

**Long-term changes in community composition and exotic species invasion in a restored wetland in North Carolina**

by

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May 2012

Master's project submitted in partial fulfillment of the requirements for the Master of Environmental Management degree in the Nicholas School of the Environment of Duke University  
2012

## Abstract

Wetland systems are highly productive and provide an estimated \$33 trillion per year in ecosystem services. However, wetland ecosystems are one of the most anthropogenically altered natural systems in the United States, with an estimated 342,700 acres of tidal and freshwater wetlands drained and developed in North Carolina alone. Wetland restoration is a growing industry in the United States, but parameters of restoration success are often poorly defined with few clear baselines available for comparison.

This study surveys a restored site in the Duke SWAMP to determine whether the site has successfully maintained robust species diversity seven years after restoration. Particularly, this study investigates whether increased species diversity has successfully resisted invasion by Japanese Stiltgrass (*Microstegium vimineum*). The current plant community was analyzed using three metrics. The first goal was to determine whether the diversity-invasibility hypothesis holds true at this site, meaning plots with higher species diversity would have less *Microstegium* by weight. The second goal was to find what the changes in plant community dynamics have been over time. This was analyzed by comparing the persistence and abundance of the 10 originally planted species with new species that have invaded subsequent to restoration. The third and final goal was to determine whether edge effects are affecting the community composition.

The results indicate that the diversity-invasibility hypothesis does not hold true for this site. There was no statistically significant difference in *Microstegium* biomass weights between the four diversity treatments of 0, 1, 4 or 8 planted species.

A total of 40 species including *Microstegium* were found in the plots, including 32 new species, seven of which were invasive species and four were obligate wetland species. A comparison of species importance by plot presence and total biomass indicated that *Microstegium* was by far the most abundant species at the site. However, several originally planted species have persisted and spread. Additionally, new native species including several tree species have established, and the site appears to be undergoing succession into a bottomland hardwood forest. Edge effects were evident, although not statistically significant, for new non-invasive species. It appears that edge effects are not strongly influencing the spread of *Microstegium*, although *Microstegium* is an extremely strong, competitive invasive regardless of planting treatments.

This study contains several suggestions for further study and *Microstegium* management strategies, including selection of competitive native species, prioritizing active restoration over natural species recruitment, and continued monitoring over time.

## Contents

Abstract .....	2
List of Figures .....	4
List of Tables .....	5
Introduction: wetland restoration .....	6
Objectives: .....	7
Background: .....	8
Methods.....	11
Survey.....	11
Statistical analysis .....	12
Results.....	12
Diversity-invasibility hypothesis .....	12
Current community composition.....	16
Edge effects.....	19
Discussion.....	20
Diversity-invasibility hypothesis .....	20
Current plant community composition .....	21
Edge effects.....	22
Study limitations .....	23
Recommendations .....	24
Appendix .....	27
Thanks .....	30
Bibliography .....	31

## List of Figures

Figure 1. Aerial overview of SWAMP .....	9
Figure 2. Planting plan for the diversity plots at SWAMP .....	10
Figure 3. Change in <i>Microstegium</i> biomass over time.....	13
Figure 4. Trends in <i>Microstegium</i> biomass and species richness .....	14
Figure 5. Trends in <i>Microstegium</i> biomass and species richness, with edge effects .....	15
Figure 6. Trends in <i>Microstegium</i> biomass and species richness, with tree presence .....	15
Figure 7. Location of outlying plots in SWAMP.....	16
Figure 8. Results of the 2011 plant biomass survey in SWAMP plots.....	17
Figure A. Trends in <i>Microstegium</i> biomass and species richness, showing diversity treatment .....	29

## List of Tables

Table 1. Species used in site restoration plantings.....	10
Table 2. Occurrences of planted species in SWAMP plots .....	17
Table 3. Most abundant species in 2011 plant survey in SWAMP.....	18
Table 4. Invasive species found in 2011 survey .....	19
Table 5. Statistical comparison of edge effects on <i>Microstegium</i> biomass by year .....	19
Table A. Wetland Indicator status for plants .....	27
Table B. 2011 survey results .....	28
Table C. Statistical comparison of <i>Microstegium</i> biomass by plot treatment.....	29
Table D. Statistical comparison of edge effects on all new plant species .....	29

## Introduction: wetland restoration

Wetland systems are highly productive, contributing to flood control, improvement of water quality, wildlife habitat and many more ecological services (Richardson et al., 2011). Some scientists have suggested that wetlands provide more services to people than any other natural system (Mitsch et al., 2012) with an estimated \$33 trillion per year in ecosystem services provided by wetlands (Costanza et al., 1998). And yet, wetland ecosystems are one of the most anthropogenically altered natural systems in the United States, with approximately half of the 220 million acres of wetlands thought to have existed lost to agricultural and urban development (Aveny, 2012). In North Carolina alone, an estimated 342,700 acres of tidal and freshwater wetlands were drained and developed (NC DENR).

It was not until we began to understand the essential services wetlands provide that the United States made the preservation and restoration of wetlands a priority, most notably with the No Net Loss policy mandating no net loss of wetland area within the United States (Heimlich, 1998). North Carolina has made the protection, restoration, or enhancement of coastal wetlands a priority pursuant to section 309 of the Coastal Zone Management Act, and since 2006 has made a net gain of 17 acres of tidal vegetated wetlands and 30,275 acres of non-tidal/freshwater wetlands (NC DENR, 2011).

Where natural wetlands cannot be preserved, they must be created or restored. Wetland restoration, or the recovery of an ecosystem to pre-disturbance functions (NRC, 1992) is a growing industry in the United States, and is undertaken by state governments and private industry. Because of the high stakes, both economically and ecologically, there is significant public interest in positive restoration outcomes. A recent Triangle News and Observer cover story took the North Carolina Ecosystem Enhancement Program to task for spending \$140 million dollars on projects that were failing or did not solve the issues they were supposed to address (Kane, 2011). Accordingly, a key question in wetland restoration ecology today is: how can we measure success? One commonly used parameter is the establishment of native plant communities (Kentula, 2000; Zedler, 1999), with species richness used as a benchmark of ecosystem function and health (DeMeester & Richter, 2010; Mitsch & Wilson, 1996; Osland et al, 2009).

However, one major complication in determining whether a system has regained the intended functionality is the lack of clear goals and baselines. A post-restoration evaluation of restored wetlands is limited by the lack of long-term, detailed data sets, insufficient monitoring periods, limited number of services measured, and few peer-reviewed publications of results (Craft, 2003; Zedler, 2000). A recent study (Bernhardt et al., 2005) found that 20 percent of river restoration projects did not list a goal, and

that only 10 percent of projects indicated that some assessment or monitoring occurred, with most projects failing to provide any analysis of restoration consequences.

This lack of clear baseline conditions is further complicated by undesirable post-restoration events, such as invasion by exotic species (Brooks, 2004; Seabloom & van der Valk, 2003). Invasive species are viewed as a significant component of global change, with the cost of invasive species estimated to range from millions to billions annually (Sakai, 2001). Because chemical and mechanical methods of managing invasive species require ongoing costly inputs of time and money, managers are interested in finding self-sustaining methods of treating and preventing invasions in restored wetlands. This study looks at one approach: reducing the invasibility of a restored wetland by increasing the diversity of native plantings.

The concept of using plant community dynamics to resist invasion began with C.S. Elton's diversity-invasibility hypothesis (Elton, 1958). The hypothesis is fairly straightforward: if an ecosystem has a diverse native community, there are fewer resources and fewer niches available, and fewer opportunities for exotic species, like *Microstegium vimineum* (Japanese Stiltgrass), to invade. However, before we can understand whether a management technique works, we must first know what was there to begin with.

In the Duke Stream and Wetland Assessment and Management Park (SWAMP) in Durham, North Carolina, the plant community has been carefully established and monitored since restoration in 2005. Because several years of survey data are available depicting clear baseline conditions, the Duke SWAMP offers a unique opportunity to track changes in a restored wetland plant community over time and see whether the diversity-invisibility hypothesis is supported.

## **Objectives:**

This study looks at a restored site in the Duke SWAMP to determine whether the site has successfully maintained robust species diversity seven years after restoration. Particularly, this study investigates whether increased species diversity has successfully resisted invasion by Japanese Stiltgrass. The current plant community was analyzed using three metrics. The first goal was to determine whether the diversity-invasibility hypothesis holds true at this site. If so, it would be expected that plots with higher species diversity (four or eight planted species) would have less *Microstegium* by weight than lower diversity plots (zero or one species). This was analyzed by comparing the persistence and abundance of the 10 originally planted species with new species that have invaded subsequent to

restoration. The second goal was to find what the changes in plant community dynamics have been over time. This was analyzed by comparing the persistence and abundance of the 10 originally planted species with new species that have invaded subsequent to restoration. The third and final goal was to determine whether edge effects (i.e. plots established near the edge of the site) are affecting the community composition and invasions.

## **Background:**

Japanese Stiltgrass (*Microstegium vimineum*) is an invasive annual C4 grass. *Microstegium* was accidentally introduced from Asia in the early 1900s and was first identified in Knoxville, TN in 1919 (Barden, 1987). Since that time, it has spread through all states east of the Mississippi, and establishes in disturbed areas (ibid).

*Microstegium* is especially productive in moist environments, can tolerate a wide range of light availability, and produces self- and cross-pollinating seeds (Heubner, 2011). An individual plant can generate up to 1,000 seeds (Barden, 1987). After it establishes, *Microstegium* displaces native vegetation by establishing dense monoculture stands (Tu, 2000). *Microstegium* may alter natural soil conditions, as one study found that invaded sites had a higher soil pH and thinner litter and organic soil horizons (Kourtev et al., 1998) and may affect soil nitrification and mineralization as well as soil arthropod diversity (Huebner 2011). These impacts threaten to inhibit tree establishment and alter successional trajectories (DeMeester & Richter, 2010; Flory, 2010). *Microstegium* is listed as a noxious weed in three states and is on invasive plant lists throughout the eastern US (USDA NRCS, 2012). Due to all of these negative impacts, managers and restoration ecologists are interested in controlling this highly invasive and harmful weed. *Microstegium* is well established across mesic environments in the North Carolina Piedmont, and is commonly found throughout Duke Forest and this study's project site, the Duke SWAMP.

SWAMP is located within the Upper Sandy Creek floodplain, a headwater stream located within the Cape Fear watershed. The site contains a two ha (five acre) restored wetland area, as well as 609 m (2,000 feet) of restored stream (Richardson & Pahl, 2005). This portion of Upper Sandy Creek drains 480 hectares, including portions of Duke University and the adjacent residential area (Osland et al., 2009). The site includes an integrated five-phase restoration project (Figure 1). Currently, phases 1-4 have been completed and Phase 5 is being designed. The study site was historically a hardwood bottomland forest, and prior to restoration the native species community was composed predominantly of Red

Maple (*Acer rubrum*), Tulip Poplar (*Liriodendron tulipifera*), American elm (*Ulmus Americana*) and Sweetgum (*Liquidambar styraciflua*) although it was also severely invaded by the exotic Chinese Privet (*Ligustrum sinense*) and *Microstegium* (Watts, 2000).

**Figure 1. Aerial overview of SWAMP**

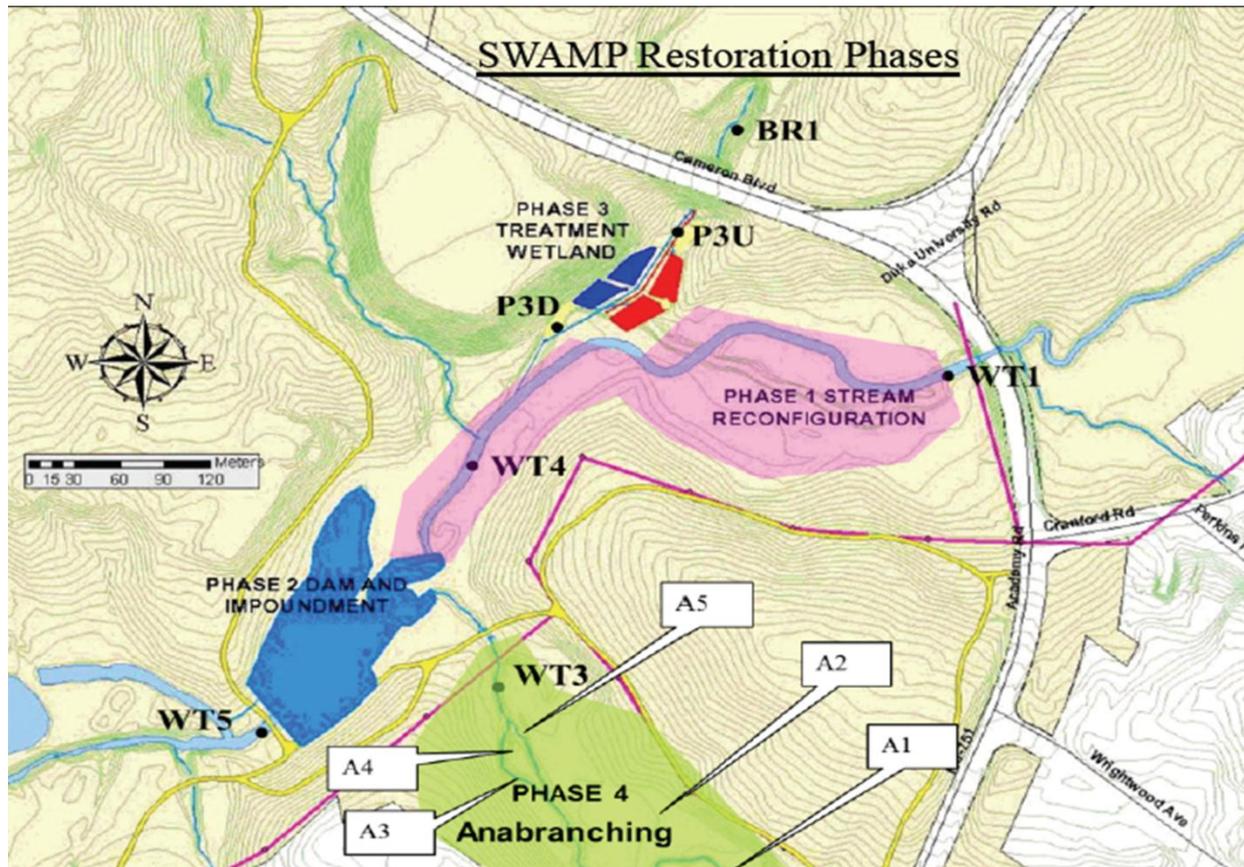


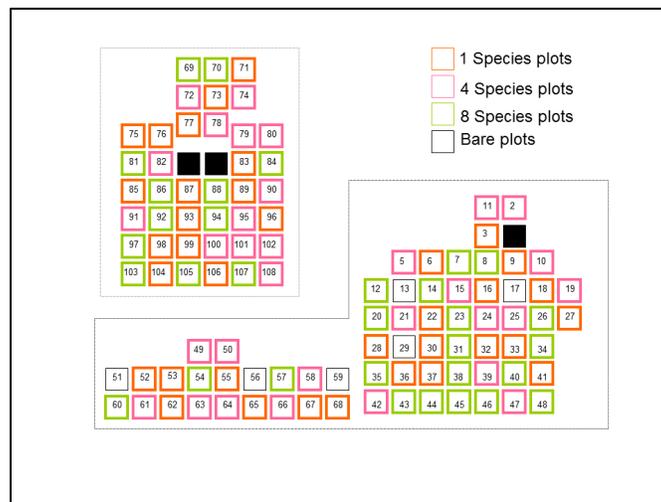
Image courtesy of Duke University Wetlands Center, 2012.

The study site consists of three different areas (Figure 2) consisting of replicated 2 x 2 meter plots adjacent to the SWAMP restored stream in a ‘high bench’ area, raised 30 centimeters above the adjoining stream and graded to a constant elevation (Sutton-Grier, 2008). In May 2005, the plots were planted with 100 seedlings of 1, 4 or 8 species from a pool of 10 species, chosen from a list of recommended plants for NC stream restoration. Six plots were left unplanted as control plots (ibid). Within each plot, species were planted in equal densities and were equally spaced, but were randomly sited within each plot. Surveys were conducted in 2005 and 2006 by Dr. Ariana Sutton-Grier, and in 2007 by Dr. Justin Wright.

The initial species planted plant selections were based on commercial availability and diversity of functional traits (Sutton-Grier, 2008). This created a plant community with a variety of wetland

indicators (WI). A plant’s WI status indicates the probable range (calculated as frequency of occurrence) of a species occurring in a wetland as opposed to a non-wetland. Accordingly, a plant whose WI is ‘obligate’ (OBL) almost always occurs in wetlands under natural conditions, approximately 99 percent of the time (NRCS, 2012). A positive or negative sign means that it tends toward the higher or lower end of the probability range. So, a plant with a WI of ‘FACW+’ means that it is found primarily in wetlands 67 to 99 percent of the time, but with a frequency closer to 99 percent than 67 percent. The species selected in this study include a number of species such as New York Ironweed (*Vernonia noveboracensis*) that are frequently found in both wetland and non-wetland habitats, indicating that they are tolerant of drier habitats (See Appendix: Table A.)

**Figure 2. Planting plan for the diversity plots at SWAMP**



Courtesy of Sutton-Grier, 2008.

**Table 1. Species used in site restoration plantings**

<i>Species</i>	<i>Wetland indicator status</i>
<i>Asclepias incarnata</i>	OBL
<i>Carex crinita</i>	FACW+
<i>Carex lurida</i>	OBL
<i>Eupatorium fistulosum</i>	FAC+
<i>Chasmanthium latifolium</i>	FAC-
<i>Juncus effusus</i>	FACW+
<i>Lobelia cardinalis</i>	FACW+
<i>Microstegium vimineum</i>	FAC+
<i>Panicum virgatum</i>	FAC+
<i>Scirpus cyperinus</i>	OBL
<i>Vernonia noveboracensis</i>	FAC+

Adapted from Sutton-Grier, 2008.

## Methods

### Survey

A plant survey of 34 two-meter by two-meter plots was conducted to establish the existing plant composition and species diversity in September 2011. The plots were randomly selected using R (R Core Development Team, 2011). Three substitutions were made in the field; in all three cases, the selected plots were either dominated by tree species too large to be cut down with the available tools, or were entirely dominated by Swamp Blackberry (*Rubus argutus*). In accordance with prior surveys conducted earlier by Sutton-Grier (unpublished data, 2006) and Wright (unpublished data, 2007), two 0.25 square meter areas were selected at random within each plot and harvested for a total of 0.5 square meter sample per plot. All biomass rooted within the plot was harvested to within 6 cm above ground. Vegetation not rooted within the plot was excluded to the best extent possible. All samples were sorted in the Wetland Lab, dried for a minimum of two days, at 60° C in a forced air drier and the dry biomass weight was recorded.

All weights were scaled up to grams per one square meter by multiplying the weights by two in accordance with standard sampling techniques and to make it easier to interpret results. Samples were considered independent because one total weight was measured per plot with all plants not rooted within the plots excluded; it was not possible for one plant or weight per plot to be measured twice.

This study analyzes species diversity using two metrics: first, the amount of a particular species found in each surveyed plot by dry biomass weight, and the number and location of each plot a species was found in. Other common methods of measuring plant populations such as density, or the number of individual stems found in a survey area, were not utilized in prior surveys. Additionally, measurements of density or coverage are difficult to quantify when the plant community contains a large number of rhizomatous grasses or other plants which spread via runner, such as this project area (Elzinga et al., 1998).

Unfortunately, previous studies did not identify all plant species found in the survey area. All species originally planted in the survey plot and all *Microstegium* were identified, dried and weighed. Any species not originally planted within the survey plot were dried and weighed, and grouped together as 'other'. While researchers sometimes noted other species found in the plots, this information was not consistently recorded. Accordingly, analysis of *Microstegium* spread across survey years breaks data down into 'planted,' meaning planted in a specific plot and found there in subsequent years, 'Microstegium,' and 'other.' Thus, this study first analyzes the change in plant community

composition over time generally, but without reference to changes in specific species or groups i.e. native or invasive species). The study then looks at the results of the 2011 survey specifically, identifying changes in specific plant presence by plots and the number and type of species found.

## Statistical analysis

Statistical analyses were conducted in R using the RCMDR GUI interface (R Core Development Team 2011) and Microsoft Excel. The data was not normally distributed for all years or for treatment within years, and it did not have constant variance within treatments or years.

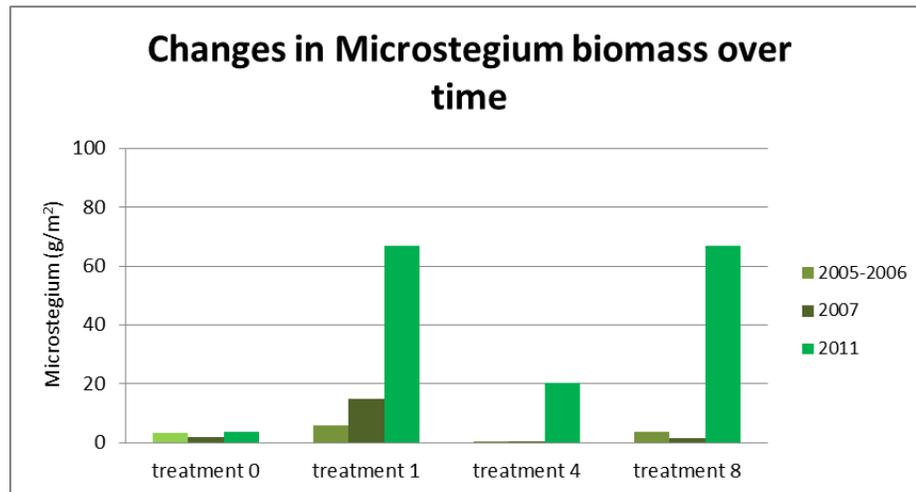
As the data was not normally distributed and did not have constant variance, it did not meet the assumptions for a t-test or ANOVA (MacDonald, 2009). Log transformations of the data rendered the results less skewed, but still not normally distributed. However, since the mean and variance are known, it is possible to use the non-parametric analogues of the t-test and ANOVA, the Mann-Whitney-Wilcoxon rank-sum test (analogue to the t-test) and the Kruskal-Wallis rank-sum test (analogue to ANOVA). Nonparametric analyses are performed by replacing observations with their ranks on an ordinal scale (Dowdy, 2004). While non-parametric tests are considered less robust than the t-test and ANOVA, they are not subject to error due to violations of the assumptions of normality and variance required for t-tests and ANOVA.

## Results

### Diversity-invasibility hypothesis

The diversity-invasibility hypothesis did not hold true for the diversity treatments. There was no significant difference in the amount of *Microstegium* between treatments for any year (2005-2006:  $p=0.3498$ ; 2007:  $p=0.06693$ , 2011:  $p=0.6182$ ). A comparison of *Microstegium* presence over time indicates that the plant has spread quickly since restoration in 2005 (Figure. 3).

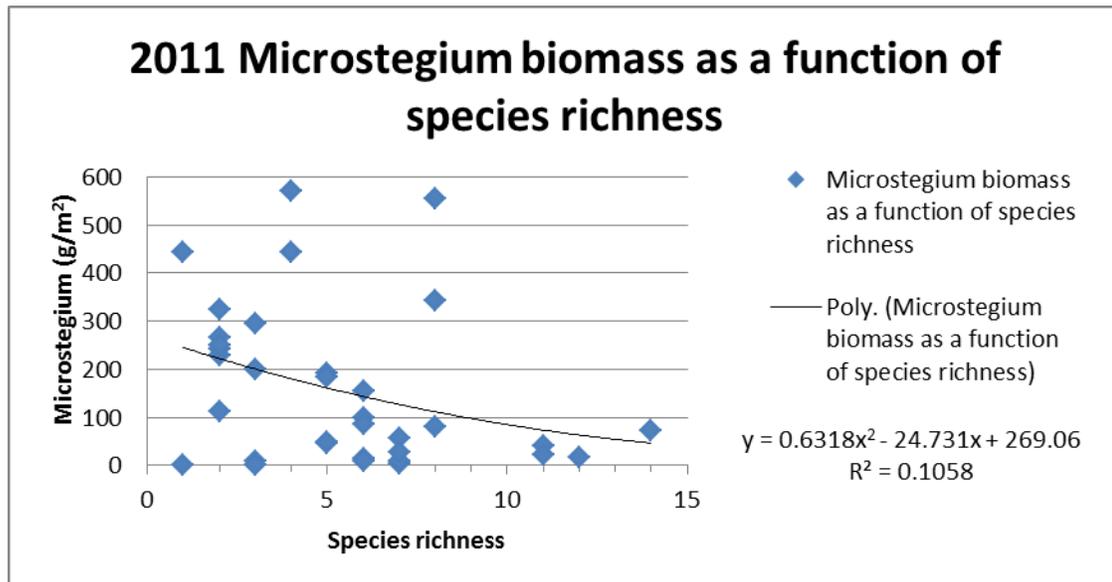
Figure 3. Change in *Microstegium* biomass over time



*Microstegium* presence was low in the first two years post-restoration (2005-2006), and made small gains in 2007 across all treatments. By 2011, the total *Microstegium* biomass had increased by several orders of magnitude. A comparison of the four treatments over time shows that initially the unplanted plots had the largest amount of *Microstegium*, but by 2011 the other three treatments had higher amounts of *Microstegium* by weight.

Total *Microstegium* biomass in each treatment was compared for each year, and with all years combined to see if there was an overarching trend. No significant differences were found (See Appendix, Table C). When the 2005-2006 and 2007 data are compared with a Mann-Whitney nonparametric test, the difference between the two years is close but not statistically significant ( $p=0.053$ ), indicating there was an important surge in *Microstegium* growth during this time.

Unlike most experiments which test the diversity-invasibility hypothesis, this site was not weeded and had no applications of pesticide or mowing since restoration. As a result, several new species have established in the plots. When the total species richness is compared to *Microstegium* biomass, an interesting trend is revealed. As shown in Figure 4, once the species richness exceeds 10 species *Microstegium* biomass variance decreases steeply. All four plots with species richness greater than 10 species have *Microstegium* biomasses of less than 100 grams per square meter. Accordingly, it is possible that the original planted diversity did not have a significant effect on *Microstegium*, but total species diversity may.

Figure 4. Trends in *Microstegium* biomass and species richness

A polynomial regression line fitted to the data shows a very low correlation coefficient, with only ten percent of the variation in *Microstegium* biomass explained by the total species richness. Accordingly, this data was then analyzed for other factors that could be influencing the outliers which exhibited very high species diversity and low *Microstegium* biomass. Three factors were analyzed: original diversity treatment, edge effects, and tree presence. Original diversity treatment was selected to determine whether the original plantings influenced the results. Edge effects and tree presence were chosen because these are both known to influence species composition (Watkins et al., 2003; Schulte et al., 2011).

While no trends were evident for original diversity planting treatments (See Appendix, Figure A), all outlying plots showing reduced *Microstegium* and high species diversity were edge plots (Figure 5) and had trees present (Figure 6).

Figure 5. Trends in *Microstegium* biomass and species richness, with edge effects

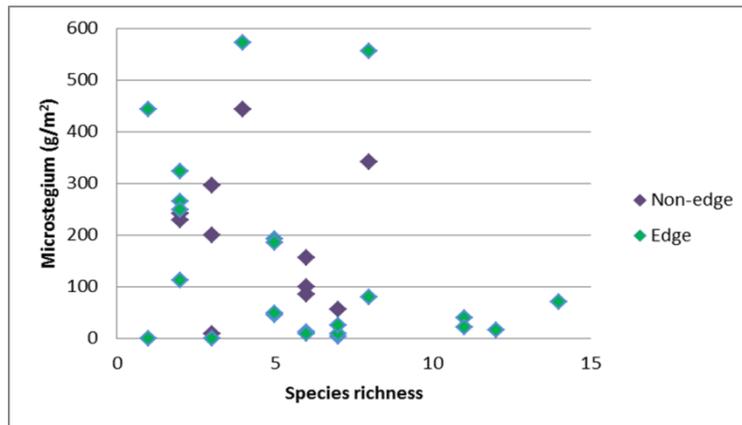
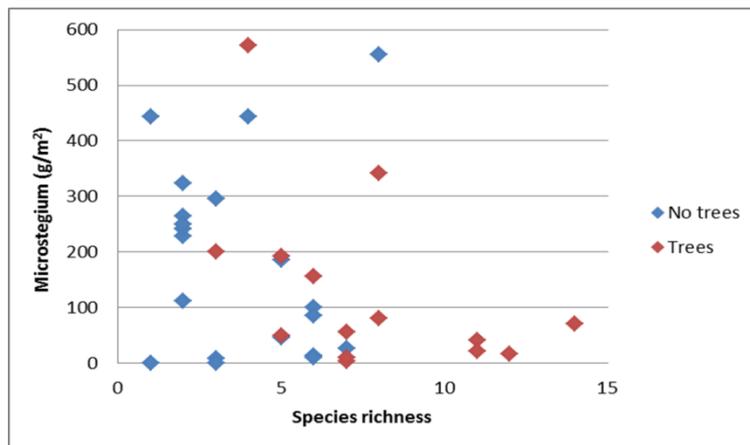
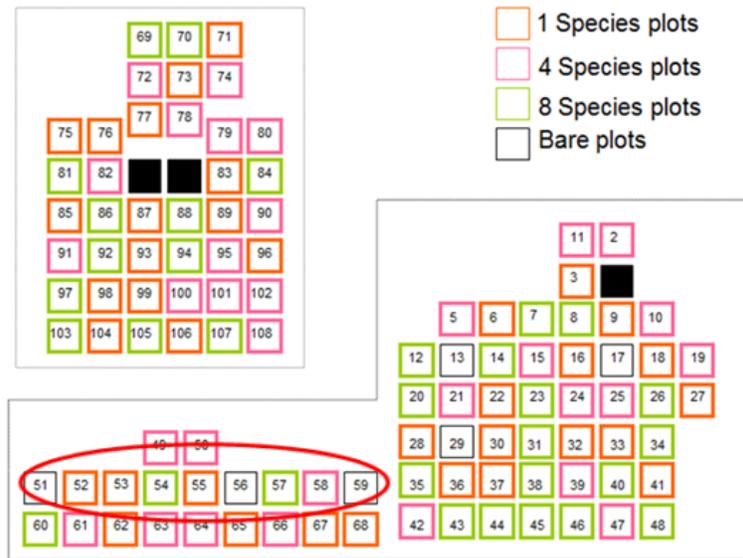


Figure 6. Trends in *Microstegium* biomass and species richness, with tree presence



All of the outlying plots with high species richness and low *Microstegium* biomass were from one portion of the restored site (Figure 7). So, these results could be influenced by the location as well as any one of a number of factors or combination of factors.

**Figure 7. Location of outlying plots in SWAMP**



Courtesy of Sutton-Grier, 2008.

Clearly, species which have invaded the site are playing an important role in plant community dynamics. This leads to this study’s second objective: what changes have occurred in plant community composition over time. In order to analyze this objective, the planted species (2005) composition is compared to the species composition as of 2011.

### **Current community composition**

Figure 8 shows the results of the 2011 survey, as broken down by originally planted species, *Microstegium*, and all other new species. This figure indicates that the unplanted (Treatment 0) plots have very little *Microstegium* and very few planted species, but the majority of the biomass are new species. The other three treatments show that the planted species have persisted well in terms of total biomass, but *Microstegium* now plays an important role, although reduced in the 4 and 8 treatment plots compared to the 1 treatment plot.

Figure 8. Results of the 2011 plant biomass survey in SWAMP plots

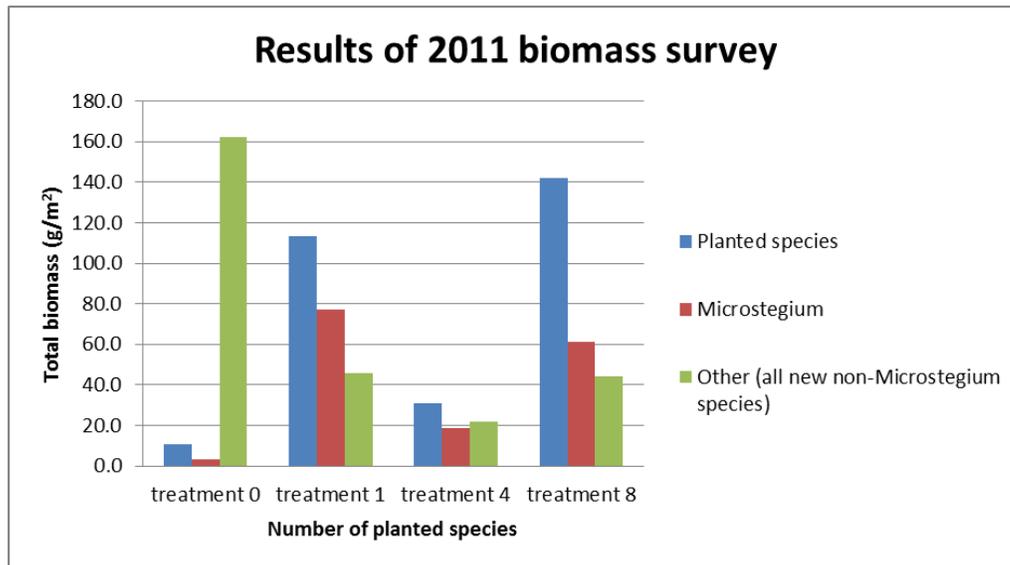


Table 2 shows the original species composition of the plots and their current plot presence. Ideally, planted species would persist in the plots they were planted in and spread to other plots. As shown in Table 2, three species, Cardinal Flower (*Lobelia cardinalis*), Shallow Sedge (*Carex lurida*), and Swamp Milkweed (*Asclepias incarnata*) were not found in the 2011 survey. While there is evidence that *Lobelia cardinalis* was eradicated by herbivory (Sutton-Grier, 2008), it is unknown whether *Carex lurida* and *Asclepias* were eradicated from the plots or simply were not captured by the survey. New York Ironweed (*Vernonia noveboracensis*) and Joe Pye Weed (*Eupatoriadelphus fistulosum*) both spread vigorously. Most other species, including Switchgrass (*Panicum virgatum*), River Oats (*Chasmanthium latifolium*), and Common Rush (*Juncus effusus*) decreased in plot presence but maintained a relatively robust presence.

Table 2. Occurrences of planted species in SWAMP plots

<i>Planted species</i>	<i>Number of plots planted with each species</i>	<i>Number of plots found with each species</i>
<i>Panicum virgatum</i>	17	8
<i>Lobelia cardinalis</i>	15	0
<i>Juncus effusus</i>	14	8
<i>Scirpus cyperinus</i>	14	1
<i>Chasmanthium latifolium</i>	13	11
<i>Vernonia noveboracensis</i>	13	18
<i>Carex crinita</i>	11	3
<i>Eupatoriadelphus fistulosum</i>	11	15
<i>Carex lurida</i>	10	0
<i>Asclepias incarnata</i>	2	0

A total of 40 species were found in the plots, with 32 new species (including *Microstegium*) (See Appendix, Table B). Of the 32 new species, 7 were invasive species and 4 were obligate wetland species.

A table of the most abundant species found in the 2011 survey, by weight and by plot presence, shows striking differences (Table 3). Of the ten most abundant species by plot presence, five are originally planted species. However, *Microstegium* occupies almost twice as many plots as the second-most abundant species (*Vernonia*). Additionally, the 10 most abundant plant species by plot presence are all grasses, vines, and forbs, with the sole exception of Red Maple (*Acer rubrum*).

When comparing presence by weight, four of the 10 most abundant species are originally planted and *Microstegium* is almost twice the biomass of the second-most abundant species. Late Goldenrod (*Solidago altissima*) was also found in several plots, although it was not a significant presence in terms of biomass (See Appendix, Table B). Additionally, it is clear that the site is experiencing a successional change. Several large, well-established trees were encountered in the plots, namely River Birch, American Elm, Loblolly Pine, and American Persimmon (*Diospyros virginiana*). Seedlings of Red Maple (*Acer rubrum*) and Tulip Poplar (*Liriodendron tulipifera*) have spread quickly. Additionally, Boxelder (*Acer negundo*), River Birch (*Betula nigra*) and American Elm are all obligate or facultative-wetland indicator species, meaning they always or almost always are found in wetlands. Their presence indicates that the site is truly functioning as a wetland.

**Table 3. Most abundant species in 2011 plant survey in SWAMP**

<b>10 most abundant species by plot presence</b>		<b>10 most abundant species by biomass *</b>		
<b>Species name</b>	<b>Number of plots present</b>	<b>Species name</b>	<b>Biomass (g)</b>	<b>Percent of total biomass</b>
<i>Microstegium vimineum</i>	32	<i>Microstegium vimineum</i>	5446.7	21.5
<i>Vernonia novaboracensis</i>	18	<i>Vernonia novaboracensis</i>	3926.2	15.5
<i>Eupatoriadelphus fistulosum</i>	15	<i>Betula nigra</i>	3701.2	14.6
<i>Chasmanthium latifolium</i>	11	<i>Eupatoriadelphus fistulosum</i>	3232.5	12.8
<i>Acer rubrum</i>	9	<i>Pinus taeda</i>	1944.2	7.7
<i>Juncus effusus</i>	8	<i>Panicum virgatum</i>	1467.3	5.8
<i>Panicum virgatum</i>	8	<i>Chasmanthium latifolium</i>	1417.8	5.6
<i>Lonicera japonica</i>	7	<i>Ulmus americanus</i>	1040.2	4.1
<i>Rubus argutus</i>	7	<i>Rubus argutus</i>	720.9	2.9
<i>Carex vulpinoidea</i>	6	<i>Diospyros virginiana</i>	470.0	1.9

\*Biomass is calculated as a total of all surveyed plots

Aside from the widespread dominance of *Microstegium* in the plots, the amounts and variety of invasive species were surprisingly low in 2011 (Table 4). Invasive species (excluding *Microstegium*) were found in 9 of 11 edge plots, and were not found outside of edge plots. Aside from *Microstegium*, invasive presence surprisingly low. There was no statistically significant difference in the biomass or frequency of presence of any non-*Microstegium* invasive by plot treatment ( $p=0.5$ ).

**Table 4. Invasive species found in 2011 survey**

<i>Species</i>	<i>Number of plots found</i>	<i>Total Biomass (g)</i>	<i>Percent total biomass</i>
<i>Microstegium vimineum</i>	32	5446.7	21.5
<i>Lonicera japonica</i>	7	82.3	0.3
<i>Ligustum sinense</i>	3	31.5	0.1
<i>Persicaria longiseta</i>	1	4.4	Less than 0.01
<i>Dioscorea oppositifolia</i>	1	4.1	Less than 0.01
<i>Kummeria striata</i>	1	2.1	Less than 0.01
<i>Gratiola virginiana</i>	1	0.5	Less than 0.01
<i>Albizia julibrissin</i>	1	0.3	Less than 0.01

## Edge effects

The results were analyzed to determine whether edge plots were significantly different than non-edge plots in terms of species richness and invasive species presence. Invasive species were identified using the USDA Natural Resource Conservation Service Plants Database (USDA NRCS, 2012) and were confirmed as invasive in the North Carolina Piedmont by Dr. Mengchi Ho, resident botanist and wetland plant specialist at the Duke Wetlands Center. New species were found in 26 of the 34 (76%) surveyed plots. 17 of the 26 plots containing new species (65%) are edge plots. Of the 32 plots where *Microstegium* was found, 21 (66%) were edge plots. The most important conclusion from this analysis was that excluding *Microstegium*, all occurrences of invasive species ( $n=8$ ), were located in edge plots.

There was no statistically significant difference in *Microstegium* biomass between edge and non-edge plots for any survey year (Table 5). Thus once it invaded it grew equally well in all plots, except where tree species invaded.

**Table 5. Statistical comparison of edge effects on *Microstegium* biomass by year**

<i>Year</i>	<i>P</i>	<i>Reject H<sub>0</sub>?</i>
2005-06	0.4394	No
2007	0.5133	No
2011	0.2381	No

When the relationship between edge effects and all new plant species was analyzed, a Mann-Whitney two-sample non-parametric test returned a statistically significant result ( $P=0.017$ ), resulting in the rejection of the null hypothesis. Because the three treatment 0 plots were also all edge plots, the test was conducted again without the treatment 0 groups to determine whether the results were influenced by confounding factors (See Appendix, Table D). This resulted in an almost but not quite significant value ( $p=0.059$ ), which indicated a difference in the median ranked value, but which was not great enough to be considered statistically significant, although the result is likely biologically significant.

The planted species diversity treatments and the number of new, non-invasive species were compared using a Kruskal-Wallis nonparametric test. However, it failed to reject the null hypothesis that there was no difference between treatment group medians ( $p=0.16$ ).

These results provide important information about *Microstegium*'s capacity as a highly invasive species. Ecosystem edges are widely understood to be highly invasible (Schulte et al., 2011). This study indicates that the study site's edges are highly invasible, with all observed occurrences of invasive species excepting *Microstegium* confined to edge plots. Further, there was an observed (although not statistically significant) difference in new, non-invasive species diversity between edge and non-edge plots. However, there was no statistically significant difference in the amount of *Microstegium* between edge and non-edge plots. This indicates that *Microstegium* is a 'strong' invader (Fargione & Tilman, 2005) and is, in essence, the most aggressive plant in the restored floodplain sites.

## Discussion

### Diversity-invasibility hypothesis

Economically, invasive species are a disaster: approximately 50,000 alien-invasive species have been introduced over the course of the United States' history (Pimentel, 2005), with a reported \$603 million in damages from only 15 exotic plant species from 1906 to 1991 (OTA, 1993). Invasive species also have serious ecological impacts. Fifty-seven percent of the 1,055 plant species listed under the Endangered Species Act are considered to be at risk because of competition with or predation by exotic species (Wilcove, et al., 1998) and alien weeds are invading approximately 46 acres of wildlife habitat per year (Babbitt, 1998). Invasive species have been proven to have deleterious effects on a range of ecosystem functions, including altering wildlife habitat, altering fire regimes and increasing the intensity and frequency of fires (Brooks, 2004). Invasives also establish monocultures, excluding native species, and affect nutrient allocation and availability (Seabloom & van der Valk, 2003). All in all, invasive species like *Microstegium* are taking a significant toll on the environment and the cost of wetland restoration.

Although there are several grass-specific herbicides which have proven effective (Tu, 2000) an undesirable potential consequence of herbicides is that native plant regeneration is inhibited as well (Flory, 2010). Hand weeding has proven successful in the short-term in reducing *Microstegium* and allowing recovery of the native plant community. However, it appears that hand-weeded sites quickly lose their gains in species richness and become re-invaded within a few seasons after treatment ceases (DeMeester & Richter, 2010).

Invasibility is defined as an environment's susceptibility to the colonization and establishment of new species that are not part of the resident community (Davis, et al., 2005). There is currently not a widely accepted hypothesis as to how natural systems are invaded, what makes an ecosystem more or less vulnerable to invasion, or how to predict what species may become invasive in the future (Dietz & Edwards, 2006). Certain traits and taxonomic groups have been identified as common or frequent markers of an invasive species; including sexual and asexual reproductivity, rapid growth, high seed or propagule production, height, and adaptation to a wide variety of habitats (Sakai, 2001). Invasive plants worldwide are more likely to belong to a particular suite of higher taxonomic groups including Asteraceae, Caryophyllidae, and Commelinidae, Amaranthaceae, Brassicaceae, and Poaceae (Richardson et al 2005; Sakai, 2001). It is also widely understood that disturbed or fragmented landscapes are known to be more vulnerable to colonization by invasive species (Sakai, 2001).

As this study shows, the diversity-invasibility hypothesis does not hold true for this site. Given the number of uncontrolled factors at the site, this is not surprising. However, a number of other studies on the diversity-invasibility hypothesis have had similar conclusions. Most of the available data on the diversity-invasibility hypothesis comes from experiments using synthetic assemblages that vary in diversity; and although some studies have shown a negative relationship between diversity and invasibility (Fargione et al., 2003; Fargione & Tilman, 2005; Sakai, 2001), most of these studies have been on a very small scale or only report results for a short timeframe of one to two growing seasons (J. Fargione et al., 2003). In contrast, large scale studies have mostly shown a positive correlation between diversity and invasibility (Maron & Marler, 2007; Richardson & Pysek, 2006) because what is good for natives habitat-wise is also good for aliens (Richardson & Pysek, 2006).

## **Current plant community composition**

Because so many factors were uncontrolled at the site, including nutrient availability, invading species, and herbivory, it is not surprising that the diversity-invasibility hypothesis was not fully

supported. However, as an example of a restored wetland, this site is unique in its long-term monitoring and provides some encouraging results for wetland restorationists.

As discussed previously, restoration projects frequently do not set clear goals or conduct thorough site assessments or monitoring (Bernhardt et al., 2005; Craft, 2003; Zedler, 2000) although this information is essential for understanding how the site has changed over time. A prior study (DeMeester & Richter, 2010) found that plant biodiversity decreased within a few growing seasons after *Microstegium* treatment was completed. However, at this site, seven years after treatment was instituted, we are seeing the spread of planted native species and the entrance of new species. Further, these new species are not solely invasive, but include a number of native trees and forbs. In the SWAMP site, as time goes on and these species mature it is likely that we will see the maturation of this site into bottomland hardwood forest, which may have further effects on *Microstegium* presence through shading and resource competition.

The survey plots of interest in this study were planted on bare soil after re-contouring of the stream channel on high-bench plots of approximately the same elevation, and were planted with seedlings propagated by Dr. Sutton-Grier in 2005. Many other restorations utilize a combination of plantings and seed mixes (Flory, 2010), making it challenging to compare the species composition changes over time. In contrast, the SWAMP site provides a unique opportunity to study long-term changes as the post-restoration plant community was carefully planted and documented.

The new wetland indicator species found at the site include primarily drier indicators (FAC and FACU, as well as a few species that are not wetland indicators) but also, surprisingly, some new obligates (*Carex vulpinoidea*, *Gratiola virginiana*, *Ludwigia alternifolia*, and *Lycopus virginicus*). This is consistent with the findings of Watt (2000), which found that the native floodplain community trended towards drier wetland indicators, but provides evidence that the site is being regularly inundated and is achieving its intended functions as a high-bench wetland area.

## **Edge effects**

Although this study did not find a significant difference in new species richness between edge and non-edge plots, invasive species (excepting *Microstegium*) are confined to edge plots alone. Future studies may find invasive species spreading and intruding into the plots, but it appears that the spread of invasion may have been slowed by some combination of species competition and site conditions. It may be that the two-meter wide edge plots are acting as a buffer for interior plots, slowing down the impacts of invasive species including *Microstegium*. While edge effects were evident, although not

statistically significant, for new non-invasive species, it appears that edge effects are not strongly influencing the spread of *Microstegium*. As *Microstegium* was found in nearly all surveyed plots and *Microstegium* biomass has greatly increased since 2007, it appears that *Microstegium* is an extremely strong, competitive invasive.

## Study limitations

Since 2005, the plots have not been weeded to maintain the original species composition. This means that species such as *Vernonia noveboracensis* (New York Ironweed) have spread from their originally planted plots across the entire study area, distorting the analysis of different treatments of planted species on *Microstegium* growth. Fast-growing tree species particularly *Betula nigra*, *Diospyros virginica*, and *Acer rubrum*, may have affected species interactions, resource competition, and shading.

Regarding abiotic factors, as previously discussed, the different treatment groups often have different shapes and scales. Because the Kruskal-Wallis test assumes that the shape and scale of the distribution of each group is the same, it is possible that this also affected the power of using the Kruskal-Wallis nonparametric test on the four treatment groups (McDonald 2009). Log transformations of the data did not render the data normal enough to use a t-test or ANOVA. It is possible that a larger sample size, capturing more of the 108 plots at the site, would reduce some of the variability and skewness of the data. Additionally, in the years 2005 through 2006, the plots had just recently been installed and plant communities were still establishing themselves.

In 2007, North Carolina experienced one of the worst droughts on record (Osland et al., 2009) which likely affected plant biomass across the board, including *Microstegium*; The increase in biomass from 2007 to 2009 and 2011 is marked, and may be due to the 2007 drought. This event may have negatively impacted the growth of some species; while several Tulip Poplar (*Liriodendron tulipifera*) seedlings have established in the plots, no mature Tulip Poplars were found at the site, unlike other tree species.

The study also did not control for herbivory. *Lobelia cardinalis* was one of the originally planted treatment species and was planted in several monoculture (1 treatment) plots, *Lobelia* was completely eradicated from the plots within a few growing seasons by deer predation (Sutton-Grier, 2008). This probably increased the likelihood of *Microstegium* invasion by creating more available niches for colonization. It is unknown what impact herbivory has had on other species in the plots, although evidence of deer herbivory on *Solidago altissima* was found during sampling in 2011. Additionally,

previous technicians appear to have selected samples randomly from plots, and did not sample from a different area each time. This may have had some impact on sample results as well.

## Recommendations

There are some promising indications for how managers can address *Microstegium* management in the future. First, it appears that planted species may have been more successful at repelling *Microstegium* invasion than leaving the site bare would have been, based on other studies that relied solely on natural recruitment (DeMeester & Richter, 2010). This indicates that if managers wish to reduce *Microstegium* invasion, 'active' restoration through planting may have better long-term results than 'passive' restoration through natural recruitment as the site overall showed an increased species diversity over time. As the results of this study show, the most abundant species by plot presence were predominantly planted species (Table 3).

It may also be that one species or taxonomic group in the site is a 'strong native' that is better at competing with an aggressive exotic due to its similar characteristics or resource needs (Fargione et al., 2003; Fargione & Tilman, 2005; Maron & Marler, 2007). Another study in the Duke SWAMP looked at the efficacy of a strong native, Giant Cane (*Arundinaria gigantea*) in competing with Chinese Privet (*Ligustrum sinense*). At the conclusion of the study it appeared that the Giant Cane was thriving and spreading despite invasive species encroachment (Osland et al., 2009).

New York Ironweed (*Vernonia noveboracensis*), and Joe Pye Weed (*Eupatoriadelphus fistulosum*) were two of the most dominant species by plot presence and by biomass weight. Late Goldenrod (*Solidago altissima*) was one of the more abundant new species by plot presence. All three species belong to the family Asteraceae. Many invasive plant species belong to this family (Richardson & Pysek, 2006) and all three of these species have 'weedy' traits: they are tall, shady, grow quickly and produce lots of seeds. Managers looking for suitable restoration species should select for native species that have weedy traits that are similar to those of *Microstegium* so that they are similarly or better able to compete for resources.

Finally, only time will tell whether this plant community will become dominated by *Microstegium*, maintain its originally planted diversity, or undergo succession into a bottomland hardwood forest. Although this site has been surveyed for much longer than many restoration sites, seven years is a brief moment in the lifetime of a wetland. Most wetland experts agree that successional processes can span decades or centuries (Seabloom & van der Valk, 2003) and that created and restored wetlands need

more than the current standard of five years of monitoring to evaluate ecosystem function. Another study (Mitsch & Wilson, 1996) estimates that freshwater wetlands need at least 15 to 20 years of establishment before restoration success is judged, while Zedler and Calloway (1999) argue for 20 to 100 years. There also appears to be a time lag before constructed or restored wetlands provide the same level of functions as natural wetlands (Craft, 2003). Any one survey is only going to capture a snapshot of the biotic community, and does not capture the full range of variation over time that the ecosystem will undergo. In order to accomplish successful restorations, scientists should seek to identify a range of ecosystem outcomes that accommodate the variability of natural systems (Hughes, 2005). Further study of SWAMP will shed more light on this question.



## Appendix

**Table A. Wetland Indicator status for plants**

<i>Wetland Indicator (WI)</i>	<i>Interpretation</i>
OBL	Probability of naturally occurring in wetlands =99%
FACW	Probability of naturally occurring in wetlands =67-99%; sometimes found in non-wetlands
FAC	Probability of naturally occurring in wetlands =34-66%; equally likely in wetlands and non-wetlands
FACU	Probability of naturally occurring in wetlands=1-33%; sometimes found in wetlands
UPL	Probability of naturally occurring in wetlands =1%; but occurs in wetlands in another region

Adapted from NRCS PLANTS Database, 2012.

Table B. 2011 survey results

<i>Species name</i>	<i>Plot presence</i>	<i>Percent of total biomass</i>
<i>Microstegium vimineum</i>	32	21.54
<i>Vernonia novaboracensis</i>	18	15.53
<i>Betula nigra</i>	3	14.64
<i>Eupatoriadelphus fistulosum</i>	15	12.78
<i>Pinus taeda</i>	3	7.69
<i>Panicum virgatum</i>	8	5.80
<i>Chasmanthium latifolium</i>	11	5.61
<i>Ulmus americanus</i>	2	4.11
<i>Rubus argutus</i>	7	2.85
<i>Diospyros virginiana</i>	1	1.86
<i>Solidago altissima</i>	6	1.48
<i>Fraxinus pennsylvanica</i>	1	1.20
<i>Carex crinita</i>	3	1.14
<i>Campsis radicans</i>	3	0.97
<i>Acer rubrum</i>	9	0.74
<i>Carex vulpinoidea</i>	6	0.34
<i>Lonicera japonica</i>	7	0.33
<i>Ludwigia alterniflora</i>	3	0.32
<i>Impatiens capensis</i>	3	0.28
<i>Juncus effusus</i>	8	0.16
<i>Persicaria punctata</i>	3	0.15
<i>Ligustum sinense</i>	3	0.12
<i>Symphotrichum pilosum</i>	2	0.10
<i>Toxicodendron radicans</i>	1	0.06
<i>Boehmeria cylindrica</i>	1	0.04
<i>Liriodendron tulipifera</i>	5	0.03
<i>Acer negundo</i>	3	0.03
<i>Lycopus virginicus</i>	2	0.02
<i>Vitis rotundifolia</i>	2	0.02
<i>Persicaria longiseta</i>	1	0.02
<i>Dioscorea oppositifolia</i>	1	0.02
<i>Kummerowia striata</i>	1	0.01
<i>Pluchea camphorata</i>	1	0.01
<i>Cryptotaenia canadensis</i>	1	0.01
<i>Panicum dichotomiflorum</i>	2	Less than 0.01
<i>Sisyrinchium angustifolium</i>	1	Less than 0.01
<i>Gratiola virginiana</i>	1	Less than 0.01
<i>Scirpus cyperinus</i>	1	Less than 0.01
<i>Acalypha chomboidea</i>	1	Less than 0.01
<i>Albizia julibrissin</i>	1	Less than 0.01

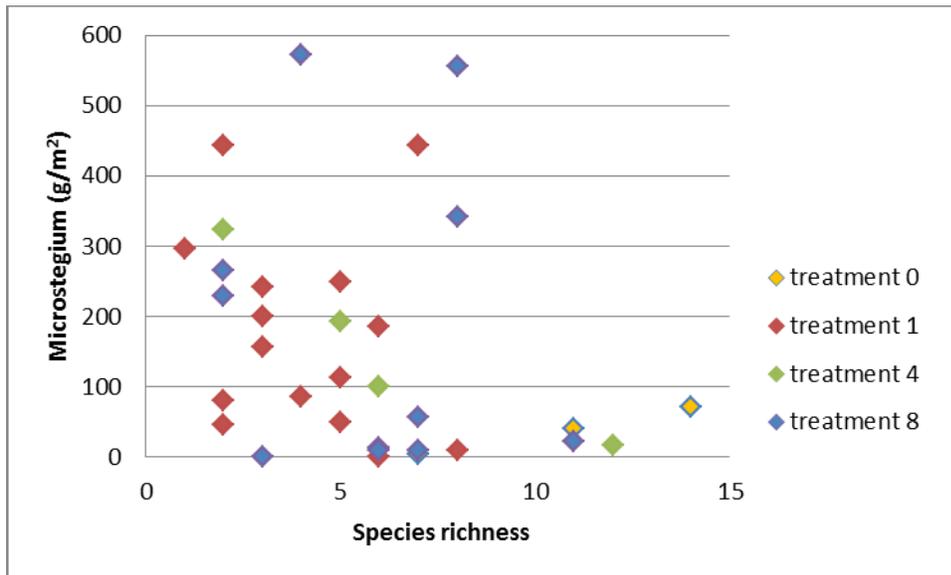
**Table C. Statistical comparison of *Microstegium* biomass by plot treatment**

	<i>P</i>	<i>Reject H<sub>0</sub>?</i>
All years combined	0.2292	No
2005-06	0.3498	No
2007	0.0669	No
2011	0.6182	No

**Table D. Statistical comparison of edge effects on all new plant species**

	<i>P</i>	<i>Reject H<sub>0</sub>?</i>
With all four treatment groups	0.01678	Yes
With Trt.0 removed	0.059	No

**Figure A. Trends in *Microstegium* biomass and species richness, showing diversity treatment**



## Thanks

This Masters Project would not have been possible without the help and support of many people at Duke University. I would like to thank my advisor, Dr. Curt Richardson, for his support of my research and guidance as I navigated a wealth of information. The comparison of vegetation data over several years was made possible by Dr. Ariana Sutton-Grier and Dr. Justin Wright, who allowed me to use their survey data in this project and provided helpful feedback and suggestions. Dr. Mengchi Ho provided extensive help with plant identification and survey design, and Dr. Dean Urban provided feedback on statistical analysis. I would also like to thank Wes Willis and the Wetlands Lab staff for their support.

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