Resource Protection Group
RFP #8

Invasive Species Research in Non-tidal Compensatory Mitigation

FINAL REPORT

SUBMITTED TO:

Resource Protection Group, Inc.
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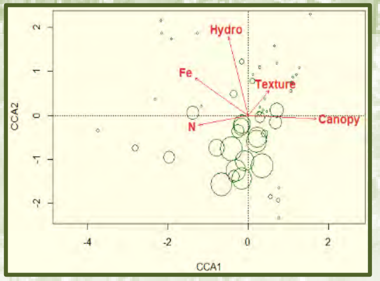

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Abstract

One of the most important and pervasive contemporary issues in the field of ecological restoration is that of biological invasion. From first principles, we know that disturbance represents a mode of introduction for invasive species, and ecological restoration sites can be particularly susceptible to biological invaders because the practices used to create, restore, or enhance ecological conditions are often the same types of disturbances that leave a site vulnerable to invasion. This is especially true of compensatory wetland and stream mitigation sites, where invasive plant species present one of the greatest challenges to managers, designers, and agency reviewers alike. The capital outlay for invasive plant management on mitigation sites has increased considerably over the past couple of decades, and in some cases it can represent the largest investment of money and resources during post-construction maintenance. Despite these circumstances, a comprehensive review of the literature reveals a conspicuous lack of applied research on invasive plant species in compensatory mitigation.

The purpose of this study was to evaluate impacts of plant species invasion and characterize important environmental factors that contribute to invasion on mitigation sites. We sampled vegetation and environmental variables (site hydrology, light availability, soil physiochemistry, site age) across invasion gradients at multiple wetland and stream mitigation sites in the Coastal Plain and Piedmont physiographic provinces of Virginia. Data analysis involved a multimetric statistical approach combining correlation, AIC, and CCA to arrive at a plausible model for invasion risk by species. On wetland sites, we targeted *Arthraxon hispidus* (joint-head grass), *Microstegium vimineum* (Japanese stiltgrass), and *Typha* spp. (cattail); on stream sites, we studied *Lespedeza cuneata* (sericea lespedeza), *Lonicera japonica* (Japanese honeysuckle), and *M. vimineum* invasions. Our analysis revealed species-specific environmental drivers of invasion with a few factors consistently important across all targeted invaders – notably, canopy cover, hydrology, and a handful of important physiochemical variables. The results of this research have been used to develop recommendations for ecological performance standards, as well as a suite of best practices that can be implemented at the outset of a stream or wetland mitigation project to reduce the risk of invasion.

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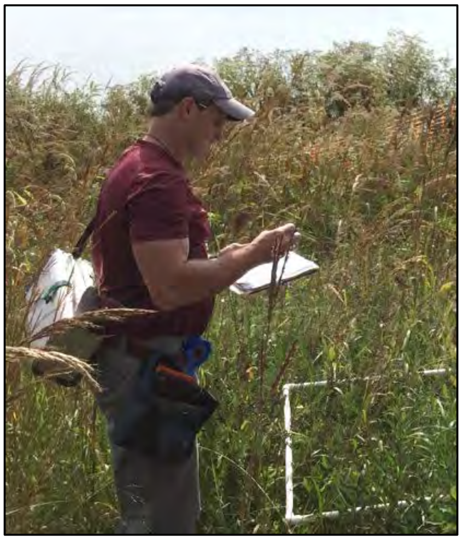

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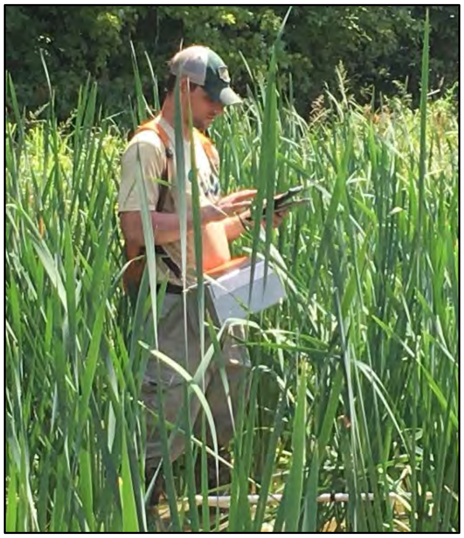

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Executive Summary

This report presents the findings of a three-year study on invasive plant species in non-tidal compensatory mitigation led by William & Mary and completed under grant RFP #08 from the Resource Protection Group, Inc. (RPG). The purpose of the study was to evaluate impacts of plant species invasion and characterize important environmental factors that contribute to invasion on mitigation sites in Virginia. This was accomplished through completion of four main tasks: 1) literature review; 2) field study, compensatory wetland mitigation; 3) field study, compensatory stream mitigation; and, 4) greenhouse experiment. A summary of each task is provided below.

Literature Review: The literature review component of this study was completed in April 2018 as an annotated bibliography, which is available at Invasive Species Research in Non-Tidal Compensatory Mitigation – Annotated Bibliography.

Field Study – Wetland Mitigation: The field study for the wetland mitigation component of this project was completed over the 2017 and 2018 growing seasons. Out of 30 mitigation sites evaluated for inclusion in the study, 23 met suitability criteria and were selected for sampling. Sites ranged in age from 1 to 23 years post-construction and were evenly distributed across the Piedmont (11 sites) and Coastal Plain (12 sites) in Virginia. Most sites were either privately owned mitigation banks or in-lieu fee sites developed under the Virginia Aquatic Resources Trust Fund. Five invasive plant taxa were screened for inclusion in the study, with *Arthraxon hispidus* (joint-head grass), *Microstegium vimineum* (Japanese stilt grass), and *Typha* spp. (cattail) being selected based on their distribution and abundance on sample sites. For purposes of this report, the term “invasion gradient” signifies the transition from high to low abundance of a target invader, which was evaluated in this study using plots arrayed on transects across the gradient. During the site screening phase, we chose to sample invasive populations where the *apparent* change in environmental conditions was negligible from the invaded end of the gradient to the uninvaded end (e.g., same relative elevations, same apparent hydrology regime, etc.). By doing this, we were able to study the conditions that “tip the scale” in favor of invasion on wetland mitigation sites in the absence of apparent environmental variation (i.e., to answer the question: When two sites *appear* to be similar, what factors lead to invasion on one and not the other?). A brief description of the sampling design follows.

Methods: Wetlands

- Within representative populations of each target invader, linear transects were established across the invasion gradient from “completely invaded” (i.e., dominant, or greater than 20% relative cover) to “uninvaded” (i.e., less than 5% relative cover).
- Five plots were arranged along each transect using a randomization procedure to determine plot centers and transect direction. Plot A corresponded to “completely invaded,” Plot C approximated the “edge” of the invasive population, and Plot E was at the “uninvaded” end of the transect. Plots B and D were established in sequence.

- The sample area at each plot was $4m^2$ and comprised of four $1m^2$ sampling frames arranged in the four quadrants surrounding the plot center (vertex). In each plot, absolute cover values were recorded for all plant species as the average of the four $1m^2$ sampling frames. Cover estimates were based on a cover class scale.
- A soil sample was extracted from the center of each plot and sent to the Virginia Tech Soil Testing Laboratory for chemical analysis, and *in situ* soil texture was approximated using established field procedures.
- Canopy cover was evaluated by taking a skyward photograph at each plot using a 180-degree hemispheric lens. Photographs were post-processed using imaging software and converted into a percent cover value.
- Hydrology was evaluated by calculating the prevalence index value for each plot.[1]
- Data were evaluated for monotonic relationships using the Spearman rank-order correlation. Community composition was characterized by the Sørenson similarity index, analysis of similarity (ANOSIM), Floristic Quality Index (FQI), species accumulation curves, and Rényi diversity profiles across the invasion gradient. Community-environment models were developed using Canonical Correspondence Analysis (CCA), with final model selection following optimization procedures using Akaike's Information Criterion (AIC).

On wetland sites, 170 total plots were sampled across 34 transects: 50 plots for *Arthraxon*; 50 plots for *Microstegium*; and, 70 plots for *Typha*. For each invasive species, plant community data were synthesized into an abundance matrix with the following dimensions: 124 species by 50 plots for *Arthraxon*; 116 species by 50 plots for *Microstegium*; and, 106 species by 70 plots for *Typha*. The corresponding environmental matrix for each target taxon included 15 environmental variables synthesized from the soil physiochemical data, canopy cover, site age, and hydrology. The key results of the data analysis are summarized below.

Results: Wetlands

- *Species composition was similar between moderately invaded plots (~5-10% relative dominance of invader) and uninvaded plots.* Analysis of similarity (ANOSIM) tests showed that only the highest levels of invasion impacted species composition.
- *Across the invasion gradient on wetland mitigation sites, native species richness and FQI were highest at moderate levels of invasion.* These results were also supported by species accumulation curves and diversity profiles, reinforcing the above finding that the invaders in this study do not appear to *exclude* native species at moderate levels of invasion.
- *Wetland hydrology showed a strong monotonic correlation with the invasion gradient of all target species on wetland sites.* Hydrology was the only environmental variable that was significantly correlated with the abundance of all three invaders along transects. Based on correlation coefficients, drier sites favored *Arthraxon* and *Microstegium* (i.e., negative correlation with hydrology), whereas wetter sites favored *Typha* (i.e., positive correlation).

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[1] For the purposes of this report, the term "hydrology" is used synonymously with "wetness."

- *Community ordination using CCA resulted in statistically significant and parsimonious models for all target taxa, revealing important environmental factors structuring plant communities along the invasion gradient on wetland sites.* These are summarized below [direction of relationship annotated with (+) for positive and (-) for negative].
  - *Arthraxon*: hydrology (-), canopy cover (-), texture (-), carbon:nitrogen ratio (-), phosphorus (+)
  - *Microstegium*: hydrology (-), canopy cover (-), texture (-), nitrogen (+), iron (-)
  - *Typha*: hydrology (+), canopy cover (-), manganese (-), site age (+)
- Consistent with its importance in the linear correlations, site hydrology emerged as a key environmental factor in all community models and was negatively correlated with *Arthraxon* and *Microstegium* and positively correlated with *Typha*. Canopy cover (light availability) was important in all models as well (negatively correlated with all invaders, indicating that shade limits invader abundance). The relative positions of macronutrients varied among taxa but were important particularly in the case of nitrogen (+) and phosphorus (+), the latter reflected in direct measurements or indirectly through relative concentrations of metal oxides that can affect the availability of phosphorus in these systems.

Field Study – Stream Mitigation: The field study for the stream mitigation portion of this project was completed over the 2018 and 2019 growing seasons, with 21 sites chosen for sampling from 30 initially screened. Sites ranged in age from 1 to 19 years post-construction and were evenly distributed across the Piedmont (10 sites) and Coastal Plain (11 sites) in Virginia. Six (6) invasive plant taxa were evaluated for inclusion in the study, with *Lespedeza cuneata* (sericea lespedeza), *Lonicera japonica* (Japanese honeysuckle), and *Microstegium vimineum* (Japanese stilt grass) being selected for sampling. A brief description of the sampling methods is provided below.

Methods: Streams

- For the stream sites, sampling design and approach follow the wetland methods outlined above with one exception: instead of using a randomly defined direction to establish a straight line transect, plots were randomized at each location along a transect that meandered roughly parallel to the nearest streambank to maintain a consistent relative elevation in the floodplain. The purpose for this modification was to ensure that landscape position within the floodplain was similar for each plot along the invasive gradient.
- All other sampling and statistical analysis methods were as noted above for the wetland plots. Likewise, the same environmental factors were evaluated; however, wetland hydrology was excluded *a priori* from the stream environmental dataset as it was not anticipated to be an important environmental factor in the analysis (i.e., all plots were in uplands within the riparian corridor).

Using the above methods, 145 total plots were sampled across 29 transects: 40 plots for *Lespedeza*; 50 plots for *Lonicera*; and, 55 plots for *Microstegium*. Plant community data were synthesized into abundance matrices with the following dimensions: 148 species by 40 plots for

Lespedeza; 167 species by 50 plots for *Lonicera*; and, 191 species by 55 plots for *Microstegium*. The corresponding environmental matrix for each target taxon included 14 environmental factors from the soil physiochemical data, canopy cover, and site age. The key results of the data analysis are summarized below.

Results: Streams

- As with the wetland study, *species composition was similar between moderately invaded plots (~5-10% relative dominance of invader) and uninvaded plots.*
- *Across the invasion gradient on stream mitigation sites, native species richness and FQI were highest at moderate levels of invasion.* As with the wetland results, species accumulation curves and diversity profiles supported these findings for all invasive taxa.
- *Canopy cover showed a strong monotonic correlation with the invasion gradient of all target species on wetland sites.* For all three target species, canopy cover was negatively correlated with invasive species abundance along transects, indicating that shadier sites tended to be less invaded.
- *Community ordination using CCA resulted in statistically significant and parsimonious models for all target taxa, revealing important environmental factors structuring plant communities along the invasion gradient on stream sites as summarized below* [direction of relationship annotated with (+) for positive and (-) for negative].
    - ➢ *Lespedeza*: canopy cover (-), soil texture (+), nitrogen (-), potassium (-), pH (-)
    - ➢ *Lonicera*: canopy cover (-), soil texture (+), nitrogen (-), iron (-), magnesium (-)
    - ➢ *Microstegium*: canopy cover (-), nitrogen (-), manganese (-), potassium (-)
- Consistent with its importance in the linear correlations, canopy cover was a key environmental factor in all community models as represented by the CCA biplots for the stream dataset. The other factors influencing stream mitigation plant communities were soil physiochemical variables consistent with the overall stress-disturbance dynamic (as described below).

Greenhouse Experiment: Multispecies mesocosms planted with representative invaders from the field studies (*Arthraxon*, *Lespedeza*, *Microstegium*) and several native species were grown under controlled conditions in the W&M greenhouse during summer and fall of 2020. Due to a heater malfunction, several plants were lost to early frost in October. The experiment is being repeated in winter/spring 2021, the results of which will be provided in an addendum to this report when the experiment is complete.

Invasives Species Performance Standard: A recommended threshold to trigger remediation in both wetland and stream compensatory mitigation is 10% relative abundance of invasive species. Based on the data, a 10% invasive species standard would be a sensible target for ecological performance that strikes a balance between proactive management and indiscriminate loss of desirable species. Invasive species abundance should be calculated from monitoring data collected using methods that conform with ecological sampling theory and for which sample adequacy has been demonstrated. The standard should be tracked by community type (or planting zone), and detailed mapping of invasive species populations is also highly recommended.

Recommended Best Practices: Environmental drivers of plant invasion on mitigation sites suggest a suite of best practices that could be implemented on mitigation sites to attenuate the risk of biological invasion while remaining consistent with most aquatic resource function goals.

Best Practice #1: Plant larger trees. Planting trees from larger stock types would promote canopy development and hasten canopy cover, a factor that ended up being an important environmental driver across the invasion gradient in all data sets. To address expense, alternative vegetation performance standard like Stem Area at Groundline (SAG) could be used instead of density, allowing larger stock to be incorporated in a planting plan without undue expense.

Best Practice #2: Plant trees at a higher density. A higher density of young trees could achieve the same results as #1 above at a reduced cost. To address the higher risk of mortality using small stock sizes, trees should be sufficiently hardened by the grower and planted while dormant in the fall if possible.

Best Practice #3: Plant early successional trees. Early successional species are fast-growing, more likely to facilitate canopy closure, and can function as a nurse crop for late successional species on mitigation sites. Planting early successional trees can increase the survivability of late successional trees while reducing risk of invasion through canopy development.

Best Practice #4: Plant a diverse seed mix at a high application rate. A diverse seed mix with high percentage of rapid-germinating annuals combined with high species richness of perennials and tree seed will maximize potential for rapid germination and ecosystem resiliency, advantaging native species via competitive benefits promoted by early establishment.

Best Practice #5: Make wetland hydrology manipulable. Given the importance of hydrology as a driver of environmental conditions on wetland mitigation sites, water control structures should be designed to allow for proactive manipulation of the wetland hydrology regime during the first several years of site development. Decisions about how and when to proactively modify hydrology on mitigation sites should be informed by vigilant surveillance over the first several years post-construction, as well as an understanding of the stress-disturbance dynamic affecting vegetation development at the site.

Best Practice #6: Understand the stress-disturbance dynamic. Invaders tend to prefer high disturbance/low stress (resource rich) habitats. The "disturbance" half of the stress-disturbance dynamic is unavoidable on mitigation sites due to construction practices, but there may be alternative approaches that would allow mitigation designers and managers to manipulate the "stress" half by *imposing* environmental stress to reduce risk of invasion.

Best Practice #7: Map invasive species annually. In addition to the relative percent cover recommendations for invasive species, it is in all mitigation practitioners' best interest to annually map the extent of invasive species on mitigation sites. This practice, in combination with diligent review of plot-based vegetation data, will help to reduce invasion risk while also identifying local "hot spots" where biological invasion can be targeted for future management.

Future Research: Field trials are recommended for testing experimental approaches as alternatives to non-selective herbicide use. Examples include soil amendments with a high carbon:nitrogen ratio, addition of metal oxides to immobilize phosphate, experimental plantings, and field manipulation of hydrology.

1 Introduction

This report presents the results of a three-year study on invasive plant species in compensatory wetland and stream mitigation. The research team for this project consisted of Principal Investigator Doug DeBerry from William & Mary's Environmental Science and Policy (ENSP) program, Dakota Hunter, who completed a master's degree in Biology at William & Mary (W&M) over the course of the study, and the Virginia Tech (VT) Soil Testing Laboratory. This project was completed under a grant from the Resource Protection Group, Inc. (RPG).

Per the original grant proposal, final award (RFP #08), and one addendum, the project was executed in four phases: 1) literature review; 2) field study, compensatory wetland mitigation; 3) field study, compensatory stream mitigation; and, 4) greenhouse experiment. This report provides results from the first three; the final phase (greenhouse experiment) is ongoing, and results from that effort will be submitted as an addendum to this report later in 2021.

Each major project task is addressed as a separate chapter in this report, followed by chapters on ecological performance standards for invasive species and a synthesis of the overall study with recommended best practices. The final chapter discusses future work, including status of the greenhouse experiment and recommendations for other studies that would complement this research project.

For the purposes of this report, "non-tidal compensatory wetland and stream mitigation" will be referred to collectively as "compensatory mitigation" or simply "mitigation." When addressed separately, the terms "wetland mitigation" and "stream mitigation" will be used. In addition, as it is used in this report the term "invasion gradient" signifies the transition from high to low abundance of a target invader, which was evaluated in this study using plots arrayed on transects across the gradient.

1.1 Background

One of the most important and pervasive contemporary issues in the field of ecological restoration is that of biological invasion (Blossey 1999). Invasive species are organisms that are successful at colonizing new sites and, once established, are able to engage in explosive population growth in combination with a highly competitive life history strategy (Miller 2003, Bryson and Carter 2004). This is problematic because invaders can quickly preempt space that could otherwise be occupied by desirable species (Zedler and Kercher 2004). Disturbance (defined in Chapter 2) represents a mode of introduction for invasive species, and ecological restoration sites can be particularly susceptible to biological invasion because the practices used to create, restore, or enhance ecological conditions are often the same types of disturbances that leave a site vulnerable to invasion (e.g., site clearing and grading, etc.; Shea and Chesson 2002, DeBerry et al. 2010).

On compensatory mitigation sites in the Mid-Atlantic Region, invasive plant species present one of the greatest challenges to mitigation managers, designers, and natural resource agency reviewers (Brooks and Gebo 2013). The capital outlay for invasive species management on compensatory mitigation sites has increased considerably over the past couple of decades, and in some cases it can represent the largest investment of money and resources in terms of post-construction maintenance on these sites (Bergdolt et al. 2005). The problem with this practice is that it is not clear that the issue merits the investment. Biological invasion is a relatively new subject of study to science, deriving many of its first principles from agricultural or other commodity-based disciplines (e.g., mariculture, silviculture, etc.) (Pimentel 2011). In these fields of research, the emphasis has been on studying biological invasion to derive management programs that will maximize values (i.e., attributes beneficial to mankind), with less emphasis on maximizing ecological functions. Although there has been some research that addresses biological invasion and ecological function on mitigation sites (e.g., Brooks et al. 2005, Matthews and Endress 2008, Matthews et al. 2009b, Mitsch et al. 2012), in most cases, invasive species have been ancillary to the primary research objectives in mitigation studies.

Perhaps even more important is the issue of performance standards for invasive species in compensatory mitigation. Performance standards are established to ensure that aquatic resource functions are maximized on mitigation sites, but it is unclear how invasive species standards accommodate this goal. For example, a standard that is set low (like a 5% threshold for invasive species cover) often necessitates the use of targeted or broadcast herbicides, a practice that introduces foreign chemicals into natural systems and can result in collateral damage to desirable species (D'Antonio and Meyerson 2002, Kettenring and Adams 2011, Lawrence et al. 2016). Although the ecological impacts of invasive species in compensatory mitigation have been reviewed by others (Brooks et al. 2005, Matthews and Endress 2008, DeMeester and Richter 2010b, Dee and Ahn 2012, Brooks and Gebo 2013), the specific topic of invasive species performance standards in mitigation programs has received relatively little attention in the literature.

The few scientific studies aimed at addressing the appropriateness of invasive species performance standards have produced variable results. For example, in a study evaluating 76 compensatory wetland mitigation sites in Illinois, Matthews and Endress (2008) noted: “Although most sites failed to meet the often-required performance standard specifying that exotic and or weedy species should not be dominant at a site, this standard does not seem inappropriate or overly stringent.” The standard in this case was that exotic or weedy species could not be dominant over the total vegetation abundance measure for the site, and it was not restricted to the concept of non-native invasive species (i.e., native “weedy” species were also considered undesirable). A similar study on 11 wetland mitigation sites in Michigan noted that sites failing the Michigan Department of Environmental Quality’s 10% standard for invasive species abundance did not fail other performance standards (Kozich and Halvorsen 2012). Although the subtext of this study was that invasive species performance standards were inconsistent and perhaps unreliable when compared with other standards, the authors did not explicitly make this claim. Likewise, in an exhaustive review of Washington State mitigation sites, the Washington State Department of Ecology (WSDE 2002, 2006) found that invasive species performance standards provided results that were inconsistent with other standards, implicating a general lack of well-defined and realistic goals in many of the projects reviewed. WSDE (2006) concluded that "[s]etting unrealistically low standards will usually increase costs by requiring extensive control efforts after the site is established."

Figure 1-1 Typha latifolia *(background) and Lespedeza cuneata (foreground) dominant on a non-tidal wetland mitigation site in Virginia. Among 15 wetland mitigation projects reviewed throughout the eastern portion of the state, DeBerry (2006) documented the highest species richness at this site.*

In Virginia, recent studies on vegetation ecology in non-tidal wetland mitigation sites have demonstrated some relevant trends. For example, Perry et al. (2009) summarized cattail (*Typha* spp.) research on Virginia Department of Transportation (VDOT) mitigation sites, concluding that the standard rationale for cattail removal in Virginia – namely, that cattails reduce species richness and diversity within the vegetative community – is not supported by the research. Further, although DeBerry (2006) did not focus specifically on invasive species, data sets from this study of fifteen created wetlands in the Piedmont and Coastal Plain of Virginia showed that sites where certain invasive species were dominant (e.g., *Typha latifolia*, *Microstegium vimineum*, and *Lespedeza cuneata*) also had among the highest species richness values (Figure 1-1). Interpreting similar data from Dee and Ahn (2012) for the Northern Virginia Piedmont, species richness and diversity index values for mitigation sites with non-native invasive species (e.g., *M. vimineum* and *Murdannia keisak*) were not statistically different from the same indices calculated for sites with no invasives.

DeBerry and Perry (2015) discuss a consideration in compensatory mitigation design and construction related to biogeochemical dynamics in the early stages of site development. Their research found that nearly all vegetation community indices calculated for a chronosequence of wetland mitigation sites in Virginia were correlated with certain soil physiochemical variables (namely, bioavailable phosphorus and texture). They related these correlations to soil nutrient status during the post-establishment phase of vegetation succession (e.g., "autogenic dominance" *sensu* Noon 1996) and the resultant proliferation of aggressive plants. Although there is less applied research on soil nutrient status and vegetation development in stream mitigation, studies in riparian corridor restoration suggest similar ideas (Audet et al. 2015). In another Virginia study, Ahn and Dee (2011) highlighted the importance of hydrologic control on vegetation development in young mitigation sites, a conclusion that is consistent with other research in similar systems (Matthews et al. 2009a, Mitsch et al. 2012). Of interest is the potential for compensatory mitigation design and management to benefit from approaches that actively control environmental factors like soil nutrient status and hydrology on young sites (i.e., during the first 5 years) to reduce risk of invasion.

1.2 Purpose

This research program was designed to address the above considerations through observation and experimentation, with an emphasis on answering the following questions: 1) What are the implications of invasive species in terms of the ecosystem functions of vegetation communities on compensatory mitigation sites? 2) Are existing invasive species performance standards appropriate and, if not, are there other standards that are more congruent with the magnitude of the problem? 3) Are there certain environmental conditions on compensatory mitigation sites that render them more susceptible to biological invasion in comparison with other sites? 4) Are there best practices that can be used in compensatory mitigation to reduce the risk of invasion?

2 Literature Review

The literature review component of this study was submitted to RPG in April 2018 as an annotated bibliography. That document can be found on the RPG website at Invasive Species Research in Non-Tidal Compensatory Mitigation – Annotated Bibliography. For the purposes of this chapter, a detailed synthesis of the literature here would be redundant with that effort and repetitive with statements and literature citations elsewhere in this report. For convenience, the text below focuses on important ideas in the overall development of this research program that have not been adequately reviewed in other chapters.

Invasive Species Definition: Although characteristics of an invasive species – e.g., successful at colonizing, rapid population growth, superior competitive ability once established, negative ecological consequences – are reasonably consistent in the literature, there is much disagreement about how to define the term (Lockwood et al. 2013). In the U.S., the federal definition (EO 13112) requires a species to be "alien" or "non-native" for it to be classified as invasive (Beck et al. 2008). A strict interpretation of this definition would exclude some wetland species that are traditionally considered native to the eastern U.S.[1] but are currently managed as invaders (e.g., *Typha latifolia*; Perry et al. 2009). To limit confusion, some definitions focus specifically on the invaded ecosystem itself as the domain for "native" or "non-native", which restricts the geographic application of these concepts to the level of a site or a specific habitat (Lockwood et al. 2013). This seems to be the inherent meaning in the federal definition as well, which specifies that an invasive species must be "non-native to the ecosystem under consideration" (Beck et al. 2008). Perhaps because of these subtleties, the native/non-native debate in the literature is rife with controversy (Shackleford et al. 2013), making it difficult to home in on a practical definition of invasive species for regulatory purposes. In Virginia, the current Interagency Review Team (IRT) Mitigation Banking Template (USCOE and VDEQ 2018) has dealt with this problem by expanding the concept of problematic species to any that could be classified as "invasive, nuisance, or undesirable" (INU), thereby circumventing the issue of defining an invasive species on the basis of whether or not it was introduced by humans from some other part of the globe. From this idea, it is reasonable to justify a mitigation-focused definition of invasive species that captures the general *characteristics* and *impacts* of invaders without restricting "membership" to only non-native species. For our purposes, then, we define an invasive plant species as one that enters an area that it did not previously occupy, rapidly expands in space once there, *and* has negative consequences for the species already in the space that it enters (Alpert et al. 2000).

Invasive Species Research in Mitigation: A conspicuous result of the literature review for this project was the overwhelming lack of applied research on invasive plant species in mitigation. If a research or review paper on mitigation addressed invasive species, it typically characterized the issue as a component of a larger research question such as overall performance standards,

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[1] "Native" is another term about which there is much debate, but most sources for the U.S. equate native species with those that were present on the North American continent prior to European contact (e.g., Weakley et al. 2020).

general vegetation community dynamics, floristic quality, ecosystem services, aquatic resource functions, etc. (e.g., Brooks et al. 2005, Kozich and Halvorsen 2012, Mitsch et al. 2012, DeBerry and Perry 2015). One notable exception is Jeff Matthews's wetland mitigation-focused research at University of Illinois (e.g., Matthews and Endress 2008, Matthews et al. 2009b). The restoration ecology literature in general has more to offer on plant invasion, and there are some comprehensive reviews of invasive plants in wetlands (e.g., Zedler and Kercher 2004), but these treatments are rarely focused on the unique contemporary field of compensatory mitigation. These overall trends in the scientific literature identify plant invasion in mitigation as a research gap, underscoring the importance of the applied research in this project.

Invasion Ecology and the "Stress-Disturbance Dynamic": From our review of the foundational literature on invasion ecology, we believe that theoretical concepts developed around the resource strategies of plants have the most explanatory value in mitigation contexts (Craine 2009). Although many theories about novel phenotypes and lack of natural enemies have been advanced to explain how non-native species become invasive [e.g., novel weapons (Callaway and Ridenour 2004), introgression (Galatowitsch et al. 1999), enemy release (Keane and Crawley 2002), etc.], those invoking environmental factors and the relationship between stress and disturbance on sites are, in our opinion, the most compelling. As these terms are typically applied in plant ecology, *stress* refers to an environmental or biological factor that causes a negative physiological response resulting in a reduction in fitness or growth (e.g., nutrient limitation or drought), whereas *disturbance* refers to a change in the environment that results in a removal of biomass (e.g., mowing or bulldozing a site) (Grime 1979, Hobbs and Huenneke 1992, Bazzaz 1996, Lichtenthaler 1996). Levels of stress and disturbance vary in space and time, and the interactions between the two can often be used to predict plant responses to environmental conditions (Craine 2009). In evaluating this "stress-disturbance dynamic" on mitigation sites, we find strong evidence to support the notion that sites with high levels of disturbance – which is the case for most recently constructed mitigation sites – combined with low levels of environmental stress (i.e., high resource availability) are the ones that are most susceptible to invasion (Alpert et al. 2000). These ideas are represented conceptually in Figure 2-1.

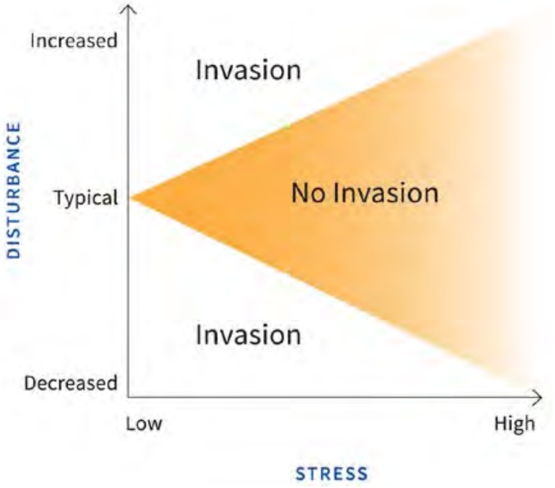


***Figure 2-1** Conceptual model showing the relationship between stress, disturbance, and invasion (adapted from Alpert et al. 2000). Source: VHB, Inc., used with permission.*

In the case of most plant invaders, low stress or high resource availability typically refers to soil nutrient status and, in particular, levels of bioavailable nitrogen and phosphorus (Chiang et al. 2000, Woo and Zedler 2002, Tuchman et al. 2009, De Jager et al. 2015, Rojas and Zedler 2015). Disturbed sites that are high in these essential nutrients have been shown to be prone to invasion (Davis 1991, Alpert et al. 2000, Kercher and Zedler 2004, Ehrenfeld 2010). However, in wetlands, hydrology can represent an important source of stress for plants because microbially-mediated chemical reduction renders saturated soils anaerobic and therefore depleted of molecular oxygen required for respiration (Mitsch and Gosselink 2015). Further, on stream sites, hydrology can function as a mode of disturbance for many plant species during episodic events like storms, where the flood-prone area around a stream channel is exposed to the biomass-altering energy of floodwaters that can uproot vegetation or smother plants with sediment (Bendix and Hupp 2000, Richardson et al. 2007). For still other species, the limiting resource factor in forested ecosystems is light availability, and a disturbance event that opens the canopy and allows light to enter the understory will effectively remove stress and facilitate invasion (Robertson et al. 1994, Woo and Zedler 2002, Schierenbeck 2004, Warren et al. 2011). In each of these scenarios, disturbance is the mode of entry, and high resource availability ensures success for the invader. Once established in a new habitat, the mechanisms used to outcompete native species are unique to each invader (e.g., allelopathy, autogenic control, rapid nutrient acquisition and slow decomposition, etc.), but it is typically the case that invaders will not be able to compete in stressful environments (Lockwood et al. 2013). In restoration ecology, if the important factors that control invasion are known, the interplay of stress and disturbance can potentially be controlled to minimize risk of invasion (Perry et al. 2004), and on mitigation sites this would be most critical during the first several years of vegetation development (Noon 1996, DeBerry and Perry 2015).

"Intrinsic Floristic Quality Parameters" and Ecosystem Function: This study evaluates the impact of invasive species on ecosystem functions related to vegetation community properties, so a brief review of these concepts bears mentioning. DeBerry and Perry (2015) describe the use of "intrinsic floristic quality parameters" such as native species richness, species evenness, and species diversity as indicators for community-based functions in mitigation sites. These ideas reflect the notion that high values of these community properties are positively correlated with functions such as habitat complexity and ecosystem resiliency (Huston 1994, Gunderson 2000). In addition, species composition – which accounts for the *identity* of species present irrespective of abundance – has been directly linked to ecosystem functions such as biogeochemical cycling of nutrients (Hooper and Vitousek 1997), ostensibly due to the fact that different species perform different functional roles in mediating ecosystem processes. Species composition has been identified as an important factor in determining structure and function of developing plant communities on mitigation sites (DeBerry and Perry 2004, 2012), so our use of composition in evaluating impacts of invasion in this study is consistent with these ideas. Floristic Quality Assessment (FQA) has also been shown to reflect ecosystem function on mitigation sites (DeBerry and Perry 2015), owing mostly to the versatility of the "species conservatism" concept embedded in the approach (for a comprehensive review of FQA see DeBerry et al. 2015). For our purposes, the most commonly used metric in FQA – the Floristic Quality Index (FQI) – is applied in this study [$FQI = \bar{C}\sqrt{N}$, where $\bar{C}$ = mean Coefficient of Conservatism (C-value) and N=native species richness; DeBerry and Perry 2015]. C-values for FQI calculations in this study were taken from the most recent list for Virginia (DeBerry 2020b).

3 Field Study – Wetland Mitigation

The field study for the wetland mitigation component of this project was completed over the 2017 and 2018 growing seasons. The primary goal of this project phase was to evaluate the relationships between invasive species, community properties, and environmental variation on compensatory wetland mitigation sites. This was accomplished by establishing transects across invasion gradients of three known wetland mitigation invaders in Virginia: *Arthraxon hispidus* (joint-head grass), *Microstegium vimineum* (Japanese stilt grass), and *Typha* spp. (cattail). These taxa were selected as representative organisms based on initial consultation with several wetland mitigation bankers and/or site managers as well as a field screening of available study sites. Along transects, we documented relative abundance of species within the plant community across the invasion gradient and collected data on environmental variables. We anticipated that wetland hydrology and light availability would be important factors (Barden 1987, Zedler and Kercher 2004, Oswalt et al. 2007, Ahn and Dee 2011, Warren et al. 2011). Further, evidence from prior studies suggested that phosphorus could also be significant (Chiang et al. 2000, Woo and Zedler 2002, DeBerry and Perry 2015).

Fieldwork was completed in two stages: 1) initial site screening, and 2) sampling. During screening, candidate invasive species populations were mapped at potential study sites, and the final detailed sampling was conducted by Dakota Hunter during peak growing season in 2018 as described below.

3.1 Species Descriptions – Wetlands

During the initial screening phase, five invasive plant taxa were evaluated for inclusion in the study. Taxa reviewed but excluded from the study included *Murdannia keisak* (marsh dewflower) and *Phalaris arundinacea* (reed canary grass). Although these two species were present on some sites, their distribution and abundance were determined to be insufficient for the study design. The three species retained for the study are described below.

Arthraxon hispidus (Thunb.) Makino [Poaceae] (hereafter "*Arthraxon*"), is an annual grass from east Asia that has received little attention in the literature but is listed as moderately invasive in Virginia and throughout the Mid-Atlantic Region (Swearingen et al. 2010, Heffernan et al. 2014). Reports from mitigation bankers across Virginia suggest that the species merits greater concern and further scientific examination (Figure 3-1). Although

Figure 3-1 Arthraxon *dominant on a wetland mitigation site in Northern Virginia.*

infestations on study sites were observed more commonly in the Piedmont physiographic province, *Arthraxon* was present on Coastal Plain sites as well. Most mitigation sites colonized by this species were adjacent to active farmland or within the floodplains of major rivers, and invasion was commonly observed on wetland "edges" where localized disturbance was more prevalent. These observations are consistent with the limited information on *Arthraxon* available from research on other continents (e.g., White et al. 2020).

Microstegium vimineum (Trin.) A. Camus [Poaceae] (hereafter "*Microstegium*"), is an annual grass native to Asia that grows in a variety of wetland and upland habitats. *Microstegium* has a known tolerance for shading (Barden 1987, Oswalt et al. 2007; Figure 3-2) and flooding (Warren et al. 2011). Because of its environmental tolerances and prolific seeding capabilities, *Microstegium* has been identified as a highly invasive plant species in Virginia (Heffernan et al. 2014). It has been documented to reduce native plant diversity (Oswalt et al. 2007, Adams and Engelhardt 2009) and alter insect community structure (Marshall and Buckley 2009). Its ability to disperse high numbers of viable seeds into a persistent seed bank makes it difficult for land managers to treat (Miller and Matlack 2010, Ziska et al. 2015), but post-emergence herbicide application can be effective within a single year (Judge et al. 2005, Flory 2010). Despite the abundance of research on this species, consensus on the specific environmental conditions that stimulate invasion by *Microstegium* has not been reached; however, high nutrient loads and light availability have both been identified as likely candidates (Warren et al. 2011).

Figure 3-2 Microstegium *demonstrating tolerance to shade in a disturbed forest understory in southeastern Virginia.*

***Typha* spp.** [Typhaceae] (hereafter "*Typha*") is a group of two cattail species (*Typha latifolia* L. and *Typha angustifolia* L.) and a hybrid of those species (*Typha* x *glauca* Godron) that are native to the U.S. but regulated as invasive species on wetland mitigation sites (Perry et al. 2009). *Typha* typically inhabits lower, wetter areas within mitigation sites (Figure 3-3), and association with changes in nutrient cycling and surface flow have also been documented where *Typha* is present (Woo and Zedler 2002, Zedler and Kercher 2004, Angeloni et al. 2006, Wiltermuth and Anteau 2016). Recent studies suggest that species in the genus

Figure 3-3 *Typical habitat condition for* Typha *on study sites (co-author D. Hunter pictured).*

may be increasing in prevalence due to anthropogenic activities related to runoff and sedimentation in wetlands (Angeloni et al. 2006, Sullivan et al. 2010). However, little evidence exists that *Typha* directly inhibits native plant diversity, and potential for positive species responses to the presence of *Typha* have been demonstrated (Green and Galatowitsch 2001, Perry et al. 2009). Nevertheless, herbicide application is regularly used to combat *Typha* growth, often with only temporary results (Brandon et al. 2004, Lawrence et al. 2016).

3.2 Site Selection and Study Area – Wetlands

Representative field sites were chosen from a pool of over 30 available sites based on location and size of invasive species populations, common native plant assemblages, site layout, and accessibility. Only non-tidal wetland mitigation sites displaying dominant patches of target organisms were used. Field sites were assigned age classes consistent with DeBerry and Perry (2012) due to the documented importance of age for plant community structure on wetland mitigation sites. On multi-user sites, distinct "phases" or areas constructed during separate time periods were treated independently so long as they fell into different age classes (this was a common condition on study sites operating as mitigation banks). The age classes were determined from site records on the number of complete growing seasons after site construction and included: 1-2 years old; 3-5 years old; 6-10 years old; 11-15 years old; and >15 years old.

Among the sites screened in 2017 and early 2018, 23 met suitability criteria and were selected for the study. Site ages ranged from 1 to 23 years post-construction and were evenly distributed across the Piedmont (11 sites) and Coastal Plain (12 sites) in

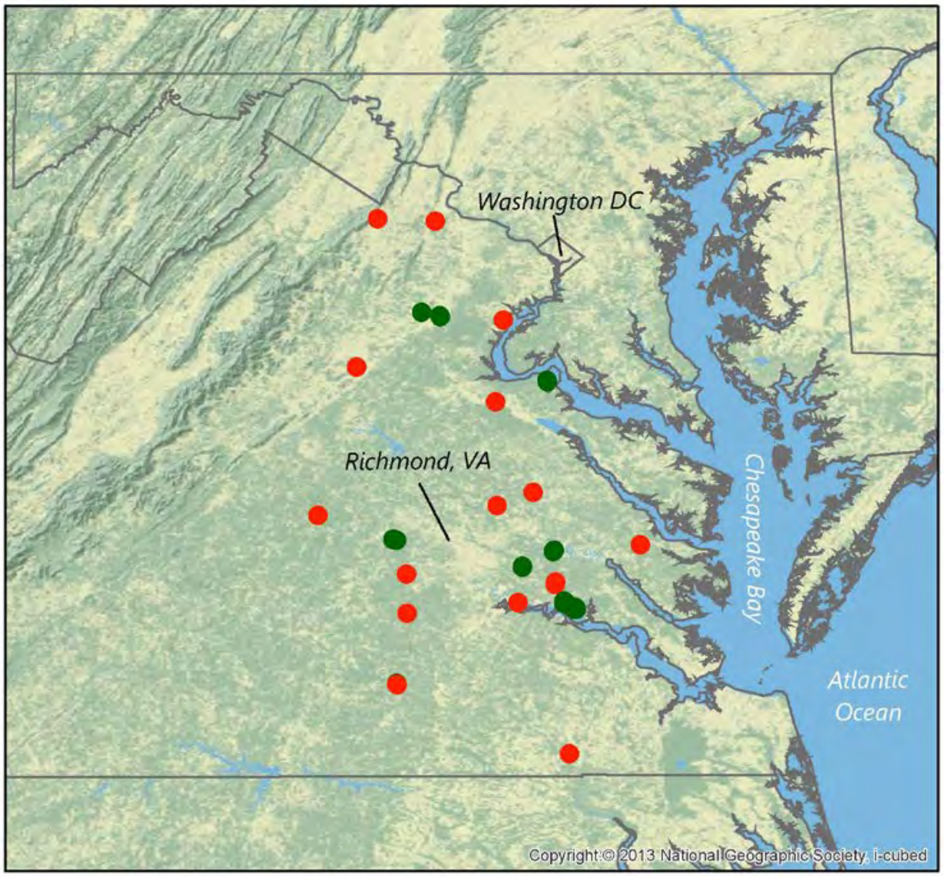


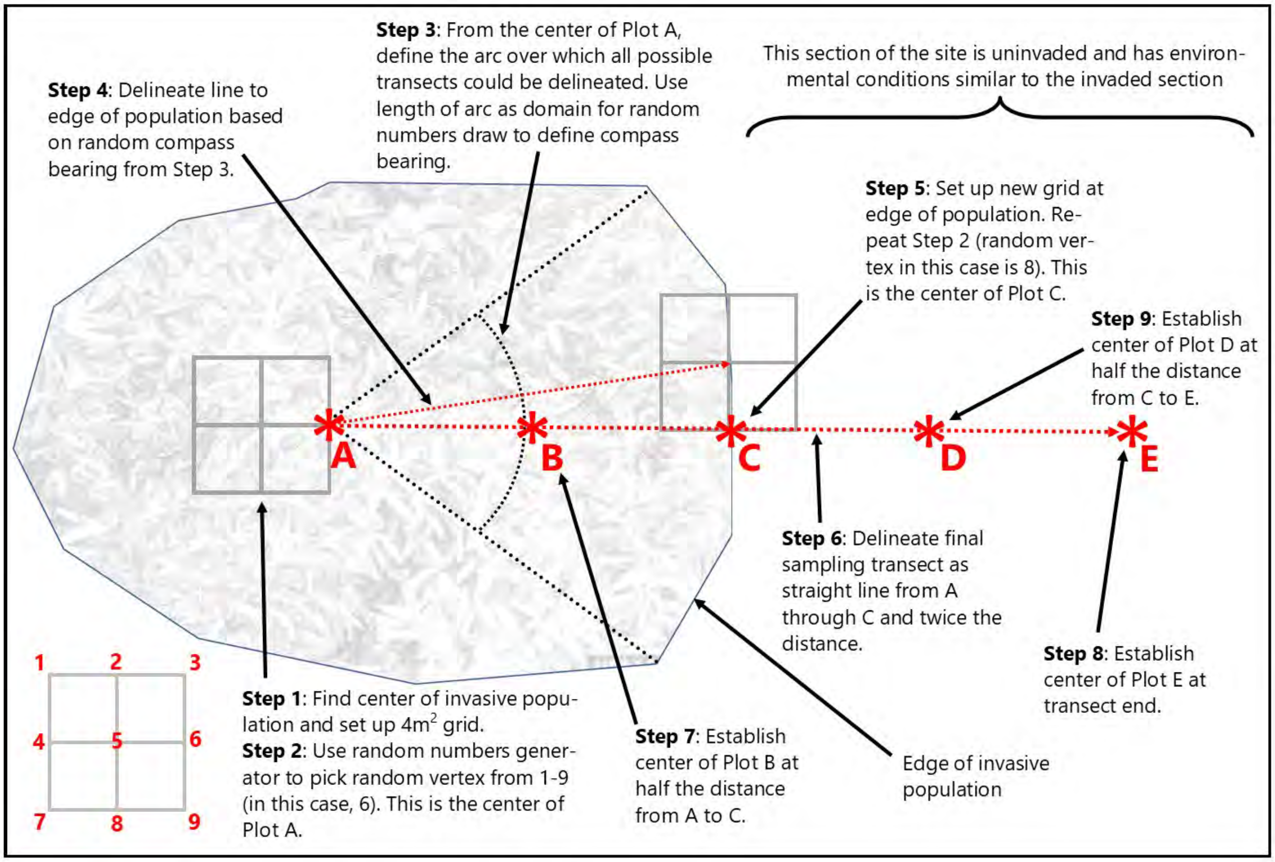
***Figure 3-4** Wetland mitigation study site locations. Red symbols indicate sites in which more than one phase was sampled; green symbols represent single-phase sample sites.*

Virginia (Figure 3-4), with the northernmost site in Loudoun County and the southernmost in Southampton County. The four main riverine watersheds in Virginia – Potomac, Rappahannock, York, and James – were all included within the scope of the study, as well as the Nottoway River in southeastern Virginia. Most sites were either mitigation banks or in-lieu fee sites (i.e., sites established under the Virginia Aquatic Resources Trust Fund).

3.3 Methods – Wetlands

At each mitigation site, populations of invasive species were sampled along a single transect. Transects consisted of five identical 4m^2 (2m x 2m) vegetation plots, randomly assigned to an area that captured the gradient from completely invaded (i.e., the invasive species was considered dominant, or comprising at least 20% of the overall relative dominance of the community) to uninvaded (i.e., the invasive species was absent or not comprising more than 5% relative dominance).

Transect Configuration and Plot Locations: The randomization procedure for transect/plot layout involved identifying the center of an invasive species population within a given site and establishing a 4m^2 grid with 9 vertices (Figure 3-5). Using a random numbers generator, a random number between 1 and 9 was selected, and its location on the grid was defined as the



Step 1: Find center of invasive population and set up 4m^2 grid.

Step 2: Use random numbers generator to pick random vertex from 1-9 (in this case, 6). This is the center of Plot A.

Step 3: From the center of Plot A, define the arc over which all possible transects could be delineated. Use length of arc as domain for random numbers draw to define compass bearing.

Step 4: Delineate line to edge of population based on random compass bearing from Step 3.

Step 5: Set up new grid at edge of population. Repeat Step 2 (random vertex in this case is 8). This is the center of Plot C.

Step 6: Delineate final sampling transect as straight line from A through C and twice the distance.

Step 7: Establish center of Plot B at half the distance from A to C.

Step 8: Establish center of Plot E at transect end.

Step 9: Establish center of Plot D at half the distance from C to E.

This section of the site is uninvaded and has environmental conditions similar to the invaded section

Figure 3-5 *General layout of wetland study design and transect configuration.*

center of the vegetation plot for the most invaded site (Plot A). From that point, the direction of the transect was initially determined by defining an arc through which all possible transects could be delineated that would lead toward an uninvaded section of the site with similar environmental conditions. The length of this arc was taken as the domain for another random numbers draw, this time with the value representing the compass bearing from the center of Plot A to the edge of the invasive species population. At the edge of the population, another $4m^2$ grid was established and another random vertex was drawn, this one representing the center of Plot C. From this point, a straight line was defined from the center of Plot A to the center of Plot C and then an equivalent distance beyond the edge of the invasive population to delineate the final sampling transect. The center of Plot B ("second most invaded") was then defined at half the distance between Plots A and C. The center of Plot E (uninvaded) was established at the far end of the transect, and the center of Plot D ("second least invaded") was established at half the distance between C and E. This procedure resulted in five plots along the invasion gradient from most invaded (Plot A) to edge of invasion (Plot C) to uninvaded (Plot E) (Figure 3-5). Transect length varied among sites but typically ranged from 50 to 100 meters.

Soil Sampling: The center of each plot was GPS-located in the ESRI-based Collector application for iPad, then a soil sample was taken to a depth of 10cm using a 6cm-diameter soil corer. Soil samples were textured on-site using field methods (Ritchey et al. 2015), then shipped to the Virginia Tech Soil Testing Lab where soil chemical variables were measured with Mehlich extractions for P, K, Ca, Mg, CEC, Zn, Mn, Cu, Fe, and B, and Elementar high-temperature combustion for total values of C and N. Finally, an automated pH analyzer was used to measure pH values of wet samples at a 1:1 soil:water ratio (Maguire and Heckendorn 2019).

Canopy Cover: Canopy cover was measured by taking a skyward, hand-leveled photograph from the center of each plot using a 180-degree hemispheric lens adapter for iPad. Photographs were taken from 1 meter above the ground in *Arthraxon* and *Microstegium* plots, and from 2 meters above the ground in *Typha* plots. These photograph heights allowed us to capture canopy cover skyward of the target organisms while avoiding any potential effects of self-shading. Photographs were analyzed using ImageJ (Rueden et al. 2017) and the package Hemispherical 2.0 (Beckschäfer 2015) to obtain a ratio of open sky to canopy cover (see also Appendix B).

Vegetation Sampling: We quantified vegetation abundance using cover estimates for all species within each of the four $1m^2$ subplots nested in the $4m^2$ plots. Cover estimates were based on a modified Daubenmire cover class scale with midpoints used for analysis (Mueller-Dombois and Ellenberg 1974). The cover classes, with midpoints in parentheses (rounded to the nearest whole integer), included: 0-1% (1%); 1-5% (3%); 5-25% (15%); 25-50% (38%); 50-75% (63%); 75-95% (85%); and, 95-100% (98%). Cover classes were recorded for each species and then averaged across the four $1m^2$ subplots. Identifications of all vascular plants were either obtained onsite or samples were gathered and preserved for later verification. Intact collections were deposited at the College of William & Mary Herbarium (WILLI) following confirmation of identity by a senior botanist. Nomenclature follows Weakley et al. (2020). Native/non-native status was based on Virginia Botanical Associates (2020) and Weakley et al. (2020).

Hydrology: Following transcription of the vegetation data, prevalence index (PI) values were calculated for use as a proxy of relative wetness (hydrology) between wetland sites (Atkinson et al. 1993, Tiner 2017). PI values are calculated from the wetland indicator status values for all species recorded within a plot. Wetland indicator status values are numbers assigned to wetland indicator status codes in accordance with the National Wetland Plantlist (Lichvar et al. 2016). The values include: 1=obligate wetland species (OBL); 2=facultative wetland species (FACW); 3=facultative species (FAC); 4=facultative upland species (FACU); and, 5=obligate upland species (UPL). Each species' indicator status value is multiplied by the relative abundance of that species within the plot then summed to produce a weighted average index between 1 and 5. Plots closer to 1 are considered to have wetter conditions, and plots closer to 5 are drier (Tiner 2017).

Statistical Analysis: Data analysis was completed using R version 4.0.3 (R Core Team 2020) including the packages vegan, Hmisc, and BiodiversityR (Kindt and Coe 2005, Borcard et al. 2018, Harrell et al. 2020, Oksanen et al. 2020). The datasets for each invasive study species were analyzed separately due to expected variation in their relative tolerances for environmental stressors and discrepancies among growth requirements (Zedler and Kercher 2004, Swearingen et al. 2010). Across the invasion gradient, changes in species composition were assessed with the Sørensen similarity index (Mueller-Dombois and Ellenberg 1974), the significance of which was tested via analysis of similarity (ANOSIM) (Kindt and Coe 2005). Floristic Quality Index (FQI) was calculated based on DeBerry and Perry (2015) using the most recent Coefficients of Conservatism (*C*-values) for the Virginia flora (DeBerry 2020b). Community properties were evaluated with species accumulation curves (species richness) and Rényi profiles (species diversity) (Kindt and Coe 2005). The correlation between relative abundance of each invader and variables in the environmental matrix was calculated using the nonparametric Spearman rank-order correlation test. The Spearman test was chosen due to its robustness to deviations from normality, as well as its ability to detect both linear and monotonic relationships, without appreciable loss of statistical power in comparison with parametric tests (Legendre and Legendre 2012).

Canonical Correspondence Analysis (CCA) (ter Braak 1986) was used to evaluate the overall community response to environmental variation along the invasion gradient. Prior to CCA analysis, rare species were removed from the abundance matrix of each dataset due to the outsized influence that rare species have on the X^2 distance used in CCA (Legendre and Gallagher 2001, Peck 2016). Rare species reduction followed the Borcard method, which uses a stepwise approach based on the correspondence analysis (CA) component of CCA to evaluate the effect of progressive species removals (Legendre and Legendre 2012). Final CCA models were chosen with a combination of forward and backward model selection using the *ordistep()* function of vegan, which eliminates environmental variables based on significance of permutation tests in combination with the Akaike Information Criterion (AIC) (Borcard et al. 2018), in addition to variance inflation factor (VIF) to identify and remove highly correlated variables (Legendre and Legendre 2012). This procedure results in a parsimonious model when all environmental variables retained in the model are statistically significant and the adjusted R^2 for the final model doesn't exceed the adjusted R^2 for the global model (global model = all environmental parameters included) (McCune and Grace 2002, Borcard et al. 2018). All permutation tests of significance were set at 1000 iterations. All statistical analyses were evaluated at α = 0.05.

3.4 Results – Wetlands

One hundred ninety-four (194) species were documented in the overall wetland mitigation field study across 23 sites, 34 transects, and 170 plots sampled. A checklist of species encountered is included in Appendix A. Community and environmental data are summarized below for each of the three target invasive species.

3.4.1 Species Composition – Wetlands

***Arthraxon*:** In the *Arthraxon* community dataset, 124 species were sampled from 50 plots along 10 transects. *Arthraxon* comprised 19.5% of the overall relative abundance within the community matrix. Co-dominants[1] included *Leersia oryzoides* (8.2%), *Symphyotrichum racemosum* var. *racemosum* (6.1%), *Juncus effusus* (5.2%), *Salix nigra* (3.8%), *Fraxinus pennsylvanica* (3.6%), *Platanus occidentalis* (2.7%), and *Eleocharis tenuis* var. *tenuis* (2.6%). The Sørensen similarity matrix for the *Arthraxon* dataset showed that community composition was somewhat similar across the invasion gradient (Table 3-1a), with all values close to a similarity cutoff of 0.5 for the index as defined by Mueller-Dombois and Ellenberg (1974). Analysis of similarity (ANOSIM) suggested a weak but statistically significant between-group difference based on permutations (R=0.132, p=0.003). From inspection of the ANOSIM boxplots (Figure 3-6a), nearly all between-group variation was attributable to the A (most invaded) group, but the B (second most invaded), C (moderately invaded), D (second least invaded), and E (uninvaded) groups were strongly aligned with between-group similarity and therefore compositionally similar.

Table 3-1. Sørenson similarity matrices for wetland data sets across the invasion gradient from A (most invaded) to E (uninvaded).

a. *Arthraxon*

	B	C	D	E
A	0.57	0.59	0.67	0.44
B		0.61	0.60	0.57
C			0.56	0.46
D				0.50

b. *Microsteg.*

	B	C	D	E
A	0.48	0.56	0.52	0.58
B		0.48	0.64	0.50
C			0.52	0.51
D				0.56

c. *Typha*

	B	C	D	E
A	0.65	0.49	0.64	0.57
B		0.51	0.57	0.54
C			0.56	0.57
D				0.70

***Microstegium* (wetlands):** The *Microstegium* wetland community dataset included 116 species sampled from 50

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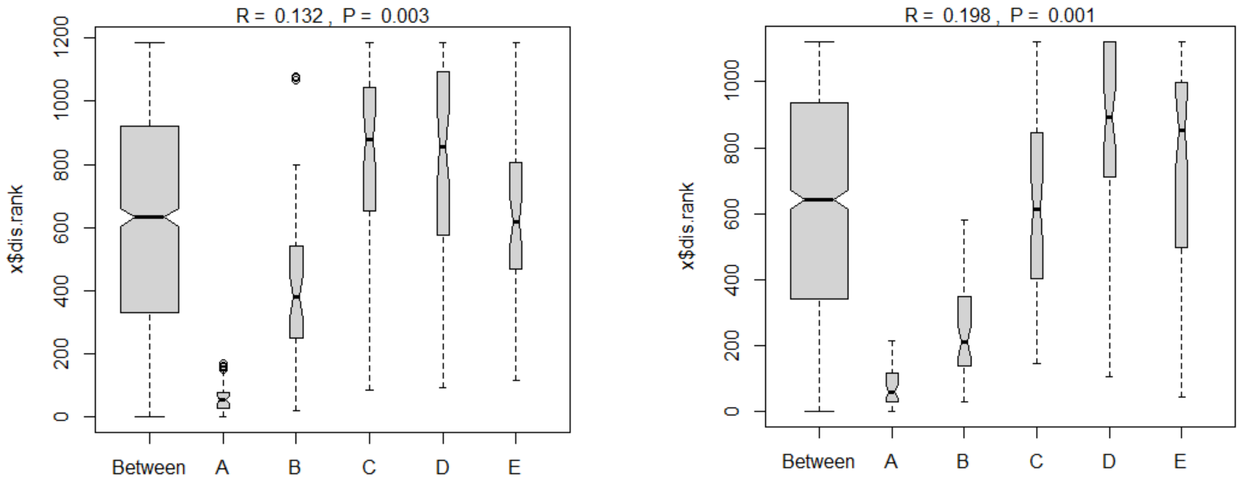
[1] Dominants calculated using the 50/20 rule (Tiner 2017).

plots across 10 transects. *Microstegium* comprised 20.6% of the overall relative abundance within the community matrix. Co-dominants included *Acer saccharinum* (7.7%), *Scirpus cyperinus* (5.9%), *Fraxinus pennsylvanica* (5.7%), *Pinus taeda* (5.4%), *Betula nigra* (4.4%), and *Juncus effusus* (3.7%). As with the *Arthraxon* analysis, the *Microstegium* Sørensen matrix showed marginal compositional similarity across the invasion gradient (Table 3-1b). ANOSIM results demonstrated a weak but statistically significant between-group difference (R=0.198, p=0.001), and boxplots indicated that this difference was due to the invaded groups (A and B), with C, D, and E groups compositionally similar (Figure 3-6b).

***Typha*:** The *Typha* community matrix included 106 species sampled from 70 plots across 14 transects. *Typha* accounted for 19.5% of the overall relative abundance, with co-dominants *Persicaria hydropiperoides* (11.6%), *Juncus effusus* (10.8%), *Leersia oryzoides* (7.7%), and *Scirpus cyperinus* (4.9%). As above, the *Typha* community matrix showed marginal similarity in species composition across the invasion gradient based on Sørensen index values (Table 3-1c). ANOSIM results showed a weak but significant between-group variation (R=0.09, p=0.003), and boxplots indicated that nearly all between-group variation was due to the most invaded group (A), with the remaining groups showing overlap and compositional similarity (Figure 3-6c).

a. *Arthraxon* ANOSIM boxplot.

b. *Microstegium* ANOSIM boxplot (wetlands).



c. *Typha* ANOSIM boxplot

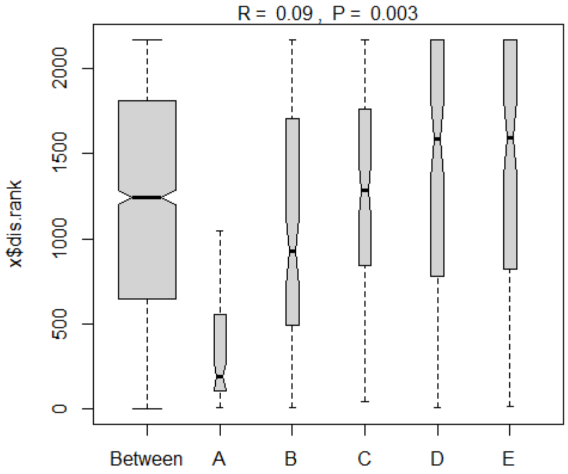


Figure 3-6 *ANOSIM boxplots for the wetland datasets showing distribution of compositional similarity among groups across the invasion gradient from most invaded (A) to uninvaded (E). For each dataset, differences in species composition from the ANOSIM statistic are attributed to groups A and B* (Microstegium) *or group A only* (Arthraxon, Typha), *with moderately invaded (C) sites showing compositional affinity to the uninvaded end of the gradient and strong overlap with between-group similarity. Boxplot width is proportional to number of observations per group ("Between" being the largest as it includes all plots across groups). Notch corresponds to group median, and whiskers show group distribution (outliers greater than 1.5 times the interquartile range are plotted as points).*

3.4.2 Community Properties – Wetlands

***Arthraxon*:** In the *Arthraxon* community matrix, native species richness peaked at moderate levels of invasion (group C) and no invasion (group E) across the gradient, and FQI was highest at moderate levels of invasion (Table 3-2). These results accord with species accumulation curves and Rényi diversity profiles, which showed moderately invaded plots (group C) among the highest levels of species richness (Figure 3-7a), and consistently highest in diversity and evenness (Figure 3-7d). It is important to note that in the case of the *Arthraxon* dataset, species richness by itself provided only marginal differentiation among groups along the invasion gradient from A (most invaded) to E (uninvaded), as the accumulation curves for most groups were close and somewhat overlapping (Figure 3-7a). However, the Rényi diversity profiles, which account for species richness, evenness, and diversity, indicate that moderate levels of invasion (C) correspond to the highest levels of these community metrics (Figure 3-7d). All results in the *Arthraxon* dataset confirmed that the highest levels of *Arthraxon* invasion (group A) negatively affected species richness, diversity, and evenness.

***Microstegium* (wetlands):** Similar to *Arthraxon*, native species richness and FQI were highest at moderate levels of invasion for *Microstegium* (Table 3-2). Likewise, species accumulation curves showed a clear pattern of species richness values where moderately invaded plots (group C) corresponded to the highest levels of richness across the dataset (Figure 3-7b). Rényi diversity profiles suggested similar results, although group C diversity values overlapped with group D (second least invaded) and group E (uninvaded) values (Figure 3-7e). These results also confirmed that the highest levels of *Microstegium* invasion (group A) negatively affected community properties.

***Typha*:** As above, the *Typha* community matrix showed highest native species richness and FQI values at moderate levels of invasion (Table 3-2). Species accumulation curves and Rényi profiles for the *Typha* dataset coincided with these results, showing that the moderately invaded group (C) was clearly differentiated as the most species-rich and most diverse along the invasion gradient

Table 3-2. Mean native species richness, FQI, and mean relative abundance of invader across invasion gradient from A (most invaded) to E (uninvaded) on wetland mitigation sites. Moderate invasion (C, red typeface) corresponds to the highest values of native species richness and FQI in the data sets of all three invaders.

Mean Native Species Richness

Invasion Gradient:	A	B	C	D	E
Arthraxon	**8.6**	**11.9**	**12.1**	**9.5**	**12.4**
Microstegium	**5.7**	**8.3**	**9.8**	**8.6**	**8.4**
Typha	**6.1**	**5.4**	**9.0**	**5.9**	**7.2**

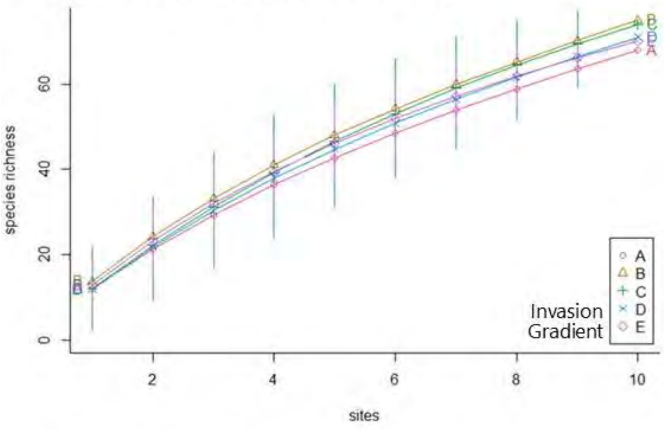
Floristic Quality Index (FQI)

Invasion Gradient:	A	B	C	D	E
Arthraxon	**10.4**	**12.7**	**12.8**	**11.3**	**12.2**
Microstegium	**9.4**	**11.0**	**12.3**	**11.8**	**11.6**
Typha	**8.5**	**7.6**	**10.1**	**9.3**	**9.1**

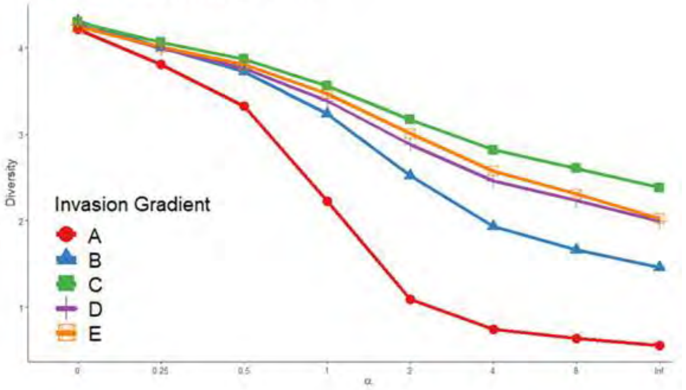
Mean Relative Abundance of Invader

Invasion Gradient:	A	B	C	D	E
Arthraxon	**66.4**	**23.2**	**6.6**	**0.7**	**0.0**
Microstegium	**58.9**	**28.8**	**8.1**	**0.9**	**0.0**
Typha	**58.2**	**26.6**	**5.1**	**0.0**	**0.0**

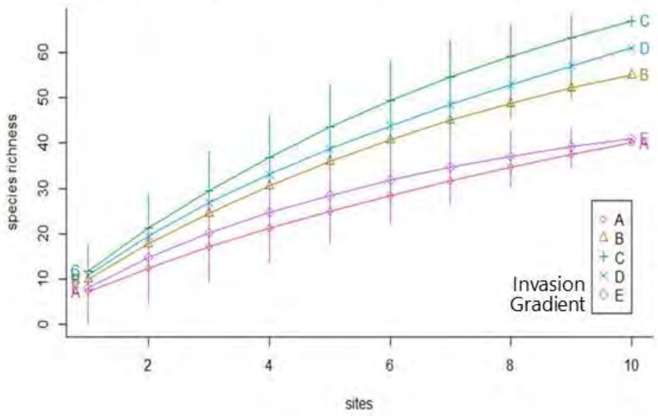
a. *Arthraxon* species accumulation curves.



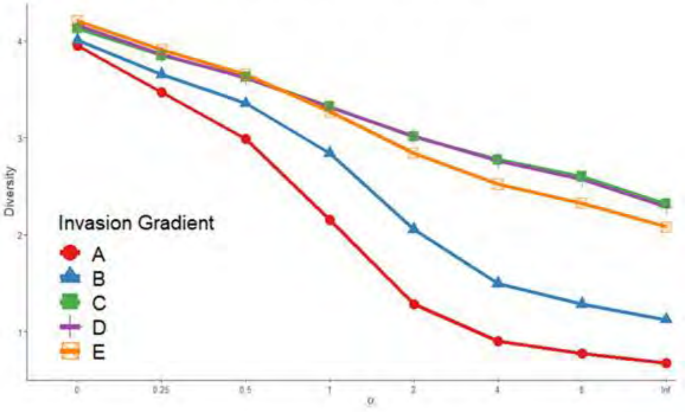
d. *Arthraxon* Rényi profiles.



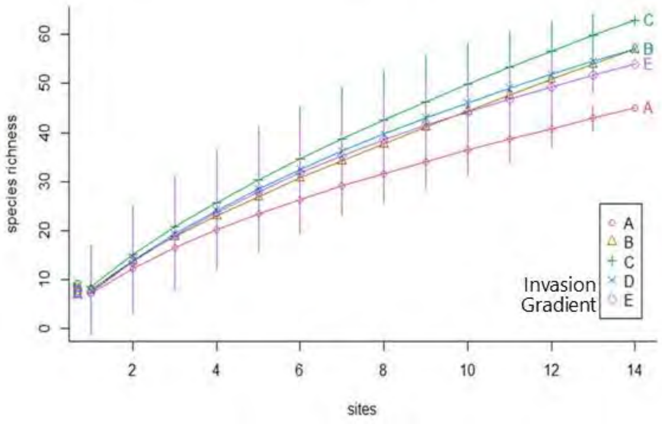
b. *Microstegium* species accumulation curves (wetlands).



e. *Microstegium* Rényi profiles (wetlands).



c. *Typha* species accumulation curves.



f. *Typha* Rényi profiles.

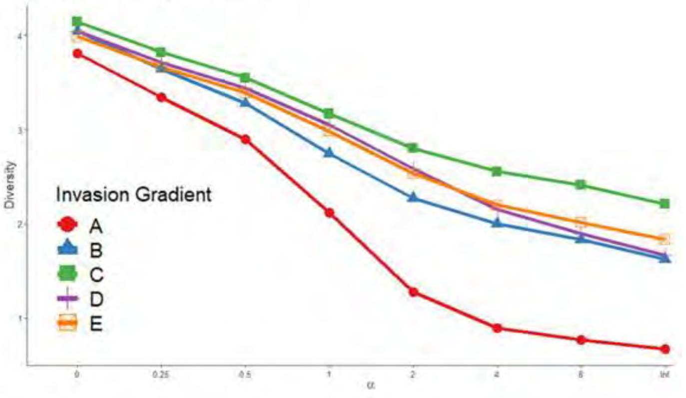


Figure 3-7 *Species accumulation curves and Rényi profiles for the wetland datasets. In each graph, the invasion gradient is represented by the different curves from A (most invaded) to E (uninvaded). The highest curves on the species accumulation and Rényi graphs represent the highest species richness and diversity values, respectively. The x-axis on the Rényi graphs is a unitless diversity ordering scale referred to as alpha (α). It represents species richness (α=0, left hand side), Shannon diversity index (α=1, center), Simpson diversity index (α=2, center), and species evenness (α=inf., right hand side), all of which represent transformed values of those original metrics to make them proportional and thus representable on one graph.*

(Figure 3-7c,f). As with *Arthraxon* and *Microstegium*, the highest levels of *Typha* invasion (group A) corresponded with the lowest levels of these community metrics.

3.4.3 Environmental Variation and Community Modeling – Wetlands

Spearman rank-order correlations were calculated for all variables in the environmental matrix as well as abundance of the target invasive species. Pre-transformation of the data was considered unnecessary because of the non-parametric approach (Legendre and Legendre 2012).[2] After the Spearman analysis, CCA ordinations were completed for the target invasive species, each one being initiated with the reduced community matrix (rare species removed) and the global model of explanatory variables. Model reduction followed the processes outlined in Section 3.3, resulting in a final parsimonious CCA model for each invasive species that included the most significant environmental variables based on permutations and AIC. Compared with the correlations, the final CCA models provided a more robust evaluation of environmental drivers in invaded communities, and ordination biplots included a visualization of the invasion gradient with symbols for each site sized according to invader abundance. Results of these analyses are outlined for the three target species below.

Arthaxon: Spearman results showed *Arthraxon* abundance significantly correlated with canopy cover (r_s=-0.295, p=0.037) and hydrology (r_s= -0.363, p=0.010). In both cases, the relationship was negative, i.e., *Arthraxon* was more prevalent in areas with less canopy cover and relatively drier conditions. No other environmental variables were significantly correlated with *Arthraxon* abundance in the Spearman test. The CCA model for *Arthraxon* was based on a community matrix with 46 dataset-rare species removed, leaving 78 species from the original dataset in the ordination. The final parsimonious CCA model included five environmental variables – hydrology, canopy cover, texture,

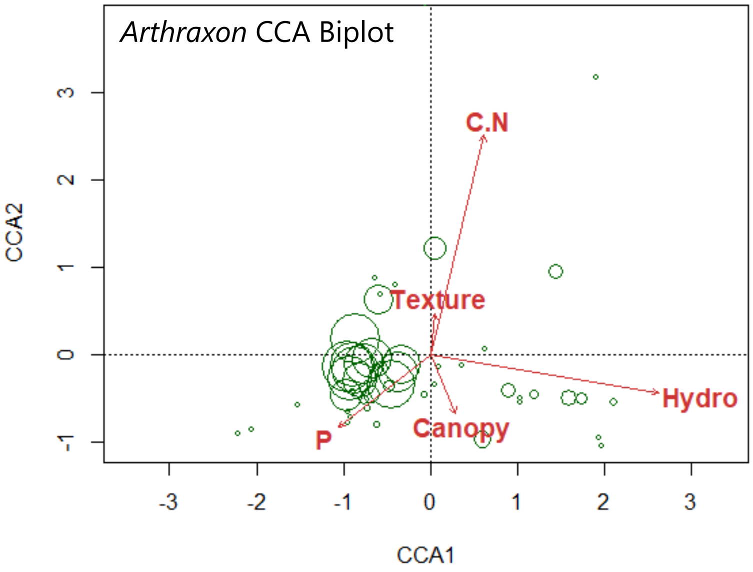


Figure 3-8 *CCA biplot for* Arthraxon *dataset. Red arrows are eigenvectors for environmental variables. Vector length indicates strength of correlation and vector direction indicates positive (pointing toward) or negative (pointing away) relationship to the plots, which are shown as circles with size corresponding to abundance of* Arthraxon *(i.e., larger circles = higher abundance). Plot relationships with environmental vectors are interpreted as perpendicular projections from green circles to red arrows.*

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[2] One exception was prevalence index, which was multiplied by the scalar (-1) to reorient the index for hydrology in a more intuitive direction (i.e., so that higher values corresponded with "wetter", and vice-versa).

carbon:nitrogen ratio (C:N), and phosphorus (P) – which accounted for 23% of the total inertia[3] in the ordination. All environmental factors (eigenvectors) were significant at $p<0.001$ with the exception of P ($p=0.046$). The ordination biplot (Figure 3-8) displays red arrows as eigenvectors for environmental variables, with the vector length corresponding to strength of correlation and vector direction indicating either a positive or negative relationship (e.g., plots aligned in the direction of and projected perpendicularly to an arrow are positively correlated with that environmental variable, and vice-versa). Circles on the biplot represent plots, and circle size corresponds to the absolute abundance of *Arthraxon* within that plot (i.e., larger circles have higher abundance values). The first two ordination axes explained over 53% of the variation in the CCA and thus were retained for the biplot. As Figure 3-8 shows, hydrology and C:N were strong environmental factors that appeared negatively correlated with higher *Arthraxon* abundance, while P appeared positively correlated. Texture was less important as an explanatory variable in the first two axes of the ordination, but based on the eigenvector direction texture was negatively correlated with *Arthraxon* abundance (i.e., texture values in the dataset were arranged on an ordinal scale from fine to coarse, so plots with higher *Arthraxon* abundance tended to be associated with lower texture values and, therefore, finer textured soils). Finally, increasing canopy cover appeared to be more aligned with plots that had low abundance values for *Arthraxon*.

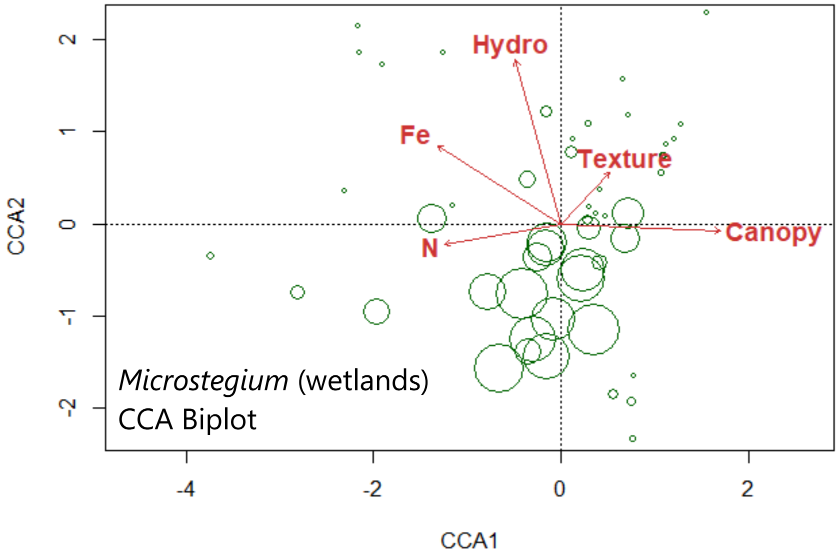

Figure 3-9 *CCA biplot for* Microstegium *(wetlands) dataset. See* Arthraxon *text and Figure 3-8 caption for notes on interpretation.*

***Microstegium* (wetlands):** Spearman results indicated that *Microstegium* abundance in the wetland dataset was positively correlated with cation exchange capacity (CEC) ($r_s=0.337$, $p=0.017$) and negatively correlated with hydrology ($r_s=-0.602$, $p<<0.001$). For the CCA analysis, the *Microstegium* community matrix was reduced by 41 dataset-rare species, leaving 75 species from the original dataset in the ordination. The final parsimonious *Microstegium* CCA model included five environmental variables – hydrology, canopy cover, texture, nitrogen (N), and iron (Fe) – which accounted for 22% of the total inertia in the ordination. All environmental factors (eigenvectors) were significant at $p<0.001$ with the exception of Fe ($p=0.005$). The first two ordination axes displayed in Figure 3-9 explained over 51% of the CCA

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[3] *Inertia* can be thought of as the total amount of variance in the model. In ordination approaches like CCA, "constrained" inertia represents the amount of variance explained by the environmental variables. For multivariate ecological data, values +/-20% like those reported here are common (McCune and Grace 2002, Borcard et al. 2018).

variation. As with *Arthraxon*, hydrology, canopy cover, and texture were negatively correlated with *Microstegium* abundance. The other two important factors, N and Fe, showed positive and negative relationships with *Microstegium* abundance, respectively.

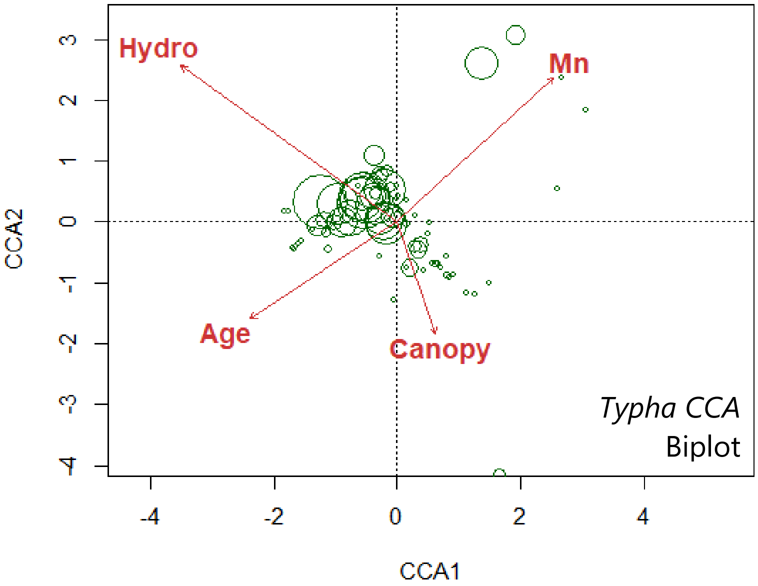

Figure 3-10 *CCA biplot for* Typha *dataset. See* Arthraxon *text and Figure 3-8 caption for notes on interpretation.*

Typha: Spearman correlations showed that hydrology was positively correlated with *Typha* abundance (r_s=0.374, *p*=0.001). No other environmental variables were significantly related to *Typha* in the correlation matrix. The CCA analysis used a *Typha* community matrix reduced by 27 dataset-rare species, leaving 79 species from the original dataset. The final parsimonious *Typha* CCA model included four environmental variables – hydrology, canopy cover, site age, and manganese (Mn) – which accounted for 16% of the total inertia in the ordination. All environmental factors were significant in the model at *p*<0.001 except canopy cover (*p*=0.003). The first two ordination axes explained 65% of the CCA variation. As Figure 3-10 demonstrates, hydrology and canopy cover were important factors in the analysis, and canopy cover was negatively correlated with *Typha* abundance similar to the other two wetland invaders. However, unlike *Arthraxon* and *Microstegium*, hydrology was *positively* associated with the invasion gradient, as indicated by the coalignment between the hydrology eigenvector and the plots where *Typha* was most dominant. Finally, site age and Mn were identified as important factors in the parsimonious model, with the former being positively associated with the invasion gradient and the latter antagonistic.

3.5 Discussion – Wetlands

The body of ecological research on invasive species in wetlands supports the notion that invaders preempt space that would otherwise be occupied by native species, thereby reducing wetland ecosystem function and biological diversity (Galatowitsch et al. 1999, Lavergne and Molofsky 2004, Zedler and Kercher 2004). Although this phenomenon has only been studied tangentially on compensatory wetland mitigation sites (e.g., Matthews and Endress 2008, Ahn and Dee 2011, DeBerry and Perry 2012), one can derive similar conclusions from those resources. This phase of our study was focused explicitly on the invasion gradient in compensatory wetland mitigation, with the intent of clarifying community properties and environmental factors related to some of the most common invasive species being managed on mitigation sites in our region. Revisiting the primary objectives of our study, the main questions being addressed on wetland mitigation sites were:

1. How do invasive species impact ecosystem functions related to native plant composition, richness, floristic quality, and diversity on compensatory wetland mitigation sites?
2. Are current invasive species performance standards in wetland mitigation aligned with #1 above?
3. What environmental conditions favor invasion on wetland mitigation sites?
4. With respect to #3, are there best practices that can be implemented on wetland mitigation sites to reduce the risk of invasion?

This discussion focuses on questions #1 and #3 above; #2 and #4 will be addressed in Chapter 5 and Chapter 6, respectively.

3.5.1 Invasion Effects on Composition, FQI, Richness, and Diversity – Wetlands

Composition: One of the most interesting results from this study was that the invasion gradient did not reflect the types of changes in species composition that we would have anticipated based on the invasion literature (Ehrenfeld 2010, Lockwood et al. 2013). It is important to remember that composition just looks at the *identity* of the species present and does not consider numbers of species or their relative abundances, both of which will be addressed below. However, composition has been identified as an important factor in ecosystem function (Hooper and Vitousek 1997) and vegetation development on mitigation sites (DeBerry and Perry 2004, 2012), so attention to this aspect of the community across the invasion gradient is warranted.

Sørensen similarity coefficients were consistently near or above a “rule-of-thumb” threshold of 0.5 for this index across all datasets (Mueller-Dombois and Ellenberg 1974), yet we had expected the pairings between the invaded and uninvaded ends of the gradient to be closer to zero. A similarity index close to zero would have indicated that invasion had changed the composition of species present due to density-dependent effects or habitat modification by the dominant invader, which is not what we found. The index, however, was also not close to one in any of the intergroup pairings (i.e., no evidence of high compositional similarity), so we needed the more rigorous computational analysis of ANOSIM to detect statistical differences that the similarity index by itself might have missed. As the ANOSIM results showed, there was a weak but significant difference between groups, but that difference was attributable to the most invaded plots (A in the case of *Arthraxon* and *Typha*; A and B in the case of *Microstegium*). This result suggests that a “threshold of dominance” needs to be exceeded before *species composition* is affected by the presence of an invader. As Table 3-2 indicates, that threshold could be high for *Arthraxon* and *Typha* (group A relative abundance = 66.4% and 58.2%, respectively), and reasonably high for *Microstegium* (group B relative abundance = 28.8%). We can conclude, therefore, that invasion does reduce ecosystem functions related to species composition on wetland mitigation sites, but perhaps at a higher level of invasion than previously thought. This conclusion accords with studies indicating that *Arthraxon* (Dee and Ahn 2012, White et al. 2020) and *Typha* (Green and Galatowitsch 2001, Atkinson et al. 2005, Perry et al. 2009) do not impact species composition when present at intermediate levels of abundance.

Irrespective of the dominance threshold concepts noted above, one clear result from our analysis is that *moderate levels of invasion do not change species composition* on wetland mitigation sites. In all cases, group C (moderately invaded) was compositionally similar to groups D and E (low/no invasion). This suggests that moderate levels of invasion (ca. 5-10%) do not preclude other species from "participating" in the community.

Richness, FQI, and Diversity: Species richness and diversity are commonly thought of as intrinsic indicators of ecosystem function, in that higher richness and diversity values generally coincide with other important properties such as habitat complexity and ecosystem resiliency (Huston 1994, Gunderson 2000). Species richness measures the total number of species present irrespective of identity (Kindt and Coe 2005), so it is qualitatively different than species composition. Unlike diversity, richness does not account for the relative abundances of species, but it can be conveniently subdivided into subsets or categories such as *native species richness*. Because native species richness is a metric that is generally regarded as important in evaluating wetland mitigation performance (Matthews and Endress 2008, DeBerry and Perry 2015, Van den Bosch and Matthews 2017), it was a focal point for our research on community properties and invasion. In addition, FQI has been shown to reflect ecosystem function on wetland mitigation sites in Virginia (DeBerry and Perry 2015); therefore, FQI was accorded importance in our analysis.

Our finding that moderate levels of invasion (group C) coincided with maximum native species richness, FQI, and species diversity for all three invaders was unexpected (Table 3-2 and Figure 3-7). Although the literature on plant invasion in wetlands is limited with respect to invasion gradients, from the information that is available (e.g., Galatowitsch et al. 1999, Zedler and Kercher 2004) we would have expected a monotonic increase in richness, FQI, and diversity from the invaded to uninvaded ends of the gradient, not a peak in the middle as found. The reasons for high values of these indicators on the fringes of invasive populations are not clear, but we suspect that localized stress-disturbance dynamics from environmental variation combine to keep more "players in the game" at intermediate levels of invasion. As discussed in Chapter 2, disturbance is a factor on nearly all wetland mitigation sites given the nature of the activities that are typically used to modify landforms and augment hydrology regimes (DeBerry et al. 2004). Although difficult to study directly, there are likely localized "disturbance gradients" that coincide with effects from construction or management practices, e.g., staging areas and haul roads can result in increased soil compaction, stormwater discharge points can increase sedimentation and nutrient availability, etc. If these types of localized phenomena were present and able to be diagnosed on our sites, then the arrival and establishment of invaders could have been predicted and even pinpointed based on the literature (Brooks et al. 2005, Bradley et al. 2010). We will discuss environmental factors further in the next section, but it is tempting to view the hump-shaped relationship between floristic quality indicators and the center of the invasion gradient as a localized expression of the intermediate disturbance hypothesis, i.e., that species richness and diversity are maximized at intermediate levels of disturbance (Connell 1978, Hobbs and Huenneke 1992), or a variant thereof that includes the interposition of stress and disturbance (Alpert et al. 2000; see Figure 2-1).

Regardless of ultimate cause, it is clear from our results that moderate levels of invasion coincide with high levels of native richness, diversity, and floristic quality. We can conclude, then, that the presence of invasive species on wetland mitigation sites does affect ecosystem functions related to species richness, diversity, and floristic quality, but it only *reduces* these functions at higher levels of invasion. This means that "low threshold" invasive species performance standards, i.e., setting very low tolerances for invasive species performance like 5%, are not advisable based on our results. These concepts will be discussed further in Chapter 5.

3.5.2 Environmental Drivers of Plant Invasion – Wetlands

Along the invasion gradients sampled in this study, variables relating to soils, hydrology, and light availability (canopy cover) all emerged as drivers of plant community structure on wetland mitigation sites. Of these drivers, hydrology and canopy cover persistently showed a strong relationship with invasive species prevalence. As is common in analyses of complex ecological data, no single environmental variable materialized as the most important, although hydrology did show strong correlation with the abundance of all three invaders in both the Spearman analysis and the CCA ordinations (Figures 3-8 through 3-10). This is not surprising given the importance of hydrology in structuring wetland plant communities (van der Valk 1981), and from our modeling it is evident that hydrology works synergistically with other environmental factors to influence community dynamics along the invasion gradients on our wetland sites. These factors are discussed for each invader below.

Arthraxon**:** The monotonic relationships between *Arthraxon* abundance and both hydrology and canopy cover were expected. *Arthraxon* is not a shade-tolerant grass (White et al. 2020), so the significant negative correlation with canopy cover was consistent with other studies. Likewise, although *Arthraxon* occurs across a broad range of moisture conditions, we consistently observed its distribution along the edges of wetlands where microtopography raised the relative elevation of the invaded area and created drier microhabitats. Following the discussion of "disturbance gradients" above, dense populations of *Arthraxon* on our sites may also have been associated with localized disturbance conditions, which could have been secondarily associated with soil texture. Soil mixing and removal can modify texture (Petru et al. 2013), and we believe that there is evidence for an effect on our sites based on the negative relationship between texture and *Arthraxon* abundance in the CCA model (e.g., *Arthraxon* was associated with finer textured soils; Figure 3-8). CCA modeling also showed that bioavailable P was positively correlated with *Arthraxon* abundance (Figure 3-8), and this result is consistent with the other wetland studies that have found P availability important in regulating invasive populations (Chiang et al. 2000, Woo and Zedler 2002), as well as the potential for a P-limiting condition to be attenuated by the chemical reduction sequence in developing wetland soils (see discussion under *Microstegium* below).

Finally, the antagonistic relationship between C:N and *Arthraxon* abundance was not expected, but is consistent with recent research on plant invasion. Conceptually, a high C:N condition would stimulate increased microbial activity, and as microbes oxidize low-nitrogen organic substrates for energy additional nitrogen sources will be required for protein synthesis, thus depleting nitrogen from the soil and causing an N-limitation (Iannone et al. 2008). Most invaders do not compete well under nutrient limitation (Bedford et al. 1999, Olde Venterink et al. 2003, Perry et al. 2004), so the negative correlation here is plausible. High C:N soil amendments have recently been reviewed as a potential invasive species control mechanism on some wetland invaders (Iannone et al. 2008, Hazelton et al. 2014).

***Microstegium* (wetlands):** As with *Arthraxon*, the significant negative correlation between *Microstegium* abundance and hydrology was anticipated. Although *Microstegium* tolerates periodic flooding (Touchette and Romanello 2010), in wetland environments it tends to inhabit moist, well-drained soils of floodplains and wetland edges (Warren et al. 2011) and does not appear to survive under long-term inundation (Tu 2000, Nord et al. 2010). The significant positive relationship between CEC and *Microstegium* abundance in the Spearman correlation analysis was not expected; however, it is supported by results of other studies in wetland habitats (Barden 1987; but see Gibson et al. 2002 for results in uplands) and coincides with a high disturbance/high resource availability model for *Microstegium* invasion (Nord et al. 2010, Warren et al. 2011) in that CEC tends to be positively related to soil fertility (Brady and Weil 2008). We suspect that the negative relationship between soil texture and *Microstegium* abundance in the CCA model (Figure 3-9) could be related to the CEC gradient as finer-textured soils would support an increase in cation adsorption sites, although we also suspect that texture could be related to localized disturbance gradients as discussed for *Arthraxon* above. The positive relationship between soil N and *Microstegium* abundance shown in the CCA analysis (Figure 3-9) is consistent with the findings of other wetland studies (DeMeester and Richter 2010a, Warren et al. 2011) and may reflect the positive feedback between enhanced N-uptake rates and increased nitrification rates under influence of a dominant *Microstegium* population (Ehrenfeld 2003, Kourtev et al. 2003).

Of interest is the negative relationship between *Microstegium* abundance and canopy cover in the CCA analysis (Figure 3-9), which seems counterintuitive given the documented shade-tolerance of this invader (Horton and Neufield 1998). However, in most studies *Microstegium* has been shown to have a positive relationship with available light (Gibson et al. 2002, Nord et al. 2010, Schramm and Ehrenfeld 2010, Warren et al. 2011), so it ends up being the *relative* amount of light across the invasion gradient that is most important. *Microstegium* is competitive in lower light conditions due in part to the reduction of other herbaceous competitors imposed by shade (Oswalt et al. 2007), but also to superior photosynthetic efficiency when light becomes available (Horton and Neufeld 1998). On forested mitigation sites, increasing canopy closure results in a reduction in shade-intolerant herbaceous species (DeBerry and Perry 2012), and this can create a more suitable competitive environment for *Microstegium* as long as light levels are *high enough* to promote expansion (Gibson et al. 2002, Warren et al. 2011).

We believe that the negative relationship between Fe and *Microstegium* abundance in the CCA model is an indirect indication of bioavailable P, which could be explained by the soil reduction sequence on developing wetland sites (DeBerry and Perry 2015). Although soil redox potential does not directly affect P transformations, an indirect effect may occur in the presence of metal oxides such as iron, manganese, and aluminum oxide, which immobilize otherwise bioavailable P by chemical precipitation (Ponnamperuma 1972, Mohanty and Dash 1982). As anoxia proceeds in saturated soils, metal oxide-bound P may be released as bioavailable phosphate during the chemical reduction sequence mediated by anaerobic microbial respiration on wetland mitigation sites (Stauffer and Brooks 1997, Hogan et al. 2004), in which case lower levels of Fe would indicate higher levels of P and support the high disturbance/high resource availability discussion above. Conversely, Fe-rich soils have been shown to increase the presence of P-sorption sites in restored wetlands even under reducing conditions (Hogan et al. 2004), suggesting that areas that are high in Fe are likely to be low in bioavailable P. Although we measured soil P directly on our sites, there was a large degree variability in the P levels – over two orders of magnitude difference – and neither data transformations nor outlier analysis were able to rectify the variance in the correlations or the CCA analysis. Although the source of P variability was not clear, but it seemed to be related to a few sites in the dataset and therefore could have been the result of different management techniques.

***Typha*:** The positive correlation between wetland hydrology and *Typha* abundance was expected based the extensive body of literature on this taxon (see Bansal et al. 2019 for a comprehensive review). *Typha* uses pressurized ventilation to induce convective throughflow of gases through its extensive rhizome network, thereby delivering oxygen to the roots and allowing *Typha* to persist in deeper water for prolonged periods of time (Tornbjerg et al. 1994). Further, species in the genus are generally considered shade-intolerant (Bansal et al. 2019), so the negative relationship between canopy cover and *Typha* abundance in our CCA model was not surprising (Figure 3-10).

We did not expect the negative relationship between soil Mn and *Typha* that emerged from the CCA analysis; however, we attribute this to the metal oxide/P-sorption dynamics described under *Microstegium* above. Oxidized Mn functions in much the same way as other metal cations to immobilize bioavailable P (Reddy et al. 2005), so we suspect that the Mn eigenvector in Figure 3-10 is an indirect reflection of P dynamics across the invasion gradient (with the same caveat regarding variability in P measurements noted under the *Microstegium* discussion above).

Finally, that site age was positively related to *Typha* abundance was also unexpected (Figure 3-10). *Typha* has been shown to exhibit autogenic control over its own habitat through detritus accumulation (Vaccaro et al. 2009, Larkin et al. 2012), owing mostly to the refractory nature of *Typha* litter (Álvarez and Bécares 2006). Under this scenario, we expected accumulated litter to increase substrate elevations, create a localized drying effect, and encourage recruitment of other species that could more successfully compete with and eventually eliminate *Typha* over time (Perry et al. 2009, DeBerry and Perry 2012). However, studies on *Typha* invasion have suggested that litter production serves to suppress competition and promote invasion (Vaccaro et al. 2009, Tuchman et al. 2009, Larkin et al. 2012), and on wetland mitigation sites this has been referred as a "autogenic dominance" in reference to plant community development (Noon 1996, DeBerry and Perry 2012). Although several of our *Typha* sites were 15+ years old, autogenic dominance has been noted by others on created wetland sites as old as 20 years in Virginia (Atkinson et al. 2005). If this is happening on our sites, it would explain the positive relationship between *Typha* dominance and age, and presumably indicate that *Typha* populations on younger sites had not yet developed a litter layer sufficient to suppress competitors.

3.6 Summary – Wetlands

The field study for the wetland mitigation component of this project was focused on sampling the vegetation community and environmental variation across the invasion gradient on multiple mitigation sites in the Coastal Plain and Piedmont of Virginia. Our investigation into intrinsic floristic quality parameters on mitigation sites shed new light on how invasive species impact ecosystem functions derived from the vegetation community. The finding that moderate levels of invasion (e.g., between 5-10% relative dominance of invader) did not impact vegetation community functions leads us to believe that current invasive species performance standards (e.g., 5% threshold) may be too low. This topic will be evaluated in detail in Chapter 5.

During the site screening phase, we chose to sample invasive populations where the *apparent* change in environmental conditions was negligible from the invaded end of the gradient to the uninvaded end (e.g., same relative elevations, same apparent hydrology regime, etc.). By doing this, we were able to rephrase our environmental variation question in a more meaningful way: When there is no *apparent* difference in site conditions across an invasion gradient, what "tips the scale" in favor of invasion on wetland mitigation sites? The answers we found – hydrology, light, soil – were not surprising based on the literature. What was surprising was how difficult these differences would have been to diagnose at a reconnaissance level or during routine performance monitoring. This study involved an extensive amount of fieldwork and analysis, and it would be neither practical nor cost-effective for mitigation bankers, consultants, managers, or agency reviewers to expect to exert the same level of effort in diagnosing relative risk of invasion and preemptively managing for it. However, our results do suggest a suite of *proactive* best practices that could be implemented in the early stages of a mitigation project to reduce or eliminate the amount of *retroactive* invasive species remediation required to meet performance standards in later years. These best practices are addressed in Chapter 6.

4 Field Study – Stream Mitigation

The field study for the stream mitigation component of this project was completed over the 2018 and 2019 growing seasons. As with the wetland mitigation study, the goal was to assess plant species invasion in the context of community properties and environmental variation. This was accomplished by establishing transects across invasion gradients of three known stream mitigation invaders in Virginia: *Lespedeza cuneata* (sericea lespedeza), *Lonicera japonica* (Japanese honeysuckle), and *Microstegium vimineum* (Japanese stilt grass). Criteria for species selection and overall sampling strategy were similar to the wetland study described in Chapter 3, with some modifications to account for the different landscape setting. For example, the stream sites were all in upland floodplains adjacent to restored stream channels, so wetland hydrology was not included in the environmental analysis. All other environmental variables in the stream dataset were the same as the wetland study. Canopy cover (light availability) was expected to be an important factor in our analysis (Robertson et al. 1994, Horton and Neufeld 1998, Brandon et al. 2004, Cummings et al. 2007, Warren et al. 2011). Further, because sedimentation and erosion are dynamic in active floodplains, soil texture was anticipated to have an effect (Hupp and Osterkamp 1996, Tickner et al. 2001). Finally, nitrogen was expected to be important due to its influence on invasion gradients in upland environments (Ehrenfeld 2003, 2010), and due to the general importance of riparian zones in nitrogen transformations (Naiman and Décamps 1997).

Fieldwork was completed in two stages: 1) initial site screening, and 2) sampling. During screening, candidate invasive species populations were mapped at potential study sites, and the final detailed sampling was conducted by Doug DeBerry during peak growing season in both years (2018 and 2019) as described below.

4.1 Species Descriptions – Streams

During the initial screening phase, six invasive plant taxa were evaluated for inclusion in the study. The taxa reviewed but excluded from the study were *Arthraxon hispidus* (joint-head grass), *Perilla frutescens* (beefsteak plant), and *Sorghum halepense* (Johnson grass). Although these three species were present on some sites, their distribution and abundance were determined to be insufficient for the study design. The three species retained for the study are described below.

Lespedeza cuneata (Dum.-Cours.) G. Don [Fabaceae] (hereafter "*Lespedeza*"), is a warm season perennial legume from east Asia that grows to a height of 1-2 meters and can form dense populations where established (Cummings et al. 2007; Figure 4-1). It was introduced to the U.S. in the 1890s to be tested for agricultural production and erosion control and has escaped into many natural habitats since its introduction (Swearingen et al. 2010). *Lespedeza* is classified as an invasive species in most states where it has naturalized, and in Virginia it is listed among species with the highest risk of invasion (Heffernan et al. 2014). Like most legumes, *Lespedeza*

Figure 4-1 Lespedeza *dominant on a riparian buffer restoration site in central Virginia.*

benefits from symbiosis with nitrogen-fixing bacteria, enabling it to inhabit nutrient-poor conditions and eroded soils that are typically inhospitable to other plants; however, it is shade-intolerant and does not establish well under a dense canopy (Brandon et al. 2004). With an extensive taproot, *Lespedeza* can survive extended drought conditions in the well-drained soils that it frequents, and it can also survive in a wide range of soil pH conditions from strongly acid to slightly basic (Cummings et al. 2007). Modes of invasion in this species have been studied, with general consensus that *Lespedeza* is able to modify its environment and facilitate localized dominance (Coykendall and Houseman 2014, Reichenborn et al. 2020). The species has a high level of tannins in its tissues, making it unpalatable to wildlife and thus resistant to herbivory (Kalburtji et al. 1999, Eddy et al. 2003). In addition, phenolic compounds emitted into the soil through root exudates or decomposing plant residues have been shown to have allelopathic properties, increasing its competitive ability by altering nutrient uptake efficiency and decreasing germination in other species (Cummings et al. 2007, Reichenborn et al. 2020). *Lespedeza* can reduce nutrient availability for other species through rapid acquisition of essential nutrients (e.g., phosphorus, potassium) and slow release back to the soil via lower rates of decomposition than other plants, a phenomenon mediated by secondary compounds (Kalburtji et al. 1999). All of these properties contribute to localized *Lespedeza* dominance, making the species a nuisance in ecosystem restoration and a particularly problematic invader of riparian restoration projects (Steele et al. 2013, Reichenborn et al. 2020).

Figure 4-2 *Dense* Lonicera *population on a stream mitigation site in Northern Virginia.*

Lonicera japonica Thunb. [Caprifoliaceae] (hereafter “*Lonicera*”) is a perennial vine turning woody with age from eastern Asia that was introduced to the U.S. in the early 1800s as a garden plant (Swearingen et al. 2010). Its ability to escape into natural habitats was first noticed along the Potomac River near Washington DC in 1882, and since that time it has expanded and become invasive in nearly every state east of the Mississippi River (Schierenbeck 2004). In Virginia, it is listed among species that pose the highest risk of invasion (Heffernan et al. 2014). Like most successful invaders, *Lonicera* colonization is greatly enhanced by disturbance (Surrette and Brewer 2008), with seeds being dispersed primarily by birds that forage on the fruits (Swearingen et al. 2010). Although it is a prolific seeder, it does not form persistent seedbanks and thus populations typically expand vegetatively (Hidayati et al. 2000, Shelton and Cain 2002). *Lonicera* inhabits a range of habitat types, soil moistures, and pH conditions, but is generally found in the well-drained, circumneutral pH soils of forests, edges, and clearings. Due to high transpiration rates, it does not tolerate prolonged drought and therefore tends to prefer mesic habitats, making riparian zones, streambanks, and floodplains particularly susceptible to invasion (Miller 2003, Schierenbeck 2004). Although it can live in shady habitats, partial or full shade has been shown to inhibit *Lonicera* growth, and studies consistently highlight its preference for open environments where it can aggressively exploit canopy gaps or clearings, grow in a dense thickets, and effectively smother other species (Robertson et al. 1994, Schierenbeck 2004; Figure 4-2). Consensus on factors contributing to invasiveness in *Lonicera* has not been reached, but all of the following functional traits have been implicated in the literature: allelopathy (Skulman et al. 2004); no known enemies and a strong compensatory response to herbivory (Shierenbeck et al. 1994); autogenic control through modification of environmental conditions and community structure, including nitrogen and carbon pools and the relative availability of nutrients for other species (Ward et al. 2020); and, morphological plasticity of leaf area and growth habit, allowing increased resiliency and expansion under disturbed conditions (Schweitzer and Larson 1999). All of these factors make *Lonicera* a considerable threat to restoration projects, particularly in streams and riparian zones (Sweeney and Czapka 2004).

Microstegium vimineum (hereafter "*Microstegium*") is described in detail under Section 3.1. The factors that contribute to its invasion potential in wetlands are as (if not more) important in streams, floodplains, and riparian zones (Figure 4-3). This is due to the fact that flowing water is a dispersal mechanism for *Microstegium* seeds, so floodwaters in unidirectional lotic systems contribute greatly to its expansion and distribution in watersheds. Much attention has been given to studying *Microstegium* in these types of habitats (Barden 1987, Flory 2010, Warren et al. 2011), and its invasion potential on stream restoration sites is of particular importance in the Mid-Atlantic Region (DeMeester and Richter 2010b).

Figure 4-3 Microstegium *infestation on a stream mitigation site in southeastern Virginia.*

4.2 Site Selection and Study Area – Streams

Representative field sites were chosen from a pool of 30 available sites based on the same selection criteria outlined in Section 3.2. Like the wetland study, some of the stream mitigation sites had different phases that had been completed at different times or in spatially separated areas of a larger mitigation bank. Phases were treated as individual sites for sampling purposes if spatial or temporal separation were deemed sufficient based on the criteria in Section 3.2. Among the stream sites screened in 2018 and 2019, 21 met suitability criteria and were selected for the study. Site ages ranged from 1 to 19 years post-construction and were evenly distributed across the Piedmont (10 sites) and Coastal Plain (11 sites) in Virginia (Figure 4-4), with the northernmost site in Fairfax County and the southernmost in Dinwiddie County. As with the wetland sites, all the main riverine watersheds in Virginia were represented, as was the Nottoway River in southeastern Virginia. Most sites were either mitigation banks or single-user sites. In the latter case, sites had either been restored as a mitigation requirement under a federal and/or state environmental permit, or as a part of a locality-sponsored stormwater management or capital improvement program.

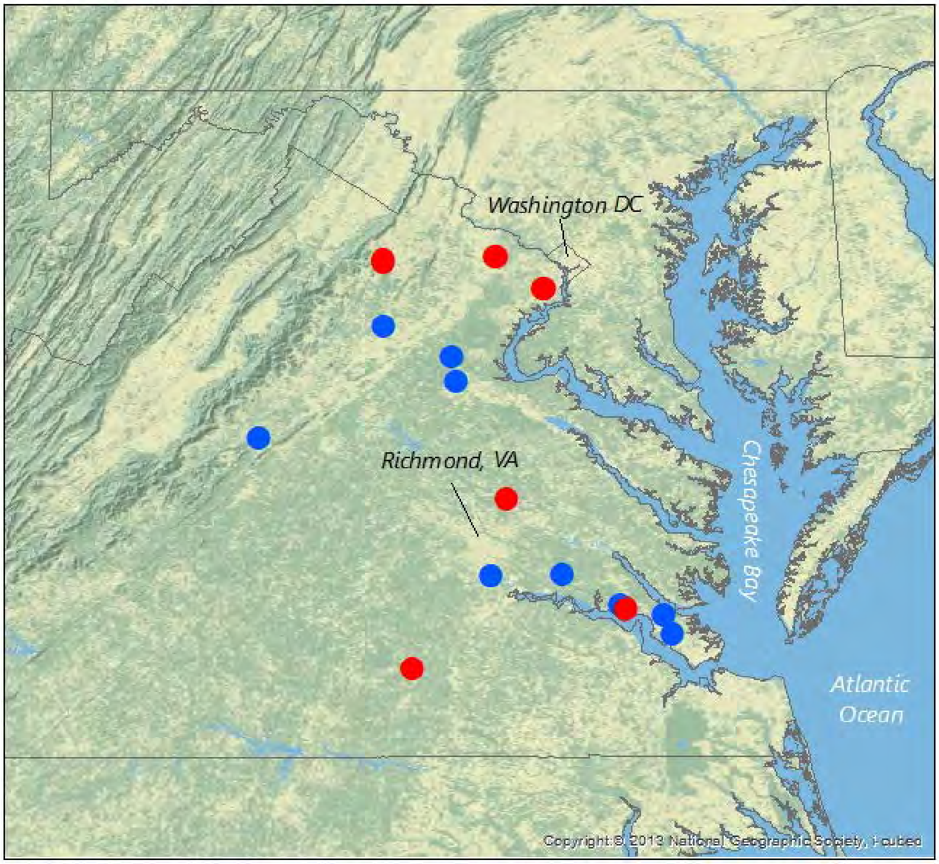

Figure 4-4 *Stream mitigation study site locations. Red symbols indicate sites in which more than one phase was sampled; blue symbols represent single-phase sample sites.*

4.3 Methods – Streams

Methods for the stream field study followed those described in Section 3.3, with modifications as outlined below. The primary differences were in the transect orientation and randomization procedures used to determine plot locations.

Transect Configuration and Plot Locations: Unlike wetland mitigation sites, which are frequently constructed with gradual changes in relative elevation to target specific hydrologic regimes (DeBerry et al. 2004), stream mitigation typically uses approaches that are designed to follow the geomorphology and natural contours of the surrounding landscape (Shields et al. 2003). This means that relative elevations can change abruptly in the cross-sectional dimension of the stream valley from channel to floodplain and from floodplain to riparian buffer. To minimize the amount of *apparent* environmental variation across the invasion gradient, transects were aligned longitudinally with respect to the valley axis and positioned on one side of and parallel to the stream. Each of the five plots on a single transect were established at approximately the same distance away from the top-of-bank of the stream channel, so that the elevation of each plot *relative to the channel* was approximately the same. The purpose for this modification was to ensure that landscape position within the floodplain was similar for each plot along the invasive gradient. In other words, this configuration avoided the scenario of a randomly-defined, straight-line transect with invaded plots down along the channel at one end of the gradient and uninvaded plots up on the ridgeline above the sidewalls of the valley (or *vice-versa*) – an orientation that would have yielded easily detectable but *unmeaningful* environmental variation in the context of study objectives.

The first plot location (Plot A, completely invaded) was determined using the 4m^2 random vertex grid approach described in Section 3.3. The distance from the center of Plot A to the top-of-bank of the stream channel was recorded, and this distance was used to define a new 4m^2 grid centered at the edge of the invasive species population and offset the same distance from the channel bank. A third grid was established using the same offset distance from the stream bank, but this time within the invasive species population and equidistant from the center of Plot A and the center of the invasive edge grid. Using these same distances, the last two 4m^2 grids

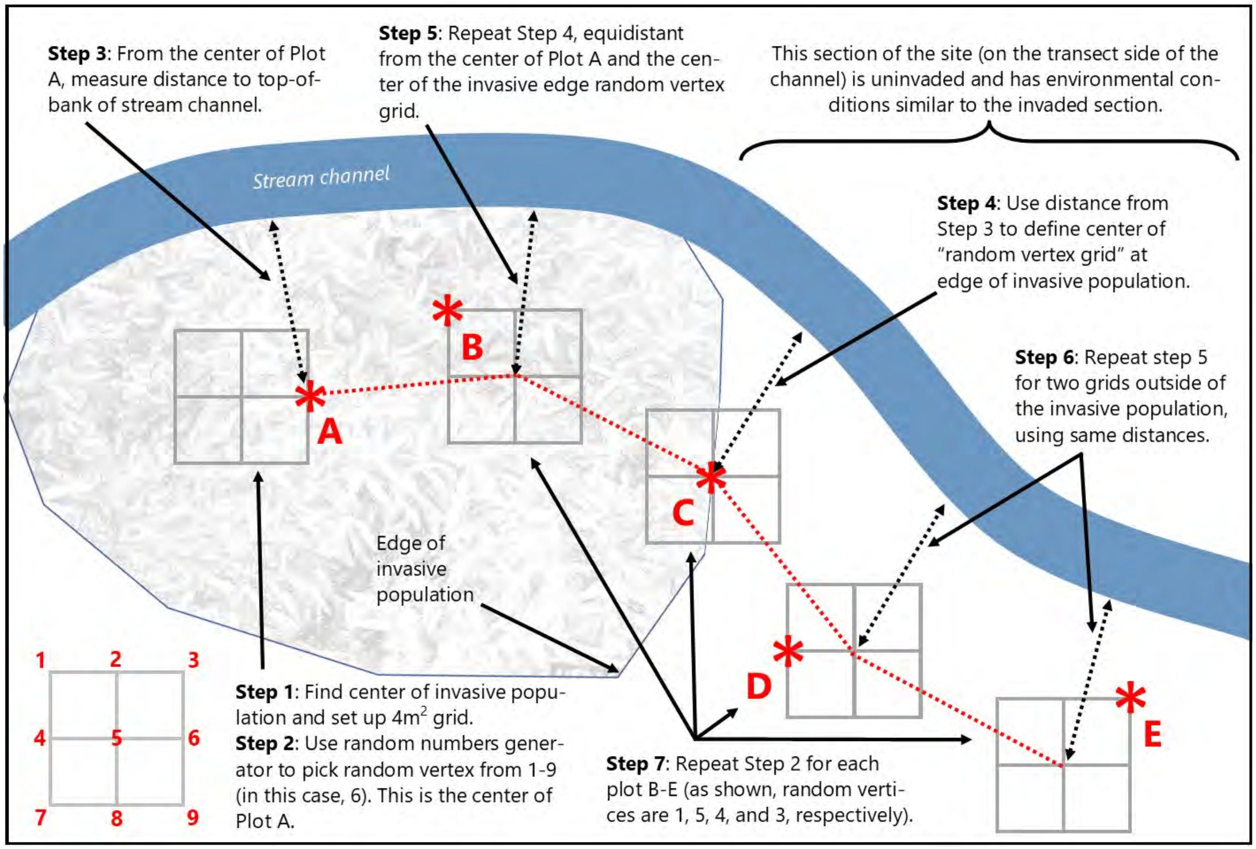

Figure 4-5 *General layout of stream study design and transect configuration.*

were laid out at the uninvaded end of the transect. For each of the remaining plots (B, C, D, and E), random numbers between 1 and 9 were drawn to determine which vertex on each respective grid would be the center of each plot (Figure 4-5). This process ensured that all five plots along the invasion gradient were approximately the same distance from the channel (+/- 1m) with the exact location of each plot being randomized.

Soil sampling, canopy cover, vegetation sampling[1], and statistical analysis methods all followed the same procedures outlined in Section 3.3. Hydrology methods were omitted from the stream study since hydrology was not evaluated as an environmental factor in the upland floodplains of the stream study sites.

4.4 Results – Streams

Two hundred eighty-six (286) species were documented in the overall stream mitigation field study across 21 sites, 29 transects, and 145 plots sampled. A checklist of species encountered is included in Appendix A. Community and environmental data are summarized below for each of the three target invasive species.

4.4.1 Species Composition – Streams

Table 4-1. Sørenson similarity matrices for the stream data sets across the invasion gradient from A (most invaded) to E (uninvaded).

a. *Lespedeza*

	B	C	D	E
A	0.61	0.57	0.58	0.69
B		0.70	0.67	0.66
C			0.67	0.62
D				0.66

b. *Lonicera*

	B	C	D	E
A	0.58	0.56	0.48	0.46
B		0.64	0.52	0.53
C			0.63	0.55
D				0.65

c. *Microsteg.*

	B	C	D	E
A	0.56	0.51	0.49	0.53
B		0.54	0.55	0.50
C			0.62	0.55
D				0.56

***Lespedeza*:** In the *Lespedeza* community dataset, 148 species were sampled from 40 plots along 8 transects. *Lespedeza* comprised 17.5% of the overall relative abundance within the community matrix. Co-dominants[2] included *Sorghastrum nutans* (7.0%), *Carex lurida* (5.9%), *Juncus effusus* (5.7%), *Panicum virgatum* var. *virgatum* (4.3%), *Eupatorium capillifolium* (3.6%), *Symphyotrichum racemosum* var. *racemosum* (3.6%), and *Solidago altissima* var. *altissima* (3.5%). The Sørensen similarity matrix for the *Lespedeza* dataset showed that community composition was somewhat similar across the invasion gradient (Table 4-1a), with all values

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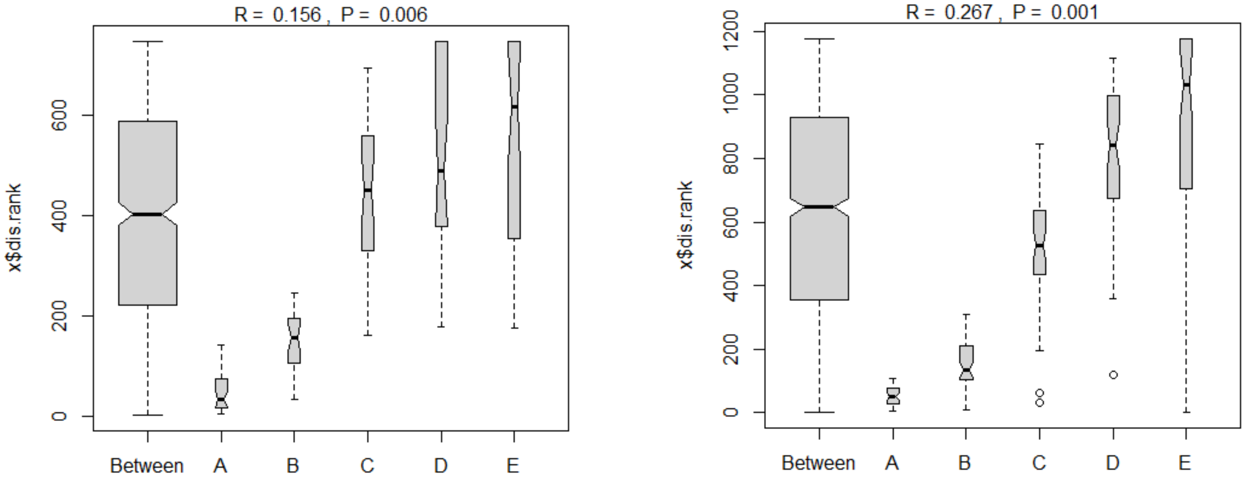
[1] One minor procedural difference: due to the prevalence of mature overstory canopies on the stream sites, cover estimates for tree species in plots were limited to a height of 3m to avoid including abundance for taller trees growing outside of the transect study area. Canopy influence was assumed to be captured by canopy cover data.

[2] Dominants calculated using the 50/20 rule (Tiner 2017).

above a similarity cutoff of 0.5 as defined by Mueller-Dombois and Ellenberg (1974). ANOSIM suggested a weak but statistically significant between-group difference based on permutations (*R*=0.156, *p*=0.006). From inspection of the ANOSIM boxplots (Figure 4-6a), nearly all between-group variation was attributable to the A (most invaded) and B (second most invaded) groups, but C (moderately invaded), D (second least invaded), and E (uninvaded) groups were strongly aligned with between-group similarity and therefore compositionally similar.

***Lonicera*:** The *Lonicera* community dataset included 167 species sampled from 50 plots across 10 transects. *Lonicera* comprised 21.9% of the overall relative abundance within the community matrix. Co-dominants included *Liquidambar styraciflua* (5.1%), *Andropogon virginicus* var.



a. *Lespedeza* ANOSIM boxplot.

b. *Lonicera* ANOSIM boxplot.

c. *Microstegium* ANOSIM boxplot (streams).

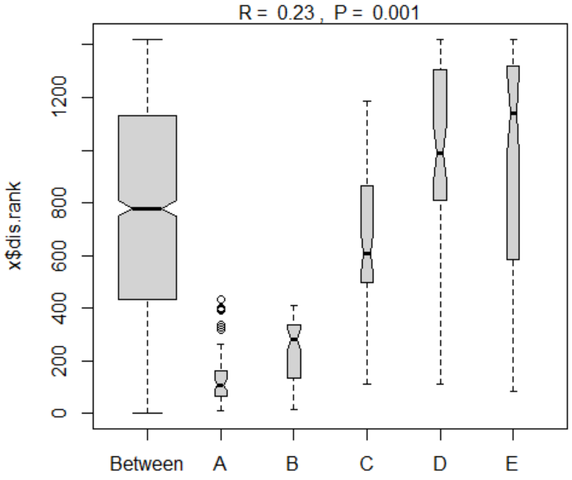


Figure 4-6 *ANOSIM boxplots for the stream datasets showing distribution of compositional similarity among groups across the invasion gradient from most invaded (A) to uninvaded (E). For each dataset, differences in species composition from the ANOSIM statistic are attributed to groups A and B, with moderately invaded (C) sites showing compositional affinity to the uninvaded end of the gradient and strong overlap with between-group similarity. Boxplot width is proportional to number of observations per group ("Between" being the largest as it includes all plots across groups). Notch corresponds to group median, and whiskers show group distribution (outliers greater than 1.5 times the interquartile range are plotted as points).*

virginicus (5.0%), *Rubus pensilvanicus* (4.7%), *Dichanthelium clandestinum* (4.5%), *Juncus effusus* (4.2%), *Parathelypteris noveboracensis* (4.1%), and *Lindera benzoin* (4.1%). As with the *Lespedeza* analysis, the *Lonicera* Sørensen matrix showed marginal compositional similarity across the invasion gradient (Table 4-1b). ANOSIM results demonstrated a weak but statistically significant between-group difference (*R*=0.267, *p*=0.001), and boxplots indicated that this difference was due to the invaded groups (A and B), with C, D, and E groups compositionally similar (Figure 4-6b).

***Microstegium* (streams):** The *Microstegium* stream community matrix included 191 species sampled from 55 plots across 11 transects. *Microstegium* accounted for 30.0% of the overall relative abundance, with co-dominants *Dichanthelium clandestinum* (11.5%), *Solidago altissima* var. *altissima* (4.8%), and *Carex lurida* (4.6%). As above, the *Microstegium* community matrix showed marginal similarity in species composition across the invasion gradient based on Sørensen index values (Table 4-1c). ANOSIM results showed a weak but significant between-group variation (*R*=0.230, *p*=0.001), and boxplots indicated that nearly all between-group variation was due to groups A and B, with the remaining groups showing overlap and compositional similarity (Figure 4-6c).

4.4.2 Community Properties – Wetlands

***Lespedeza*:** In the *Lespedeza* community matrix, native species richness and FQI peaked at moderate levels of invasion (group C) across the gradient (Table 4-2). These results accord with species accumulation curves and Rényi diversity profiles, which showed moderately invaded sites (group C) with the highest levels of species richness (Figure 4-7a), diversity, and evenness (Figure 4-7d). All results in the *Lespedeza* dataset confirm that the highest levels of *Lespedeza* invasion negatively affect community properties.

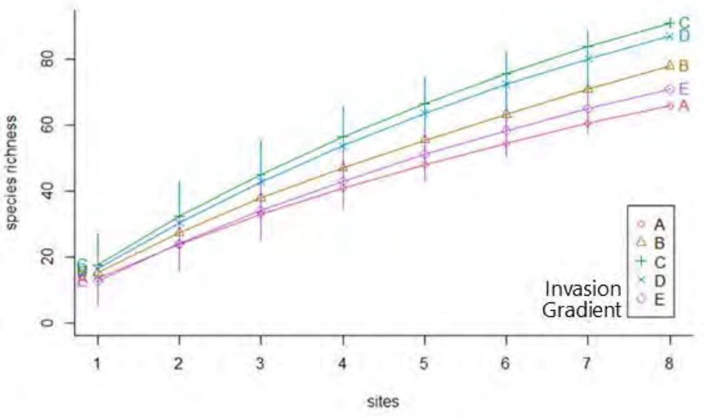
Table 4-2. Mean native species richness, FQI, and mean relative abundance of invader across invasion gradient from A (most invaded) to E (uninvaded) on stream mitigation sites. Moderate invasion (C, red typeface) corresponds to the highest values of native species richness and FQI in the data sets of all three invaders.

Mean Native Species Richness					
Invasion Gradient:	A	B	C	D	E
Lespedeza	**11.4**	**13.3**	**15.1**	**14.4**	**10.6**
Lonicera	**10.3**	**10.7**	**12.4**	**8.6**	**10.1**
Microstegium	**9.8**	**9.5**	**12.1**	**11.9**	**9.0**

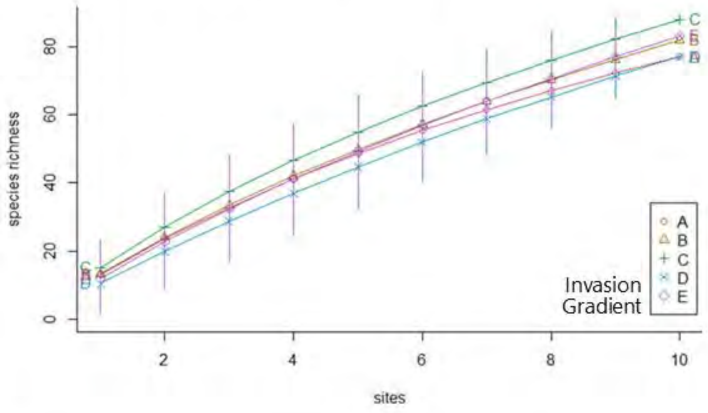
Floristic Quality Index (FQI)					
Invasion Gradient:	A	B	C	D	E
Lespedeza	**11.1**	**12.8**	**14.0**	**11.5**	**12.6**
Lonicera	**10.7**	**12.4**	**13.5**	**10.2**	**11.3**
Microstegium	**11.1**	**11.1**	**12.9**	**12.2**	**10.7**

Mean Relative Abundance of Invader					
Invasion Gradient:	A	B	C	D	E
Lespedeza	**48.4**	**26.1**	**3.2**	**0.6**	**0.2**
Lonicera	**48.6**	**38.5**	**6.5**	**1.1**	**0.4**
Microstegium	**56.9**	**44.1**	**14.0**	**2.6**	**0.3**

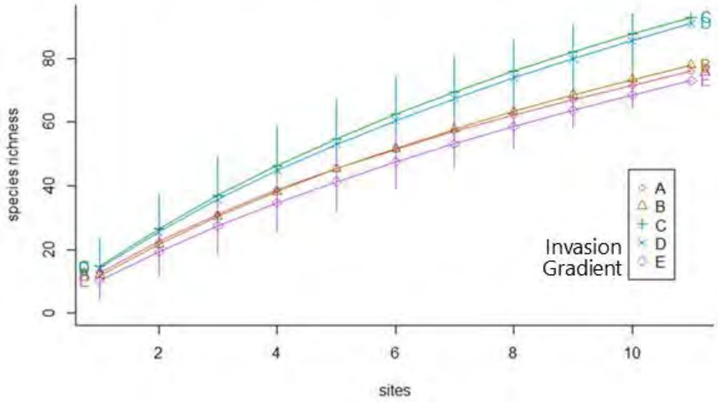
a. *Lespedeza* species accumulation curves.



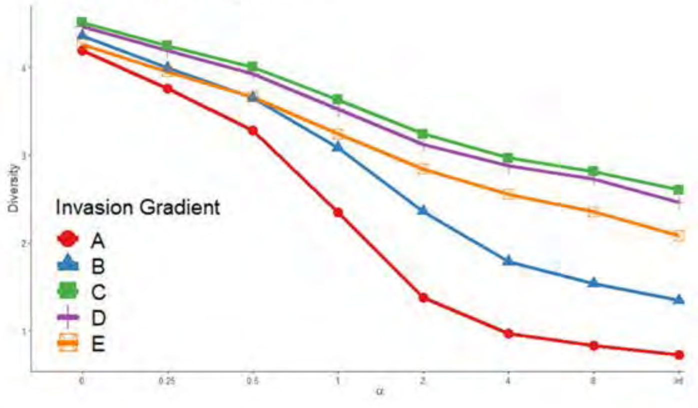
b. *Lonicera* species accumulation curves.



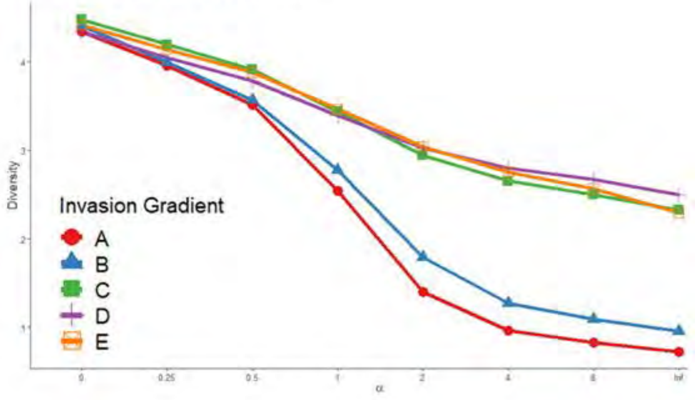
c. *Microstegium* species accumulation curves (streams).



d. *Lespedeza* Rényi profiles.



e. *Lonicera* Rényi profiles.



f. *Microstegium* Rényi profiles (streams).

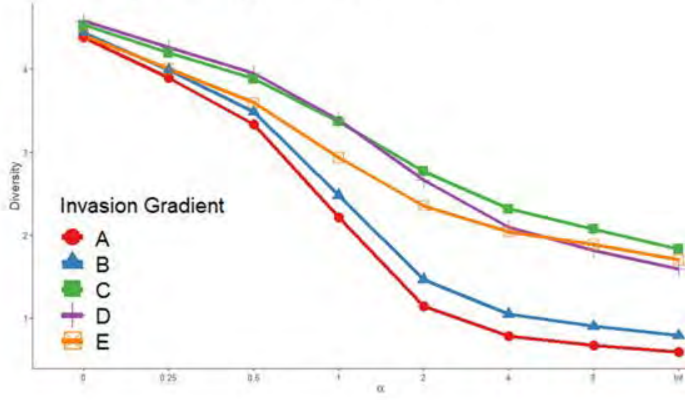


Figure 4-7 *Species accumulation curves and Rényi profiles for the stream datasets. In each graph, the invasion gradient is represented by the different curves from A (most invaded) to E (uninvaded). The highest curves on the species accumulation and Rényi graphs represent the highest species richness and diversity values, respectively. The x-axis on the Rényi graphs is a unitless diversity ordering scale referred to as alpha (α). It represents species richness (α=0, left hand side), Shannon diversity index (α=1, center), Simpson diversity index (α=2, center), and species evenness (α=inf., right hand side), all of which represent transformed values of those original metrics to make them proportional and thus representable on one graph.*

Lonicera: Like *Lespedeza*, native species richness and FQI were highest at moderate levels of invasion for *Lonicera* (Table 4-2). Likewise, species accumulation curves showed a clear pattern of species richness values with moderately invaded sites (group C) corresponding to the highest levels of richness across the dataset (Figure 4-7b). Rényi diversity profiles suggested similar results, although group C diversity values overlapped with group D (second least invaded) and group E (uninvaded) values (Figure 4-7e). These results also confirm that the highest levels of *Lonicera* invasion negatively affect community properties.

***Microstegium* (streams):** As above, the *Microstegium* community matrix showed highest native species richness and FQI values at moderate levels of invasion (group C; Table 4-2). Species accumulation curves and Rényi profiles for the *Microstegium* dataset coincided with these results, showing the moderately invaded group (C) differentiated as the most species-rich and most diverse along the invasion gradient (Figure 4-7c,f). As with *Lespedeza* and *Lonicera*, the highest levels of *Microstegium* invasion corresponded to the lowest levels of these community metrics with the exception of group E (uninvaded), which had the lowest species richness profile.

4.4.3 Environmental Variation and Community Modeling – Streams

For the stream datasets, Spearman rank-order correlations and CCA ordinations were calculated for all variables in the environmental matrix as well as abundance of the target invasive species as described in Section 3.4.3. Results of these analyses are outlined for the three target species below.

Lespedeza: Spearman results showed *Lespedeza* abundance significantly correlated with canopy cover (r_s=-0.690, $p << 0.001$). The relationship was negative, indicating that *Lespedeza* was more prevalent in areas with less canopy cover. No other environmental variables were significantly correlated with *Lespedeza* abundance in the Spearman analysis. The CCA ordination was based on a community matrix with 11 dataset-rare species removed, leaving 148 species from the original matrix in the ordination. The final parsimonious CCA model for *Lespedeza* included five environmental variables – canopy cover, soil texture, nitrogen (N), potassium (K), and pH – which accounted for 25% of the total inertia[3] in the ordination. All environmental factors (eigenvectors) were significant at $p \leq 0.01$ based on permutations. The ordination biplot (Figure 4-8) displays red arrows as eigenvectors for environmental variables, with the vector length corresponding to strength of correlation and vector direction indicating either a positive or negative relationship (e.g., plots aligned in the direction of and projected perpendicularly to an arrow were positively correlated with that environmental variable, and *vice-versa*). Circles on the biplot represent plots, and circle size corresponds to the absolute abundance of *Lespedeza* within that plot (i.e., larger circles have higher abundance values). The first two ordination axes explained over 49% of the variation in the CCA model and thus were retained for the biplot.

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[3] *Inertia* can be thought of as the total amount of variance in the model. In ordination approaches like CCA, "constrained" inertia represents the amount of variance explained by the environmental variables. For multivariate ecological data, values +/-20% like those reported here are common (McCune and Grace 2002, Borcard et al. 2018).

Figure 4-8 shows that, in general, canopy cover, pH, N, and K were negatively related to *Lespedeza* abundance, although there is some variability in the final model. Soil texture appears positively correlated with *Lespedeza* abundance, suggesting that the invader tended to be on sites with coarser textures (i.e., texture values in the dataset were arranged on an ordinal scale from fine to coarse, so higher values corresponded to coarser-textured substrates).

***Lonicera*:** Spearman results indicated that *Lonicera* abundance in the wetland dataset was positively correlated with potassium (K) (r_s=0.298, p=0.035) and negatively correlated with canopy cover (r_s=-0.749, p<<0.001). For the CCA analysis, the *Lonicera* community matrix was reduced by 9 dataset-rare species, leaving 158 species from the original matrix in the ordination. The final parsimonious *Lonicera* CCA model included five environmental variables – canopy cover, texture, N, magnesium (Mg), and iron (Fe) – which accounted for over 20% of the total inertia in the ordination. All environmental factors (eigenvectors) were significant at p<0.01 except texture (p=0.04). The first two ordination axes displayed in Figure 4-9 explained 52% of the CCA variation. As with *Lespedeza*, canopy cover and N were negatively correlated with *Lonicera* abundance, and

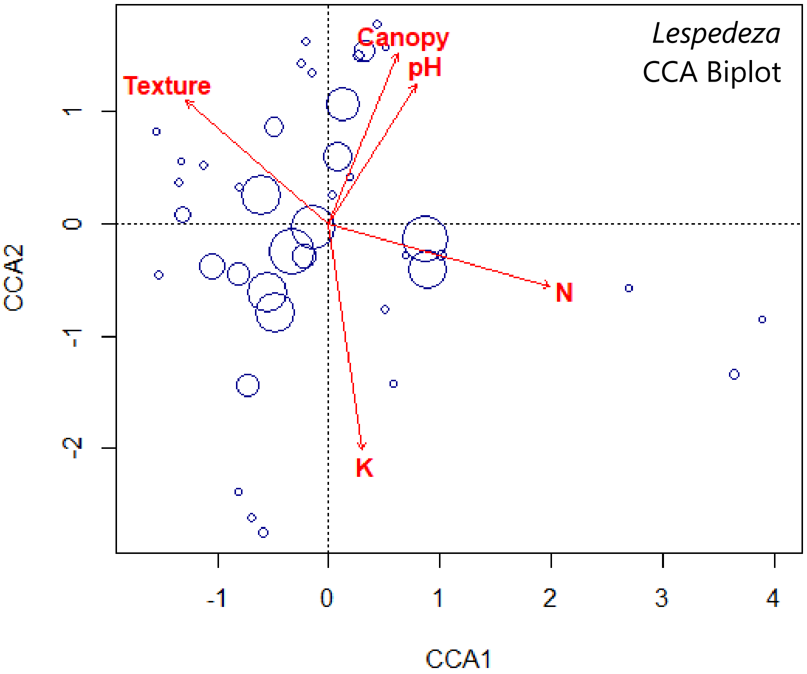

Figure 4-8 *CCA biplot for* Lespedeza *dataset. Red arrows are eigenvectors for environmental variables. Vector length indicates strength of correlation and vector direction indicates positive (pointing toward) or negative (pointing away) relationship to the plots, which are shown as circles with size corresponding to abundance of* Lespedeza *(i.e., larger circles = higher abundance). Plot relationships with environmental vectors are interpreted as perpendicular projections from blue circles to red arrows.*

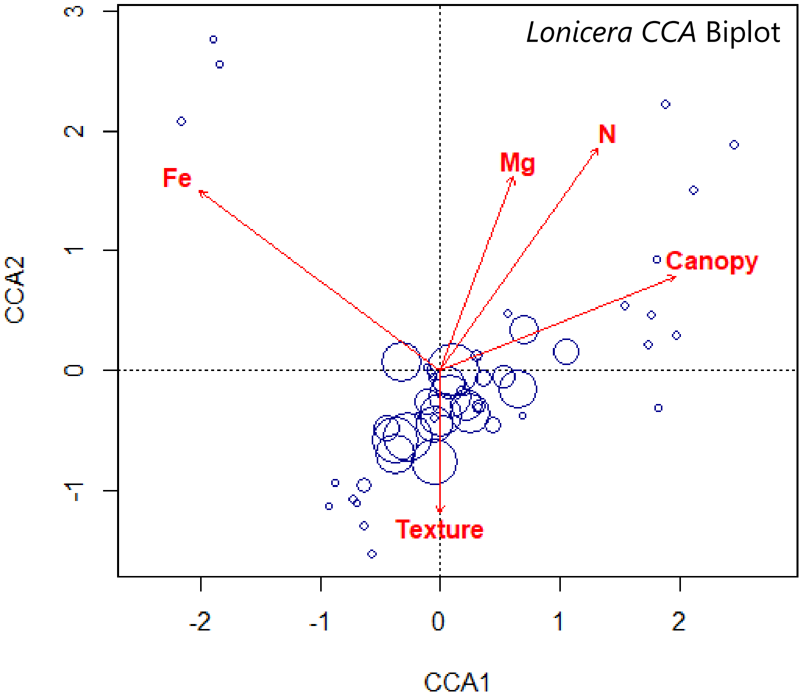

Figure 4-9 *CCA biplot for* Lonicera *dataset. See* Lespedeza *text and Figure 4-8 caption for notes on interpretation.*

texture showed a positive relationship with highly invaded sites. The other two important factors, Mg and Fe, both showed negative relationships with *Lonicera* abundance.

***Microstegium* (streams):** Spearman correlations showed that canopy cover was negatively correlated with *Microstegium* abundance in the stream dataset (r_s=-0.467, p<0.001). No other environmental variables were significantly related to the *Microstegium* invasion gradient in the correlation matrix. The stream CCA analysis for *Microstegium* used a community matrix reduced by 24 dataset-rare species, leaving 167 species from the original matrix. The final parsimonious *Microstegium* CCA model included four environmental variables – canopy cover, N, K, and manganese (Mn) – which accounted for 14% of the total inertia in the ordination. Canopy cover was significant in the model at p<0.001; all other environmental factors were significant at p<0.05. The first two ordination axes explained 57% of the CCA variation. As Figure 4-10 demonstrates, all four environmental variables showed a negative relationship with *Microstegium* abundance in the stream dataset.

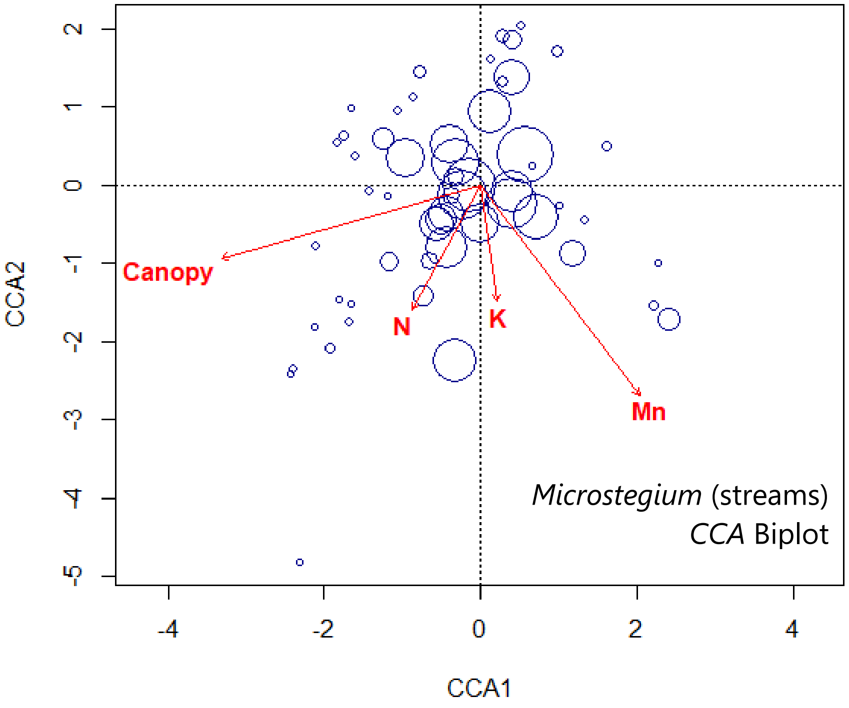

Figure 4-10 *CCA biplot for* Microstegium *(streams) dataset. See* Lespedeza *text and Figure 4-8 caption for notes on interpretation.*

4.5 Discussion – Streams

Stream channels and riparian zones are dynamic environments with open energy cycles (Naiman and Décamps 1997). The environmental drivers that structure plant communities in riparian zones – such as nutrient availability, sediment budgets, flood frequency and duration, and light availability – are all highly variable and subject to the pulsing forces that correspond to prevailing climate conditions and event-driven anomalies such as storms (Hupp and Osterkamp 1996, Hupp 2000). Floodplains are the "melting pots" of organic and inorganic matter that is shed from the upstream catchment, including the propagules of plants that use water or gravity as dispersal vectors in reproduction (Bendix and Hupp 2000). For mitigation practitioners, this makes stream corridors one of the most challenging settings within which to develop planting plans and vegetation management strategies, because the plant community is subject to continuous propagule pressure from upstream sources (Hughes et al. 2005). These factors are all the more challenging in view of biological invasion, because for many invasive plants watersheds and drainage networks are the main conduits of dispersal (Richardson et al. 2007).

Recognizing that most stream restoration projects will only be able to control environmental factors *within* project boundaries, the primary objectives of this study were directly related to the *localized* conditions within a stream mitigation project that correlate with invasion gradients; i.e., the factors over which mitigation designers and managers can have an influence. Within the dynamic equilibrium (*sensu* Shields et al. 2003) ecosystem targets for a stream restoration project, there may be design and management considerations that can then help to build ecosystem resilience and resistance against future invasion (Lockwood et al. 2013). With this in mind, the primary questions being addressed in this phase of the study were the same as those outlined in Section 3.5. Paraphrased here in the context of stream mitigation, they are: 1) How do invasive plants impact community functions? 2) Are current invasive species performance standards appropriate? 3) What are the environmental drivers of invasion? 4) Are there best practices to reduce invasion risk? As with Section 3.5, the following discussion focuses on questions #1 and #3 above; #2 and #4 will be addressed in Chapter 5 and Chapter 6, respectively.

4.5.1 Invasion Effects on Composition, FQI, Richness, and Diversity – Streams

Composition: As interesting as the composition results were for the wetland study (see Section 3.5.1), it is perhaps even more interesting that the same trends were observed in the stream analyses. Sørensen similarity coefficients showed marginal similarity for nearly all inter-group pairings across the invasion gradients in all datasets, but interpretation was limited as most values were not significantly different from 0.5 (Meuller-Dombois and Ellenberg 1974). As with the wetland study, further analysis with the ANOSIM statistic for the stream datasets identified a weak but significant difference between groups was that attributable to the most invaded end of the gradient (groups A and B). Thus for streams, like wetlands, there appears to be a threshold of dominance beyond which *species composition* is affected by the presence of an invader. Table 4-2 shows that this threshold could be relatively high for the target invaders (group B relative abundance = 26.1%, 38.5%, and 44.1% for *Lespedeza*, *Lonicera*, and *Microstegium*, respectively). From these analyses, the conclusions are the same as noted in Section 3.5.1 – invasion impacts species composition at high levels of invasion, but not at moderate or low levels. Like the wetland results, there was compositional similarity between group C (moderately invaded) and groups D and E (low/no invasion) in the stream datasets. Although the range of group C invader abundance was larger for the stream study (3.2% for *Lespedeza* to 14.0% for *Microstegium*), the average condition still suggests that a 5-10% rule-of-thumb definition for "moderate level of invasion" is reasonable based on both the wetland and stream analyses.

Richness, FQI, and Diversity: As with the wetland study, moderate levels of invasion (group C) coincided with maximum native species richness, FQI, and diversity for all three invaders in the stream analyses (Table 4-2 and Figure 4-7). The effects of localized disturbance could be implicated in stream mitigation settings; if so, the open energy cycles (Naiman and Décamps

1997), variability of cross-sectional and longitudinal gradients (Hupp and Osterkamp 1996), and allochthonous influences from watershed inputs (Bendix and Hupp 2000) would perhaps make construction phase diagnosis of localized disturbance – and attendant invasion risk – more challenging in stream mitigation scenarios vs. wetland sites. Regardless, the stress-disturbance dynamic discussion in Section 3.5.1 is plausible for stream sites, and likely even more relevant given the expected return intervals for disturbance-inducing events like floods (Hughes et al. 2005). Likewise, it is equally tempting to view pattern and process in community assembly corresponding to the influences of intermediate disturbance, at least at a local scale within the riparian zone of a stream mitigation project (Biswas and Mallik 2010, Catford et al. 2012). Irrespective of environmental factors (discussed below), the results of the stream study are the same as the wetland study: invasive species do impact ecosystem functions related to species richness, diversity, and floristic quality, but only at higher levels of invasion.

4.5.2 Environmental Drivers of Plant Invasion – Streams

The invasion gradients sampled in this study corresponded to light availability (canopy cover) and soil physiochemical variables, with canopy cover emerging as the most important factor across all datasets. Canopy cover was a significant variable in both the Spearman correlations and the CCA ordinations; thus, light availability exhibits a clear and distinct relationship with vegetation community development and invasive species impacts on stream mitigation sites. The influence of canopy cover and other environmental variables is discussed for each target invader below.

***Lespedeza*:** *Lespedeza* is a shade-intolerant species (Brandon et al. 2004), so the negative correlation between *Lespedeza* abundance and canopy cover in the stream study was not surprising. This result was also reflected in the CCA model (Figure 4-8), which accords with the notion that communities with *Lespedeza* as a dominant species would be more likely to have shade-intolerant associates like *Sorghastrum nutans*, *Panicum virgatum* var. *virgatum*, and *Solidago altissima* var. *altissima*, all of which were co-dominants in the community matrix. The negative relationships between *Lespedeza* abundance and soil N and K were unexpected but could have been predicted from the literature. As an “N-fixing” plant, *Lespedeza* benefits from N subsidies through a symbiotic relationship with *Rhizobium* bacteria in root nodules, which allows *Lespedeza* it to inhabit nutrient-poor soils with low available N pools (Riechenborn et al. 2020). *Lespedeza* has been shown to reduce availability of other macronutrients, including K, by rapid uptake and slow release back to the soil, a phenomenon that is apparently mediated by high levels of phenolic compounds (e.g., tannins) that reduce decomposition rates of its senescent tissues (Kalburtji et al. 1999). In dense *Lespedeza* populations on the stream sites in this study, autogenic control of nutrient availability could explain the K trends observed in the ordination.

The negative relationship between invasion and pH in the CCA model is also consistent with known ecological tolerances for *Lespedeza*, which has been documented in soils with pH as low as 4.0 (Cummings et al. 2007). On stream sites, low pH could also be an indirect reflection of reduced soil nutrient status, as acidic substrates tend to be low in exchangeable bases, N, and P (Mulholland 1992, Brady and Weil 2008). Finally, the positive relationship between *Lespedeza* dominance and coarse-textured substrates in the CCA model could signal localized disturbance in the riparian corridors of the study sites. Deposition of coarse-textured alluvium would be consistent with the type of flood-induced disturbance that could create habitat for a species like *Lespedeza* in a small stream floodplain. Anecdotally, evidence of fresh sediment accretion was noted on transects at several of the stream project sites, the consistency of which was predominantly sand (D. DeBerry, pers. obs.). A substrate like this with a low surface:volume particle ratio would likely also be low in ionic exchange sites for essential nutrients (Brady and Weil 2008), a condition that would be consistent with the types of substrates that *Lespedeza* can exploit based on the above discussion.

Of interest is *Lespedeza*'s relationship to the "high disturbance/high resource availability" model for invasion described in Chapter 2 and in Section 3.5.2. With its ability to invade nutrient-poor soils, at face value *Lespedeza* does not appear to use the "high resource" half of this strategy for dispersal and establishment. However, if the critical resource is light, then *Lespedeza*'s mode of invasion does fit the paradigm because light-limited environments clearly reduce *Lespedeza* abundance along the invasion gradient.

***Lonicera*:** As with *Lespedeza*, the significant negative correlation between *Lonicera* abundance and canopy cover was anticipated and is consistent with *Lonicera*'s aggressive growth response to available light (Robertson et al. 1994, Ward et al. 2020). The positive correlation with K was not expected but is consistent with resource availability models for aggressive species (Craine 2009). Likewise, soil texture was positively related to *Lonicera* abundance in the CCA model (Figure 4-9), which at the scale of the *Lonicera* transects (30-80 meters) most likely signals some type of localized disturbance that is probably related to floodplain deposition. The importance of soil N levels in the CCA model was expected, but the *negative* correlation between N and *Lonicera* abundance was not. Like *Lespedeza*, *Lonicera* has been shown to exhibit autogenic control over soil nutrient pools using different strategies. Morphological plasticity in *Lonicera* facilitates opportunistic expansion of plant modules into favorable microhabitats (Schweitzer and Larson 1999), where fast growing plants can exercise rapid uptake of mobile nutrients like N coupled to slow release back to the environment through modification of labile carbon pools (Ward et al. 2020). Additionally, studies have found allelopathic compounds in *Lonicera* tissues, with demonstrated effects on native species in eastern US forests (Skulman et al. 2004). The "resource hoarding" effects of rapid nutrient uptake and slow release could explain the lower ambient levels of soil N at the invaded end of the *Lonicera* gradient, and the "interference" effects of allelochemicals combined with morphological plasticity for microhabitat exploitation would mean that lowering N pools would have minimal consequences for *Lonicera* itself. If this was occurring on stream sites, it would still be consistent with the high disturbance/high resource concepts discussed above in that microsites exploited by *Lonicera* would have been characterized by both disturbance (habitat opening) and high resource availability (N, light), and only after *Lonicera* expansion and dominance would the relative availability of resources have been affected by autogenic controls.

The two remaining environmental variables that were important in the CCA model – soil Fe and Mg – are probably indirectly related to other factors influencing community structure along the invasion gradient. The most obvious one is the antagonistic relationship between Fe and bioavailable P as described in Section 3.5.2 (see Fe-P discussions under the *Microstegium* and *Typha* headings), which would indirectly indicate a positive relationship between soil P and *Lonicera* abundance based on the reciprocal relationship with Fe in the model (Figure 4-9). The stream sample sites are uplands, so the chemical reduction sequence described for wetland soils would not be relevant to Fe-P dynamics in the stream mitigation sites, but the Fe gradient would still correspond to an increase in P-sorption sites and therefore more chance for P immobilization in Fe-enriched soils. The negative association between Mg and *Lonicera*-dominant sites in the CCA model could be indirectly related to soil texture. In the Mid-Atlantic Region, Mg deficiencies are often linked to coarse-textured soils from which cations are more easily leached (Haering et al. 2015). On *Lonicera* sites, invasion was positively related to soil texture, so the Mg soil status could be a reflection of this Mg-texture antagonism. If so, Mg would be viewed as less of a community driver and more of a secondary indicator in the *Lonicera* CCA analysis. One other possible explanation for Mg gradients is geology: some of the *Lonicera* study sites were located in Triassic shale districts in Virginia that are high in diabase, a source of Mg in soil residuum (VDMME 2020), and based on inspection of the environmental matrix those sites also had higher Mg values. Such mafic substrates (i.e., soils high in Mg and Fe from weathering of diabase) can be important in structuring plant communities in Virginia (Farrell and Ware 1991, Weakley et al. 2020). The effects of source bedrock would be unlikely to emerge as a measurable gradient at the scale of the transects used in this study, but could have been detectable over short distances if the invaded end of the gradient was disturbed by low Mg allochthonous sedimentation in the floodplain as noted above.

***Microstegium*:** As with the other two study species, the negative correlation between *Microstegium* abundance and canopy cover is consistent with the literature. From the discussion in Section 3.5.2, although *Microstegium* exhibits shade tolerance, it aggressively exploits available light and will correspond to relative light availability in forest understories (Gibson et al. 2002, Nord et al. 2010, Warren et al. 2011). The negative relationship between Mn and *Microstegium* dominance in the CCA model (Figure 4-10) was unanticipated but is thought to reflect the metal oxide/P dynamics also described in Section 3.5.2. If so, the negative correlation would relate to greater P availability in *Microstegium*-dominant sites in the same manner as described under *Lonicera* above for Fe-P complexes in upland floodplains.

As with *Lonicera*, the influence of soil N on *Microstegium* community dynamics was anticipated, but the *direction* of influence was not. Although functional traits are much different between the two species, there are similarities in their patterns of resource exploitation and habitat modification. Like *Lonicera*, *Microstegium* rapidly acquires nutrients once it expands into a new area, can modify soil nutrient content (Ehrenfeld et al. 2001), and has been shown to have allelopathic properties in competition experiments (Pisula and Meiners 2010). If this pattern of exploitation and resource depletion is characteristic of *Microstegium* on stream mitigation sites, then it could explain the negative N relationship in the CCA model, and possibly the negative K relationship as well given the mobility of both of these elements in the soil and in plant tissues (Haering et al. 2015). Admittedly, there is much variability in the overlap of the N and K vectors with *Microstegium*-dominant plots on Figure 4-10, so it is probably prudent to view these relationships as less important than the other factors (particularly canopy cover, which was monotonically correlated with *Microstegium* abundance).

4.6 Summary – Streams

As with wetlands, the field study for the stream mitigation component of this project was focused on plant communities and environmental variation across invasion gradients on Coastal Plain and Piedmont sites in Virginia. Results from analysis of community properties on stream sites were consistent with the wetland findings, leading to similar conclusions and recommendations (see Section 3.6 and Chapter 5).

Revisiting the "what tips the scale?" question posed in Section 3.6, the answer on stream sites overwhelmingly pointed to canopy cover (light availability), which was directly correlated with the abundance of all three invaders. Further, based on CCA modeling, canopy cover was also important in structuring plant communities along the invasion gradients of all target species in this project, wetlands and streams combined. Other factors emerged in community modeling for the stream data sets, the interpretation of which generally coincides with the literature on these species and on invasion ecology in general. As with the wetlands, results from the stream study suggest some best practices for mitigation planning, implementation, and management, which will be discussed in Chapter 6.

5 Invasive Species Performance Standard

Both the wetland and stream mitigation field studies indicate that ecosystem functions related to vegetation community properties (e.g., species composition, richness, floristic quality, diversity) are impacted by invasive species at high levels of invasion (see Sections 3.5.1 and 4.5.1). This was expected based on the literature and, frankly, common sense: we can *see* the effects of dominant invaders reflected in their overwhelming density on sites, and the resultant diminishment of native species in space and time. Collecting data along invasion gradients has allowed us to confirm and enumerate these impacts at the invaded end of the continuum.

What was *unexpected* in our data was the recurrence of higher levels of these intrinsic floristic quality parameters at *moderate* levels of invasion. This result was made all the more surprising by the fact that it was consistent across both the wetland and the stream datasets. For each target invader in both studies, species composition in the middle of the invasion gradient (group C in our analysis) showed greater similarity to the uninvaded end (groups D and E) than to the invaded end (groups A and B). This was a clear signal that invaders at moderate percentages (e.g., 5-10%) do not impact the *composition* of the vegetative community. Likewise, native species richness and FQI were highest at moderate levels of invasion for all taxa, suggesting that desirable native plants are not preemptively replaced by invaders at these moderate levels. All of these observations were supported by species accumulation curves and diversity profiles, which consistently showed the moderately invaded group as highest in these important community properties.

5.1 Examples of Performance Standards

The question that remains is: What is an appropriate invasive species performance standard for compensatory mitigation sites? In Virginia, a threshold of 5% invasive species cover has historically been the agency-required benchmark for this aspect of vegetation performance on mitigation sites as specified in permit conditions and/or mitigation banking instruments (D. DeBerry pers. obs.).[1] A review of Clean Water Act Section 404 (USC 33 §1344 et seq.) and analogous state water control law regulatory programs shows that established requirements vary from state to state and sometimes even within individual regulatory programs. For example, Reiss et al. (2009) reported a range of performance thresholds for invasive species from as low as 1% to has high as 10% in Florida, and Kozich and Halvorsen (2012) and WSDE (2002) documented 10% thresholds for Michigan and Washington state, respectively. In past guidance documents, Ohio set a 5% threshold for non-*Typha* invaders, but up to 10% for *Typha* spp. due to challenges in differentiating native species in that genus from hybrids (Mack et al. 2004). In some project-specific instances, Ohio mitigation banks have been established with a

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[1] In addition to his faculty position at W&M, senior author and Principal Investigator D. DeBerry has been a professional environmental consultant in Virginia since 1993 and has reviewed and/or drafted dozens of wetland and stream mitigation planning documents over that timeframe.

non-specific performance standard requiring the overall vegetative community to be "predominantly native" (Spieles et al. 2006). Similar qualitative criteria have been specified for mitigation projects in Illinois, where the invasive or nuisance species standard was "none dominant" (Matthews and Endress 2008). Maryland adopted a similar standard with a bit more specificity in requiring that mitigation sites cannot be "dominated by common reed (*Phragmites australis*) or other nuisance vegetation," a standard clearly aimed at one of the more problematic invaders in that state (MDE 2011). In their Mitigation Banking Instrument (MBI) Template, North Carolina simply stated that invasive species could not impact the "functional integrity of the target vegetative community" (USCOE and NCIRT 2016). As this is just a selection of the many regulatory programs and mitigation guidance documents nationwide, it is evident that invasive species performance in compensatory mitigation lacks consensus.

5.2 Important Considerations for Invasion Thresholds

Programs that have adopted a low threshold like "no greater than 5% cover of invasive species" have done so as a precautionary approach. At face value, the logic of this tactic seems sound: recognizing that a "zero tolerance" stance with respect to plant invaders is likely unattainable (D'Antonio and Meyerson 2002), a "low tolerance" threshold would provide some latitude for mitigation sites to meet standards while also keeping invaders below a dominance threshold to reduce risk. However, our results suggest that this approach is inherently flawed because it compels mitigation site managers to remediate a condition that is not actually impacting ecological functions related to plant community properties. The most common corrective approach is to use non-selective herbicides to control invasive populations (Kettenring and Adams 2011), but what our research has shown is that herbicide use (or other methods such as mechanical removal) to control problematic species at moderate levels of invasion will cause indiscriminate mortality of desirable native species at much higher richness, diversity, and floristic quality than previously thought. In addition, continued use of herbicides in long-term management strategies to meet aggressive performance standards have been shown to result in chronic and deleterious effects on environmental conditions such as lower soil nutrient status, decreased dissolved oxygen, acidification, and carbon imbalance, and have in some cases facilitated re-invasion of treated areas (Apfelbaum and Sams 1987, Kettenring and Adams 2011, Lawrence et al. 2016). In light of these considerations, we propose a results-based standard that is informed by our work in this project.

5.3 Results-based Recommendation for Invasive Standard

Based on our data, there appears to be an advisable threshold of invasion somewhere between the group C range (ca. 5-10% abundance of invader) and the group B range (+/- 30% abundance of invader) where impacts to ecosystem functions would be detectable. To determine a reasonable threshold within this range, we sorted each community matrix in descending order of invasive species dominance and plotted the running average of relative invasive species abundance against the running average of native species richness. Native richness was chosen because of its importance in vegetation performance standards for compensatory mitigation (Matthews and Endress 2008, DeBerry and Perry 2015, Van den Bosch and Matthews 2017), and also because native species trends were representative of the other intrinsic floristic quality parameters evaluated in our results for every dataset (namely, composition, FQI, evenness, and diversity). The "bin" size for each average calculation was equivalent to the original bin size for each group (e.g., 14 plots for *Typha*, 10 plots for *Arthraxon*, etc.). Calculated in this way, we were able to superimpose the trend in native richness over the invasion gradient and observe the point at which the "hump" in native richness began to decline on the invaded side of the gradient (this was visualized by fitting a polynomial trendline to the scatterplot of native richness data points). The results from this analysis are shown in Figure 5-1(a-f). Although there is variability among the different datasets, the 10% invasion line on the graph consistently aligns with maximum native richness or is coincident with the start of the declining limb on the richness curve for all taxa.

Therefore, **we recommend an invasive species performance standard of 10% relative abundance for invasive species on both stream and wetland compensatory mitigation sites in Virginia.** Based on our data, a 10% invasive species standard would be a sensible target for ecological performance that strikes a balance between proactive management and indiscriminate loss of desirable species and ecosystem function.

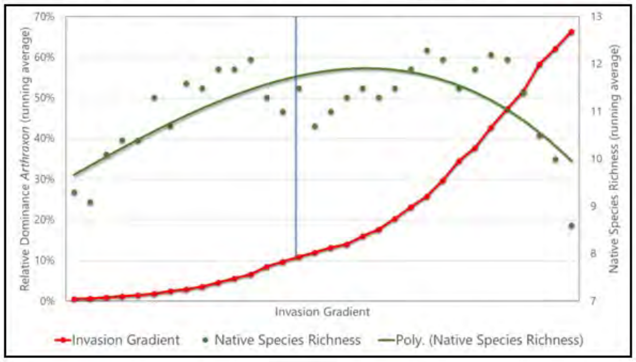
5.4 Monitoring Invasion on Mitigation Sites

In application, this performance standard should be monitored on mitigation sites by calculating the relative abundance of invasive species from vegetation monitoring data (e.g., plot-based data or equivalent), as long as the monitoring data have been collected using methods that conform to assumptions of ecological sampling theory for which sample adequacy has been demonstrated (DeBerry 2020a). This means that on most mitigation sites, invasive species will be tracked by community type or planting zone rather than by the site as a whole. On sites where more than one invasive species is present, relative abundance should be calculated as a cumulative value for the performance standard (i.e., sum of relative abundance values for all invasive species present).

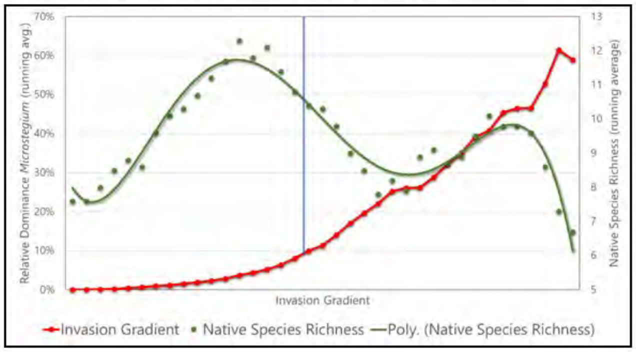
The value in assessing invasion using a randomized sample dataset that has been subjected to a sample adequacy test is that it provides an unbiased estimate of invader abundance. This type of surveillance is advisable because it discourages the habit of monitoring stationary plots from year to year and, as a consequence, only treating invasive species in localized proximity to monitored plots. However, a monitoring program that includes both random samples *and* mapping of invader populations would be the best approach to reduce risk of invasion on mitigation sites.

Combining relative invader abundance with mapping of invasive species to determine the areal extent of localized "invasion hot spots" on a site would be a judicious approach that, in our opinion, would not be excessive in comparison with typical monitoring requirements. Most compulsory mitigation monitoring is completed using plot-based sampling techniques, the results of which are summarized in data tables that can be manipulated to calculate relative abundance. Likewise, most monitoring requirements for mitigation projects stipulate updated mapping of site resources per monitoring year (e.g., jurisdictional wetland limits, vegetation communities, groundwater well and plot locations, etc.), and invasive species populations are frequently included in that effort.

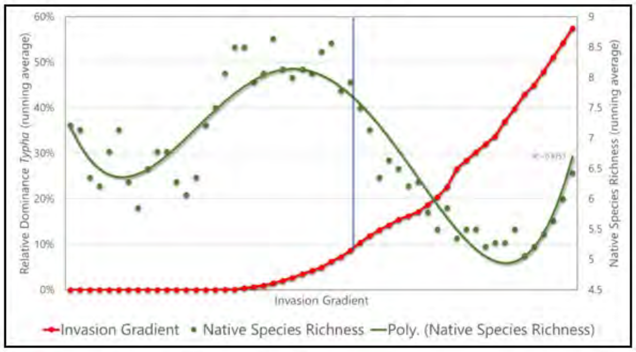
a. *Arthraxon* native richness vs. invader abundance.



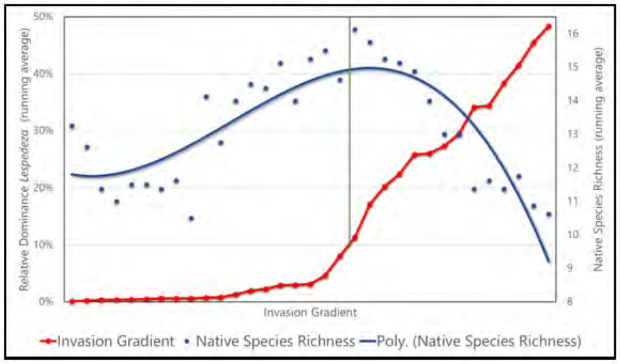
b. *Microstegium* (wet) abundance vs. native richness.



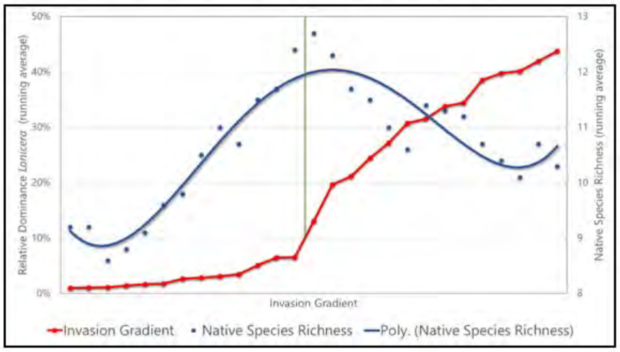
c. *Typha* abundance vs. native richness.



d. *Lespedeza* native richness vs. invader abundance.



e. *Lonicera* native richness vs. invader abundance.



f. *Microstegium* (streams) abundance vs. native richness.

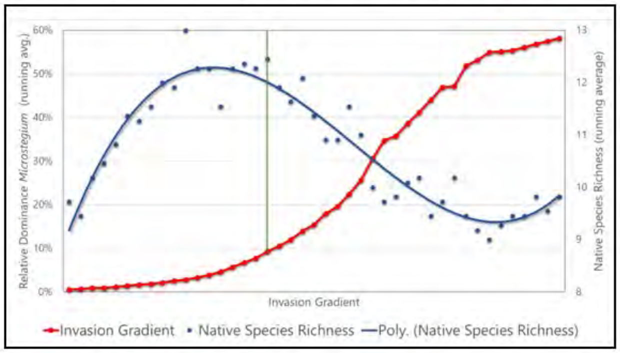


Figure 5-1 *X-Y scatterplot of mean native species richness and invasive species abundance for all taxa in both the wetland and stream studies. On each graph, the vertical "10% threshold" line is projected from the invasion gradient (red line) upward and intersecting with the native richness polynomial trendline for wetlands (green) and streams (blue). In all cases, the 10% line coincides with the peak or the start of the receding limb for the "hump" in the native richness curve.*

6 Environmental Drivers: Conclusions and Best Practices

By studying environmental variability along invasion gradients in both wetland and stream mitigation settings, we have learned that a “high disturbance/low stress” model for plant invasion – which is one of the most important unifying principles in the foundational literature on plant invasions – accords with the ecological amplitudes and general resource acquisition strategies for all of our target invaders. It bears mentioning that this is the case even though our “study group” spans a very wide range of tolerance for environmental gradients (e.g., *Typha* and *Lespedeza* living at opposite ends of the moisture continuum) and represents a diverse group in terms of habit and life history strategy (annual grasses, perennial forbs, tall emergent graminoids, woody vines).

Among environmental drivers, canopy cover (light availability) was conspicuous in that it was important across the invasion gradients of all target organisms on both wetland and stream sites. This is noteworthy because it indicates a potential confluence between theory and practice that could be actionable on mitigation sites during the construction phase (see below). This is also the case for wetland hydrology, which was a significant factor for all study species in the wetland datasets. Although our evaluation of soil chemistry produced results that varied by species, our interpretation is that the usual suspects from the literature (N and P) underlie the gradients we observed in our community models. Where soil texture was important, it seemed to signal localized disturbance, and this was judged to be a very probable consequence of legacy effects from construction-phase activities, active site management, or erosion and sedimentation dynamics in the case of stream sites. All of the above environmental drivers give us a clearer picture of the causes and consequences of invasion on mitigation sites and suggest some potential proactive measures that could be implemented to reduce risk of invasion at the outset of a mitigation project.

6.1 Best Practices to Reduce Risk of Invasion

Although the techniques used to design and construct wetland and stream mitigation sites should be selected to attain the goals and objectives established during the planning and design phase, we believe the best practices outlined below would help to attenuate the risk of biological invasion while remaining consistent with most aquatic resource function goals.

Best Practice #1: Plant larger trees.
This best practice applies to forested wetland mitigation and stream sites where the restoration goal for the riparian zone is a forest, which includes most sites. Planting trees from larger stock types would promote canopy development and hasten canopy cover, which was the preeminent environmental factor in our analysis as noted above. Larger nursery trees are expensive and including them in a planting plan is typically cost-prohibitive if the mitigation project is required

to meet a stem density performance standard (for example, 400 stems per acre). An alternative vegetation performance standard like Stem Area at Groundline (SAG; Hudson and Perry 2018) would accommodate a reduction in planting density and allow larger stock to be incorporated in a planting plan without undue expense.

Best Practice #2: Plant trees at a higher density.
This best practice is consistent with the goal of #1 above but achieved through planting higher densities of younger stock to balance the costs of buying trees in higher quantities. The inherent risk in this approach is that younger stock types are more susceptible to event-driven mortality (e.g., drought or prolonged inundation) (Roquemore et al. 2014). These issues may be attenuated to some degree by ensuring that purchased trees have been properly hardened (i.e., environmentally conditioned against climatic changes) and planted at an appropriate time of year (e.g., fall planting of dormant stock, which allows trees to acclimate over winter and establish stronger root systems during the following spring).

Best Practice #3: Plant early successional trees.
This best practice is also supplementary to the goals of #1 above. Early successional species are fast-growing and more likely to facilitate canopy closure on mitigation sites. In the past, this approach has been difficult to implement because early successional species have traditionally been eschewed in the regulatory approval process in favor of late successional species, presumably with the goal of promoting ecosystem functions related to community composition.[1] However, prior research suggests that early successional species can function as a nurse crop for late successional species (Figure 6-1; McLeod et al. 2001, DeBerry and Perry 2012); therefore, planting early successional trees can increase the survivability of late successional trees while performing other important ecosystem functions, namely, reduced risk of invasion through canopy development.

Figure 6-1 Salix nigra *on a wetland mitigation site in eastern Virginia. This species is an early-successional tree that performs an important "nurse crop" function for other species on mitigation sites. In only its third growing season, this tree already has a crown diameter approaching 20 feet.*

Best Practice #4: Plant a diverse seed mix at a high application rate.
This recommendation is consistent with research dating back to the early 1990s on mitigation sites (e.g., Reinartz and Warne 1993), has been revisited frequently in the mitigation literature since that time (e.g., Brown and Bedford 1997, Stauffer and Brooks 1997), and is still an

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[1] Although note that some regulators in Virginia have allowed use of nurse crops with positive results (M. Rolband, pers. comm.).

important conclusion of many contemporary studies (e.g., William and Ahn 2015). Because of this, it is surprising to hear of mitigation designers or managers still “cutting corners” by applying a low-diversity seed mix at the lowest recommended application rates (D. DeBerry, pers. obs.). Native seed mixes can be expensive, and this is perhaps one of the overriding considerations. A better approach would be to customize a seed mix with a high percentage of fast-growing annual species supplemented with high richness of perennials and early successional tree seeds. This type of application would maximize potential for rapid germination and ecosystem resiliency, advantaging the native species via the competitive edge promoted by early establishment.

Best Practice #5: Make wetland hydrology manipulable.

At face value, this idea seems to contradict the notion that wetland mitigation sites should be self-sustaining ecosystems and, if designed and constructed properly, should be “hands-off” with respect to management. The wetland mitigation literature often refers to this philosophy as “self-design”, i.e., ensuring that all the “pieces are in place” and letting the ecosystem create or restore itself over time (Mitsch et al. 2012). There is value in this approach because it accords with our understanding of ecological succession; however, we believe that proactive management of hydrology during the establishment phase (1-5 years post construction) could help to reduce risk of invasion without countermanding sustainability goals (DeBerry and Perry 2015). Ideally, tools like Wetbud (Stone et al. 2017) will allow designers to plan for a hydrology regime that can be facilitated by a “light footprint,” i.e., less of an engineered solution and more of a natural design for water storage onsite. However, given the importance of hydrology as a driver of environmental conditions that can either deter or enhance biological invasion, we believe that water control structures should be designed to allow for proactive manipulation of the wetland hydrology regime during the first several years of site development. This idea is species-specific: the overall strategy for manipulating hydrology to control invasive species would depend on the tolerances of the target invaders (for example, in our study wetter conditions reduced *Arthraxon* and *Microstegium* but favored *Typha*).[2] Decisions about how and when to proactively modify hydrology on mitigation sites should be informed by vigilant surveillance over the first several years post-construction, as well as an understanding of the stress-disturbance dynamic affecting vegetation development at the site.

Best Practice #6: Understand the stress-disturbance dynamic.

As noted above, we believe that the most instructive model for understanding plant invasions on mitigation sites is one that combines environmental stress and disturbance to predict trends in resource acquisition and competition. Our observations over the course of this project have convinced us that risk of invasion increases with increased disturbance and decreased stress, which is consistent with the literature on biological invasion and resource strategies of wild plants (Craine 2009, Lockwood et al. 2013). Stress in mitigation is experienced by plants in various ways, but disturbance is more or less the same: sites are cleared and graded as necessary to establish appropriate elevations and desired landforms, and soil conditions are

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[2] Note that flooding, in combination with cutting or removal of aboveground biomass, has been used as an effective *Typha* management strategy (Bansal et al. 2019).

often manipulated through excavation, tilling, or addition of soil amendments to improve conditions for target plant communities. Thus, the types of activities used in the construction sequence for a mitigation site are also the types of activities that typically “open the door” for biological invasion. If the “disturbance” half of the stress-disturbance dynamic is an unavoidable consequence of the construction sequence, there may be alternative approaches that would allow mitigation designers and managers to manipulate the “stress” half to reduce risk of invasion. *Imposing* stress on a mitigation site seems like a counterintuitive management strategy, but our research suggests that it could be used to increase native species richness and reduce the risk of invasion (Alpert et al. 2000, Bryson and Carter 2004), particularly in the establishment phase of a mitigation site (DeBerry and Perry 2015). Factors that could be controlled to induce environmental stress include hydrology (see #3 above), nutrient availability and/or limitation, and light availability. Examples of some techniques are discussed in Chapter 7.

Best Practice #7: Map invasive species annually.

In addition to the relative abundance metrics described in this report, we feel that the most effective approach for monitoring invasive species on mitigation sites is to combine abundance measures with annual mapping of invasive populations. As noted in Chapter 5, the combination of statistically valid measurements of abundance with mapping of invasive populations is advisable because it discourages the habit of monitoring stationary plots from year to year and, as a consequence, only treating invasive species in localized proximity to monitored plots. Mapping provides an extra level of security, and mitigation practitioners are therefore encouraged to “keep their eyes open” for localized “hot spots” of invasion using mapping techniques.

7 Future Work

7.1 Greenhouse Experiment

As a component of this research program (see Chapter 1), multispecies plant mesocosms containing *Arthraxon hispidus*, *Lespedeza cuneata*, *Microstegium vimineum*, and several native species were grown from seed in a controlled environment over the course of several months in late summer and early fall 2020. Once individual plants were old enough to identify from vegetative or reproductive characters, mesocosm pots were subjected to different treatments designed to replicate varying environmental conditions (e.g., soil nutrient content, wetland hydrology, canopy cover). Due to a malfunction in the heating system of the greenhouse where the experiment was staged, the pots were exposed to excessively low overnight temperatures in the fall of 2020, many plants were lost, and the experiment could not be completed. The research team is in the process of completing a new greenhouse study that will run during winter and spring of 2021. Results from that study will be provided as an addendum to this report when available.

Figure 7-1 *Multispecies mesocosms in greenhouse experiment ca. late August 2020 (prior to heater malfunction). Experiment is being repeated in winter/spring of 2021 and results will be submitted as an addendum to this report.*

7.2 Other Research Initiatives

The results of this project suggest that there may be alternatives to the traditional invasive species management techniques of non-selective or targeted herbicide use, mechanical removal, and disking or plowing. New strategies like the stress induction methods described below are relatively untested in applied settings, so field trials would be instructive not only for mitigation practitioners but also for any land managers for whom invasive species remediation is a priority.

One example of a potentially low-cost stress induction method would be to use soil amendments with a high carbon:nitrogen ratio (e.g., sawdust, wood chips, etc.). High carbon:nitrogen ratio materials have been shown to stimulate microbially-mediated removal of nitrogen from the soil, thereby inducing a nitrogen limitation (stress) that could potentially favor native species over invaders (Perry et al. 2004, Iannone and Galatowitsch 2008). Other examples include addition of metal oxides such as aluminum and iron oxide to the soil. Metal oxides and other cationic forms with strong anion exchange capacities are known to complex with and immobilize phosphate, thus reducing its bioavailability and potentially inducing a phosphorus limitation (stress) (Stauffer and Brooks 1997, Hogan et al. 2004). Industrially manufactured forms such as alum are reasonably affordable in bulk and have been used for this same purpose in freshwater lakes (Douglas et al. 2016). Field trials with these amendments and other strategies (see comments on hydrology and tree stock types in Chapter 6) could be completed in large-scale experiments on sites where invaders are already present.

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Appendix A

Checklists of Species Sampled

A1. Wetland Dataset
A2. Stream Dataset

Appendix A1. Checklist of Species Sampled (Wetland Dataset)

D. Hunter (sites sampled during peak growing season, 2018)

Scientific Name	**Common Name**	**Family**	**Introduced**[1]
Acer negundo var. *negundo*	Eastern Boxelder	Sapindaceae	
Acalypha rhomboidea	Common Three-seeded Mercury	Euphorbiaceae	
Acer rubrum	Red Maple	Sapindaceae	
Acer saccharinum	Silver Maple	Sapindaceae	
Agrostis gigantea	Redtop	Poaceae	*
Agrimonia parviflora	Small-flowered Agrimony	Rosaceae	
Agalinis purpurea	Purple False Foxglove	Orobanchaceae	
Alnus serrulata	Smooth Alder	Betulaceae	
Alisma subcordatum	Southern Water-plantain	Alismataceae	
Ambrosia artemisiifolia	Common Ragweed	Asteraceae	
Ampelopsis brevipedunculata	Porcelain-berry	Vitaceae	*
Amphicarpaea bracteata	Hog-peanut	Fabaceae	
Ammannia coccinea	Scarlet Ammannia	Lythraceae	
Anagallis arvensis ssp. *arvensis*	Scarlet Pimpernel	Primulaceae	*
Andropogon gerardii	Big Bluestem	Poaceae	
Antennaria plantaginifolia	Plantain-leaved Pussytoes	Asteraceae	
Andropogon virginicus var. *virginicus*	Broomsedge	Poaceae	
Apocynum cannabinum	Indian Hemp	Apocynaceae	
Arthraxon hispidus var. *hispidus*	Joint-head Grass	Poaceae	*
Asclepias incarnata var. *incarnata*	Swamp Milkweed	Apocynaceae	
Azolla caroliniana	Eastern Mosquito Fern	Salviniaceae	
Baccharis halimifolia	Groundsel Tree	Asteraceae	
Betula nigra	River Birch	Betulaceae	
Bidens aristosa	Tickseed Sunflower	Asteraceae	
Bidens comosa	Three-lobe Beggar-ticks	Asteraceae	
Boehmeria cylindrica	False Nettle	Urticaceae	
Carex comosa	Bottlebrush Sedge	Cyperaceae	
Carex complanata	Hirsute Sedge	Cyperaceae	
Carex crinita var. *crinita*	Long-fringed Sedge	Cyperaceae	
Carex frankii	Frank's Sedge	Cyperaceae	
Carex grayi	Gray's Sedge	Cyperaceae	
Carex lupulina	Hop Sedge	Cyperaceae	
Carex lurida	Sallow Sedge	Cyperaceae	
Campsis radicans	Trumpet-creeper	Bignoniaceae	
Carex scoparia var. *scoparia*	Broom Sedge	Cyperaceae	
Carex squarrosa	Squarrose Sedge	Cyperaceae	
Carex swanii	Swan's Sedge	Cyperaceae	
Carex tribuloides var. *tribuloides*	Blunt Broom Sedge	Cyperaceae	
Carex vulpinoidea	Fox Sedge	Cyperaceae	
Celtis occidentalis	Common Hackberry	Cannabaceae	
Cephalanthus occidentalis	Buttonbush	Rubiaceae	
Chasmanthium laxum	Slender Spikegrass	Poaceae	
Cinna arundinacea	Common Wood Reedgrass	Poaceae	
Cicuta maculata var. *maculata*	Water-hemlock	Apiaceae	
Cornus amomum	Silky Dogwood	Cornaceae	

Appendix A1. Checklist of Species Sampled (Wetland Dataset)

D. Hunter (sites sampled during peak growing season, 2018)

Scientific Name	**Common Name**	**Family**	**Introduced**[1]
Coleataenia anceps ssp. *anceps*	Beaked Panic Grass	Poaceae	
Conoclinium coelestinum	Mistflower	Asteraceae	
Coleataenia stipitata	Redtop Panic Grass	Poaceae	
Cuscuta gronovii	Common Dodder	Convolvulaceae	
Cyperus bipartitus	Slender Flatsedge	Cyperaceae	
Cyperus difformis	Variable Flatsedge	Cyperaceae	*
Cyperus iria	Rice-field Flatsedge	Cyperaceae	*
Cyperus pseudovegetus	Green Flatsedge	Cyperaceae	
Cyperus strigosus	Straw-colored Flatsedge	Cyperaceae	
Desmodium paniculatum var. *paniculatum*	Narrow-leaf Tick-trefoil	Fabaceae	
Dichanthelium clandestinum	Deer-Tongue Grass	Poaceae	
Dichanthelium commutatum var. *commutatum*	Variable Panic Grass	Poaceae	
Dichanthelium dichotomum var. *dichotomum*	Small-fruited Panic Grass	Poaceae	
Dichanthelium scoparium	Velvet Panic Grass	Poaceae	
Diodia teres	Common Buttonweed	Rubiaceae	
Digitaria villosa	Shaggy Crabgrass	Poaceae	
Diodia virginiana	Virginia Buttonweed	Rubiaceae	
Diospyros virginiana	Common Persimmon	Ebenaceae	
Echinochloa muricata var. *microstachya*	Rough Barnyard Grass	Poaceae	
Echinochloa muricata var. *muricata*	Rough Barnyard Grass	Poaceae	
Eclipta prostrata	False Daisy	Asteraceae	
Eleocharis acicularis	Needle Spikerush	Cyperaceae	
Eleocharis obtusa	Blunt Spikerush	Cyperaceae	
Eleocharis quadrangulata	Square-stem Spikerush	Cyperaceae	
Eleocharis tenuis	Slender Spikerush	Cyperaceae	
Elymus virginicus	Virginia Wild Rye	Poaceae	
Epilobium coloratum	Purple-leaved Willow-herb	Onagraceae	
Saccharum giganteum	Giant Plumegrass	Poaceae	
Erechtites hieraciifolius	Fireweed	Asteraceae	
Eupatorium capillifolium	Dog-fennel	Asteraceae	
Euthamia caroliniana	Slender Flat-top Goldenrod	Asteraceae	
Euthamia graminifolia	Grass-leaved Goldenrod	Asteraceae	
Eupatorium perfoliatum	Boneset	Asteraceae	
Eupatorium serotinum	Late Thoroughwort	Asteraceae	
Fimbristylis autumnalis	Slender Fimbry	Cyperaceae	
Fraxinus pennsylvanica	Green Ash	Oleaceae	
Galium tinctorium	Three-lobed Bedstraw	Rubiaceae	
Geum virginianum	Cream Avens	Rosaceae	
Hamamelis virginiana var. *virginiana*	Witch Hazel	Hamamelidaceae	
Hibiscus moscheutos	Swamp Rose-mallow	Malvaceae	
Hypericum mutilum var. *mutilum*	Dwarf St. John's-wort	Hypericaceae	
Hypericum virginicum	Virginia Marsh St. John's-wort	Hypericaceae	
Ilex verticillata	Winterberry	Aquifoliaceae	
Impatiens capensis	Spotted Jewelweed	Balsaminaceae	
Ipomoea lacunosa	Small White Morning Glory	Convolvulaceae	

Appendix A1. Checklist of Species Sampled (Wetland Dataset)

D. Hunter (sites sampled during peak growing season, 2018)

Scientific Name	**Common Name**	**Family**	**Introduced**[1]
Itea virginica	Virginia Sweetspire	Iteaceae	
Juncus acuminatus	Sharp-fruited Rush	Juncaceae	
Juncus canadensis	Canadian Rush	Juncaceae	
Juncus effusus	Soft Rush	Juncaceae	
Juncus marginatus	Grass-leaved Rush	Juncaceae	
Juncus tenuis	Path Rush	Juncaceae	
Juniperus virginiana var. *virginiana*	Eastern Redcedar	Cupressaceae	
Kummerowia stipulacea	Korean-clover	Fabaceae	*
Landoltia punctata	Dotted Duckmeat	Araceae	*
Lespedeza cuneata	Sericea Lespedeza	Fabaceae	*
Lemna minor	Common Duckweed	Araceae	
Leersia oryzoides	Rice Cutgrass	Poaceae	
Lindernia dubia var. *anagallidea*	Long-stalked False Pimpernel	Linderniaceae	
Lindernia dubia var. *dubia*	False Pimpernel	Linderniaceae	
Liquidambar styraciflua	Sweetgum	Altingiaceae	
Liriodendron tulipifera	Tulip-tree	Magnoliaceae	
Lonicera japonica	Japanese Honeysuckle	Caprifoliaceae	*
Ludwigia alata	Winged Seedbox	Onagraceae	
Ludwigia alternifolia	Alternate-leaved Seedbox	Onagraceae	
Ludwigia decurrens	Wing-leaved Primrose-willow	Onagraceae	
Ludwigia glandulosa	Cylindric-fruited Primrose-willow	Onagraceae	
Ludwigia palustris	Marsh Seedbox	Onagraceae	
Lysimachia nummularia	Moneywort	Primulaceae	*
Lythrum salicaria	Purple Loosestrife	Lythraceae	*
Lycopus virginicus	Virginia Bugleweed	Lamiaceae	
Mimulus alatus	Winged Monkeyflower	Phrymaceae	
Mikania scandens	Climbing Hempweed	Asteraceae	
Microstegium vimineum	Japanese Stiltgrass	Poaceae	*
Morella cerifera	Wax Myrtle	Myricaceae	
Murdannia keisak	Marsh Dewflower	Commelinaceae	*
Nyssa sylvatica	Black Gum	Nyssaceae	
Onoclea sensibilis var. *sensibilis*	Sensitive Fern	Onocleaceae	
Oxalis stricta	Common Yellow Wood-sorrel	Oxalidaceae	
Paspalum laeve	Field Paspalum	Poaceae	
Parthenocissus quinquefolia	Virginia-creeper	Vitaceae	
Panicum verrucosum	Warty Panic Grass	Poaceae	
Panicum virgatum var. *virgatum*	Switchgrass	Poaceae	
Persicaria arifolia	Halberd-leaf Tearthumb	Polygonaceae	
Persicaria glabra	Dense-flowered Smartweed	Polygonaceae	
Persicaria hydropiperoides	Mild Water-pepper	Polygonaceae	
Persicaria pensylvanica	Pennsylvania Smartweed	Polygonaceae	
Persicaria sagittata	Arrow-leaf Tearthumb	Polygonaceae	
Peltandra virginica	Arrow-arum	Araceae	
Phegopteris hexagonoptera	Broad Beech Fern	Thelypteridaceae	
Pinus rigida	Pitch Pine	Pinaceae	

Appendix A1. Checklist of Species Sampled (Wetland Dataset)

D. Hunter (sites sampled during peak growing season, 2018)

Scientific Name	**Common Name**	**Family**	**Introduced**[1]
Pinus taeda	Loblolly Pine	Pinaceae	
Plantago major	Common Plantain	Plantaginaceae	*
Platanus occidentalis	Sycamore	Platanaceae	
Pluchea odorata	Salt Marsh Fleabane	Asteraceae	
Poa annua	Annual Bluegrass	Poaceae	*
Pontederia cordata var. *cordata*	Pickerelweed	Pontederiaceae	
Poa trivialis	Rough Bluegrass	Poaceae	*
Quercus alba	White Oak	Fagaceae	
Quercus bicolor	Swamp White Oak	Fagaceae	
Quercus michauxii	Swamp Chestnut Oak	Fagaceae	
Quercus nigra	Water Oak	Fagaceae	
Quercus palustris	Pin Oak	Fagaceae	
Quercus phellos	Willow Oak	Fagaceae	
Rhynchospora corniculata	Short-bristled Horned Beaksedge	Cyperaceae	
Rhynchospora glomerata var. *glomerata*	Clustered Beaksedge	Cyperaceae	
Rhexia mariana var. *mariana*	Maryland Meadow Beauty	Melastomataceae	
Rhynchospora microcephala	Small-headed Bunched Beaksedg	Cyperaceae	
Rotala ramosior	Toothcup	Lythraceae	
Rubus flagellaris	Common Dewberry	Rosaceae	
Rudbeckia laciniata var. *laciniata*	Cut-leaf Coneflower	Asteraceae	
Rubus pensilvanicus	Pennsylvania Blackberry	Rosaceae	
Saururus cernuus	Lizard's-tail	Saururaceae	
Sagittaria latifolia	Broad-leaved Arrowhead	Alismataceae	
Salix nigra	Black Willow	Salicaceae	
Scirpus atrovirens	Dark Green Bulrush	Cyperaceae	
Scirpus cyperinus	Woolgrass	Cyperaceae	
Scirpus georgianus	Georgia Bulrush	Cyperaceae	
Scutellaria integrifolia	Hyssop Skullcap	Lamiaceae	
Scutellaria lateriflora	Mad-dog Skullcap	Lamiaceae	
Schoenoplectus mucronatus	Bog Bulrush	Cyperaceae	
Schoenoplectus purshianus	Blunt-scale Bulrush	Cyperaceae	
Schoenoplectus tabernaemontani	Soft-stem Bulrush	Cyperaceae	
Setaria faberi	Nodding Bristlegrass	Poaceae	*
Setaria parviflora	Knotroot Bristlegrass	Poaceae	
Setaria pumila ssp. *pumila*	Yellow Bristlegrass	Poaceae	*
Smilax rotundifolia	Common Greenbrier	Smilacaceae	
Smilax walteri	Coral Greenbrier	Smilacaceae	
Solidago altissima ssp. *altissima*	Tall Goldenrod	Asteraceae	
Solanum carolinense var. *carolinense*	Horse-nettle	Solanaceae	
Sorghum halepense	Johnson Grass	Poaceae	*
Solidago rugosa	Wrinkle-leaf Goldenrod	Asteraceae	
Symphoricarpos orbiculatus	Coralberry	Caprifoliaceae	
Symphyotrichum racemosum var. *racemosum*	Small White Aster	Asteraceae	
Taxodium distichum	Baldcypress	Cupressaceae	
Toxicodendron radicans var. *radicans*	Poison Ivy	Anacardiaceae	

Appendix A1. Checklist of Species Sampled (Wetland Dataset)

D. Hunter (sites sampled during peak growing season, 2018)

Scientific Name	Common Name	Family	Introduced[1]
Tridens flavus	Purpletop	Poaceae	
Trifolium pratense	Red Clover	Fabaceae	*
Typha latifolia	Broadleaf Cattail	Typhaceae	
Ulmus rubra	Slippery Elm	Ulmaceae	
Utricularia geminiscapa	Two-flowered Bladderwort	Lentibulariaceae	
Utricularia gibba	Humped Bladderwort	Lentibulariaceae	
Vernonia glauca	Upland Ironweed	Asteraceae	
Verbena hastata	Blue Vervain	Verbenaceae	
Vitis aestivalis var. *aestivalis*	Summer Grape	Vitaceae	
Viburnum dentatum var. *dentatum*	Arrow-wood	Adoxaceae	
Vitis rotundifolia var. *rotundifolia*	Muscadine Grape	Vitaceae	
Viola sororia	Common Blue Violet	Violaceae	
Woodwardia areolata	Netted Chain Fern	Blechnaceae	
Xanthium strumarium	Common Cocklebur	Asteraceae	

[1] Introduced (non-native) species determined in accordance with Weakley et al. (2020)

Appendix A2. Checklist of Species Sampled (Stream Dataset)

D. DeBerry (sites sampled during peak growing seasons, 2018 and 2019)

Scientific Name	Common Name	Family	Introduced[1]
Acalypha rhomboidea	Common Three-seeded Mercury	Euphorbiaceae	
Acer rubrum	Red Maple	Sapindaceae	
Acer saccharinum	Silver Maple	Sapindaceae	
Aegopodium podagraria	Bishop's Goutweed	Apiaceae	*
Agalinis purpurea	Purple False Foxglove	Orobanchaceae	
Agrimonia parviflora	Small-flowered Agrimony	Rosaceae	
Agrostis perennans	Autumn Bentgrass	Poaceae	
Ailanthus altissima	Tree-of-heaven	Simaroubaceae	*
Albizia julibrissin	Mimosa	Fabaceae	*
Alliaria petiolata	Garlic Mustard	Brassicaceae	*
Allium canadense var. *canadense*	Wild Onion	Amaryllidaceae	
Alnus serrulata	Smooth Alder	Betulaceae	
Ambrosia artemisiifolia	Common Ragweed	Asteraceae	
Ambrosia trifida	Giant Ragweed	Asteraceae	
Amelanchier arborea	Downy Serviceberry	Rosaceae	
Ampelopsis brevipedunculata	Porcelain-berry	Vitaceae	*
Amphicarpaea bracteata	Hog-peanut	Fabaceae	
Andropogon gerardii	Big Bluestem	Poaceae	
Andropogon virginicus var. *virginicus*	Broomsedge	Poaceae	
Antennaria plantaginifolia	Plantain-leaved Pussytoes	Asteraceae	
Artemisia vulgaris	Common Mugwort	Asteraceae	*
Arthraxon hispidus var. *hispidus*	Joint-head Grass	Poaceae	*
Asclepias incarnata var. *pulchra*	Swamp Milkweed	Apocynaceae	
Asclepias syriaca	Common Milkweed	Apocynaceae	
Asimina triloba	Pawpaw	Annonaceae	
Athyrium asplenioides	Southern Lady Fern	Woodsiaceae	
Baccharis halimifolia	Groundsel Tree	Asteraceae	
Betula nigra	River Birch	Betulaceae	
Bidens aristosa	Tickseed Sunflower	Asteraceae	
Bidens bipinnata	Spanish Needles	Asteraceae	
Bidens frondosa	Devil's Beggar-ticks	Asteraceae	
Bignonia capreolata	Cross-vine	Bignoniaceae	
Boehmeria cylindrica	False Nettle	Urticaceae	
Botrypus virginianus	Rattlesnake Fern	Ophioglossaceae	
Callicarpa americana	American Beauty-berry	Lamiaceae	
Calystegia sepium	Hedge Bindweed	Convolvulaceae	
Campsis radicans	Trumpet-creeper	Bignoniaceae	
Cardamine hirsuta	Hairy Bittercress	Brassicaceae	*
Carduus nutans	Musk Thistle	Asteraceae	*
Carex amphibola	Eastern Narrow-leaved Sedge	Cyperaceae	
Carex blanda	Eastern Woodland Sedge	Cyperaceae	
Carex debilis	White-edged Sedge	Cyperaceae	
Carex frankii	Frank's Sedge	Cyperaceae	
Carex lurida	Sallow Sedge	Cyperaceae	
Carex retroflexa	Reflexed Sedge	Cyperaceae	

Appendix A2. Checklist of Species Sampled (Stream Dataset)

D. DeBerry (sites sampled during peak growing seasons, 2018 and 2019)

Scientific Name	**Common Name**	**Family**	**Introduced**[1]
Carex squarrosa	Squarrose Sedge	Cyperaceae	
Carex swanii	Swan's Sedge	Cyperaceae	
Carex tribuloides var. *tribuloides*	Blunt Broom Sedge	Cyperaceae	
Carex vulpinoidea	Fox Sedge	Cyperaceae	
Carpinus caroliniana	Ironwood	Betulaceae	
Carya cordiformis	Bitternut Hickory	Juglandaceae	
Carya glabra	Pignut Hickory	Juglandaceae	
Celastrus orbiculatus	Oriental Bittersweet	Celastraceae	*
Celtis occidentalis	Common Hackberry	Cannabaceae	
Cephalanthus occidentalis	Buttonbush	Rubiaceae	
Cercis canadensis var. *canadensis*	Eastern Redbud	Fabaceae	
Chaerophyllum tainturieri	Hairy-fruit Chervil	Apiaceae	
Chamaecrista fasciculata var. *fasciculata*	Common Partridge-pea	Fabaceae	
Chasmanthium latifolium	River Oats	Poaceae	
Chasmanthium laxum	Slender Spikegrass	Poaceae	
Chimaphila maculata	Spotted Wintergreen	Ericaceae	
Cinna arundinacea	Common Wood Reedgrass	Poaceae	
Circaea canadensis ssp. *canadensis*	Enchanter's Night-shade	Onagraceae	
Clematis virginiana	Virgin's-bower	Ranunculaceae	
Clethra alnifolia	Sweet Pepperbush	Clethraceae	
Coleataenia anceps ssp. *anceps*	Beaked Panic Grass	Poaceae	
Coleataenia rigidula ssp. *rigidula*	Tall Flat Panic Grass	Poaceae	
Commelina communis	Asiatic Dayflower	Commelinaceae	*
Conoclinium coelestinum	Mistflower	Asteraceae	
Conyza canadensis var. *canadensis*	Common Horseweed	Asteraceae	
Coreopsis lanceolata	Long-stalk Coreopsis	Asteraceae	
Cornus amomum	Silky Dogwood	Cornaceae	
Cornus florida	Flowering Dogwood	Cornaceae	
Corylus americana	American Hazelnut	Betulaceae	
Crepis capillaris	Smooth Hawksbeard	Asteraceae	*
Cryptotaenia canadensis	Honewort	Apiaceae	
Cyperus strigosus	Straw-colored Flatsedge	Cyperaceae	
Dendrolycopodium obscurum	Common Tree-clubmoss	Lycopodiaceae	
Desmodium glabellum	Dillenius' Tick-trefoil	Fabaceae	
Desmodium paniculatum var. paniculatum	Narrow-leaf Tick-trefoil	Fabaceae	
Dichanthelium clandestinum	Deer-Tongue Grass	Poaceae	
Dichanthelium commutatum var. *commutatum*	Variable Panic Grass	Poaceae	
Dichanthelium dichotomum var. *dichotomum*	Small-fruited Panic Grass	Poaceae	
Dichanthelium dichotomum var. *ramulosum*	Branched Panic Grass	Poaceae	
Dichanthelium laxiflorum	Open-flower Panic Grass	Poaceae	
Dichanthelium scoparium	Velvet Panic Grass	Poaceae	
Digitaria ciliaris	Southern Crabgrass	Poaceae	
Digitaria sanguinalis	Northern Crabgrass	Poaceae	*
Diodia virginiana	Virginia Buttonweed	Rubiaceae	
Dioscorea villosa	Wild Yam	Dioscoreaceae	

Appendix A2. Checklist of Species Sampled (Stream Dataset)

D. DeBerry (sites sampled during peak growing seasons, 2018 and 2019)

Scientific Name	Common Name	Family	Introduced[1]
Diospyros virginiana	Common Persimmon	Ebenaceae	
Echinochloa crusgalli var. *crusgalli*	Barnyard Grass	Poaceae	*
Echinochloa muricata var. *muricata*	Rough Barnyard Grass	Poaceae	
Eclipta prostrata	False Daisy	Asteraceae	
Elephantopus carolinianus	Carolina Elephant's-foot	Asteraceae	
Eleusine indica	Indian Goosegrass	Poaceae	*
Elymus hystrix	Bottlebrush Grass	Poaceae	
Elymus virginicus	Virginia Wild Rye	Poaceae	
Erechtites hieraciifolius	Fireweed	Asteraceae	
Euonymus alatus	Winged Euonymus	Celastraceae	*
Euonymus americanus	Strawberry-bush	Celastraceae	
Euonymus fortunei	Winter Creeper	Celastraceae	*
Eupatorium capillifolium	Dog-fennel	Asteraceae	
Eupatorium perfoliatum	Boneset	Asteraceae	
Eupatorium rotundifolium	Roundleaf Thoroughwort	Asteraceae	
Eupatorium serotinum	Late Thoroughwort	Asteraceae	
Euthamia graminifolia	Grass-leaved Goldenrod	Asteraceae	
Eutrochium fistulosum	Hollow Joe-pye-weed	Asteraceae	
Eutrochium purpureum var. *purpureum*	Sweet-scented Joe-pye-weed	Asteraceae	
Fraxinus pennsylvanica	Green Ash	Oleaceae	
Galium circaezans	Forest Bedstraw	Rubiaceae	
Galium tinctorium	Three-lobed Bedstraw	Rubiaceae	
Galium triflorum	Sweet-scented Bedstraw	Rubiaceae	
Geum canadense	White Avens	Rosaceae	
Glechoma hederacea	Ground-ivy	Lamiaceae	*
Helenium autumnale	Common Sneezeweed	Asteraceae	
Hydrocotyle umbellata	Marsh Water-pennywort	Araliaceae	
Hylodesmum nudiflorum	Naked-Flowered Tick-trefoil	Fabaceae	
Hypericum hypericoides	St. Andrew's Cross	Hypericaceae	
Hypericum mutilum var. *mutilum*	Dwarf St. John's-wort	Hypericaceae	
Hypericum punctatum	Spotted St. John's-wort	Hypericaceae	
Ilex decidua var. *decidua*	Deciduous Holly	Aquifoliaceae	
Ilex glabra	Inkberry	Aquifoliaceae	
Ilex opaca var. *opaca*	American Holly	Aquifoliaceae	
Ilex verticillata	Winterberry	Aquifoliaceae	
Impatiens capensis	Spotted Jewelweed	Balsaminaceae	
Ipomoea purpurea	Common Morning Glory	Convolvulaceae	*
Juglans nigra	Black Walnut	Juglandaceae	
Juncus coriaceus	Leathery Rush	Juncaceae	
Juncus dichotomus	Forked Rush	Juncaceae	
Juncus effusus	Soft Rush	Juncaceae	
Juncus scirpoides var. *scirpoides*	Needle-pod Rush	Juncaceae	
Juncus tenuis	Path Rush	Juncaceae	
Juniperus virginiana var. *virginiana*	Eastern Redcedar	Cupressaceae	
Kummerowia striata	Japanese-clover	Fabaceae	*

Appendix A2. Checklist of Species Sampled (Stream Dataset)

D. DeBerry (sites sampled during peak growing seasons, 2018 and 2019)

Scientific Name	Common Name	Family	Introduced[1]
Laportea canadensis	Wood Nettle	Urticaceae	
Leersia oryzoides	Rice Cutgrass	Poaceae	
Leersia virginica	White Grass	Poaceae	
Lespedeza cuneata	Sericea Lespedeza	Fabaceae	*
Ligustrum sinense	Chinese Privet	Oleaceae	*
Lindera benzoin	Spicebush	Lauraceae	
Liquidambar styraciflua	Sweetgum	Altingiaceae	
Liriodendron tulipifera	Tulip-tree	Magnoliaceae	
Lobelia siphilitica var. *siphilitica*	Great Blue Lobelia	Campanulaceae	
Lonicera japonica	Japanese Honeysuckle	Caprifoliaceae	*
Lonicera morrowii	Morrow's Honeysuckle	Caprifoliaceae	*
Ludwigia alternifolia	Alternate-leaved Seedbox	Onagraceae	
Lycopus americanus	American Bugleweed	Lamiaceae	
Lycopus virginicus	Virginia Bugleweed	Lamiaceae	
Melia azedarach	Chinaberry	Meliaceae	*
Melothria pendula	Creeping Cucumber	Cucurbitaceae	
Microstegium vimineum	Japanese Stiltgrass	Poaceae	*
Mikania scandens	Climbing Hempweed	Asteraceae	
Mimulus alatus	Winged Monkeyflower	Phrymaceae	
Mimulus ringens var. *ringens*	Square-stemmed Monkeyflower	Phrymaceae	
Miscanthus sinensis	Chinese Silvergrass	Poaceae	*
Mitchella repens	Partidge-berry	Rubiaceae	
Morella cerifera	Wax Myrtle	Myricaceae	
Morus rubra	Red Mulberry	Moraceae	
Muhlenbergia schreberi	Nimblewill	Poaceae	
Murdannia keisak	Marsh Dewflower	Commelinaceae	*
Nyssa sylvatica	Black Gum	Nyssaceae	
Onoclea sensibilis var. *sensibilis*	Sensitive Fern	Onocleaceae	
Osmorhiza longistylis	Aniseroot	Apiaceae	
Osmundastrum cinnamomeum	Cinnamon Fern	Osmundaceae	
Oxalis dillenii	Southern Yellow Wood-sorrel	Oxalidaceae	
Oxalis stricta	Common Yellow Wood-sorrel	Oxalidaceae	
Packera aurea	Golden Ragwort	Asteraceae	
Panicum dichotomiflorum var. *dichotomiflorum*	Fall Panic Grass	Poaceae	
Panicum virgatum var. *virgatum*	Switchgrass	Poaceae	
Parathelypteris noveboracensis	New York Fern	Thelypteridaceae	
Parthenocissus quinquefolia	Virginia-creeper	Vitaceae	
Paspalum floridanum	Florida Paspalum	Poaceae	
Paspalum laeve	Field Paspalum	Poaceae	
Passiflora incarnata	Purple Passionflower	Passifloraceae	
Penstemon digitalis	Foxglove Beard-tongue	Plantaginaceae	
Penthorum sedoides	Ditch Stonecrop	Penthoraceae	
Perilla frutescens	Beefsteak Plant	Lamiaceae	*
Persicaria hydropiperoides	Mild Water-pepper	Polygonaceae	
Persicaria lapathifolia	Dock-leaf Smartweed	Polygonaceae	

Appendix A2. Checklist of Species Sampled (Stream Dataset)

D. DeBerry (sites sampled during peak growing seasons, 2018 and 2019)

Scientific Name	**Common Name**	**Family**	**Introduced**[1]
Persicaria longiseta	Bristly Lady's-Thumb	Polygonaceae	*
Persicaria pensylvanica	Pennsylvania Smartweed	Polygonaceae	
Persicaria punctata	Dotted Smartweed	Polygonaceae	
Persicaria sagittata	Arrow-leaf Tearthumb	Polygonaceae	
Persicaria virginiana	Jumpseed	Polygonaceae	
Phegopteris hexagonoptera	Broad Beech Fern	Thelypteridaceae	
Phryma leptostachya var. *leptostachya*	Lopseed	Phrymaceae	
Phyllanthus caroliniensis ssp. *caroliniensis*	Carolina Leaf-flower	Phyllanthaceae	
Phytolacca americana var. *americana*	Common Pokeweed	Phytolaccaceae	
Pilea pumila	Clearweed	Urticaceae	
Pinus taeda	Loblolly Pine	Pinaceae	
Platanus occidentalis	Sycamore	Platanaceae	
Pluchea camphorata	Camphorweed	Asteraceae	
Polygonatum biflorum var. *biflorum*	Solomon's-seal	Ruscaceae	
Polystichum acrostichoides	Christmas Fern	Dryopteridaceae	
Populus deltoides var. *deltoides*	Eastern Cottonwood	Salicaceae	
Potentilla canadensis var. *canadensis*	Canada Cinquefoil	Rosaceae	
Potentilla indica	Indian-strawberry	Rosaceae	*
Prunella vulgaris	Heal-all	Lamiaceae	
Prunus avium	Sweet Cherry	Rosaceae	*
Prunus serotina var. *serotina*	Black Cherry	Rosaceae	
Pseudognaphalium obtusifolium	Sweet Everlasting	Asteraceae	
Pueraria montana var. *lobata*	Kudzu	Fabaceae	*
Pycnanthemum incanum var. *incanum*	Hoary Mountain-mint	Lamiaceae	
Pycnanthemum tenuifolium	Narrow-leaf Mountain-mint	Lamiaceae	
Pycnanthemum verticillatum var. *verticillatum*	Whorled Mountain-mint	Lamiaceae	
Pyrus calleryana	Callery Pear	Rosaceae	*
Quercus alba	White Oak	Fagaceae	
Quercus bicolor	Swamp White Oak	Fagaceae	
Quercus falcata	Southern Red Oak	Fagaceae	
Quercus michauxii	Swamp Chestnut Oak	Fagaceae	
Quercus montana	Chestnut Oak	Fagaceae	
Quercus palustris	Pin Oak	Fagaceae	
Quercus phellos	Willow Oak	Fagaceae	
Quercus rubra	Northern Red Oak	Fagaceae	
Ranunculus recurvatus var. *recurvatus*	Hooked Buttercup	Ranunculaceae	
Rhexia mariana var. *mariana*	Maryland Meadow Beauty	Melastomataceae	
Rhexia virginica	Virginia Meadow Beauty	Melastomataceae	
Rhynchospora glomerata var. *glomerata*	Clustered Beaksedge	Cyperaceae	
Robinia pseudoacacia	Black Locust	Fabaceae	
Rosa multiflora	Multiflora Rose	Rosaceae	*
Rosa virginiana	Virginia Rose	Rosaceae	
Rubus discolor	Himalayan Blackberry	Rosaceae	*
Rubus flagellaris	Common Dewberry	Rosaceae	
Rubus hispidus	Bristly Dewberry	Rosaceae	

Appendix A2. Checklist of Species Sampled (Stream Dataset)

D. DeBerry (sites sampled during peak growing seasons, 2018 and 2019)

Scientific Name	**Common Name**	**Family**	**Introduced**[1]
Rubus occidentalis	Black Raspberry	Rosaceae	
Rubus pensilvanicus	Pennsylvania Blackberry	Rosaceae	
Rudbeckia hirta	Black-eyed Susan	Asteraceae	
Rumex obtusifolius	Bitter Dock	Polygonaceae	*
Salix nigra	Black Willow	Salicaceae	
Sambucus canadensis	Common Elderberry	Adoxaceae	
Sanicula canadensis	Black Snakeroot	Apiaceae	
Saururus cernuus	Lizard's-tail	Saururaceae	
Sceptridium dissectum	Cut-leaf Grape Fern	Ophioglossaceae	
Schizachyrium scoparium var. *scoparium*	Little Bluestem	Poaceae	
Scirpus cyperinus	Woolgrass	Cyperaceae	
Scirpus georgianus	Georgia Bulrush	Cyperaceae	
Scirpus polyphyllus	Leafy Bulrush	Cyperaceae	
Scutellaria integrifolia	Hyssop Skullcap	Lamiaceae	
Senna marilandica	Maryland Wild Senna	Fabaceae	
Setaria parviflora	Knotroot Foxtail	Poaceae	
Sicyos angulatus	Bur Cucumber	Cucurbitaceae	
Sida spinosa	Prickly Mallow	Malvaceae	*
Silene stellata	Starry Campion	Caryophyllaceae	
Smilax glauca	Catbrier	Smilacaceae	
Smilax rotundifolia	Common Greenbrier	Smilacaceae	
Solanum carolinense var. *carolinense*	Horse-nettle	Solanaceae	
Solidago altissima ssp. *altissima*	Tall Goldenrod	Asteraceae	
Solidago patula var. *patula*	Rough-leaved Goldenrod	Asteraceae	
Solidago rugosa	Wrinkle-leaf Goldenrod	Asteraceae	
Sorghastrum nutans	Indian Grass	Poaceae	
Sorghum halepense	Johnson Grass	Poaceae	*
Stellaria media	Common Chickweed	Caryophyllaceae	*
Symphoricarpos orbiculatus	Coralberry	Caprifoliaceae	
Symphyotrichum lanceolatum var. *lanceolatum*	Panicled Aster	Asteraceae	
Symphyotrichum lateriflorum	Calico Aster	Asteraceae	
Symphyotrichum novae-angliae	New England Aster	Asteraceae	
Symphyotrichum novi-belgii	New York Aster	Asteraceae	
Symphyotrichum pilosum var. *pilosum*	Frost Aster	Asteraceae	
Symphyotrichum puniceum var. *puniceum*	Purple-stem Aster	Asteraceae	
Symphyotrichum racemosum var. *racemosum*	Small White Aster	Asteraceae	
Symphyotrichum undulatum	Wavy-leaved Aster	Asteraceae	
Teucrium canadense	Canada Germander	Lamiaceae	
Thelypteris palustris var. *pubescens*	Marsh Fern	Thelypteridaceae	
Toxicodendron radicans var. *radicans*	Poison Ivy	Anacardiaceae	
Tridens flavus	Purpletop	Poaceae	
Trifolium repens	White Clover	Fabaceae	*
Tripsacum dactyloides var. *dactyloides*	Eastern Gamagrass	Poaceae	
Ulmus alata	Winged Elm	Ulmaceae	
Ulmus americana	American Elm	Ulmaceae	

Appendix A2. Checklist of Species Sampled (Stream Dataset)

D. DeBerry (sites sampled during peak growing seasons, 2018 and 2019)

Scientific Name	Common Name	Family	Introduced[1]
Ulmus rubra	Slippery Elm	Ulmaceae	
Uvularia perfoliata	Perfoliate Bellwort	Colchicaceae	
Vaccinium pallidum	Early Lowbush Blueberry	Ericaceae	
Verbena hastata	Blue Vervain	Verbenaceae	
Verbena urticifolia	White Vervain	Verbenaceae	
Verbesina alternifolia	Wingstem	Asteraceae	
Verbesina occidentalis	Yellow Crownbeard	Asteraceae	
Vernonia noveboracensis	New York Ironweed	Asteraceae	
Viburnum dentatum var. *dentatum*	Arrow-wood	Adoxaceae	
Viburnum plicatum	Japanese Snowball	Adoxaceae	*
Viburnum prunifolium	Black Haw	Adoxaceae	
Viola sororia	Common Blue Violet	Violaceae	
Vitis labrusca	Fox Grape	Vitaceae	
Vitis rotundifolia var. *rotundifolia*	Muscadine Grape	Vitaceae	
Woodwardia areolata	Netted Chain Fern	Blechnaceae	
Xanthium strumarium	Common Cocklebur	Asteraceae	

[1] Introduced (non-native) species determined in accordance with Weakley et al. (2020)

Appendix B

Representative Photographs

Wetland Study: ***Arthraxon*** Plot Examples

INVADED → UNINVADED

3-year-old site

8-year-old site

20-year-old site

Wetland Study: ***Microstegium*** Plot Examples

INVADED → UNINVADED

3-year-old site

8-year-old site

20-year-old site

Wetland Study: ***Typha*** Plot Examples

INVADED ⟶ UNINVADED

2-year-old site

8-year-old site

14-year-old site

Wetland Study: **Canopy Cover** Examples

INVADED → UNINVADED

Arthraxon 6-year-old site

Arthraxon 11-year-old site

Microstegium 6-year-old site

Microstegium 20-year-old site

Typha 13-year-old site

Typha 20-year-old site

Stream Study:[1] *Lespedeza* Plot Examples

INVADED → UNINVADED

2-year-old site

4-year-old site

9-year-old site

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[1] Note: For the stream study, nearly all plot images were recorded during transect layout prior to sampling, so sampling frames do not appear in most photos.

Stream Study: ***Lonicera*** Plot Examples

INVADED → UNINVADED

4-year-old site

9-year-old site

11-year-old site

Stream Study: ***Microstegium*** Plot Examples

INVADED → UNINVADED

2-year-old site

4-year-old site

8-year-old site

Stream Study: **Canopy Cover** Examples

INVADED → UNINVADED

Lespedeza 3-year-old site

Lespedeza 11-year-old site

Lonicera 4-year-old site

Lonicera 8-year-old site

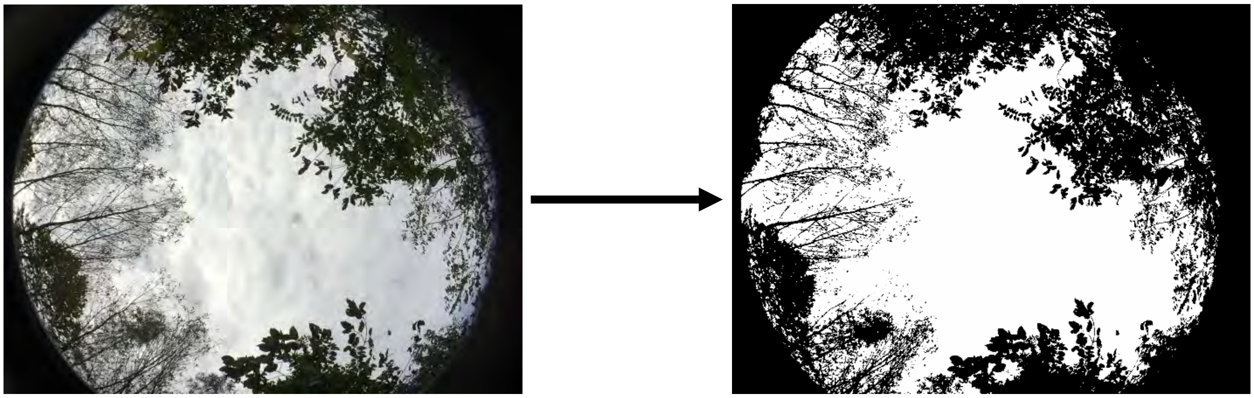
Microstegium 1-year-old site

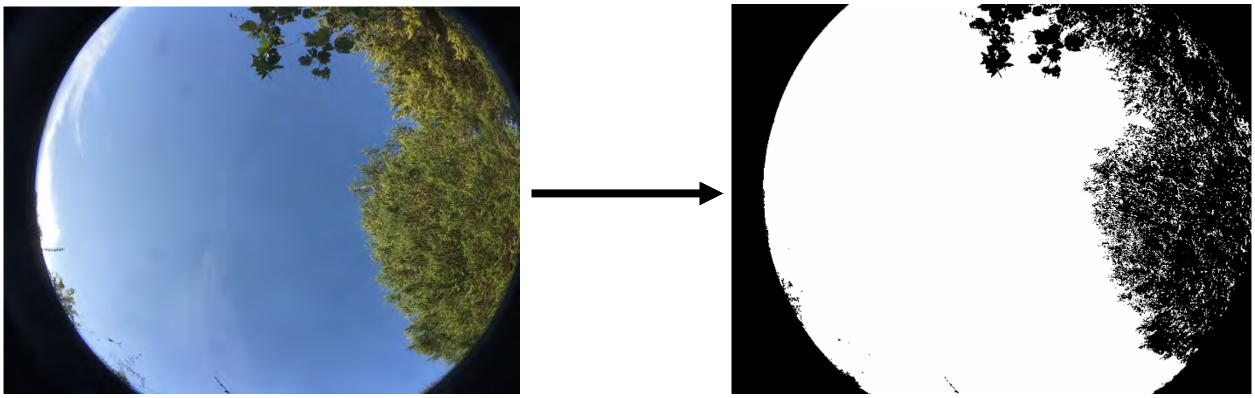
Microstegium 10-year-old site

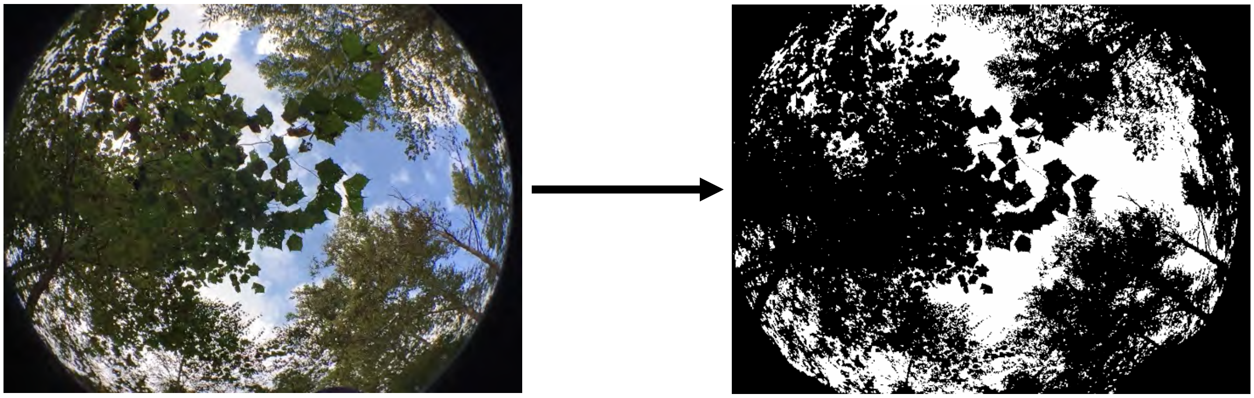
ImageJ Binary Conversion Examples

The images below are a few examples of the original true-color hemispheric photos taken skyward from the center of every plot in this study (left side), and the binary (black and white) images resulting from ImageJ conversions (right side). Binary images were used to calculate pixel density, which was converted into canopy cover based on the ratio of black to white pixels (the image conversion routine subtracts out any "non-photo" space around the perimeter). The examples below demonstrate the versatility of the method over a range of cloud cover conditions.

True Color **Binary**







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