities in Maryland and are aesthetically pleasing

Several plants were identified as meeting these requirements during the ride along with Mary Travaglini in Montgomery County, Maryland (Table 3.1). Additionally, in the Silver Spring survey (see objective 2 below), E. dubium *was found to be a good candidate for bioretention in Maryland in terms of survivorship, cover, and an extensive root system with some thick roots*. The other originally planted species that had survived for more than seven years in the cell were: *Solidago rugosa, Cephalanthus occidentalis, Lobelia siphilitica*, and *Cornus amomum. In the greenhouse study (Chapters four and five), in contrast to the Silver Spring study results*, E. dubium *struggled with survival and did not put on as much biomass over the study as the other two species*. Juncus effusus *was the most successful plant in the greenhouse study in terms of growth*, adding, on average, 56 g of dry biomass in 54 weeks vis-à-vis an average of 6.5 g dry biomass in 54 weeks for the Iris and an average of 2.5 g dry biomass in 38 weeks for the Joe Pye. Iris

versicolor *in the greenhouse study was generally aesthetically pleasing (except for in the winter), but did not generate the amount of biomass that Juncus did.*

Characterize the overall vegetation community, and measure the root characteristics and aboveground height of successful plant species in an established bioretention cell.

The full results of the study are discussed in Chapter three. Eleven months after last maintenance, volunteer plants constituted more than half of the cell vegetation. *Eutrochium dubium* (Joe Pye weed) had the most coverage of all plant species, 43%. *The average longest root length for the three examined species was 29.1 cm*, and was not statistically different among the species. *E. dubium* had the thickest roots, with its thickest root diameter averaging 2.2 centimeters, and an extensive root structure. *E. dubium* also had the tallest aboveground biomass, averaging 88.7 cm. Based on the findings of this study, E. dubium *is recommended for bioretention vegetation due to its survivorship and root structure. Additionally, the high percentage of volunteer species suggests the importance of vegetation maintenance planning.*

Quantify N and P uptake of successful bioretention plants identified in objectives 1 and 2, under bioretention conditions. Determine if single-species plantings have different uptake than mixed-species plantings. Additionally, quantify the amount of biomass change and overall aesthetics for these plants over the course of a year, when grown in bioretention conditions.

The accumulation of N by an average all-plants treatment, over the course of the experiment, was 0.63 g N in the vegetation and 2.5 g N in the media. 0.81 g N were applied in the influent over the course of the study, and measured effluent concentrations ranged from 0.46 to 1.8 g N.

In terms of mass loading per area of bioretention, 101 kg-N ha^{-1} yr^{-1} accumulated in the vegetation and 420 kg-N ha^{-1} yr^{-1} accumulated in the media. The influent loading was 130 kg-N ha^{-1} yr^{-1}, with 73–290 kg-N ha^{-1} yr^{-1} in the effluent.

Therefore, over the course of the study the vegetation accumulated about a quarter of the N that accumulated in the media. Thus, *changes in the media N capacity will have a larger impact on N removal rates from stormwater than changes in vegetation, but both plants and media should be optimized in order to maximize N removal.*

Objective Three A

Hypothesis one: N (NO3- and NH4+) and P uptake will change over the course of the year, with vegetation growth and bloom times. Higher uptake will be found during vegetation growth and bloom times.

The statistically significant differences in N species and TP uptake are presented in Tables 6.1 and 6.2.

Table 6.1 Storm dates and N or P species in which the concentration in one or more treatments was significantly *lower* than the influent concentration.

Storm Date	Significant N or P Species	Significant Treatment(s)	Simulated Storm Depth (cm)	Applied Stormwater Depth (cm)	Influent Concentration	Effluent Concentration(s)
1/14/15, second flush	**TP**	**All treatments**	0.69 (0.35 at second flush sampling)	12 (6 at second flush sampling)	0.16 mg P L^{-1}	0.034–0.068 mg P L^{-1}
4/2/15	**TP**	**All treatments**	1.0	19	0.17 mg P L^{-1}	0.043–0.074 mg P L^{-1}
6/1/15	**NO$_3^-$**	**One-*Juncus***	0.69	12	0.21 mg NO$_3^-$-N L^{-1}	0.066 mg NO$_3^-$-N L^{-1}

Table 6.2 Storm dates and N or P species in which the concentration in one or more treatments was significantly *higher* than the influent concentration.

Storm Date	Significant N or P Species	Significant Treatment(s)	Simulated Storm Depth (cm)	Applied Stormwater Depth (cm)	Influent Concentration	Effluent Concentration(s)
7/23/14, second flush	**PON**	**No-plants**	1.4 (0.35 cm at second flush sampling)	25 (6.2 cm at second flush sampling)	0	1.6 mg PON-N L^{-1}
	TN	**No-plants**	1.4 (0.35 cm at second flush sampling)	25 (6.2 cm at second flush sampling)	0.91 mg N L^{-1}	1.7 mg N L^{-1}
10/31/14	**TN**	**Joe-Pye**	0.18	3.2	0.70 mg N L^{-1}	0.83 mg N L^{-1}
6/1/15	**NO$_3^-$**	**No-plants**	0.69	12	0.21 mg NO$_3^-$-N L^{-1}	0.72 mg NO$_3^-$-N L^{-1}
	TDN	**No-plants**	0.69	12	0.67 mg dissolved N L^{-1}	1.2 mg dissolved N L^{-1}

The only instance of vegetation causing a significant nutrient uptake is on 6/1/15, when the one-*Juncus* treatment significantly reduced the influent NO_3^- concentration, from 0.21 mg NO_3^--N L^{-1} to 0.066 mg NO_3^--N L^{-1}. This instance of uptake occurred when the vegetation was well established, and in an active growing season. Several of the *Juncus* plants bloomed in May and June 2015. The 6/1/15 storm was in week 47 of the study, and between week 1 and 53, the average *Juncus* in one-*Juncus* and all-plants treatments increased its biomass by 56 grams. Therefore, *the one-*Juncus *NO_3^- uptake does appear to change at a statistically significant level seasonally, during the first year after planting*.

P removal was significant in all treatments, planted and unplanted, in the 4/2/15 and 6/1/15 storms. Because equal removal was seen in all treatments, *plant uptake of P does not appear to be significant for any of the three species examined in this study*.

Although 6/1/15 is the only instance of a plant causing significant uptake not found in other planted or unplanted mesocosms, Table 6.2 shows that *while plants may sometimes not have created significant uptake, they did at times prevent significant export*. The only treatments that displayed export (i.e., effluent concentration significantly higher than the influent concentration) were no-plants and Joe-Pye. The Joe-Pye export on 10/31/14 is attributed to washout of media particles caused by the replanting of the Joe-Pye plants that same week, prior to the 10/31/14 storm. The other instances are cases where the plants in the planted treatments prevented the export of N that occurred in the no-plants treatment. On 7/23/14, this was export in the form of PON: 1.6 mg PON-N L^{-1} in the no-plants treatment effluent vis-à-vis 0 mg PON N L^{-1} in the influent, export which was not seen at significant levels in the planted treatments. On

6/1/15, this export was in the form of NO_3^-: 0.72 mg NO_3^--N L^{-1} vis-à-vis 0.21 mg NO_3^--N L^{-1} in the influent. This export was not seen in significant levels in planted treatments.

Thus, while significant N species removal was only found once in planted treatments, the prevention of N export by planted treatments is also an important consideration and argument for using vegetation in bioretention to prevent further degradation of water quality downstream from a bioretention cell.

Interestingly, *the significant removal of TN in planted mesocosms seen in previous bioretention vegetation studies (Henderson et al. 2007, Lucas and Greenway 2008, Read et al. 2008, Zhang et al. 2011), was not seen in this study*. Full planting and maintenance recommendations are spelled out below.

Objective Three B

Hypothesis two: N and P uptake will be consistently highest in a mixed planting, because it will include multiple plant species with differing growth and bloom times, which will maximize uptake at all times of the year.

No evidence was found that the all-plants treatment created any N or P removal that was not found in other treatments. However, data on the all-plants treatment, as planted, are only available through October 31, 2014. However, TN was measured in the 6/1/15 storm for the all-plants mesocosms that no longer had a Joe Pye plant but still had a *Juncus* and Iris plant. Given the success of the one-*Juncus* treatment in June 2015 in removing NO_3^- and NH_4^+, it might be expected to see similar results in the all plant treatment, which includes only one *Juncus* plant. No significant difference with the influent was seen in those mesocosms, despite well-established *Juncus* and Iris plants.

However, the data in Table 5.3 shows that the effluent rate for the all-plants treatment is faster than both the two and three *Juncus* treatments. The two and three *Juncus* treatments did not demonstrate the same NO_3^- and NH_4^+ removal that the one *Juncus* treatment did, perhaps due in large part to the contact time of the stormwater with the media and plants. Therefore, *these study results suggest that a mixed planting with these three species does not offer any N or P removal benefits over a single-*Juncus *system with well-established vegetation.*

Objective Three C

Hypothesis three: More densely planted treatments will remove more NO_3^- and NH_4^+ from the influent than less densely planted treatments of the same plant species.

As discussed in Chapter five, surprisingly *the data support the reverse of this hypothesis, once the vegetation is well established in June 2015*. In the 6/1/15 storm, more NO_3^- was removed in the less-densely planted one-*Juncus* treatment rather than the two- and three-*Juncus* treatments, which did not offer removal benefits. However, the two- and three-*Juncus* treatments demonstrated better removal than the no-planted control, which exported NO_3^-.

A hypothesis to explain *Juncus effusus*' success in NO_3^- removal and its better biomass growth is that its large root network allows for more nutrient uptake than the other two plant species. The average increase in dry biomass of the measured *Juncus* roots was more than four times that of the measured Iris roots, and more than 12 times that of the measured Joe Pye roots. A more extensive root network allows more surface area for absorption of nutrients, which could have contributed to the one-*Juncus*

treatment being the only treatment with statistically significant NO_3^- removal from the influent stormwater. Since the two- and three-*Juncus* treatments did not produce statistically significant NO_3^- removal, plant density is also important, at least for *Juncus*. As noted in section 5.7, with Figure 5.6, a hypothesis to explain this phenomenon is that the denser roots of the two- and three-*Juncus* treatments create fast water movement through the mesocosm, leading to reduced time for nutrient uptake by the roots. However, the NO_3^- concentration for the two- and three-*Juncus* treatments was still less than half of the no-plants treatment given similar contact times, so even dense plantings offer better nutrient removal than unplanted setups. Density effects for other plant species need to be tested to determine if a similar trend occurs.

Objective Three D

Hypothesis four: Multiple species will have differential growth and aesthetics. This will lead to differences among the species in the quantity of N incorporated into the plant biomass over the course of the year. Different aesthetics between the plant species will lead to different recommendations for each species on use for aesthetic considerations in bioretention.

Based on the greenhouse study, Juncus effusus *is recommended for its year-round greenery and small, green flowers that dry to brown.* In the greenhouse study the "messy" look of *Juncus effusus* reported in Montgomery County was not observed, but the plants may not have been given enough time to reach this state. Some people may not be as attracted to *J. effusus'* pointy look as to other broader-leaved plants with larger, more colorful and showy blooms.

The Joe Pye provide a pleasant broad-leaf look and aesthetically-pleasing flowers. The Iris is a sturdier-looking plant that produces beautiful flowers that fade quickly. As noted previously, *the lack of aboveground greenery in the winter for both the Joe Pye and Iris may be seen as a drawback*. The timing of flowering should also be considered. A staggered flowering planting scheme, such as the one created by these three species, is recommended to maximize aesthetic interest throughout the year.

In terms of growth and biomass accumulated in the greenhouse study, the Juncus *was by far the most effective of the three species*, with almost 10 times as much biomass accumulated as Iris, and more than 20 times that of Joe Pye. This has repercussions in nutrient uptake as well, as clearly seen in the amount of N that was incorporated into biomass over the course of a year. *A* Juncus *plant accumulated in its biomass an average of 65% percent (in the all-plants and* Juncus *treatments) of the TN applied in the stormwater, vis-à-vis only 8% for an average Joe Pye plant and only 9% (in the Iris treatment) or 4% (in the all-plants treatment) for an average Iris plant* (Table 5.9). With 65% of applied N accumulated in a *Juncus* plant's biomass on average, it is unclear why significant TN removal was not seen in the effluent from *Juncus* treatments, especially the one-*Juncus* treatment that had the only significant N removal caused by plants, of NO_3^- on 6/1/15.

Such a differential in biomass between tested plant species and therefore a differential in N uptake clearly supports the use of *Juncus* for maximizing nutrient holding capacity, at least during the first year after planting (as tested in this study). Shoot harvesting could be an effective means of removing the majority of this N accumulated in the plant biomass. Table 5.11 shows that shoot biomass represents 64–

90% of the total plant biomass for all of the species and treatments measured. Further study on the amount of biomass grown back after cutting and the percent N in that biomass would be needed in order to determine if the rate of N sequestration into plant biomass would be affected by cutting. Contribution of N by plant dead biomass was negligible in this study, but data on dead biomass production in subsequent years and by other plant species are needed to ensure that the overall long-term effect of plants in bioretention is net N removal rather than net N export due to dead biomass.

6.1 Summary of Vegetation Recommendation for Bioretention in Maryland

Given its success in growing well in the field and in the greenhouse in bioretention media, one instance of statistically significant NO_3^- removal from synthetic stormwater in a greenhouse study, its ability to increase its biomass by 29 fold over the course of a year and therefore to incorporate much more N into its biomass than the other two species [0.51 g (one-*Juncus* treatment) or 0.53 g (all-plants treatment) N plant^{-1} for *Juncus* vis-à-vis 0.052 g N plant^{-1} for Joe Pye and 0.047 g (Iris treatment) or 0.044 (all-plants) g N plant^{-1} for Iris] that were tested in the greenhouse study, and its pleasant year-round green aesthetic, Juncus effusus *is recommended for bioretention use in Maryland*. Additionally, it is reported to be able to tolerate bioretention inlets well (Mary Travaglini, personal communication, fall 2013). One downside is that Montgomery County has received some complaints about its "messy appearance" in later warm months and subsequently has trimmed them back. *Juncus effusus* also had by far the most extensive root system of the three species in this study (averaging 20 g dry biomass at the end of the study, vis-à-vis 1.6 g for Joe Pye and 4.8 g for Iris). Deep, extensive root systems have been recommended (Hunt et al. 2012) to increase evapotranspiration, maximize

infiltration in bioretention cells, prevent clogging, and facilitate nutrient uptake. However, this study indicates that caution may be needed in densely planting such species, if NO_3^- removal is of concern, because in the 6/1/15 storm only the one-*Juncus* and not the two- and three-*Juncus* treatments displayed significant removal of NO_3^-. The two- and three-*Juncus* treatments still had NO_3^- concentrations that were less than half of the no-plants control, so planting at any of the three *Juncus* density plantings still can improve NO_3^- removal, though not always at a statically significant level. However, for optimal nutrient removal, *it is recommended that* Juncus effusus *in particular not be planted closer than one plant per 12" on center*, and that those in charge of bioretention maintenance *consider thinning bioretention plantings to prevent very dense plantings from developing*. Additional research is called for to confirm or reject this finding for other species besides *Juncus effusus*, and in additional climates.

E. dubium *(Joe Pye) is cautiously recommended for bioretention use*. Its failure in the first 16 weeks of the greenhouse experiment may be due to factors that would not affect growth in the field, such as a lack of micronutrients (subsequently corrected) or excessive drying due to mesocosm size. The lackluster resprouting of aboveground biomass in the Spring of 2015 in the greenhouse study (<50%) also gives cause for concern, but again the size of the mesocosm may create artificial conditions not present in the field, and it is possible that given additional time to become established, the plants that did survive would put on additional biomass and spread, as they appeared to have done in the Silver Spring bioretention cell. It must also be considered that the field study is a snapshot of the vegetative community at one time, so information on the dynamics of the community are not available for comparison with the greenhouse study. For biomass

production over the course of the first year of growth, both *Iris versicolor* and *Juncus effusus* are recommended over Joe Pye. Joe Pye also did not demonstrate significant nutrient removal in the greenhouse study, but as with the other species it did prevent nutrient export seen in the no-plants control.

Iris versicolor *is recommended for aesthetic use in bioretention*. It was the only one of the three plant species in the greenhouse study that had no mortalities throughout the entire course of the study. It produced sturdy green vegetation and one plant created visually pleasing flowers. As did Joe Pye and *Juncus*, Iris also prevented the N export seen in two storms in the no-plants control. Iris' drawbacks are its untidy look in the winter when the aboveground biomass dies back, its lackluster biomass growth vis-à-vis *Juncus* (6.56 g total mass added for Iris versus 56 g for *Juncus*), and its lack of demonstrated nutrient removal.

Finally, as seen in Figure 5.12, the media accumulated about four times the amount of N during the study than the vegetation did. Thus, media optimization will have a larger impact than vegetation optimization on N removal, but ideally both should be optimized for maximum N removal. Long-term (beyond one year) data on plant survivorship, production of dead plant biomass, and regrowth rates after aboveground harvesting are needed to better inform vegetation maintenance recommendations for optimal N removal.

Appendix A: Greenhouse Study Temperature Regime

Table A.1   Greenhouse study temperature regime. *Note*: The climate system of the greenhouse matched these temperatures as closely as possible throughout the course of the study. Negative temperatures were not obtainable in the greenhouse.

Week	*1 a.m. to 5 a.m. Temperature, deg C*	*5 a.m. to 1 p.m. Temperature, deg C*	*1 p.m. to 5 p.m. Temperature, deg C*	*5 p.m. to 1 a.m. Temperature, deg C*
Jan 1-7	-3.1	1.3	5.8	1.3
Jan 8-14	-3.5	1.1	5.6	1.1
Jan 15-21	-3.9	0.6	5.6	0.6
Jan 22-28	-3.9	1.1	5.6	1.1
Jan 29 - Feb 4	-3.7	1.3	6.3	1.3
Feb 5-11	-3.2	1.9	7.0	1.9
Feb 12-18	2.7	0.0	7.9	0.0
Feb 19-25	-1.7	3.7	8.9	3.7
Feb 26 - Mar 4	-0.6	4.8	10.0	4.8
Mar 5-11	0.6	6.0	11.3	6.0
Mar 12-18	1.8	7.2	12.6	7.2
Mar 19-25	2.9	8.4	13.9	8.4
Mar 26 - April 1	3.8	9.6	15.5	9.6
April 2-8	4.9	11.0	16.9	11.0
April 9-15	6.0	12.2	18.3	12.2
April 16-22	7.1	13.3	19.6	13.3
April 23-29	8.3	14.6	21.0	14.6
April 30 - May 6	9.8	16.0	22.1	16.0
May 7-13	11.1	17.2	23.2	17.2
May 14-20	12.5	18.3	24.3	18.3

Week	1 a.m. to 5 a.m. Temperature, deg C	5 a.m. to 1 p.m. Temperature, deg C	1 p.m. to 5 p.m. Temperature, deg C	5 p.m. to 1 a.m. Temperature, deg C
May 21-27	13.9	19.6	25.5	19.6
May 28 - June 3	15.0	20.9	26.7	20.9
June 4-10	16.3	22.0	27.7	22.0
June 11-17	17.4	23.1	28.7	23.1
June 18-24	18.3	24.0	29.7	24.0
June 25 - July 1	19.2	24.8	30.3	24.8
July 2-8	20.0	25.5	31.0	25.5
July 9-15	20.6	26.0	31.3	26.0
July 16-22	20.6	26.1	31.7	26.1
July 23-29	20.6	26.1	31.7	26.1
July 30 - Aug 5	20.4	25.9	31.3	25.9
Aug 6-12	20.0	25.6	31.0	25.6
Aug 13-19	19.4	25.0	30.5	25.0
Aug 20-26	18.8	24.3	29.8	24.3
Aug 27 - Sept 2	18.2	23.7	29.0	23.7
Sept 3-9	17.4	22.7	28.1	22.7
Sept 10-16	16.3	21.7	27.0	21.7
Sept 17-23	14.8	20.3	25.7	20.3
Sept 24-30	12.9	18.7	24.4	18.7
Oct 1-7	11.0	16.9	22.8	16.9
Oct 8-14	9.1	15.2	21.3	15.2
Oct 15-21	7.6	13.8	19.9	13.8
Oct 22-28	6.3	12.4	18.3	12.4
Oct 29 - Nov 4	5.3	11.2	17.1	11.2
Nov 5-11	4.4	10.0	15.6	10.0
Nov 12-18	3.4	8.9	14.3	8.9

Week	1 a.m. to 5 a.m. Temperature, deg C	5 a.m. to 1 p.m. Temperature, deg C	1 p.m. to 5 p.m. Temperature, deg C	5 p.m. to 1 a.m. Temperature, deg C
Nov 19-25	2.5	7.6	12.8	7.6
Nov 26 - Dec 2	1.3	6.3	11.3	6.3
Dec 3-9	0.2	5.0	10.0	5.0
Dec 10-16	-0.8	4.0	8.7	4.0
Dec 17-23	-1.7	3.0	7.5	3.0
Dec 24-31	-2.6	1.9	6.4	1.9

Appendix B: Greenhouse Study Plant Biomass Data

Table B.1 Dry plant biomass sample measurements: Beginning of study

Plant Species	Sample Number	Root Beginning of Study Mass (g)	Average Root Beginning of Study Mass (g)	Standard Deviation (g)	Shoot Beginning of Study Mass (g)	Average Shoot Beginning of Study Mass (g)	Standard Deviation (g)
Joe Pye	1	1.9	**1.5**	**0.79**	0.51	**0.46**	**0.13**
	2	0.85			0.38		
	3	2.6			0.50		
	4	0.32			0.23		
	5	1.5			0.58		
	6	1.6			0.54		
Juncus	1	0.43	**0.61**	**0.28**	1.06	**1.3**	**0.38**
	2	0.63			0.87		
	3	1.0			1.31		
	4	0.77			1.62		
	5	0.20			1.08		
	6	0.63			1.86		
Iris	1	0.87	**1.1**	**0.33**	0.99	**1.3**	**0.37**
	2	0.77			0.95		
	3	1.5			1.87		
	4	1.3			1.02		
	5	1.3			1.30		
	6	0.70			1.38		

Table B.2 Dry plant biomass sample measurements: End of study

Plant Species	Mesocosm Sampled From	Root End of Study Mass (g)	Average Root End of Study Mass (g)	Average Root End of Study Mass for Plant Species (g)	Standard Deviation of Root End-of-Study Mass (g)	Shoot End of Study Mass (g)	Average Shoot End of Study Mass (g)	Average Shoot End-of-Study Mass for Plant Species (g)	Standard Deviation of Shoot End-of-Study Mass (g)
Joe Pye (from Joe-Pye treatment)	JP1	2.0	**1.6**	**1.6**	**0.97**	2.4	**2.9**	**2.9**	**0.97**
	JP2	2.3				5.4			
	JP4	0.49				0.80			
Juncus (from one *Juncus* treatment)	OJ1	35	**23**	**20**	**8.4**	43	**39**	**38**	**8.4**
	OJ2	19				47			
	OJ3	16				27			
Juncus (from all plant treatment)	ALL1	20	**16**			56	**37**		
	ALL2	17				44			
	ALL4	10				12			
Iris (from Iris treatment)	I2	7.1	**6.1**	**4.8**	**2.3**	10	**5.9**	**4.1**	**2.3**
	I3	7.6				4.3			
	I4	3.7				3.3			
Iris (from all plant treatment)	ALL1	2.0	**3.4**			1.3	**2.4**		
	ALL2	5.5				3.4			
	ALL4	2.8				2.4			

Appendix C: Greenhouse Study Plant Percent N Data

Table C.1   Plant percent N results: beginning of study

Plant Species	Sample Number	Root Percent N	Average Root Percent N	Standard Deviation of Root Percent N	Shoot Percent N	Average Shoot Percent N	Standard Deviation of Shoot Percent N
Joe Pye	1	0.61	**0.68**	**0.075**	1.0	**1.1**	**0.13**
	2	0.76			1.2		
	3	0.67			0.96		
Juncus	1	0.94	**1.2**	**0.48**	1.1	**1.4**	**0.55**
	2	0.82			1.0		
	3	1.7			2.0		
Iris	1	0.84	**0.81**	**0.031**	0.81	**0.90**	**0.17**
	2	0.78			1.1		
	3	0.82			0.80		

Table C.2   Plant percent N results: end of study

Plant Species	Mesocosm Sampled From	Root Percent N	Average Root Percent N	Standard Deviation of Root Percent N	Shoot Percent N	Average Shoot Percent N	Standard Deviation of Shoot Percent N	Average Shoot Percent N, Plant Species	Standard Deviation Percent N, Plant Species
Joe Pye (from Joe-Pye treatment)	JP1	1.1	**1.1**	**0.25**	2.0	**2.1**	**0.17**	**2.1**	**0.17**
	JP2	0.84			2.1				
	JP4	1.3			2.3				
Juncus (from one-*Juncus* treatment)	OJ1	0.52	**0.52**	**0.014**	1.1	**1.1**	**0.19**	**1.1**	**0.30**
	OJ2	0.50			0.88				
	OJ3	0.53			1.2				
Juncus (from all-plants treatment)	ALL1	0.63	**0.64**	**0.016**	1.4	**1.1**	**0.44**		
	ALL2	0.62			1.3				
	ALL4	0.66			0.62				
Iris (from Iris treatment)	I2	0.53	**0.54**	**0.047**	0.87	**0.96**	**0.10**	**0.95**	**0.14**
	I3	0.50			0.95				
	I4	0.59			1.1				
Iris (from all-plants treatment)	ALL1	0.45	**0.46**	**0.015**	1.2	**0.94**	**0.19**		
	ALL2	0.48			0.81				
	ALL4	0.46			0.86				

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