Preventative Control of the Invasive Japanese Stiltgrass in Stream Restoration

Robert Alan Sullivan

Leesburg, VA

Biology B.S., College of William & Mary, 2022

A Thesis presented to the Graduate Faculty of The College of William and Mary in
Virginia in Candidacy for the Degree of
Master of Science

Biology

The College of William and Mary in Virginia
January 2025

APPROVAL PAGE

This Thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Science

Signed by:
7CC1153FEEB0401...

Robert Alan Sullivan

Approved by the Committee, November 2024

Signed by:
Doug DeBerry
54E1B7AA353B419...

Committee Chair
Doug DeBerry, Research Assistant Professor/Affiliate Faculty, ENSP/Biology
College of William & Mary

Signed by:
Randy Chambers
5C1135DE9EE54DB...

Randolph Chambers, Professor/Keck Environmental Field Laboratory Director, Biology/ENSP
College of William & Mary

Signed by:
Joshua Puzey
0B3E82E67829458...

Joshua Puzey, Associate Professor, Biology
College of William & Mary

ABSTRACT

Microstegium vimineum (Japanese stiltgrass) is one of the most invasive species in the eastern United States and poses a constant problem to restoration ecologists. *M. vimineum* is constantly invading restored wetlands and streams, and the most common solution is to use non-selective herbicides, like glyphosate, to reduce invasion. However, herbicide often comes with its own negative consequences to the ecosystem and can even lead to reinvasion after it is applied. This project looks to study the current available alternatives to herbicide for reducing invasion and then test them in the field. According to the stress-disturbance invasion model, higher levels of stress can help reduce invasion and therefore promote native species, so we tested cultural methods that could act as stress agents to *M. vimineum*. We chose to implement four treatments: 1) canopy shade (light limitation), 2) sawdust (to stimulate a short-term nitrogen limitation), 3) wood mulch soil amendments (to stimulate a longer-term nitrogen limitation), and double seeding rates (to stimulate competition), as well as a combination of these treatments. Over our two-year field study of a restored stream corridor, we found that sawdust was the most effective at reducing *M. vimineum*, and that shade heavily promoted natives to compete with this invader. This suggests a set of best practices that stream restoration project managers should consider during the design and construction phases of a stream restoration project.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I wish to express my thanks and gratitude towards my thesis advisor Dr. Doug DeBerry for helping me make this project run smoothly, as well as my two other committee members, Dr. Randy Chambers and Dr. Josh Puzey. My colleague and fellow graduate student Matt Whalen provided a lot of help for setting up this study and has supported my research throughout our shared time at W&M.

I'd like to thank Athena Tilley and Dr. Lee Daniels at the Virginia Tech Soil labs for analysis and interpretation of our soil samples. Another thanks to Mowcow Landscaping for helping us initialize site preparation.

Dr. Cindy Smith and her amazing team of George Mason undergraduates helped us maintain our plots for the past two years, and the importance of their part in this project cannot be overstated. This includes Nayeli Arellano, Jackson Bayuk, David Castellanos, Adrian Hagarty, Sam Heath, Sydney Jackson, Lauren Kelley, Gregory Kobayashi, Jeffery Maldonado, Malia Stephens, and Ky Tran. Their help on this project was invaluable. I'm also incredibly grateful to Ben Rhoades, Watershed Manager for the Reston Association (RA), and the rest of the leadership at RA for being so accommodating and giving this project a home for over two years.

This study started with a mapping effort that was led by Kent Codding (W&M) and Ryan McIntyre (GMU), completing a joint undergraduate research project on the distribution of invasive plants in the Northern Virginia Stream Restoration Bank corridor. These two scoured miles of riparian habitat in the Snakeden and Glade watersheds mapping invasive species, and their results were instrumental in homing in on ideal sites to conduct this experiment.

Last but not least, I'd like to thank the grantor of this amazing project, Resource Protection Group, Inc. (RPG) for funding this research. Without their support none of this would be possible. Special thanks to Mike Rolband, RPG president and Chairman of the Board, for insight on project setup and coordination with RA to help get this study in the ground.

This thesis is dedicated to my mom who always believed I could do great things. I hope I can continue to prove her right.

LIST OF TABLES

LIST OF FIGURES

Chapter 1: Introduction

1.1. Current Issues with Invasive Plants

Invasive species pose an existential threat to ecosystems and economies by disrupting established natural systems and creating areas solely dominated by these introduced species. An invasive species is often defined as a plant that enters an area that it previously didn’t occupy, and then rapidly expands and has negative effects on established species (Alpert et al., 2000). In the US it is specifically defined as a species “whose introduction causes or is likely to cause economic or environmental harm or harm to human health” (Executive Order 13112). Invasive plants are often non-native, but this is not always the case as any plant that outcompetes other species and causes damage to that ecosystem is considered invasive (e.g., *Typha latifolia*; Perry et al. 2009). Invaders compete with native plants for any number of resources, including light, nutrients, water, and space, and the resultant reduction in native diversity from superior invasive competitors is among the most damaging of environmental consequences stemming from plant invasion (Wilcove et al. 1998, Pimentel et al. 2001, Pauchard and Shea 2006, Davis 2009, Powell et al. 2011). The number of invasive species has continuously increased over the past two hundred years, but the rate of increase has risen dramatically over the past several decades. Case-in-point, a third of all invasive species were introduced and identified between 1970 and 2014 (Seebens et al. 2017). The U.S. spends around $10 billion annually on controlling invasive plants, which includes using herbicides and physically removing invaders (Pimental et al. 2011).

Current dogma for dealing with invasive plants is the use of herbicides (e.g., glyphosate) to control and remove invasive species (Guynn et al. 2004). One of the biggest issues with using herbicides is that most of them used are non-selective, which results in collateral mortality of desirable native species along with the invaders being targeted (Wilson and Gerry 1995, Kettenring and Adams 2011, Lawrence et al. 2016). Killing all vegetation in the areas applied with herbicide creates a disturbance (i.e., removal of biomass) that may allow for a reinvasion (see Figure 1; Gibson et al. 2019). This follows the stress-disturbance model for invasive plants in that invasion typically occurs in areas with atypical disturbance regimes and low levels of stress (see Figure 2; Alpert et al. 2000). There are many studies that look at changing the disturbance regime to combat invasive plants (e.g., Glasgow and Matlack 2007, Flory and Lewis 2009, Flory 2010), or different temporally spaced treatments (Beam et al. 2022), but fewer studies on the effect of different stress agents, outside of herbicides, on invasive plant growth and dominance.

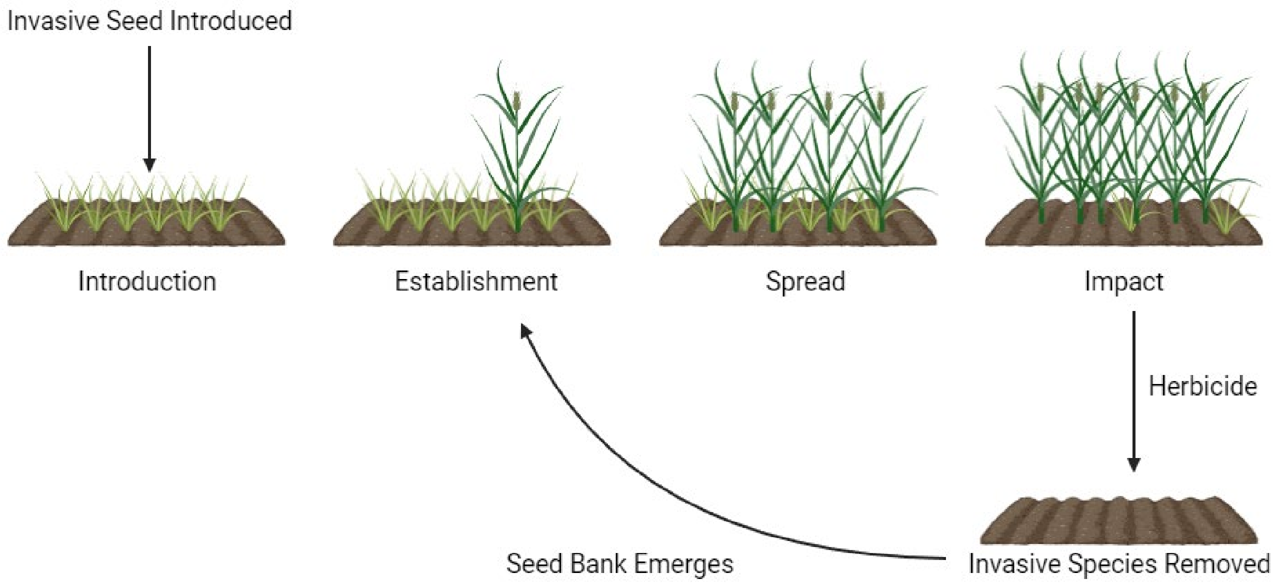


Figure 1. Reinvasion pattern of invasive plants after removal of the target species.

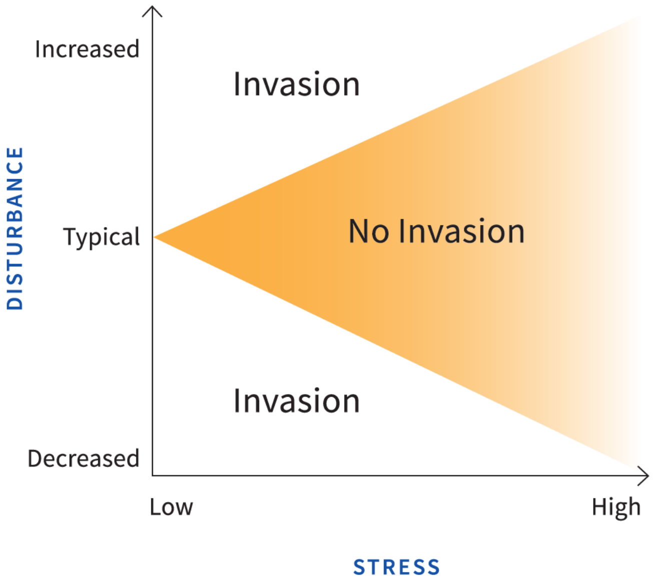


Figure 2. The relationship between stress, disturbance, and plant invasion in natural ecosystems (adapted from Alpert et al. 2000; VHB, Inc., used with permission).

1.2 Cultural Methods

A study of stress agents for controlling invasive species, such as cultural methods, is needed, however we have little understanding of their effectiveness under different ecological conditions. Cultural methods can be defined as naturally occurring methods, like shade, or agricultural methods, like mulching. Some possible methods that could be applied to invasive species include reducing available light with shade (DeBerry and Hunter 2024), reducing available nutrients with high carbon soil amendments (e.g., processed wood; Perry et al. 2004, Homyak et al. 2008), and reducing available resources through competition by increasing the number of native plants in the community (Sheley and Half 2006). Following the stress-disturbance model, reducing light availability, imposing a nutrient limitation, or increasing competition can increase the level of stress and therefore reduce the amount of invasion in a heavily disturbed area (Alpert et al. 2000). For example, high carbon-to-nitrogen (C:N) ratio materials have been shown to stimulate microbially metabolism by adding more consumable carbon (C) to the soil, which in turn creates a consumption of nitrogen (N) that is needed for growth activities (i.e. amino acid production and respiration), which leads to less available N in the soil (Perry et al. 2004, Iannone and Galatowitsch 2008, Homyak et al. 2008). These cultural treatments could be effective alternatives to herbicide use, which would be extremely useful to public and private land managers.

1.3 Stream Restoration, Compensatory Mitigation, Mitigation Banking, and the Impact of Invasion on Stream Mitigation Sites

Cultural methods could prove especially useful in combating invasive species in restored ecosystems. Streams restoration is an example of a specialized type of applied ecology where the threat of invasion is large, owing mostly to the fact that streams are dynamic systems with large watersheds and therefore open to multiple inputs (e.g., seeds of invasive plants) and potential disturbance factors (e.g., flooding) (DeBerry and Hunter 2024). Streams are often restored by filling in downcut or otherwise degraded channels and replacing them with new ones by reestablishing healthy stream pattern and profile using natural channel design principles (Shields et al. 2003, Mattern et al. 2020: Figure 3). Although necessary, the construction process creates a major disturbance which, as stated earlier, can increase the risk of invasion. By introducing cultural treatments and leveraging the stress-disturbance model to reduce invasion at the inception of a stream restoration project, stream practitioners may be able to attenuate that risk and reduce the need for more aggressive intervention later.

Restored streams or other ecosystems constructed to compensate for impacts to ecosystems elsewhere are referred to as “compensatory mitigation”. Compensatory mitigation is required for activities that will result in impacts to natural resources that are protected under the preview of the federal and state law. In the case of streams, the protection is under the auspices of Section 404 of the Clean Water Act (33 USC §1344) and analogous state water control laws. These laws require any impacted stream to be mitigated, and this is typically required as a condition of a permit received from the U.S. Army Corps of Engineers, the agency that reviews projects under Section 404. The

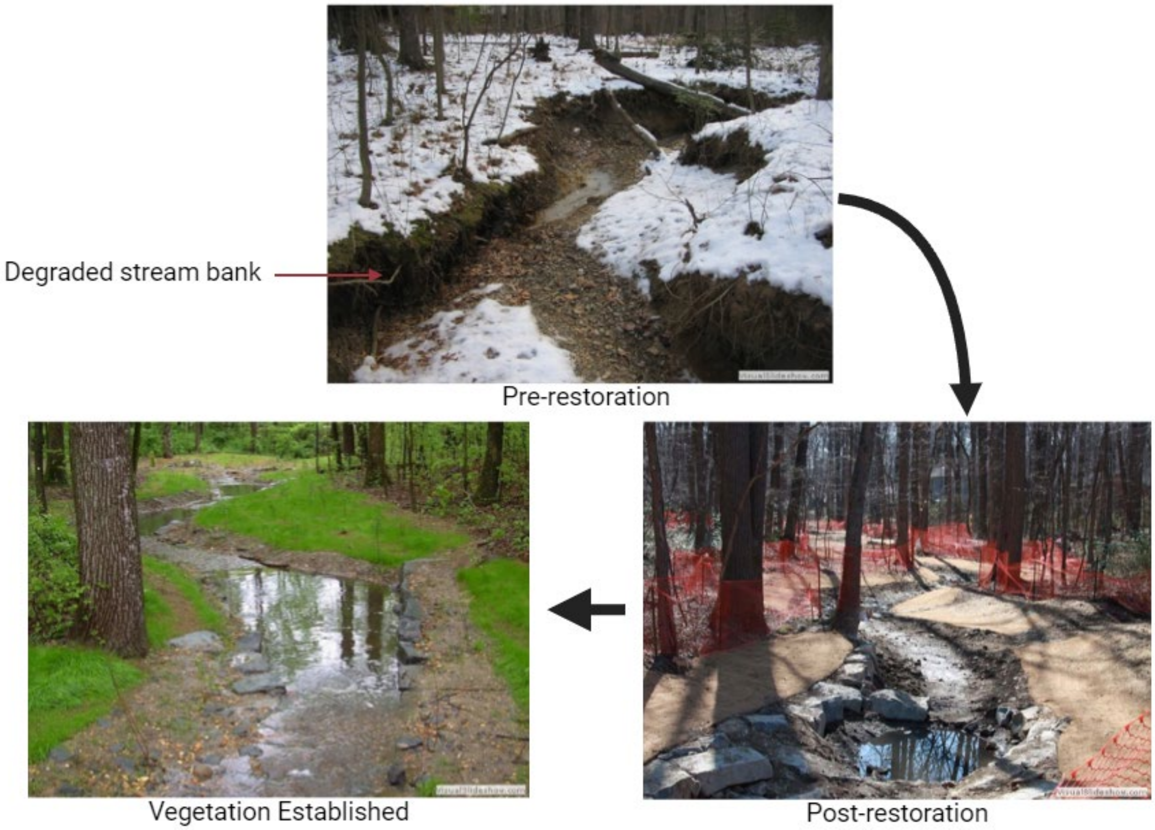


Figure 3. The restoration process from degraded streambank (left) to reconstructed stream (middle) to fully restored stream (right) at the Northern Virginia Stream Bank (NVSB; Wetland Studies and Solutions, Inc.)

permit recipient is referred to as the “permittee”, and the mitigation itself is typically accomplished through stream restoration. However, most permittees do not have the land, resources, or training required to restore streams, a situation that necessitates another viable alternative for compensatory mitigation.

In Virginia, that viable alternative is often facilitated by a third-party sponsor referred to as a “mitigation banker” who oversees the siting, design, construction, and monitoring of a “mitigation bank” from which that banker is allowed to draw revenue by sale of mitigation credits (D. DeBerry, pers. comm.). Anyone with a construction project that will impact a protected stream channel may purchase credits from the mitigation banker as compensatory mitigation for the length of stream lost due to their project. However, the above transaction can only proceed as long as: 1) the purchaser’s construction project is within close enough proximity to the mitigation bank to be considered in the same sub-watershed (i.e., equivalent to an 8-digit hydrologic unit code (HUC) as defined by the U.S. Geological Survey; Merchant 2010); 2) the federal and/or state agencies issuing the environmental permit for the project agree to the allow purchase of bank credits for mitigation; and, 3) the mitigation bank itself is meeting its “ecological performance standards,” i.e., it is a restored stream that is performing the expected aquatic resources functions of a natural stream system.

Ecological performance standards for stream mitigation banks include metrics like healthy communities of benthic macroinvertebrates, evidence of stable stream patterns and profiles, and – most importantly for this project – low to no levels of biological invasion (DeBerry and Hunter 2024). Invasive plants pose a real issue to both mitigation bankers and the restored site since the standard is usually stringent (e.g., no more than 5% cover of invasive plants; U.S Army Corps of Engineers 2018) and, as previously mentioned, stream corridors are highly susceptible to plant invasions. Also, invasion defeats the purpose of restoring an ecosystem to a more natural and stable state. If the performance standard is not met then credits can be withheld, which causes an economic loss for both the mitigation banker and the permittee who needs the banker's credits to stay in compliance with the stream protection laws.

Invasive plants pose one of the greatest challenges to mitigation sites and restoration managers in the Mid-Atlantic (Brooks and Gebo 2013). The cost of combating invaders at these sites continuously increases and accounts for the largest part of expenses at some sites (Bergdolt et al. 2005). Mitigation bankers and restoration managers could save both time and resources by using cultural treatments as preventative measures to treat invasive plants on their site. If used proactively, cultural treatments have the potential to offer inexpensive alternatives to herbicide by leveraging resources that are already available on site, such as existing canopy trees (shade) or processed wood from trees or shrubs removed during the construction process (nutrient limitation). In this way, cultural methods could reduce the likelihood of reinvasion or withheld credits compared to traditional herbicides. This would promote more investment in the healthy maintenance of these stream systems, which are not only a vital part of local ecosystems but also play an essential role in the health of larger waterways (Costenza et al. 1997, Verdenschot and Verdenschot, 2023).

1.4 Microstegium viminieum

M. vimineum is a non-native annual grass originating from East-Asia that was first recorded in Tennessee in 1919 (Fairbrothers and Gray 1972) and has rapidly spread across the eastern United States over the past 100 years (Barden, 1987). What makes this plant such an effective invader is that it is a prolific seeder, uses waterways as a vector, and once established generally dominates both sunny and partially shaded areas causing it to crowd out and outcompete important native plant species (Oswalt et al. 2007, Warren et al. 2011; as seen in Figure 4). Invasion from *M. vimineum* is often encouraged by increased levels of light and nutrients (Waren et al. 2011), which makes it a great invader of restored streams, where the canopy is often opened during construction, and flooding events can cause excess of nutrients.

Many of the outcompeted native plant species are flowering, deep-rooted plants that would otherwise provide soil stability (Rajan et al. 2015) and a source of food for native pollinators. The most efficient way to retain these ecosystem functions would be to remove *M. vimineum* without removing native species; however, the methods typically employed to remove invaders like *M. vimineum* involve use of non-selective herbicides that result in categorical mortality of all plants in the community (Wilson and Gerry 1995, Kettenring and Adams 2011, Lawrence et al. 2016). Herbicide is typically effective for the first year (Judge et al. 2005, Flory 2010), but because of its prolific seeding it often returns from an established seed bank after it has been removed, which can also result in developed resistance to herbicide (Miller and Matlack 2010, Ziska et al. 2015).

Cultural treatments as a stress agent could provide a potential alternative to herbicide that would accomplish invasive control without wholesale collateral damage to the native species community (Perry et al. 2004, Homyak et al. 2008, DeBerry and Hunter 2024). The cultural treatments mentioned earlier (shade, high C:N soil amendments, native seeding) offer an alternative to herbicide that can be effectively implemented into a stream restoration project.

Figure 4. An understory area in Reston, Virginia completely dominated by *M. vimineum*.

1.5 Overall Importance

Extensive research has been done on *M. vimineum*, specifically in stream systems where the species proliferates and can become dominant (Barden 1987, Flory 2010, Warren et al. 2011). Research specifically on *M. vimineum* in stream restoration settings is less prevalent in the literature, but available studies underscore the importance of removing this and other invaders to attenuate ecosystem deterioration and allow time and funds to be allocated to other restoration efforts (DeBerry and

Hunter 2024). *M. vimineum* continues to be of relevance in restored streams in the Mid-Atlantic specifically (DeMeester and Richter 2010), as its traits and life strategies give it a competitive advantage against other plants in the region (Williams and Brewer 2024).

The purpose of this project is to test specific cultural methods to determine level of effectiveness as preventative measures to control *M. vimineum* invasion on stream restoration sites. Based on prior research, the most promising approaches include techniques that would: 1) reduce light availability, 2) stimulate nutrient limitation, 3) increase native competitors, or 4) combinations of these. With this knowledge restoration managers and land managers in general will hopefully benefit from the ability to control invasion of *M. vimineum* and similar invaders before it occurs. The remaining chapters of this thesis will review and explain many of the different cultural methods available to managers, and how they may be effective against an invader like *M. vimineum* (Chapter 2) and present the findings of a two-year field experiment testing the above-mentioned methods on a stream restoration site in Virginia (Chapter 3).

Chapter 2: Cultural Approaches for Invasive Species Control in Stream Restoration: A Review

2.1. Introduction

Invasive plant species are a continuous problem for restoration managers and mitigation bankers (Blossey 1999). Non-selective herbicide (e.g., glyphosate) continues to be the current dogma for managing such invaders (Guynn et al. 2004) even though it has negative ecosystem repercussions (Relya 2005, Lawerence et al. 2015, Badani et al. 2023). Not only does herbicide have negative effects on the ecosystem and biodiversity, but since it causes the death of plant biomass it acts as disturbance event, which could allow for reinvasion (Alpert et al. 2000, Gibson et al. 2019). It is therefore imperative to find alternatives to herbicide through other means.

Cultural methods represent a possible alternative for removing and preventing invasive plants on a large scale. These methods tend to work by using natural and agricultural methods to apply stress to the invaders while promoting native species that have moderate stress tolerance (Alpert et al. 2000). Here, we are focusing on the stress agents of light limitation, nutrient limitation, and competition, which can be induced on ecological restoration sites by canopy tree conservation or management, soil amendments, and targeted planting techniques. These were approaches that were suggested in a recent study on environmental drivers of invasion in stream restoration by DeBerry and Hunter (2024), with a specific focus on *M. vimineum* as the target invader.

Cultural treatments such as these could be beneficial in restored stream ecosystems, which are often heavily disturbed during the restoration process and therefore become vulnerable to invaders. This can be a problem for restoration managers as there are often government mandated restoration standards, specifically invasive plant cover thresholds (e.g., > 5% cover of an invader; U.S. Army Corps of Engineers 2018). Equally problematic is the threat that some shallow-rooted invaders like *M. vimineum* pose to the structural integrity and soil stability of a restoration site. Unfortunately, the research around *M. vimineum* and other invaders often focuses on the use of herbicide, or other disturbance methods, instead of looking at stress-based methods. A better understanding of these stress focused techniques and treatments could allow for better prevention of invaders in restored stream sites or other ecosystems that are subjected to disturbance.

This review aims to expand on the research of DeBerry and Hunter (2024) by analyzing and compiling available research on the above-referenced cultural methods, with a focus on treatment of invasives in disturbed habitats like constructed streams. *M. vimineum* will be the focal invader, and streams in eastern North America will be the targeted habits for research sites (wetlands, forests, and grasslands will also be considered due to their similar ecosystem types). The purpose of this is to create a comprehensive list of possible cultural methods that can be used to develop a suite of best practices for preventative, herbicide-free management of invasive species.

2.2. Review Methodology

For this section we created a methodology to narrow the body of literature reviewed. We systematically selected papers believed to be most applicable through search methods, currentness of research, and relevance to controlling invasive plants, cultural methods and treatments, or alternatives to herbicide. Studies cited as essential methods or theories by reference were reviewed as well. Google Scholar. William & Mary Libraries, and other academic literature search engines (e.g., Scopus, EBSCOHost, ScienceDirect, ProQuest, etc.) were used to search for peer-reviewed scientific papers. Searches used keywords including “herbicide alternatives,” “cultural methods,” “invasive species management,” and “natural solutions for *Microstegium vimineum*.” We searched through the most relevant and recent papers and focused on those that looked at *M. vimineum* and/or restored streams.

2.3. Invasion Stress and Disturbance Model

The invasive plant stress-disturbance model, mentioned in Chapter 1, is based on the of Alpert et al. (2000; see Figure 2), but it draws heavily from the mid-1900s work of vegetation ecologist Philip Grime (Grime 1979, Craine 2009). Grime’s original conception of the “CSR” life history strategy triangle for vegetation recognizes three categories of wild plants: 1) competitors, 2) ruderals, and 3) stress-tolerators (Figure 5). In Grime’s view, the classical model of “r vs. K selection” (*sensu* MacArthur and Wilson 1967) was short-sighted for plants because it focused on habitat equilibrium, or lack thereof, as the primary determinant of plant community composition, classifying species as either ruderal (i.e., opportunistic, weedy) or competitive. Grime provided strong evidence for a third strategy that he termed “stress-tolerator”, referring to some plants’ ability to persist under stressful conditions (e.g., light or nutrient limitation, drought, salt stress, etc.; Grime 1979). Alpert et al. (2000) built on this model by proposing that invasion is directly linked to two main factors – disturbance and stress – with both aspects being important for understanding outcomes of competition, why

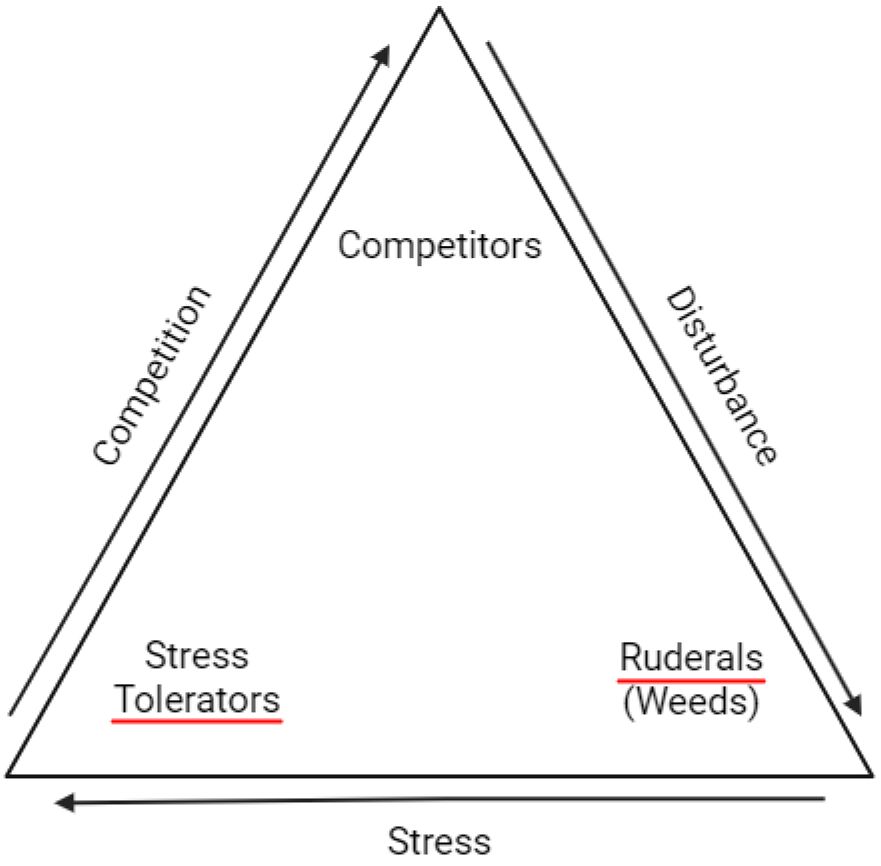


Figure 5. The Grime triangle identifying three life history strategies of wild plants (Adapted from Grime 1979).

some areas are more invaded than others, and how invaders might be reduced in areas that have increased disturbance and are therefore more vulnerable to invasion. Our research focuses on changing the stress agents in invaded environments, but first we need to understand why disturbance is important and how these disturbance regimes change and affect invasion patterns.

Disturbance is defined as “the mechanisms which limit the plant biomass by causing its partial or total destruction” (Grime 2001, p. 80). By contrast, stress is defined as “any unfavorable condition or substance that affects or blocks a plant’s metabolism, growth, or development" (Lichtenthaler 1998), with the tacit understanding that stress does not involve direct removal of biomass. In the case of disturbance, we are looking at the removal of plant biomass specifically in restored stream systems where there is often heavy construction and earth moving to restore the eroded stream banks and stream sinuosity. Through this restoration process, biomass is lost in all vegetative strata from trees to herbaceous plants, and this can lead to invasion due to the availability of newly opened habitat and the opportunistic nature of invaders (DeBerry and Hunter 2024).

D'Antonio et al. (1999) postulated that it is likely that the more an area departs from its "typical" disturbance regime the more likely it is to be invaded. An example of a typical disturbance regime for a natural ecosystem would be a 1- to 2-year flood return interval for a stream channel floodplain, with the flood representing a mode of disturbance to the plants due to flood scour, uprooting, debris damage, or smothering from deposited sediment (Marks et al. 2014). Although our focus is stream systems, this scenario is easily observed in other ecosystems. For example, in experimental studies, Smith and Knapp (1999) found that grasslands with an established fire regime saw a decrease in invasion after burning but an increase after grazing. In a similar experiment, Milchunas et al. (1989) found that grazing reduced invasion in grasslands where grazing (not fire) was the typical disturbance regime. The difference in disturbance regime explains the different outcomes: fire is often more intense but less frequent, while grazing is less intense but more frequent. In either case, the typical regime establishes the balance of life history strategies among the plants in the community, and a change in that balance could represent an open doorway for invasion. Along these same lines, Richardson & Bond (1991) found that depending on the ecosystem and environment fire can either increase or decrease invasion by pines. The result depends on whether the established native plants are adapted to such changes in the environment, and whether nearby invaders are more likely take advantage of newly opened areas and preempt space that would otherwise be occupied by native species.

These considerations are magnified in stream restoration due to the multiple vectors for seed introduction (e.g., watersheds, flooding) superimposed on the atypical disturbance regime represented by stream construction. This reality of stream restoration was underscored by DeBerry and Hunter (2024), who were working on multiple sites in the Mid-Atlantic Region. In their words: “If the ‘disturbance’ half of the stress-disturbance dynamic is an unavoidable consequence of the [stream restoration] construction sequence, there may be alternative approaches that would allow… designers and managers to manipulate the ‘stress’ half to reduce the risk of invasion.” Here, DeBerry and Hunter (2024) were referring specifically to the cultural approaches outlined in this chapter.

For this research we are focusing on resource availability as it is generally the most straightforward way to manipulate stress. Dukes and Mooney (1999) found that low stress favors invasive species because they have a superior ability to take advantage of higher resource amounts compared to native species. We are specifically interested in stress agents that affect our focus species, *M. vimineum*, which includes light (Hunter and DeBerry 2023, DeBerry and Hunter 2024), nitrogen (DeMeester 2009, DeBerry and Hunter 2024), and competition (Sheley and Half 2006). Other stress agents are either too difficult to control (e.g., temperature), too expensive (e.g., toxins), or too subjugated to the unpredictability of natural events (e.g., flooding). In the following sections we will explore specific agents of stress as cultural methods for dealing with invasion.

2.4. Negative Effects of Herbicide

Herbicides continue to be the most dominant form invasive species management across agriculture, horticulture, and especially ecological restoration (Guynn et al. 2004). Glyphosate is the most common active ingredients in non-selective herbicides, like RoundUp™, and has multiple negative effects on both ecology and possibly human health. Invasive species are currently defined in the US as a species “whose introduction causes or is likely to cause economic or environmental harm or harm to human health” (Executive Order 13112). Treating invasive species with herbicides replaces one harmful factor with another, and herbicide treatments can often lead to reinvasion. Invasion typically occurs in areas with high disturbance (Hobbs and Huenneke 1992), so in this context herbicide creates disturbance where the same or new invasive species are likely to invade (Gibson et al. 2019; see Figure 1).

Herbicide has been linked to negative effects on biodiversity and overall ecosystem health. Lawerence et al. (2015) found that the use of herbicide, specifically glyphosate, to reduce invasive cattails (*Typha* x *glauca*) resulted in a reduction of native biodiversity. This study also found that glyphosate resulted in an immediate increase of available nutrients in this ecosystem, which reduces stress and increases the likelihood of invasion. Van Brugenn et al. (2018) found that plants treated with glyphosate often do not produce secondary compounds, which includes antimicrobial phytoalexins that help defend against pathogens. If a glyphosate treatment overreaches the surrounding environment, it could effectively weaken the immune system of desirable plants and reduce diversity.

Herbicides have also been shown to result in negative effects on animals. This is especially concerning in or adjacent to active waterways, which can act as a vector for spreading the chemical beyond its intended target area. For example, Relya (2005) found that glyphosate was responsible for the death of aquatic animals such as macroinvertebrates in nearby waterways. Glyphosate, and specifically its primary commercial imprint Roundup™, resulted in a reduction of 22% aquatic animal species richness, eliminating 2 species of frogs (specifically in their tadpole form) and almost wiping out a third. Animals such as macroinvertebrates are essential to the ecosystem functions of aquatic resources like streams and can act as a bioindicator for the health of these ecosystems (Leigh et al. 2013, Lu et al. 2019). Use of herbicides countermands the goals of keeping of aquatic fauna healthy, which is a foundational objective of stream restoration.

Herbicides have also been shown to negatively affect soil microbial ecosystems. A review by Badani et al. (2023) found that glyphosate had a wide range of effects on microbial bacteria and fungi and their role in nutrient cycling. Glyphosate can negatively impact beneficial soil bacteria while also encouraging pathogenic plant fungi, altering not only the soil microbe make-up, but also the availability of nutrients for future plants and microbes (Zabaloy et al. 2012, Zhan et al. 2018). This effect on microbes and subsequent nutrient cycling is linked with excess nutrients caused by glyphosate treatments in the cattails experiment by Lawerence et al. (2016).

In addition to the above impacts on natural systems, recent research has highlighted the potential consequences of glyphosate use on human health. Neurological and neurodegenerative conditions like ADHD, Autism, Alzheimer's, and Parkinson's have been linked to exposure (Jayasumana et al., 2014, Swanson et al., 2014, Mesnage et al., 2015, Fluegge and Fluegge, 2016, Fortes et al., 2016). The mechanism for human health impairment may be glyphosate's disruption of mitochondrial function, leading to cell death (Clair et al. 2012). These systemic effects have led glyphosate to be classified by the Agency for Research on Cancer as a known human carcinogen (Séralini et al., 2014, IARC, 2015). However, this classification has been highly debated particularly by the chemical and agribusiness industries (EPA 2024), so further research is necessary to clarify the full range of negative human health effects. Regardless, glyphosate should be regarded as a chemical that can impact both ecosystem and human health.

Even though glyphosate is a common option for herbicides there are other chemicals on the market that are also used for controlling invasive species. Two examples include fluazifop, a post-emergent herbicide, and pendimethalin, a pre-emergent herbicide. Both have been shown to kill *M. vimineum* (Flory 2010). Fluazifop is sprayed on plants after they emerge, but unlike glyphosate it is a selective herbicide that only targets grasses (Lewis et al. 2016). It works by inhibiting acetyl CoA carboxylase, which disrupts fatty acid biosynthesis and lipid formation in grass species (Lewis et al. 2016). There is moderate toxicity to biodiversity and has been shown to have both cytotoxic and genotoxic effects that may cause cytological changes on non-target plants and species (Lewis et al. 2016, Ammar et al. 2022). Pendimethalin is both a pre-emergent and post-emergent herbicide, and specifically targets grasses and some broadleaf herbs (4 Farmers 2024). This herbicide works by targeting root and shoot uptake and inhibits cell division and growth, which makes it a good choice for pre-emergent spraying (4 Farmers 2024). However, if the soil is not moist then the herbicide can be ineffective at penetrating the soil and targeting roots and shoots (4 Farmers 2024). Pendimethalin has been known to persist in the soil for relatively long periods of time (Zimdahl et al. 1984) and has been linked to pancreatic cancer through the forming of nitroso-compounds, which affect the pancreas (Andreotti 2009). These two alternative herbicides aren't as well researched as the more common glyphosate but seem to have different negative side effects to ecosystem and or human health regardless of their chemical pathway and make-up.

These negative effects could be amplified in ecosystems like streams and their riparian corridors due to the potential for herbicides to be moved from the targeted site to other areas by flooding. This dynamic has been shown for other residual chemicals in floodwaters. For example, mercury released into rivers has been traced from flooding to aquatic insects to predatory terrestrial insects and ultimately to terrestrial birds, with significant bio amplification of blood mercury levels (Cristol et al. 2008). Although glyphosate is rated for low bioaccumulation risk due to its low fat solubility, it has been shown to bioaccumulate in shorter food webs, with the potential to increase bio-mobility in the presence of the surfactants that are mixed into herbicides to increase surface-to-leaf contact when spraying (Annett et al. 2014). This could lead to unforeseen consequences to the surrounding flooded area in cases where extensive glyphosate is sprayed. Taken in combination with the above considerations, these potential effects emphasize the importance of finding new alternatives to combat invasive plants in restored stream systems.

2.5. Shade Treatments on Invasives

Light is an essential resource to all photosynthetic plants; therefore, shade is a stress agent because it reduces accessibility to a critical resource. Shade can be implemented at the scale of an ecological restoration project by either removing fewer large canopy shade trees or planting larger stock of fast-growing trees. Based on restoration-focused research, this could represent a viable tool for reducing and preventing invasive plants in restored streams and other invaded ecosystems (Hunter and DeBerry 2023, DeBerry and Hunter 2024).

At the level of an individual plant, competition for light is restricted to plants that are within the same range of height growth (Craine 2009 pg. 109-111). *M. vimineum* is a fast-growing species that can establish in an area and dominate light, blocking access for desirable native herbaceous species. This competitive edge may be attenuated by shade at higher levels of community strata, such as understory shrubs and saplings or canopy trees (Warren et al. 2011). *M. vimineum* has been described as shade tolerant, and in greenhouse settings can perform similarly at 18% and 100% full sunlight and can even grow at 5% full sunlight (Winter et al. 1982). However, in competitive settings such as stream restoration sites that are under the influence of other environmental factors (e.g., soil nutrient status, temperature, moisture, etc.), *M. vimineum* invasion has been shown to be negatively correlated with shade (Nord 2011, DeBerry and Hunter 2024).

This suggests that native plants may be more competitive with *M. vimineum* in heavily shaded areas than areas with more available light.

On ecological restoration sites, shade can be leveraged as a stress factor by reducing the amount of tree clearing during construction. This approach also offers a generally cheaper alternative to herbicide if there is already a well-established canopy. However, one of the unfortunate realities of stream restoration is that relocation of the stream channel usually necessitates tree removal (Shields et al. 2008). Careful planning and conservation-focused construction techniques can reduce some of the clearing but not all, particularly in the “limits of disturbance” (LOD) zone where the active channel is being constructed. In these circumstances, it may be appropriate to plant large, fast-growing, early successional trees that will promote canopy closure after the site has been constructed (DeBerry and Hunter 2024). Examples of tree species that would be appropriate for the eastern US include red maple (*Acer rubrum*), silver maple (*A. saccharinum*), river birch (*Betula nigra*), dogwood (*Cornus* spp.), sweetgum (*Liquidambar styraciflua*), sycamore (*Platanus occidentalis*), eastern cottonwood (*Populus deltoides*), and black willow (*Salix nigra*) (Spencer et al. 2001, Diggins 2013, Marks et al. 2021).

2.6. Nutrient Limitation: High Carbon:Nitrogen Treatments

Nitrogen is the most limiting nutrient in most terrestrial ecosystems (LeBauer and Treseder 2008). Specifically, it is the relative amount of bioavailable nitrogen in soils that is the most common limiting factor, as it is often the most demanded nutrient besides readily available carbon that is required for primary production, and therefore is one of the most common stress agents to plants. Nitrogen runoff has become more common due to agricultural inputs, and in the Chesapeake Bay, it accounts for roughly 60 percent of the total nitrogen entering the Bay (Chesapeake Bay Foundation 2024). Other sources include lawn fertilizers, and unmitigated wastewater, which makes suburban areas, as well as rural areas, extremely vulnerable to excess nitrogen deposits. This excess nitrogen reduces stress in nitrogen limited ecosystems, like forests and streams, which can lead to an increased risk of invasion (Alpert et al. 2000).

Many invasive species are nitrophilous (i.e., prefer habitats with high nitrogen availability), so their resource acquisition strategies are typically more efficient for nitrogen uptake than most of the native species found in the geographies that they invade. *M. vimineum* is especially nitrophilous as it seems to do extraordinarily well with high amounts of nitrogen compared to other plant species. For example, in a study comparing relative growth rates, Ross et al. (2011) *M. vimineum* grew best at higher levels of nitrogen when compared to another invasive species (*Berberis thunbergia*) and two co-occurring native species (*Vaccinium pallidum* and *Hamamelis virginiana*). In fact, *M. vimineum* was the only plant to show a significant difference between high levels of nitrogen and medium to low levels, with an increase of almost double the relative growth in steam height. Specifically, nitrate was the bioavailable form of nitrogen that caused such high levels of growth, which is reasonable as it is the form of nitrogen entering soil through the action of nitrifying bacteria. In habitats with high nitrogen availability, this process makes nitrate more available to plants like *M. vimineum*, which can quickly assimilate nitrogen into its tissue and increase growth rates. At ground level, taller *M. vimineum* will outcompete native species for not only nutrients but also light. Gilliam

(2006) found that in forested ecosystems slow growing plants, many of which are native, prefer NH4+ to NO3-. This could mean that nitrogen-rich environments privilege fast growing invaders, like *M. vimineum*, compounding the advantage where the nitrogen cycling could be changed permanently. In ecosystems like streams where pulsed delivery of nitrogen from runoff is inevitable (Gordon et al. 2020), control of invaders like *M. vimineum* may benefit from methods that could potentially immobilize nitrate and thereby impose a nutrient limitation in the soil.

One of the most effective ways to remove nitrogen from the environment is to increase the availability of carbon to immobilize nitrogen (Perry et al. 2010; Figure 6). Carbon serves as a food source for heterotrophic microbes in the soil, so increasing it increases microbial biomass and activity (Zink Ret and Allen 1998, Baer et al. 2003). This increase in microbial activity leads to greater uptake of nitrogen, specifically NO3- and NH4+, the two most common forms of available nitrogen in the soil. Nitrogen is needed in the metabolic functions of the soil microbes, which increases when large amount of carbon is add, leading to the immobilization of nitrogen and therefore lower ambient levels of available nitrogen in the soil (Baer et al. 2003, Averett et al. 2004; Figure 7). Since microbes are better competitors for nutrients than plants (Marion et al. 1982), the nitrogen that would otherwise be used by plants is removed from the zone of root uptake. A review by Perry et al. (2010) found that among methods focused on lowering nitrogen in the soil, most only had a small effect on the total nitrogen but immobilization was far more promising, especially when low nitrogen-demanding species were in competition with the nitrophilous invaders, like *M. vimineum*.

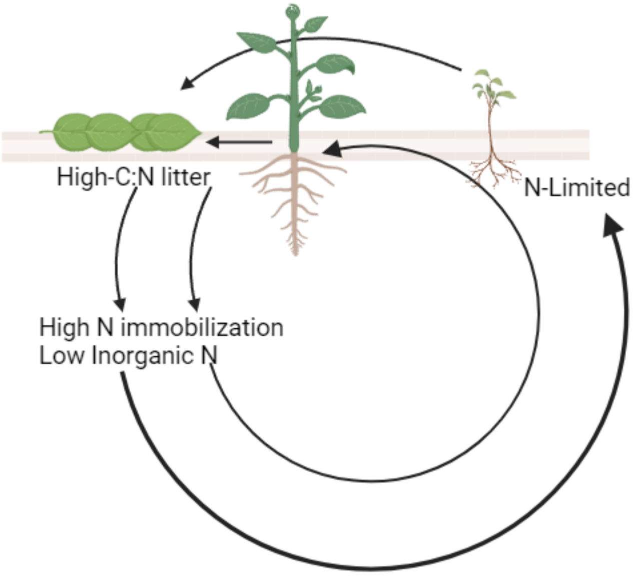


Figure 6. A low-nitrogen system where a high C:N organic source often leads to immobilization and nitrogen limitation for plants. The plant to the left represents a low-N species that is better adapted to low nitrogen environments and the plant to the right represents a nitrophilous plant that is limited by the low levels of nitrogen (adapted from Perry et al. 2010).

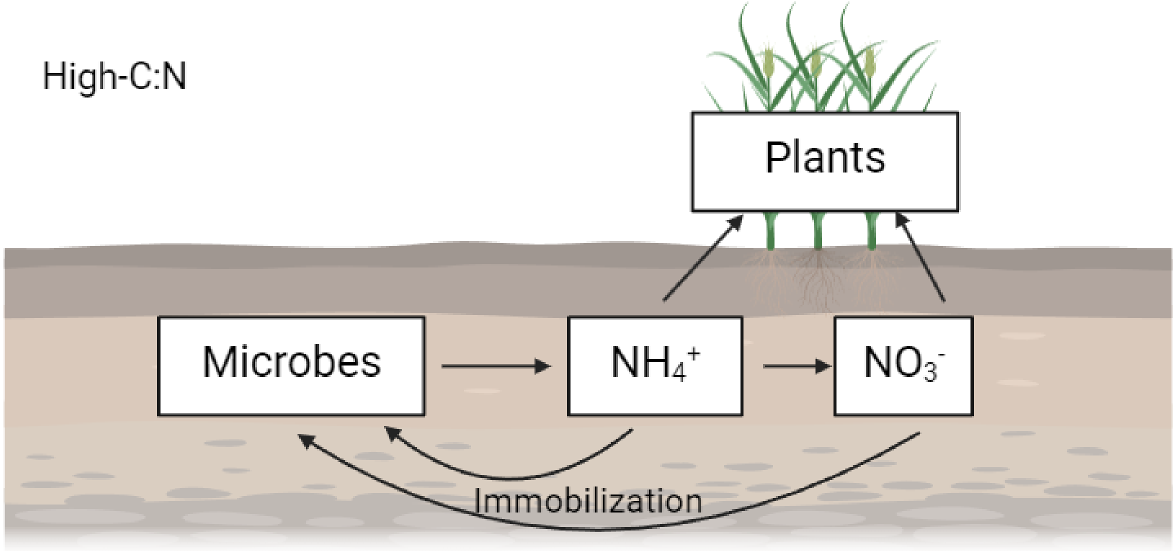


Figure 7. High C:N conditions in which microbes immobilize available nitrogen (i.e., NH4+ and NO3-), which competes with plant uptake of the nutrient (adapted from Schimel and Bennett 2004).

By adding a high carbon source to increase the carbon-to-nitrogen ratio (C:N) microbial activity could be leveraged to immobilize and reduce the overall amount of available nitrogen to invasive and native plants alike. However, since higher stress reduces invasion, native species with some amount of stress tolerance are more likely to be better adapted to an N-limiting condition (Alpert et al. 2000). There are many sources of carbon, but processed wood is the easiest form to work with and the most commercially available for restoration projects. Since processed wood can come in different shapes and forms, we will be focusing on two main forms that are likely to be the most accessible and effective, sawdust and wood mulch. Sawdust is likely to be more labile than wood mulch due to its greater surface to volume ratio (Davis and Whiting 2000). This means that microbially-mediated nitrogen immobilization should happen at a faster rate in sawdust-amended soil compared to wood mulch-amended soil. However, wood mulch may have more longevity than sawdust and may provide a longer-term effect, so the merits of both approaches are worth investigating.

Soil amendment ratio of soil to processed wood is also important to consider. Generally, soil microbes start to immobilize nitrogen at a C:N of around 20:1 or higher (Chapin et al. 2002). This means that when adding high carbon medium into the soil, it is important to add enough to reach a C:N of 20:1 or more in order to reduce available nitrogen for the desired level of plant nutrient stress. Perry et al. (2004) found that to reduce the invasive *Phalaris arundinacea* and encourage native *Carex* species in an invaded wetland a volumetric ratio of soil-to-sawdust at 2:1 (9:1 by weight) was effective. The C:N ratio of soils and processed wood might vary so it is important to take this into account when prescribing wood amendment to reduce invasion, but it seems that generally a volume ratio of 2:1 is a reasonable prescription according to the literature.

If effective, the value of these approaches is that they are cheap to implement and, more importantly for ecological restoration, the materials may be sourced onsite. Sawdust is readily available from lumber production, but its use as a soil amendment could require the extra step of mobilizing and hauling to a site (i.e., if timber sawing is not a part of the construction sequence on a restoration site, which is likely the case for most projects). By contrast, wood mulch can be processed with industrial chipper/shredder equipment directly on a restoration site. In either case, the cost differential may be negligible when compared to the expense of herbicide management.

2.7. Native Plant Competition with Invasives

Competition is defined as “the process by which two or more individuals differentially capture a potentially common, limiting resource supply” (Craine 2009 pg. 93). This means that when two plants are competing, they must “be competing for a limiting resource when the resources acquired by one plant could have been acquired by the other” (Craine 2009 pg. 93). A full-grown tree cannot compete with a grass for light because grass could never acquire the light that the tree obtains in the upper canopy, yet a plant upstream in a watershed can compete with a plant downstream for nutrients. When looking at native plants that could potentially compete with an invader like *M. vimineum*, it is therefore important to focus on species that grow on the forest floor, and especially ones that could shade this invader before or after it emerges.

As noted, *M. vimineum* is a highly competitive grass and outcompetes other species in both greenhouse (Liecht et al. 2005, Morrison et al. 2007) and field experiments (Morrison et al. 2007, Marshall et al. 2009, Moyer and Brewer 2018, Williams and Brewer 2023). *M. vimineum* is highly competitive for three main reasons: 1) it grows densely and forms a thick layer of detritus that decomposes slowly, blocking out light to other understory plants (Ehrenfeld et al. 2001, Flory 2010); 2) it produces a large number of seeds that it uses for the next generation, especially during times of disturbance (Barden 1987); and, 3) its seeds in the seedbank remains viable for at least three years in the soil, allowing it to outlast many other annual plants (Tanaka 1975, Barden 1987). *M. vimineum* also has a shallow root system allowing for lateral propagation (Templeton et al. 2020), which in turn will crowd out other plants' root systems. Craine et al. (2002) found that rhizomatous root systems tended to dominate high-nitrogen systems and had depleted levels of nitrate in the topsoil. This means that *M. vimineum*, and other shallow-rooted invaders, are dependent on initially high levels of nitrogen in the soil to quickly grow and outcompete native species, but they can only access the top level of the soil. Plants with deeper roots can likely be more competitive in low nitrogen topsoil environments, as they can reach deeper sources of nitrogen and other nutrients. However, interspecific competition is not categorically dependent on the availability of one nutrient, so other environmental factors should be considered when assessing the competitive outcomes of invasive and native species.

Huston (2004) argued that invaders will be more competitive in highly productive environments (low stress), and that diversity is a result of less competition rather than increased competitive resistance. In the case of *M. vimineum*, this is supported by experimental evidence finding that the invader is not both a threat to diversity and limited by diversity, but rather simply a threat to diversity (Williams and Brewer 2024). In the case of *M. vimineum*, and likely many other invaders, species diversity alone cannot be expected to reduce invasion but must be viewed as a metric of success for removing the invader from the environment.

Increasing competition with a native species therefore necessitates an increase in available native propagules for establishment in the disturbed condition of a restored stream corridor. Without an adequate supply of native seeds in the soil, recovery of the native community could lag, which could open the door for invasive species to become establish (Masters and Shelly 2001, Mason and French 2007, Mau-Crimmins 2007). Seed addition should replenish the seed bank and hasten native plant community recovery (Sheley and Half 2006). However, Flory (2010) found that seed additions didn’t help the recovery of native plant communities or increase overall native plant cover in areas previously invaded by *M. vimineum*, theorizing that seed predators or increasing soil pathogen activity could have inhibited seeding success.

A common management strategy for planting native seeds is doubling the recommended rate of seeding at a site to increase the amount of viable native seeds and plants (Yanelli et al. 2018), which would hopefully help the native plant community recover. However, it is unclear if this would be more effective than regular seeding rates, as regular seeding already seems to be ineffective, especially against *M. vimineum*, given the evidence. More field research is needed to fully explore and understand the effect of seeding rate on native-invasive competitive outcomes.

2.8. Other Cultural Treatments

Besides shade, soil amendments, and native seeding there are many other options for cultural treatments. Some examples include soil solarization, livestock grazing, burning, and water level manipulation (Clout and Williams 2009, Manning and Miller 2011). Soil solarization is the method by which one heats the top layer of soil by allowing high levels of solar heating on the ground and has been found to be effective at reducing invasive species through expanding the invasive seed bank (Cohen et al. 2018). However, this method would not be practical for several reasons, including removing tall trees, therefore destroying the canopy and changing the ecosystem. Grazing is another common cultural method and is often implemented through controlled grazing, such as adaptive multi-paddock grazing (AMP grazing) and rotational grazing. Grazing can have a positive effect in the right ecosystem (Milchunas et al. 1989), but in stream and other ecosystems in the eastern U.S. there is no native predation of *M. vimineum* by white-tailed deer (Knight et al. 2009) or any other recorded grazer, like goats or cattle. Burning can also be effective at removing invaders in certain ecosystems with established fire regimes (Smith and Knapp 1999), but for *M. vimineum,* Glasgow and Matlack (2007) found that controlled burns in forests allow for invasion. Also, burning is heavily controlled in the eastern U.S. and would not be a viable option for most land and restoration managers. Finally, controlling water levels can create drought and stress, but Webster et al. (2008) found that *M. vimineum* is drought resistant, and buffers against drought years through its extensive seed bank (Gibson et al. 2002). Also, controlling water levels, even in a constructed stream system, is extremely difficult and impractical to land and restoration managers.

2.9. Best Practices

When looking at the literature there seem to be three main effective cultural methods for dealing with invasive species, like *M. vimineum*. These cultural methods include canopy shade, high C:N soil amendments, and possibly increased rates of native seeding. According to the literature canopy shade and soil amendments seem to be the most effective at reducing invasion by creating high levels of stress that *M. vimineum*, and other invasive plants, aren’t well adapted to handle. It is unclear if native seeding or double seeding will be effective at increasing native species in previously invaded plots.

Given this literature review our recommendation for best practices to reduce invasion of *M. vimineum*, and similar invaders, in restored streams is to add high C:N sources (i.e., sawdust or wood mulch) to the soil in combination with shade management, the latter of which would be most effective if existing canopy tree removal could be minimized on a stream restoration site during construction. Although native seeding is important, it is unclear from the literature whether this technique would result in a beneficial and sustainable native herbaceous community that could outcompete invaders, but there is some evidence to support this approach. We plan to test these different treatments separately and in tandem to see the effectiveness of both individual and combined stress agents in a parallel field experiment. In the next chapter we will review this experiment and its outcomes and discuss how it compares to the available literature reviewed here.

Ch 3: Experimental Analysis of Cultural Methods to Reduce *Microstegium vimineum*

3.1.1. Invasive Species

Invasive plants are a constant issue for both our environment and our economies (Blossey 1999). Invaders can cause deterioration of local ecosystems, and around $10 billion is spent annually on invasive vegetation in the United States alone (Pimental 2011). Invasive plants are defined as those that enter an area that they previously didn't inhabit and cause harm to the established ecosystem and/or human health (Executive Order 13112, Alpert et al. 2000; see Section 1.1). Due to their deleterious effects, invasive plants are often treated with non-selective herbicide (e.g., glyphosate). The issue with these types of herbicides is that they don't just kill the invasive species, but also any desirable native species in the invaded community (Wilson and Gerry 1995, Kettenring and Adams 2011, Lawrence et al. 2016). This often leaves the area disturbed (i.e., removal of biomass) and with low levels of stress (e.g., high nutrient and sunlight levels), a combination of conditions that leaves sites susceptible to invasion (Alpert et al. 2000; see Figure 2). Removing invaders with herbicides often opens the treated area for reinvasion or incursion by new invaders, and ultimately may augment the invasion instead of stopping or attenuating it (Gibson et al. 2019).

3.1.2. Stream Restoration and Invasion

The above issues are especially problematic in ecological restoration, owing mostly to the fact that the practices used to create, restore, or enhance ecological conditions are often the same types of disturbances that leave a site vulnerable to invasion (Hunter and DeBerry 2023). This is particularly true of stream restoration sites, which are characterized by open energy cycles and are exposed to multiple modes of plant dispersal from the watershed (e.g., flowing water and flooding in the riparian zone) (Richardson et al. 2007). Invasive plant management on these sites has increased considerably in recent decades, and in most cases, it is compulsory, i.e., it is required as a condition of an environmental permit or a banking agreement for stream restoration after invasive plant species colonize (U.S. Army Corps of Engineers 2018). This reactive posture to management typically results in use of non-selective herbicides and significant collateral damage to native species (Kettenring and Adams 2011), but what is needed is a proactive approach to stream restoration with best practices aimed at inhibiting invasion from the start. Cultural invasive species control methods have the capacity to provide a means for such a proactive approach, but little field research has been completed to address the efficacy of these treatments.

3.1.3. Cultural Treatments

Cultural treatments are non-chemical alternatives that could be a solution for preventing invasive plants. Cultural treatments are land management approaches derived from agriculture, horticulture, and related disciplines to drive the outcomes of plant community composition toward desired goals (Manning and Miller 2011). Examples include use of non-chemical means such as prescribed burning, flooding or draining, livestock grazing, solarization, mulching, light manipulation, and strategic planting (Clout and Williams 2009). The latter three approaches – mulching, shade, and strategic planting – are of interest in stream restoration because of they are scalable to the level of an entire restoration site and would not be restricted by issues like federal, state, or local ordinances or prohibitions (fire, flooding, draining), inability to target invaders (grazing), or impracticability (solarization).

Many invaders are less correlated with heavily shaded areas (Hunter and DeBerry 2023), areas with less nitrogen (DeBerry and Hunter 2024), and areas with more competitive native plants (Sheley and Half 2006). A few cultural treatments that could reduce invasive plants include natural tree canopies to increase shade (Belote and Weltzin 2006), planting a high density of native species to increase competition with invaders (Craine 2009, Peter and Burdick 2010), and processed wood soil amendments to temporarily reduce nitrogen (Perry et al. 2004, Homyak et al. 2008). The latter technique reduces nitrogen by increasing the C:N ratio, which increases soil microbial metabolism and causes uptake and immobilization of available nitrogen that would otherwise be used by plants (Schimel and Bennett 2004). These cultural treatments offer an alternative to the short-term gains but potential long-term losses of biodiversity and ecosystem health that can result from use of herbicides. There is also little to no human risk associated with these cultural treatments compared to popular herbicides like glyphosate (e.g., RoundUp™; Zhang et al. 2019).

3.1.4. Target Species: Microstegium vimineum

Microstegium vimineum (Japanese stiltgrass) is one of the most common invaders in disturbed stream and wetland restoration sites throughout the Eastern United States (Nord 2011). *M. vimineum* is an annual grass from China that was first recorded in North America over 100 years ago and has since spread across the Eastern U.S. (Fairbrothers and Gray 1972, Barden,1987). It can grow to 105 cm tall (Nitzsche and Rector 2023) and forms dense monocultures when established (Barringer and Pannaman 2003). *M. vimineum* is also shade tolerant (Barden 1987, Oswalt et al. 2007), but it is only tolerant up to a certain level of shade (Hunter and DeBerry 2023, DeBerry and Hunter 2024). This invader is also a prolific seeder (Heffernan 2024) and has high seed viability (Miller and Matlack 2010, Ziska et al. 2015) making it highly invasive and difficult to treat with traditional methods (i.e., herbicide). Previous studies have confirmed that *M. vimineum* reduces native plant diversity (Oswalt et al. 2007, Adams and Engelhardt 2009) and can alter insect community structure (Marshall and Buckley 2009). It also has shallow roots (DeMeester 2009), which can lead to severe erosion over time at restored stream sites (Rajan et al. 2015), undoing the initial work to restore the streambanks and natural profile of a repaired or newly constructed channel. All these traits and factors make *M. vimineum* an ideal candidate for which to find effective treatments, and on which to test cultural control methods.

3.1.5. Project Purpose

The purpose of this project was to test the efficacy of cultural methods as preventative management techniques for the control of the invasive *M. vimineum* in stream restoration. This was accomplished by designing and executing a large-scale field experiment on a stream restoration site in Northern Virginia. The study design included blocked treatments that evaluated shade, high C:N soil amendments using both sawdust and wood mulch as unique treatments, and high seeding density, all of which were implemented as individual trials and in different combinations to test potential interactions. Based on the literature (see review in Chapter 2), we hypothesized that both shade and wood amendments would be effective management techniques, with the more labile sawdust providing short-term benefits and more refractory wood mulch providing longer-term effects. We further hypothesized that high native seeding density by itself might not be effective, but when combined with the other treatments would enhance control of *M. vimineum* due to positive effects of competition.

3.2. Materials and Methods

3.2.1. Study Site

Our project site is located within the Northern Virginia Stream Restoration Bank (NVSRB), positioned in Reston, VA (Figures 8 and 9). This site is an ideal location for this study as it is a restored stream bank that has areas along its bank that are heavily dominated by *M. vimineum*. It also has a mix of open and closed canopies within those invaded areas. The two main streams in the restoration bank are The Glade and Snakeden Branch ("Snakeden" hereafter), both of which are part of the Difficult Run watershed, which is a tributary of the Potomac River. Their location near walking paths and roads also made these streams easy sites to bring in the machinery necessary to set up the study plots and to continue to maintain them over the two-year study period.

The Glade and Snakeden are both urban streams that are several miles long, with The Glade eventually feeding into Snakeden. Since the development of the Reston area in the 1960s these streams have undergone straightening and channelization because of urbanization and subsequent runoff (Wetland Studies and Solutions, Inc.

2024). This resulted in severe bank erosion and a loss of native riparian vegetation throughout both streams. In 2008, The Glade was restored by Wetland Studies and Solutions, Inc. (WSSI) to improve channel stability and provide a better habitat for native plants and animals. Then in 2009 Snakeden was restored. The restoration process for both streams involved increasing stream sinuosity, creating step-pools habitat, and leveling out eroded streambanks to reduce future erosion and channelization within the streams and watershed.

The restoration process necessarily resulted in a disturbance event by removing the established biomass from the streambanks to fix the channel issues. Over the span of the next several years, the adjacent floodplain was subject to increases in invasive species like *M. vimineum*. Both systems therefore provided ideal study sites for this research project.

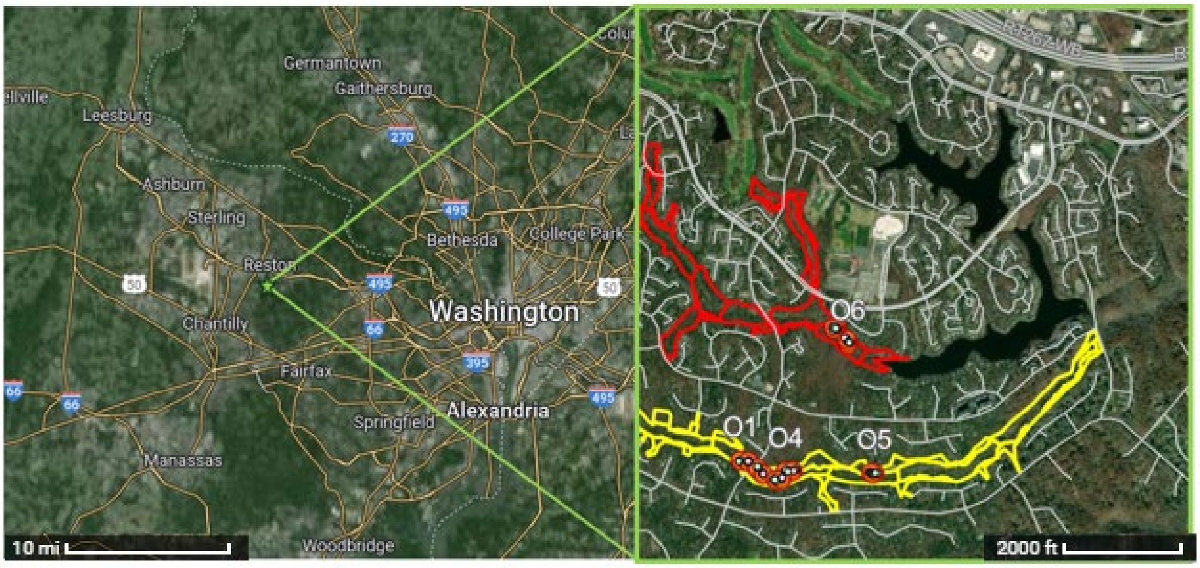


Figure 8. Study site, located within Reston, VA along the streams of Snakeden (highlighted in red) and The Glade (highlighted in yellow).

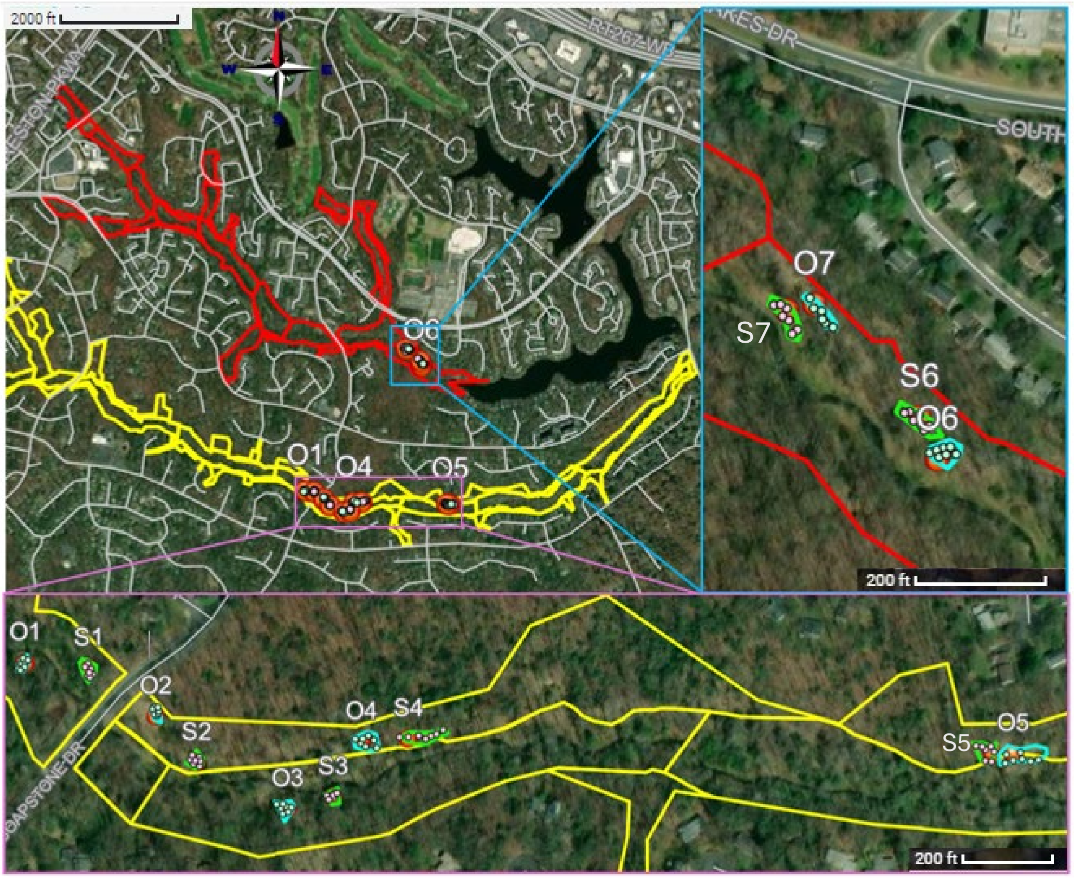


Figure 9. 14 study blocks are set up (7 shaded "S" canopies and 7 open "O" canopies) along the streams with shaded and open canopy blocks 1 through 5 located in the Glade and 6 and 7 in Snakeden.

3.2.2. Experimental Design

Areas dominated by *M. vimineum* (>50% cover) along Snakeden and The Glade were identified a year before the study (see Appendix B). Data on canopy cover, as a surrogate for light availability, and soil samples were collected from each area. The soil samples were analyzed by the Virginia Tech Soil Testing Lab for physical and chemical traits. These data were used to help determine which areas within the NVSRB corridor would be most suitable for experimental replication of the various treatments listed above. Ideal sites included open canopy areas (i.e., <50 percent canopy cover) of sufficient size to allow seven treatment plots as part of the low shade ("open") trials, paired with another site nearby under a closed canopy (i.e., >75 percent canopy cover) that was otherwise similar in soil, elevation, and hydrology conditions as part of the high shade ("shade") trails. Canopy cover was calculated using a hemispherical lens camera pointing directly at the sky from a height of 1.5 meters. The photos were then calculated using Image J with the plugin Hemispherical 2.0, which converts sky to white pixels and canopy to black and then creates a percentage of total pixels to give canopy percentage (Beckschäfer 2015).

A total of 14 blocks were selected (seven shade canopies and seven open canopies) with eight blocks in The Glade and four in Snakeden. Within each of these blocks, seven plots were randomly distributed using the ArcGIS random point feature with a plot buffer of at least one meter (Figure 9). If the points ended up on a rock, tree, or other obstacle they were moved within a reasonable distance of the original point while still maintaining the meter distance from the perimeter of any other plot. Each plot was 1.5 meters by 1.5 meters and each block had plots labeled one through seven, also randomized using GIS The seven treatments are listed in the following table (Table 1).

1.	Sawdust
2.	Sawdust and high native seed density
3.	Processed wood mulch
4.	Wood mulch and high seed density
5.	High seed density

6.	Herbicide (positive control)
7.	No treatment (negative control)

Table 1. List of treatments and correlating plot numbers

Each treatment was implemented in the spring of the first year and then left to grow for two growing seasons. The processed wood treatments were added to the first 10 cm of the soil at a 2:1 volumetric ratio for a total of 11.34 kg per plot (25 lbs. per plot, equivalent to 56 Megagrams/hectare or twenty-five tons/acre; Davis and Whiting 2013). The native seeds were spread at a rate of 67.2 kg/ha (60 lbs/ac or ¼ cup per plot) for all plots, except the high seed density treatments, which were spread at double the rate at 134.4 kg/ha (120 lbs/ac or ½ cup per plot). Native seeds were selected and provided by Ernst Conservation Seeds, with a focus on graminoids to discourage foraging by deer (for full list see Appendix C). The herbicide was applied as a positive control and an 18% glyphosate herbicide was mixed and applied in accordance with the label specifications of 0.365 grams of herbicide to 1 liter of water (3oz/gal). The different treatments were implemented in the order that a typical restoration project would occur, with mowing and tilling/earth moving plus soil amendments first, then applying native seeds, and finally applying herbicide (only in designated herbicide plots) during peak growing season after the invasive species had become well established.

On May 22, 2023, all aboveground vegetation was mowed as close to the ground surface as possible in all 14 blocks. From May 23rd to 25th the corners of all plots were marked using a 1.5 m x 1.5 m PVC frame. Each plot was also marked with a numbered flag corresponding to the treatment number of that plot. From June 2nd to 3rd the plots were hand-raked to remove debris, then tilled using a front-tine rototiller down to roughly

10 cm to simulate a disturbance event like the fine-grading implemented at the completion of a typical stream restoration site (Beauchamp et al. 2015). For wood treatments, the soils were amended with the processed wood additives then re-tilled until the soil-to-wood ratio was evenly mixed within the soil matrix. On June 4th the base application rate of the native seed mix was added to all plots and doubled for high density seed plots at the equivalent seeding rates noted above. On June 20th, all plots were corner-staked and outlined with twine to mark out each plot. From the end of June through September 2023, a minimum 1-meter buffer was maintained around each plot using a commercial-grade weed trimmer to inhibit inter-plot dispersal and to ensure that plots were not subject to plant colonization from the immediate vicinity in the floodplain. This maintenance process was repeated throughout the second growing season (2024).

3.2.3. Data Collection

Data were collected during peak growing season (late summer) from all 98 plots in 2023 and 2024, including canopy cover, soil physiochemistry, and plant species density and cover. Canopy cover was analyzed using the approach described above, with cover converted to percentage for analysis (i.e., percent cover of canopy). Soil samples were taken by coring 10 cm of soil from the center of each plot using a 5cm diameter soil corer. The soil was then bagged, labeled, and sent to the Virginia Tech Soil Testing Lab for analysis of chemical and physical traits. Chemical variables were measured with Mehlich extractions for bulk nutrients, 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).

Vegetation abundance was quantified using an importance value (IV) derived from cover and density estimates for all species occurring within a 1-meter square sampling quadrat nested within each experimental plot. 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%). Density estimates were based on McAuliffe's logarithmic density classes (McAuliffe 1990) in which the midpoint of the arithmetic interval represented by the logarithmic density class ($\log_2 N$) is recorded for analysis. The density classes, with midpoints in parentheses, include: 1 (1), 2 (2), 3-5 (4), 6-11 (8), 12-23 (16), 24-47 (32), 48-95 (64), 96-191 (128), 192-383 (256), 384-767 (512), and 768-1535 (1024). IV is calculated as the sum of relative cover and relative density (Mueller-Dombois and Ellenberg 1974).

Identifications of all vascular plants were either obtained onsite or samples were gathered and preserved for later verification. Nomenclature follows Weakley et al. (2020). Native/non-native status was based on Virginia Botanical Associates (2024) and Weakley et al. (2020).

3.2.4. Statistical Analysis

To address our different data models, we tested the measured variables using an information-theoretic approach (Burnham and Anderson 2002, Anderson 2007). All herbicide treatments along with any plot with total cover less than a quantile of the cover range (i.e., less than lower 20% of IV range) was removed from the first-year data set to remove the outsized effect of low cover plots on the abundance matrix analysis (Legendre and Legendre 2017). Block S3 was removed from both the first- and second-year data set because it was continuously washed out from flood events. Block O2 was removed from the second-year data set because there was a significant pipe burst that caused it to flood in December 2023, with deposition of heavy sediment that buried most plots within the block. The ratio of *M. vimineum* IV to native species IV (MV:Native) was modeled as a continuous variable, and first- and second-year percent canopy cover data were modeled as continuous variables based on the processed canopy photo data. To represent the effects of soil amendments we added the major soil data (i.e., nitrogen, phosphorus, C:N, other macronutrients, CEC, and pH) and modeled as continuous variables as well. The seed mix (competitive mix industry standard) was modeled as a fixed factor. The models (Table 2) were analyzed and compared using a logistic regression to evaluate relative invasion under these experimental conditions and all done in relation to the IV. Bias-corrected Akaike's information criterion (AICc) values were calculated to determine the best model using R statistical software (Version 4.4.1).

After AIC was run on all the models, a Spearman rank-order correlation was completed on both the ratio of *M. vimineum* IV to native species IV and just the *M. vimineum* IV. 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). We ran the shade data and all of the soil variable to see if there were any other unexpected correlations outside of carbon, nitrogen, and phosphorus.

Models	**Variables**
1	Shade (S)
2	Carbon:Nitrogen (C:N)
3	Nitrogen (N)
4	Phosphorus (P)
5	Seeding Rate (SR)
6	S + C:N
7	S + N
8	S + P
9	S + SR
10	C:N + P
11	C:N + SR
12	N + P
13	N + SR
14	P + SR
15	S + C:N + P
16	S + C:N + SR
17	S + N + P
18	S + N + SR
19	S + P + SR
20	C:N + P + SR
21	N + P + SR
22	S + C:N + P + SR
23	S + N + P + SR

Table 2. A list of all the models run against AICc in R.

P-values for all Spearman r were also checked at α = 0.05. The data were then checked against the previous model's AIC results for consistency. Finally, a nonmetric multidimensional scaling (NMDS) ordination of the abundance matrix in both sampling years was performed to evaluate underlying community structure across all plots, with environmental variables fit to the final ordination model. The NMDS dissimilarity matrix was based on the Bray distance, which is a robust measure for community data of this nature (Faith et al. 1987). Site scores were plotted on the final NMDS ordination graph with the *M. vimineum*-to-native-species ratio represented by the size of the plot in the graph. As with the AICc analysis, all correlation and ordination tests were performed using R (Version 4.4.1) with vegan as a modeling platform (Kindt and Coe, 2005).

3.3. Results

3.3.1. Year One Floristic Composition and Dominance

In the first growing season of this experiment, *M. vimineum*'s overall dominance was at 48.9% across the entire data set (i.e., average relative ratio of *M. vimineum* IV to all other species IV within each plot). Graphing the data, sawdust treatments seemed to be the most effective at minimizing the response variable (Figure 10a and 10b). Likewise, shaded plots had an overall lower average MV:Natives relative to open plots (Figure 11). Herbicide was not compared with the other treatments in year 1 since it completely killed all plants in the plots it was applied.

Of the native species found in our plots the most common in the first year were *Parathelypteris noveboracensis*, *Dichanthelium clandestinum*, *Bidens aristosa*, along with a few species of unidentified sedges and grasses that were not in flower at the time of sampling. The overall native species richness of the data set from the first year was 58. Native richness in individual plots ranged from 9 to 27, with an overall mean of 19.2. When a similarity index (Sorensen index; Mueller-Dombois and Ellenberg 1974) was run between the native seed mix used and the species found in the plots it showed that the plots were dissimilar from the species percent composition in the mix, indicating that many of the native species that occurred in the plots had volunteered from the surrounding areas.

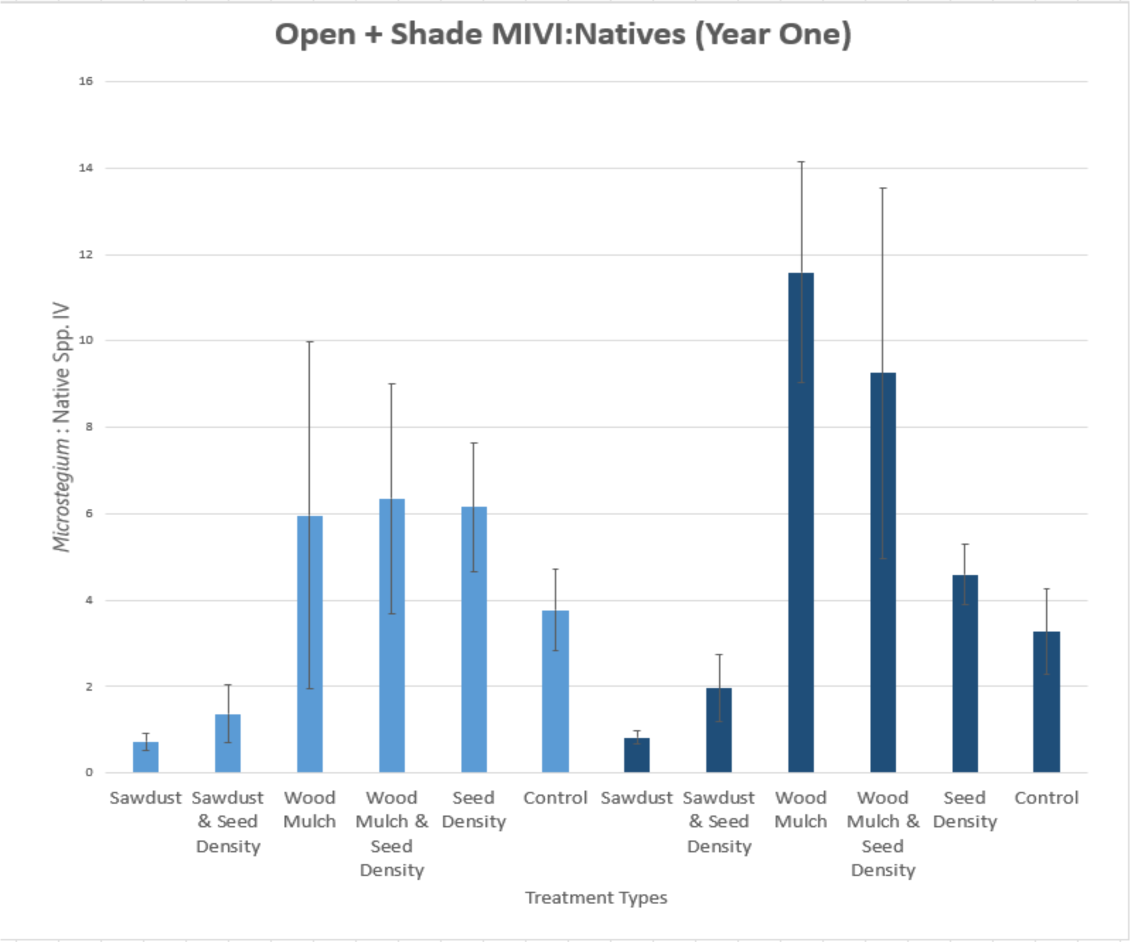


Figure 10. Year one data of MV:Natives for each treatment. Light blue represents open canopy and dark blue represents closed canopy. Lines represent standard error.

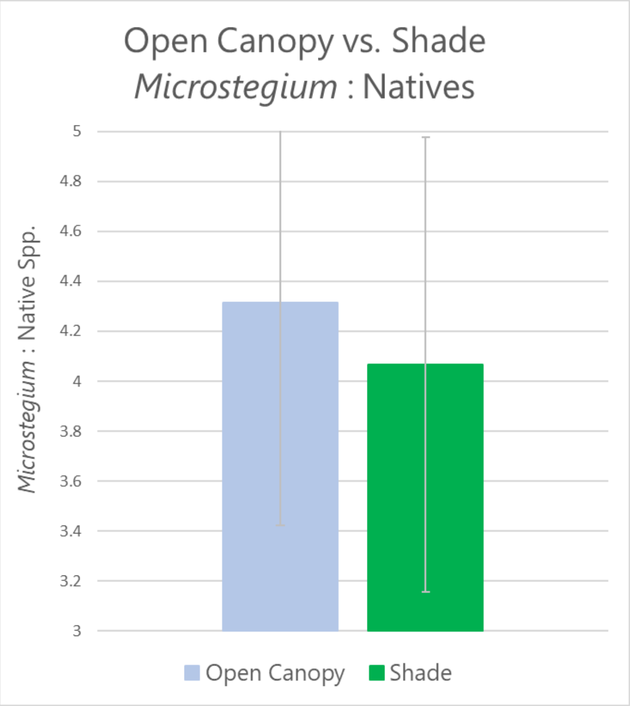


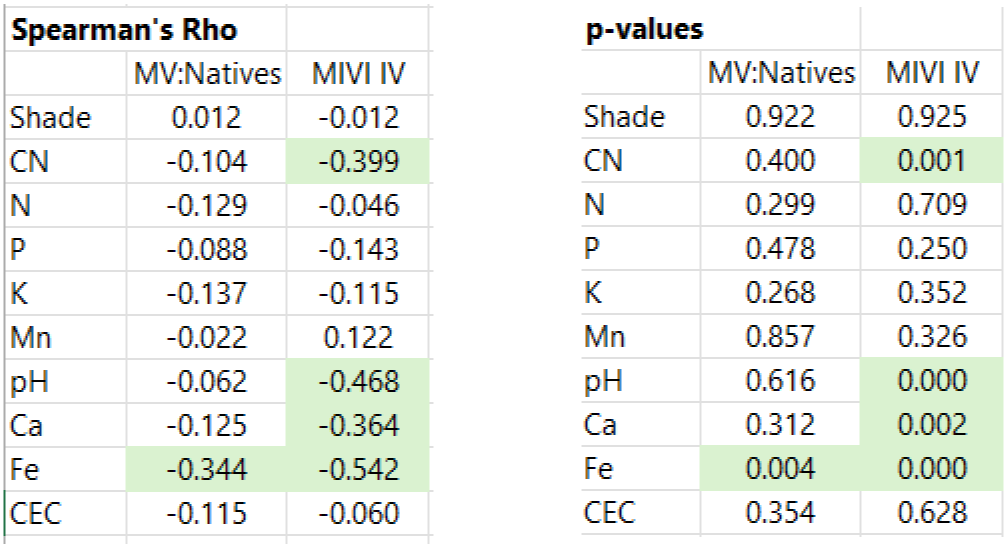
Figure 11. Year one data of MV:Natives in shaded vs. open plots. Lines represents standard error.

3.3.2. Year One Model Selection

For year one data the AIC showed that model 3 (just N) is the most concise model at predicting the response variable (MV:Natives) with a score of 372.70 and a delta of 0, but only a cumulative weight of 0.30 (See Appendix E). Model 12 (N + P) was close with a score s of 373.83 and a delta of 1.13 with a weight of 0.17. Model 2 (just C:N) performed much worse than either with a score of 377.74 and a delta of 5.04, and the weight was 0.02. One of the worse scores was from model 1 (just shade) with a score of 380.12 and a delta of 7.42 with a weight of 0.01. All other models were a score of about 375 or higher and a delta of 2 or higher with a weight of 0.11 or lower.

3.3.3. Year One Correlation

The Spearman rank-order test for the first year of data showed that C:N was negatively correlated with *M. vimineum* IV (MIVI IV), as were pH, calcium (Ca), and iron (Fe) (Tables 3 and 4). Fe was also negatively correlated with MV:Natives.



Spearman's Rho		
	MV:Natives	MIVI IV
Shade	0.012	-0.012
CN	-0.104	-0.399
N	-0.129	-0.046
P	-0.088	-0.143
K	-0.137	-0.115
Mn	-0.022	0.122
pH	-0.062	-0.468
Ca	-0.125	-0.364
Fe	-0.344	-0.542
CEC	-0.115	-0.060

p-values		
	MV:Natives	MIVI IV
Shade	0.922	0.925
CN	0.400	0.001
N	0.299	0.709
P	0.478	0.250
K	0.268	0.352
Mn	0.857	0.326
pH	0.616	0.000
Ca	0.312	0.002
Fe	0.004	0.000
CEC	0.354	0.628

Table 3 and 4. A Spearman's test rho values (left) and p-values (right) for independent variables in the first year.

3.3.4 *Year One NMDS Model*

The NMDS model for the first-year data supported the correlation analysis. The same environmental variables were significant in the ordination model, and all appear to be negatively related to MV:Natives. One departure was shade: in the final NMDS solution shade showed a significant relationship to the overall vegetation community represented by the circles on the graph, and a clear negative relationship with MV:Natives.

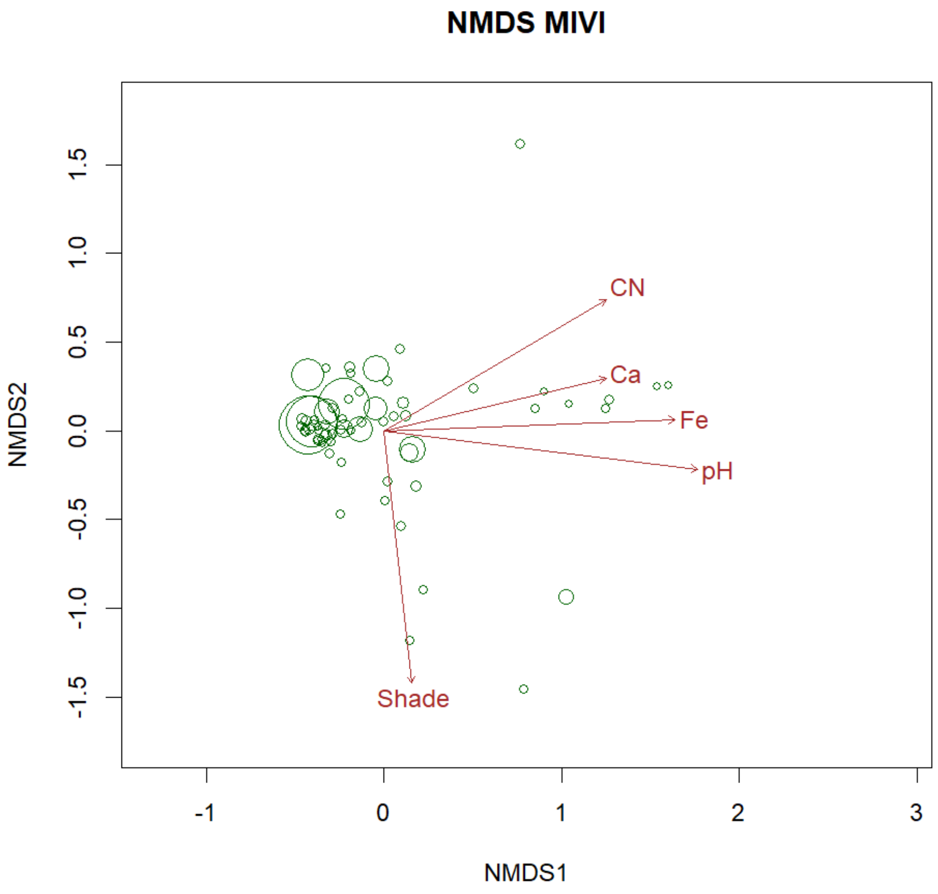


Figure 12. NMDS model of the relationship between plots (circles) and environmental variables (vectors) for the year one data. Larger circles represent plots with higher MV:Natives values, and vector length indicates strength of environmental correlation to the ordination.

3.3.5. Year Two Floristic Composition and Dominance

In the second year of the study *M. vimineum* overall dominance increased to 69.0%, most of which was attributed to dense cover in the open canopy trials. Plotting the raw data, sawdust again appeared most effective at minimizing MV:Natives in individual plots, and all of the shade trials clearly reduced the response variable relative to the open trials (Figure 12 and 13). Herbicide also seems to be the most ineffective treatment in open canopy plots and one of the most ineffective treatments in the closed canopy plots (Figure 12). For year two the most common natives were *Carex lurida*, *Dichanthelium clandestinum*, *Bidens aristosa*, and *Parathelypteris noveboracensis*. Overall native species richness remained similar to Year 1 at 60. The native richness did slightly increase in individual plots with the range from 12 to 27 and with a mean of 20.5. Once again, the similarity index showed that the seed mix and species recorded in the plots were dissimilar; however, three of the four most common natives were in the seed mix (*C. lurida*, *D. clandestinum*, and *B. aristosa*) and appeared to be competitive with *M. vimineum* in plots.

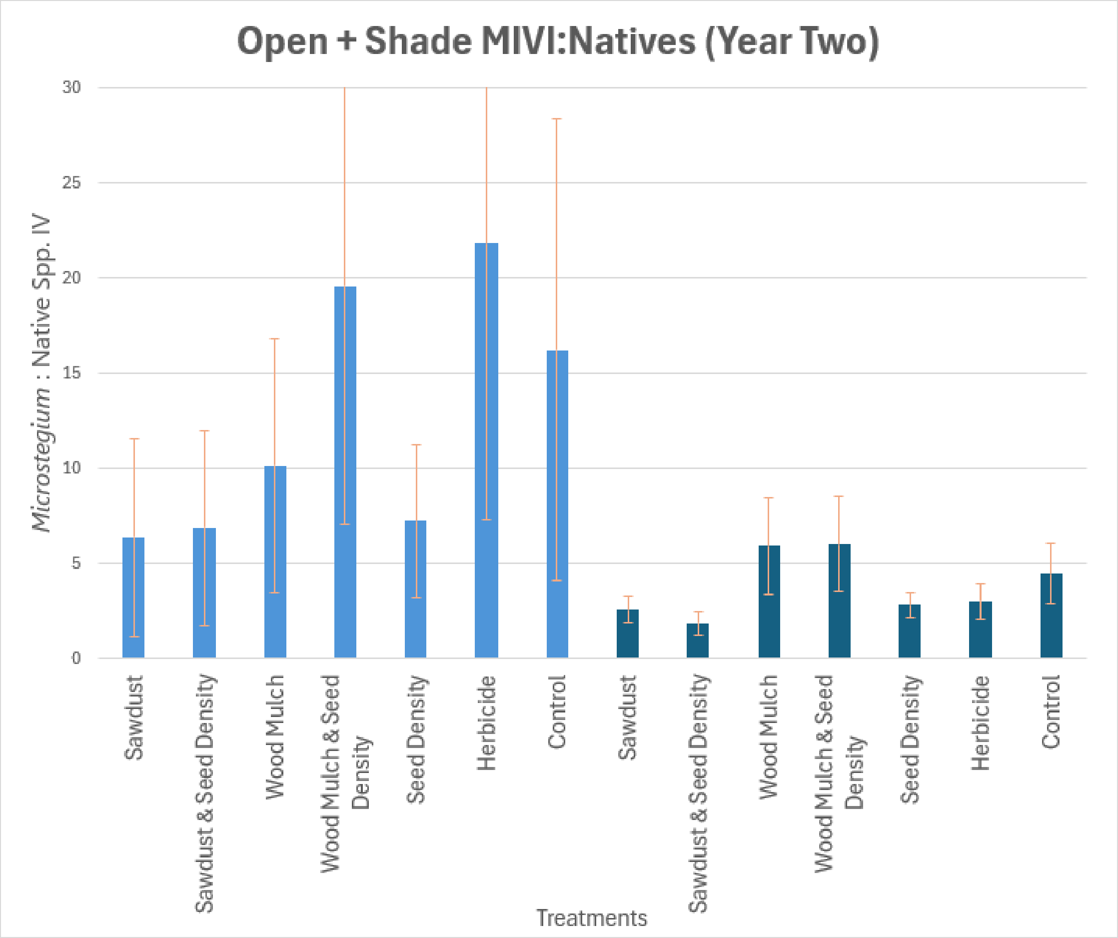


Figure 13. Year two data of MV:Natives for each treatment. See Figure 10 for explanation of the graph.

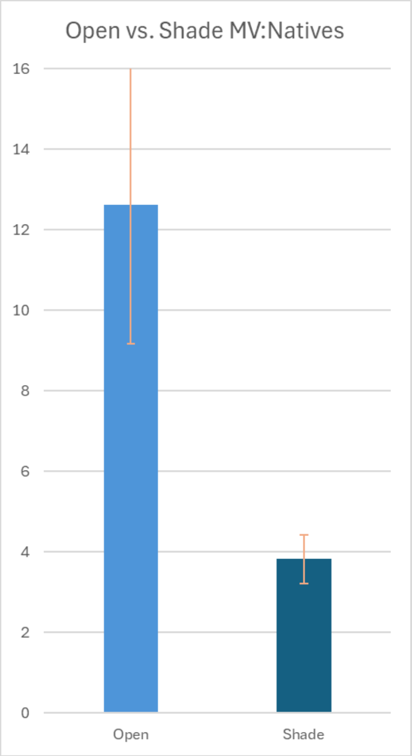


Figure 14. Year two data **of** MV:Natives in open vs. shaded plots. Line represents standard error.

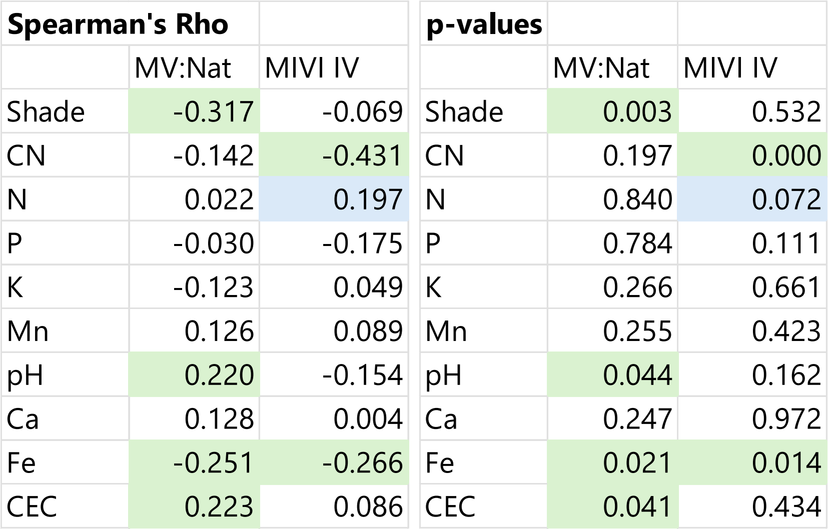
3.3.6. Year Two Model Selection

The AIC showed that model 6 (shade + C:N) is the most concise model at predicting the MV:Natives with a score of 705.89 and a delta of 0 with a weight of 0.21. Model 1 (just shade) was close with a score of 705.94 and a delta of 0.05 with a weight of 0.20. Model 2 (just C:N) performed much worse than either with a score of 713.10 and a delta of 7.21 with a weight of 0.01. Models 8 and 7(shade + P and shade + N) all had the lowest AIC, beside model 6 and 1, in that order with a score of 707.37 and 707.63 and a delta of 1.75 and 1.48 with a weight of 0.09 and 0.10. All other models scored about 708 or higher and had a delta of 2 or higher with a weight of 0.07 or lower. This tells us that shade is the best individual metric at predicting the ratio of MV:Natives, and that adding C:N to the model makes it even more concise, but not by much.

However, C:N on its own is much worse at predicting the IV ratio than most other models, so only when in tandem are they a good predictor model.

3.3.7. Year Two Correlation

Spearman correlations aligned with the AIC results for the Year 2 data. Shade was negatively correlated with MV:Natives, as was C:N with MIVI IV (Table 5 and 6). Among other soil variables, Fe was negatively correlated with both MV:Natives and MIVI IV, while pH and CEC were positively correlated with both MV:Natives and MIVI IV. Finally, the correlations showed a moderately positive relationship between soil N and MIVI IV, although the relationship was non-significant.



Spearman's Rho		
	MV:Nat	MIVI IV
Shade	-0.317	-0.069
CN	-0.142	-0.431
N	0.022	0.197
P	-0.030	-0.175
K	-0.123	0.049
Mn	0.126	0.089
pH	0.220	-0.154
Ca	0.128	0.004
Fe	-0.251	-0.266
CEC	0.223	0.086

p-values		
	MV:Nat	MIVI IV
Shade	0.003	0.532
CN	0.197	0.000
N	0.840	0.072
P	0.784	0.111
K	0.266	0.661
Mn	0.255	0.423
pH	0.044	0.162
Ca	0.247	0.972
Fe	0.021	0.014
CEC	0.041	0.434

Table 5 and 6. Spearman’s test rho values (left) and p-values (right) for shade and different soil data for year two.

3.3.8. Year Two NMDS Model

The NMDS supported the AIC and Spearman results, which is that shade, C:N, and Fe are correlated and likely cause a reduction in *M. vimineum*. As shown on Figure

14, C:N was an important variable in the NDMS model as evidenced by the length of the vector. It is also not associated with plots where MV:Natives was highest, which are delineated on the ordination biplot as larger circles. Nitrogen was also a significant variable in the ordination and showed a positive relationship with MV:Natives. This result aligns with the expected effect of higher C:N in the surface soils and explains its negative relationship with N in the model. Like C:N, Fe was a significant variable in the model and showed a negative relationship with MV:Natives. Finally, although vector length suggests less importance in the ordination, shade was negatively correlated with MV:Natives, a result that agrees with the correlation and AIC analysis.

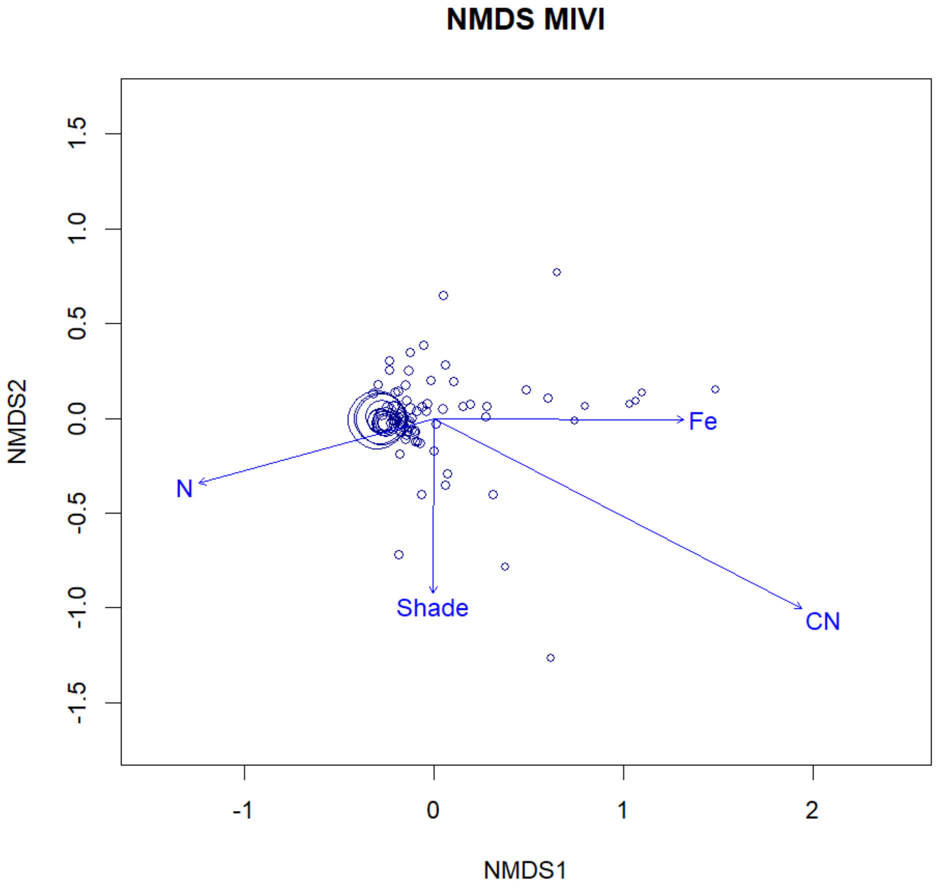


Figure 15. NMDS model for the year two data. See Figure 12 for explanation of graph.

3.4. Discussion

According to our data, sawdust amendments increased C:N in the soil which reduced *M. vimineum* in the treated plots (Table 5; Figure 12). This result seems to be reflected by the positive relationship between soil N and MIVI IV (albeit weak), suggesting that the C:N amendments were working to immobilize N in those treatments. Shade also had an impact by increasing the number and density of native species within the shaded plots relative to *M. vimineum* (Table 5; Figure 13). However, wood mulch and double seeding seemed to have a positive effect on the MV:Natives (Figure 12). These two treatments had deleterious effects on native species and seemed to encourage invasion.

3.4.1. Shade Treatment

The shade treatment was extremely effective at increasing the number of natives relative to the invader but was not able to reduce the overall amount of invasion. This makes sense given that *M. vimineum* is shade tolerant and likely still competitive at higher shade levels. Anything less than 95% shade is likely to still allow *M. vimineum* to persist (Winter et al. 1982), but from our data the higher canopy cover areas also allowed native competitors to increase. This supports the idea that tree protection and/or planting of fast-growing trees from larger stock sizes will work to inhibit invasion.

3.4.2. Soil Amendments

Of the two soil amendments, sawdust was the only one that was effective at reducing invasion in our trials. Sawdust treatments had significantly higher levels of C:N compared to wood mulch and other plots (see Appendix D). The C:N ratio for sawdust seemed to hover around 20, which is the recorded level at which soil microbes typically start to immobilize nitrogen (Chapin et al. 2002). This is likely because sawdust is much more labile than wood mulch given its surface-to-volume ratio (Davis and Whiting 2000). This data was collected over a two-year period, so given more time the wood mulch might have increased N immobilization, but it is unclear if it would have been as effective as the faster-acting sawdust.

From our data, sawdust is the most effective treatment for reducing *M. vimineum*, at least in the short term. However, sawdust treatments do not seem to be correlated with decreasing MV:Natives. Likely the lower amount of available N in the soil does prevent certain native species from growing as effectively, so it would be necessary to focus on planting either deep rooting or low-N plants in sawdust amended soils (Templeton et al. 2020). The effects of sawdust on soil needs more research, especially in stream systems, but given the current data it seems to be an effective tool at reducing invasion of a nitrophilous invader like *M. vimineum*.

Wood mulch seemed to have had the opposite intended effect and, in most cases, increased the amount of *M. vimineum* to native species (Figures 10 and 12). As mentioned earlier, this is likely because the material is less granular (lower surface:volume) and more refractory, which made it harder for the soil microbes to decompose and uptake in the N-immobilization process (Davis and Whiting 2000, Perry et al. 2004). Amending the soil with wood mulch might have resulted in a more aerated soil environment as well by changing the soil consistency compared to sawdust. This change in soil consistency might explain the positive effect wood mulch had on invasion from our shallow-rooted *M. vimineum* by providing access to more nutrients and water, which could more easily penetrate the soil during flood events compared to the more compacted soils of the other plots. Wood mulch might be useful if it is a very fine mulch and if it is added to the surface of the soil, instead of mixed in. This could both provide an extra source of carbon after the amended sawdust has been used up and reduce the amount of shade on the surface of the soil, therefore encouraging more native species to grow as well.

Iron was an unexpected result in our data since in both the first and the second year it had a high correlation with reduced levels of *M. vimineum* and the ratio of *M. vimineum* to natives (Tables 3-6). Our intention was not to change the iron in the soil, but it seems that soil with higher levels of iron had a positive effect of reducing our invader. The reason for this is likely because the oxidized form of ferric iron (Fe (III)) is known to immobilize phosphate by precipitation (Ponnamperuma 1972, Mohanty and Dash, 1982). Fe (III) has previously been correlated with lower amounts of available P (DeBerry and Perry 2015) and therefore *M. vimineum* in developing wetlands (Hunter and DeBerry 2023). Fe performs this role in its oxidized state, which was to be expected in the upland floodplains where our experiment was sited. Iron could also be deposited by flood events, like other nutrients, leaving elevated levels in surface soils that may immobilized bioavailable P once floodwaters recede.

3.4.3. Seeding Rate

Increased seeding rate also seemed to have had the opposite intended effect, with an increase in MV:Natives and MIVI IV in those trials. In a similar experiment on *M. vimineum*, Flory (2010) saw similar results with high native seeding rates reducing community productivity. The hypothesis suggested for this result was that adding seeds attracted seed predators or increased soil pathogen activity and inhibited the existing native seed bank. We did observe birds foraging in plots after seeding, although could not confirm if they were feeding specifically on the seed mix that we applied (R. Sullivan, pers. obs.). Denser seeding also may have unnecessarily increased competition and caused already germinating native seedlings to die from lack of resources in the soil, or possible alleopathic responses from some seedlings to others in the seedbank. Unfortunately, there isn't much research into this topic so more studies are needed to properly explain this result. Contrary to some studies in similar experiments (e.g., Yanelli et al. 2018), our data suggest that higher seeding rates could have unintended consequences; however, given the potential for anomalous scenarios in our experiment (e.g., bird depredation), additional research is recommended before strict conclusions can be drawn.

3.4.4. Recommendations

Given the data presented here, stream restoration practitioners looking to reduce invaders like *M. vimineum* should focus on soil amendments that will successfully reduce bioavailable N. In our study and others (e.g., Perry et al. 2004), the material recommended is processed wood with a consistency similar to sawdust. Stream restoration designers and managers could integrate sawdust at a volumetric ratio of 2:1, which worked well in our experiment. Ideally, the amendments could be sourced from felled trees and shrubs directly onsite; however, if construction crews do not have a viable option for processing wood at that fine of a consistency, then it should be readily available and cheaply sourced from local sawmills. Based on our data, these soil amendments could lead to an overall reduction in initial invasion for at least the first two years.

Restoration site managers should also focus on encouraging more shade. This can be done by removing as few large canopy shade trees as possible and by planting more canopy shade trees from larger stock sizes (e.g., containerized trees rather than bare root or tubeling sizes). Fast-growing, broad-leaved trees are best suited for re-establishment of a canopy as quickly as possible to hasten canopy closure and decrease the risk of early invasion. In addition, choosing trees that have a high C:N in their leaves would be useful at continuing to encourage N-immobilization in the soil. Unfortunately, there are no data on which trees have the highest C:N ratio in their leaves but future research could help provide some answers.

Herbicide also clearly leads to reinvasion which supports the existing literature (Gibson et al. 2019). Restoration managers should avoid using it to treat invasive species as much as possible, especially in an area where native species already have an established seed bank. In our experiment, sawdust and shade performed much better at reducing invasion than herbicide, suggesting that cultural methods can be successful without the need for excessive use of chemical treatments (Figure 12). If land managers are forced to use herbicide to remove invasive plants, they should try to focus on heavily shaded areas, where native communities seem to rebound much better than open canopy areas that are sprayed (Figure 12).

Appendices

Appendix A: Representative Photos

Figure A1. One of the experimental blocks before the experiment (Block O4). Are almost completely dominated by *Microstegium vimineum*.

Figure A2. Experimental block after mowing was completed. All vegetation was mowed down to ground level (Block O4; May 2023).

Figure A3. One of the plots is being marked out using a 1.5m x 1.5m frame and marking the corners with field paint after it was mowed (Summer 2023).

Figure A4. Plots being tilled by gas tiller (Summer 2023).

Figure A5. Plot (S1-2) after being tilled and marked with a numbered flag (Summer 2023).

Figure A6. Application of herbicide using a hand pump sprayer on the herbicide positive control plots (Summer 2023).

Figure A7. Site maintenance done by GMU undergrads using weedwhackers to mow around the plots to reduce drift from plot to plot (Summer 2024).

Figure A8. Plot (S1-2) after growing back the first year. Mostly dominated by *Microstegium vimineum* (September 2023).

Figure A9. Plot (S1-2) during the second year of the project. More biodiversity than the first year of the project (September 2024).

Figure A10. Open canopy plot (O7-7) almost completely dominated by *Microstegium vimineum* by the end of the second year (September 2024).

Figure A11. Closed canopy plot (S7-7) less dominated by *Microstegium vimineum* than the open plot by the end of the second year (September 2024).

Figure A12. An open canopy plot canopy photo (43% canopy shade) taken with a hemispherical lens (plotO2-2).

Figure A13. A closed canopy plot canopy photo (89% canopy shade) taken with a hemispherical lens (plot S6-3).

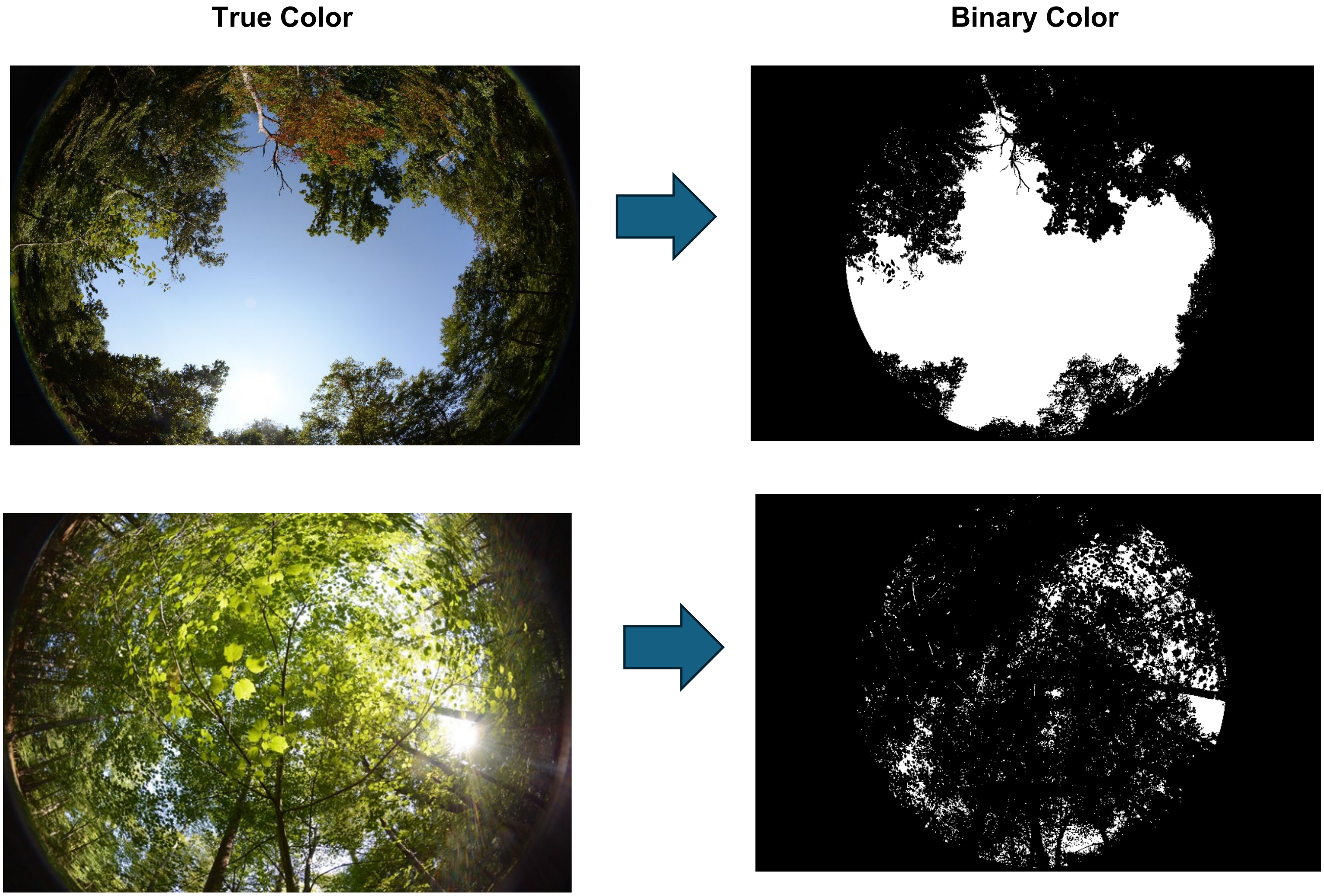


Figure A14. True color photos taken by the camera (right) are converted to black and white binary color (left) to process through Hemispherical_2.0 in Image J. The black represents canopy, such as leaves and branches, and the white represents sky. The image is cut into a circle, with a diameter of 3942 pixels, to represent the true canopy and remove any ground level parts of the photo. The white pixels are counted and a percentage of white pixels to the total number of pixels in the circle is given to give us the true canopy cover percentage.

Appendix B: Invasive Species Mapping

Invasive Plant Species Inventory Project Northern Virginia Stream Restoration Bank (NVSRB) Reston, Virginia

Doug DeBerry[1], Kent Codding[1], Ryan McIntyre[2]

[1]College of William & Mary, Environment and Sustainability Program
[2]George Mason University, Environmental Science and Policy Program

---

Introduction

Over the course of the 2022 growing season, we mapped the relative dominance of invasive plant species within the boundaries of the Northern Virginia Stream Restoration Bank (NVSRB), a 12-mile stream restoration project in the town of Reston, Virginia. The project area includes the conservation easement that surrounds the stream restoration corridor, which includes ti riparian corridors of both Snakeden Branch and The Glade, the two principal stream systems within the bank, as well as some secondary tributaries. The mapping methodology is provided below, followed by the results of the mapping project.

Mapping Techniques

Mapping was completed using the "Field Maps" ESRI application in combination with Survey123 to collect abundance data on invasive plant species. The entire project area was divided into management compartments. Compartments were designated based on the following criteria:

1. Proximity to the 100-year floodplain.
2. Ease of access (i.e., roads, paths, bridges, etc.).
3. Overall plant community properties.

Compartments were labeled "G1, G2, etc." for The Glade, and "S1, S2, etc." for Snakeden Branch. For each compartment, a visual inspection of the field conditions was conducted in which the assessor noted overall cover of invasive species within the compartment, as well as any individual invasive plant species present along with their relative abundance in the compartment. This information was recorded in a Survey123 form, along with the following data fields:

1. Current date and time
2. Compartment ID
3. Braun-Blanquet cover classes: 0-5%, 5-25%, 25-50%, 50-75%, 75-100%.
4. Invasive species present in compartment, along with each species' relative cover in the compartment using one of four qualitative abundance values: Dominant (>20% of compartment), Common (5-20% of compartment), Scattered (1-5% of compartment), Occasional (<1% of compartment).
5. Representative photos
6. Any additional notes or observations that may be relevant to the inventory (e.g., deer prevalence, human disturbance, etc.).

Results

We mapped 36 compartments in The Glade and 29 in Snakeden Branch (see attached mapping and tables). During the 2022 growing season, there were 25 invasive plants found The Glade and 22 in Snakeden. The overwhelming dominant was Japanese stiltgrass (*Microstegium vimineum*) (see attached charts). The overall invasive cover by compartment is shown on the attached map.

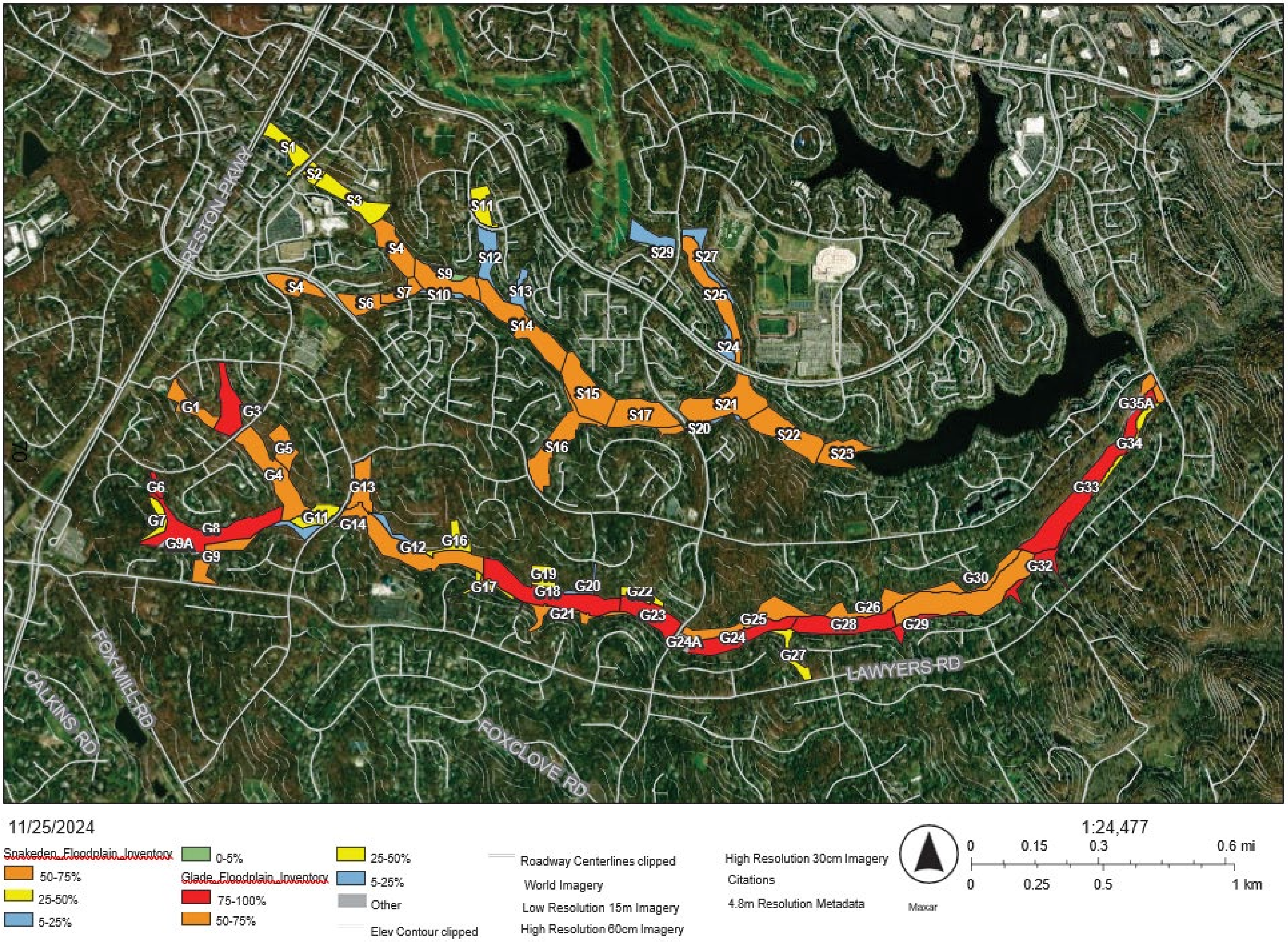


Figure B1. Map of *Microstegium vimineum* invasion throughout Snakeden and The Glade stream systems.

The Glade Invasive Species Mapping Project - Summary by Compartment

Overall Cover codes: 1=0-5%, 2=5-25%, 3=25-50%, 4=50-75%, 5=75-100%

Invasive Species Relative Dominance codes: O=Occasional (<1%), S=Scattered (1-5%), C=Common (5-20%), D=Dominant (>20%)

Compartment	OVERALL COVER	*Ailanthus altissima*	*Albizia julibrissin*	*Alliaria petiolata*	*Ampelopsis*	*Berberis thunbergii*	*Celastrus orbiculatus*	*Commelina communis*	*Dioscorea polystachya*	*Elaeagnus umbellata*	*Euonymus alatus*	*Euonymus fortunei*	*Glechoma hederacea*	*Hedera helix*	*Ligustrum obtusifolium*	*Ligustrum sinense*	*Lonicera japonica*	*Lonicera maackii*	*Microstegium vimineum*	*Paulownia tomentosa*	*Persicaria perfoliata*	*Rosa multiflora*	*Rubus phoenicolasius*	*Rumex crispus*	*Viburnum dilatatum*	*Vinca minor*
G01	4					O							O	S			O		D			O			S	
G02	5					O				O	O	O	O	S			O		D	O		O			S	S
G03	2											O		S					C						S	
G04	4					O					O		O				O	O	D			O			C	O
G05	4			O		O						O							D			O			C	O
G06	5													S		O	O		D			S			S	S
G07	3													O			O		D			O			S	S
G08	5					O					O	O	O	O		O	O		D			S			S	O
G09	4			O							O		O	O			O		D			O			S	O
G10	2										O						O		C						O	
G11	3					O						O		O			O		D			S			O	O
G12	4					O						O		S			O		D			O				
G13	4									O				O					C			O				S
G14	4																		D	O		O				S
G15	2																O		C			O				
G16	3					O						O							D							
G17	3										O						O		D							S
G18	5																		D			C				
G19	3																O		C							O
G20	2					O				O		O		O			O		S							
G21	4									O	O			S			O		D			O	O			O
G22	3																		C			O				O
G23	5							O		O		O	O	S		O	C		D	O		C	O	O	O	
G24	5					O				O				O			S	O	D		S	O	O			
G25	4					O						O		O			O	O	D		O	S	O			S
G26	4					S				O		S	O	S			O	S	D			C				S
G27	3					O							O	O			O		D			S	S		O	
G28	5												O	O			O	O	D			C		O	O	S
G29	5					O				O		O		S		O	O		D		O	S			O	S
G30	4		O			O	O					O	O	S	O		S	O	D		O	S			O	O
G31	4	O					O				O	S	O	O			O		D		O	S			O	O
G32	5				S	O	S			O	O	O		S		S	S	O	D		O	C			S	S
G33	5						O		O	S		S		O			S	O	D		S	O				S
G34	3					O	S			O	O						S		D			O	O		O	
G35	4						S			O	S			S			S	O	D			C			O	
G36	4		O		O		S			S	S					O	C	O	C			C			O	

Table B1. Mapping of different compartments of The Glade for invasive species.

Snakeden Branch Invasive Species Mapping Project - Summary by Compartment

Overall Cover codes: 1=0-5%, 2=5-25%, 3=25-50%, 4=50-75%, 5=75-100%

Invasive Species Relative Dominance codes: O=Occasional (<1%), S=Scattered (1-5%), C=Common (5-20%), D=Dominant (>20%)

Compartment	OVERALL COVER	*Alliaria petiolata*	*Berberis thunbergii*	*Celastrus orbiculatus*	*Commelina communis*	*Dioscorea polystachya*	*Elaeagnus umbellata*	*Euonymus alatus*	*Euonymus fortunei*	*Glechoma hederacea*	*Hedera helix*	*Ligustrum sinense*	*Lonicera japonica*	*Lonicera maackii*	*Microstegium vimineum*	*Murdannia keisak*	*Oplismenus hirtellus ssp. undulatifolius*	*Persicaria perfoliata*	*Pyrus calleryana*	*Rosa multiflora*	*Rubus phoenicolasius*	*Viburnum dilatatum*	*Vinca minor*
S01	3										S				S					C			
S02	3														C				O	S		O	
S03	3		O	O				O						O	C			O		S			
S04	4				S										D							O	
S05	3														D					O			
S06	4				S										C	O		S		O			
S07	4			S	O			O			S		O		D	O		C					
S08	4				S									O	D					O			
S09	1										O				O					O			
S10	2										C		S		C								
S11	3														C						S		
S12	2				O										S						S		
S13	2														C					O			
S14	4				O										D	O						O	
S15	4				S							O		O	D				O	O		S	
S16	4							O		S	O				C					O	O	S	
S17	4				O										D					S	O	C	
S19	4						S	O			O				C								S
S20	2														C						O		S
S21	4										S			O	D								
S22	4														D					O			
S23	4			O							O	O			D		O			O			
S24	2						S		S														
S25	4				O		O						O		D			O		O			
S27	2				O		O						S		S								
S28	2				O						S		S		C								S
S29	1														S								S

Table B2. Mapping of different compartments of Snakeden for invasive species.

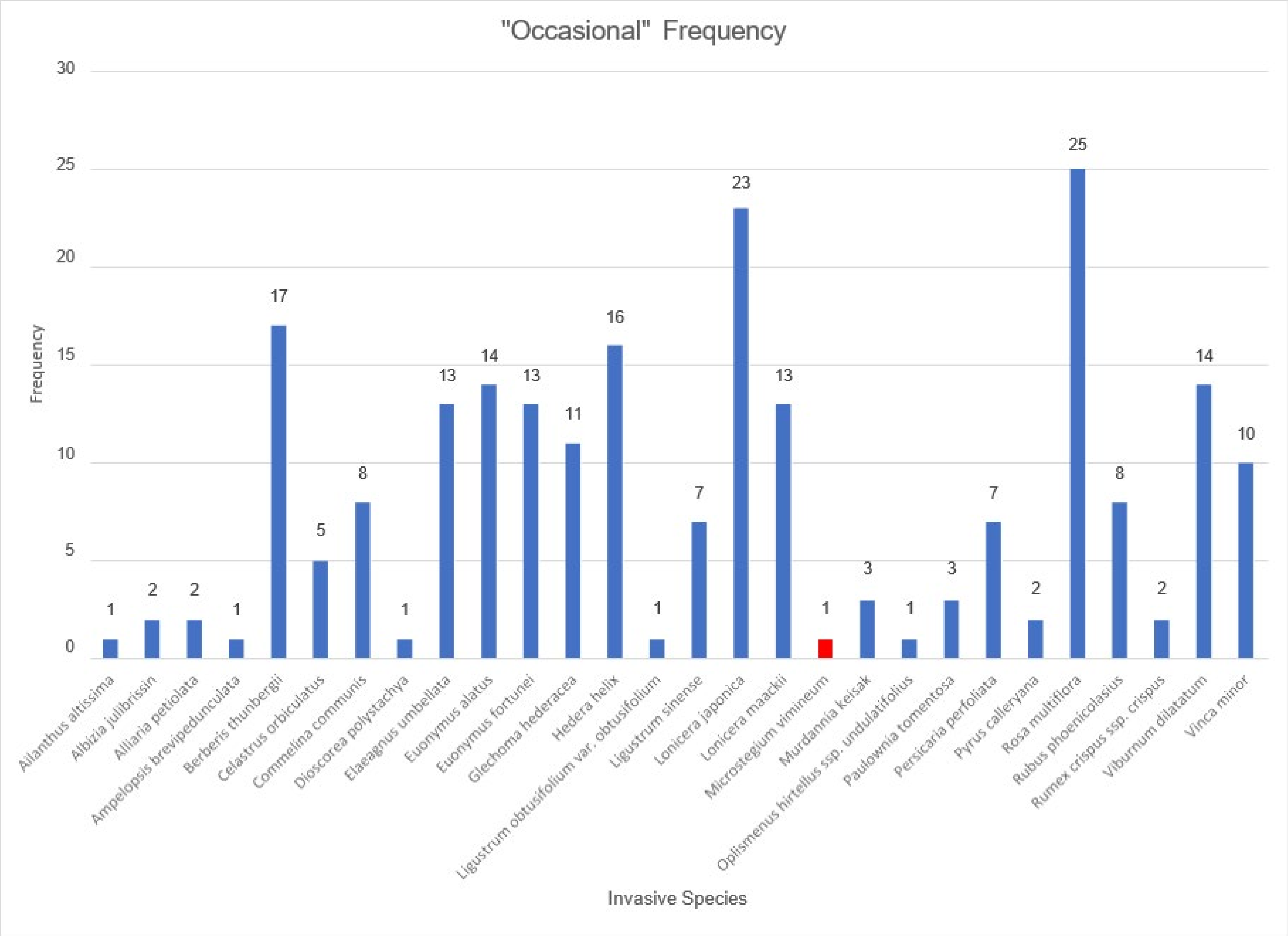


Figure B2. Occasional frequency of different invasive plants throughout the sites.

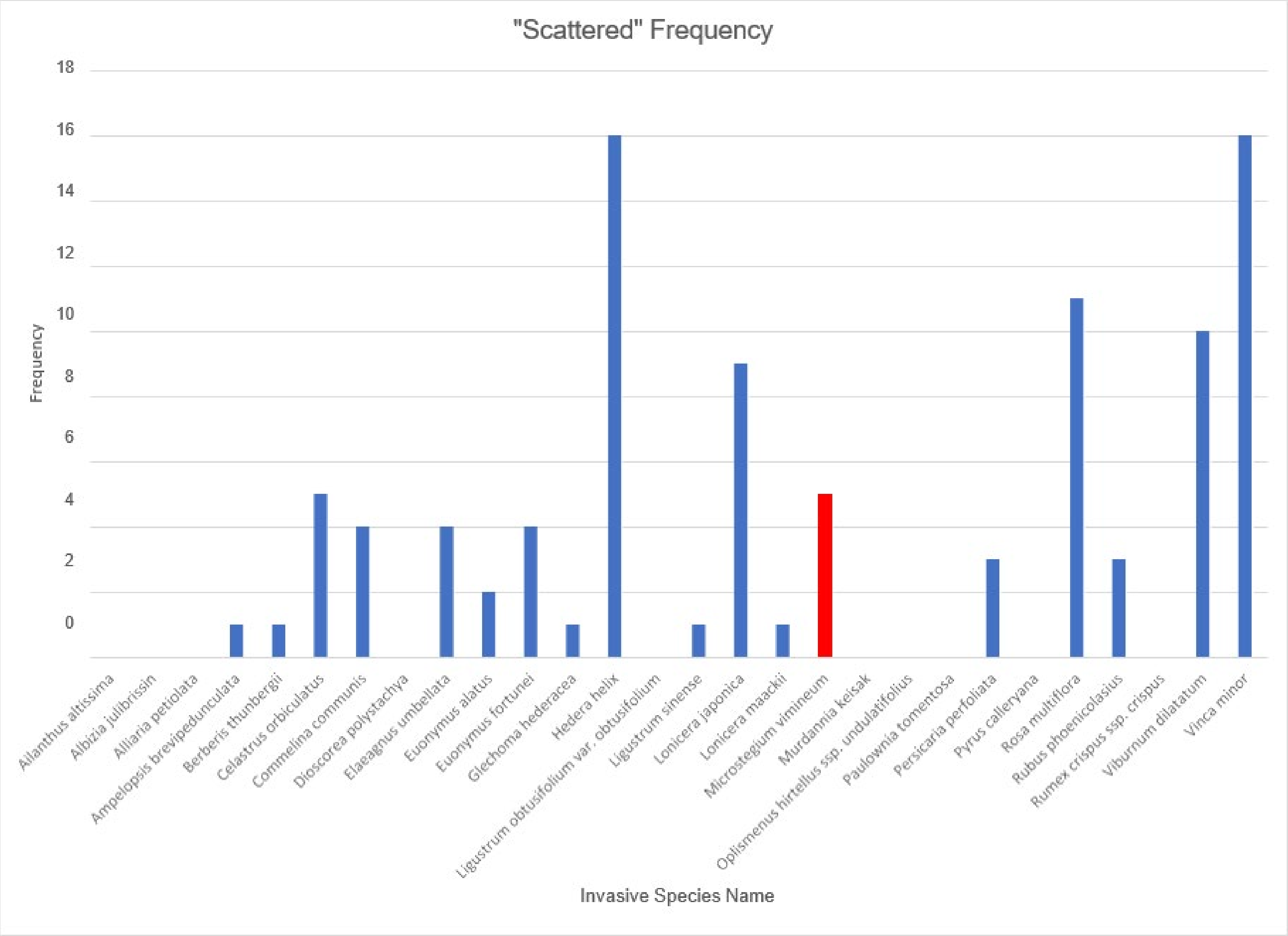


Figure B3. Scattered frequency of different invasive plants throughout the sites.

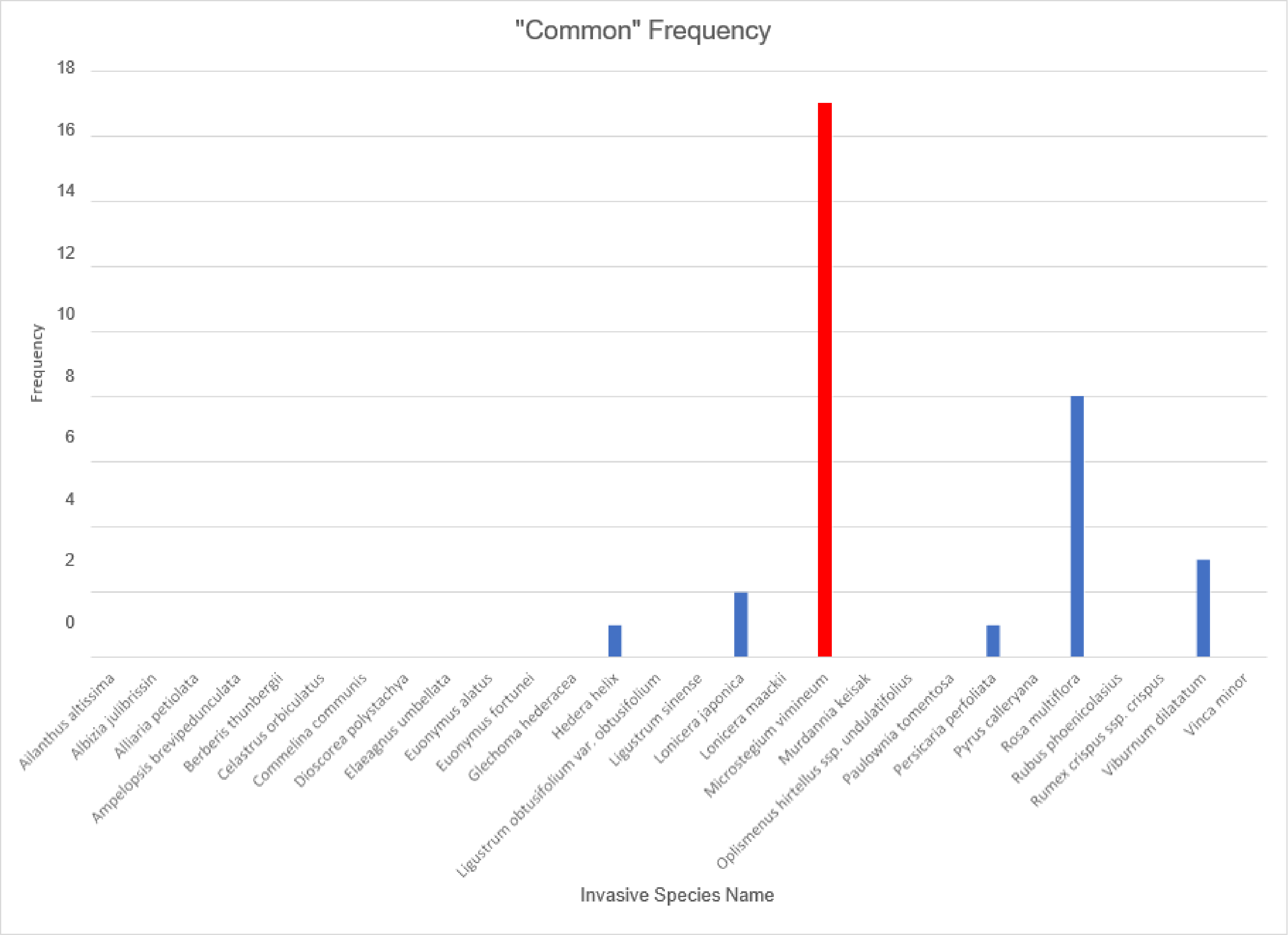


Figure B4. Common frequency of different invasive plants throughout the sites.

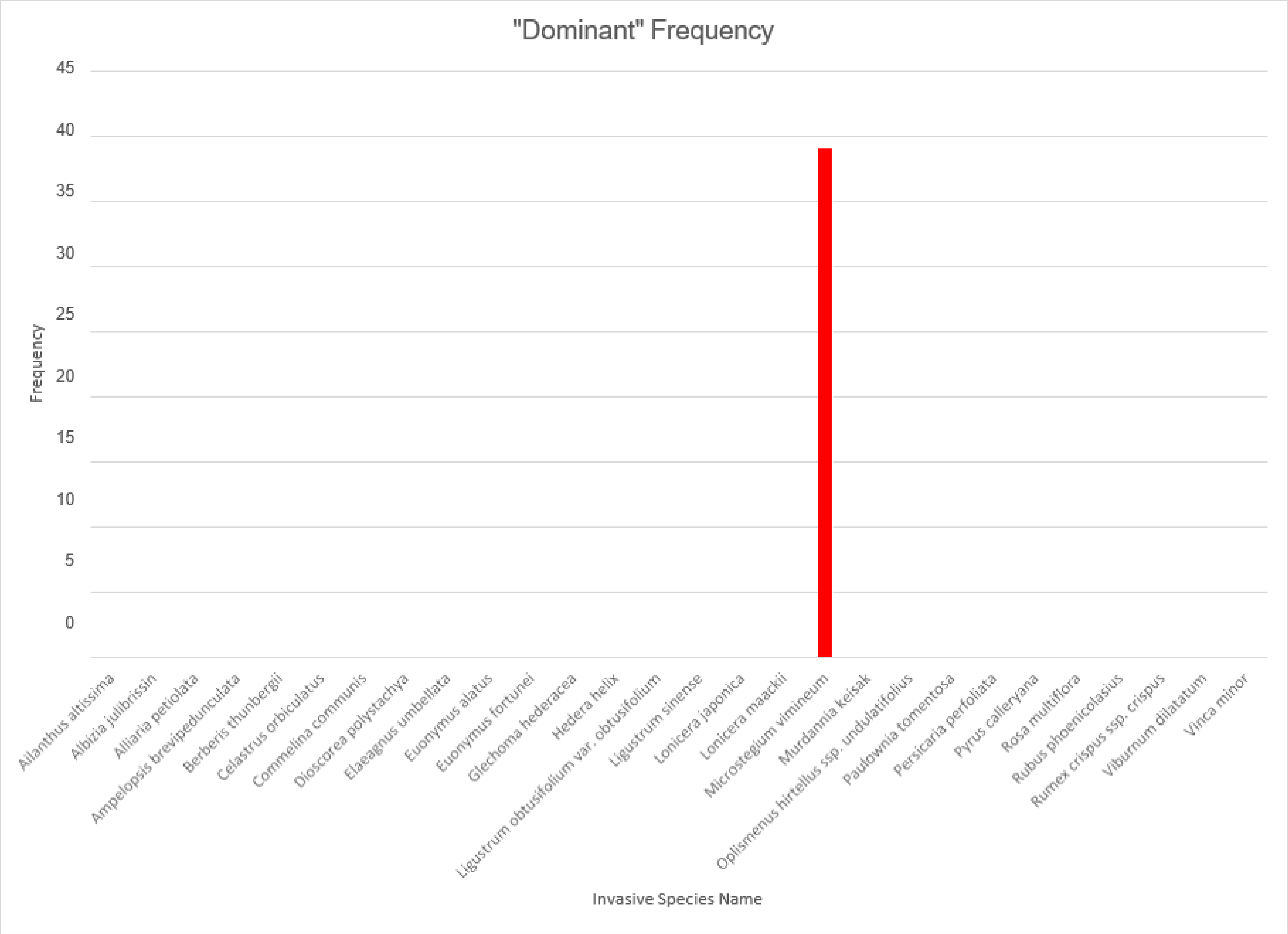


Figure B5. Dominant frequency of different invasive plants throughout the sites.

Appendix C: Species Checklist

Scientific Name	Common Name	Family	Status*	Comments
Acalypha virginica	Virginia Three-seeded Mercury, Virginia Copperleaf	Euphorbiaceae	N	Present in Year 1
Acer rubrum	Red Maple	Sapindaceae	N	Present in Year 1 & 2
Albizia julibrissin	Mimosa, Silk Tree	Fabaceae or Leguminosae	NNI	Present in Year 1
Allium canadense var. canadense	Wild Onion, Meadow Onion	Amaryllidaceae	N	Present in Year 1
Amphicarpaea bracteata	Hog-peanut	Fabaceae or Leguminosae	N	Present in Year 1 & 2
Apocynum cannabinum	Indian Hemp, Hemp Dogbane	Apocynaceae	N	Present in Year 1 & 2
Arthraxon hispidus var. hispidus	Joint-head Grass	Poaceae or Gramineae	NNI	Present in Year 1
Betula nigra	River Birch, Red Birch	Betulaceae	N	Present in Year 2
Bidens aristosa	Tickseed Sunflower	Asteraceae or Compositae	N	Present in Year 1 & 2, In Seed Mix, Dominant in Year 1 & 2
Bidens frondosa	Devil's Beggar-ticks	Asteraceae or Compositae	N	Present in Year 1
Boehmeria cylindrica	False Nettle	Urticaceae	N	Present in Year 1 & 2
Carex lurida	Sallow Sedge	Cyperaceae	N	Present in Year 1 & 2, Dominant in Year 2
Carpinus caroliniana	American Hornbeam, Ironwood	Betulaceae	N	Present in Year 2
Celastrus orbiculatus	Oriental Bittersweet	Celastraceae	NNI	Present in Year 2
Cinna arundinacea	Common Wood Reedgrass, Sweet Wood Reed-grass	Poaceae or Gramineae	N	Present in Year 2
Commelina communis	Asiatic Dayflower, Common Dayflower	Commelinaceae	N	Present in Year 1
Conyza canadensis var. canadensis	Horseweed, Common Horseweed	Asteraceae or Compositae	N	Present in Year 2

Cyperus iria	Rice-field Flatsedge	Cyperaceae	NNI	Present in Year 1
Cyperus strigosus	Straw-colored Flatsedge	Cyperaceae	N	Present in Year 1 & 2
Dichanthelium clandestinum	Deer-Tongue Grass	Poaceae or Gramineae	N	Present in Year 1 & 2, In Seed Mix, Dominant in Year 1 & 2
Dioscorea villosa	Wild Yam	Dioscoreaceae	N	Present in Year 1 & 2
Echinochloa muricata var. muricata	Rough Barnyard Grass	Poaceae or Gramineae	N	Present in Year 1
Elephantopus carolinianus	Carolina Elephant's-foot	Asteraceae or Compositae	N	Present in Year 1 & 2
Elymus virginicus	Virginia Wild Rye	Poaceae or Gramineae	N	Present in Year 2, In Seed Mix
Erechtites hieraciifolius	Fireweed, Pilewort, American Burnweed	Asteraceae or Compositae	N	Present in Year 1 & 2
Euonymus americanus	Strawberry-bush, American Strawberry-bush, Heart's-a-bustin'	Celastraceae	N	Present in Year 2
Eupatorium perfoliatum	Boneset, Common Boneset	Asteraceae or Compositae	N	Present in Year 2
Euphorbia maculata	Spotted Spurge, Milk-purslane	Euphorbiaceae	N	Present in Year 1
Fragaria virginiana	Wild Strawberry	Rosaceae	N	Present in Year 1
Galium asprellum	Rough Bedstraw	Rubiaceae	N	Present in Year 2
Geum canadense	White Avens	Rosaceae	N	Present in Year 2
Geum virginianum	Cream Avens	Rosaceae	N	Present in Year 1
Glyceria striata var. striata	Fowl Mannagrass	Poaceae or Gramineae	N	Present in Year 2
Hedera helix	Common Ivy, English Ivy	Araliaceae	NNI	Present in Year 1 & 2
Ilex opaca var. opaca	American Holly	Aquifoliaceae	N	Present in Year 1 & 2
Leersia virginica	White Grass, White Cutgrass, Virginia Cutgrass	Poaceae or Gramineae	N	Present in Year 2
Lindera benzoin	Spicebush	Lauraceae	N	Present in Year 1 & 2

Liriodendron tulipifera	Tulip-tree, Tulip-poplar, Yellow Poplar	Magnoliaceae	N	Present in Year 1 & 2
Lobelia inflata	Indian Tobacco	Campanulaceae	N	Present in Year 2
Lonicera japonica	Japanese Honeysuckle	Caprifoliaceae	NNI	Present in Year 1 & 2
Ludwigia alternifolia	Seedbox, Alternate-leaved Seedbox	Onagraceae	N	Present in Year 2
Ludwigia palustris	Marsh Seedbox, Common Water-purslane	Onagraceae	N	Present in Year 1 & 2
Lycopus virginicus	Virginia Bugleweed, Virginia Water Horehound	Lamiaceae or Labiatae	N	Present in Year 1 & 2, In Seed Mix
Mimulus alatus	Winged Monkeyflower	Phrymaceae	N	Present in Year 2
Microstegium vimineum	Japanese stiltgrass, Nepalese Brown-top, Japanese Grass	Poaceae or Gramineae	NNI	Present in Year 1 & 2, Dominant in Year 1 & 2
Nyssa sylvatica	Black Gum, Sour Gum	Nyssaceae	N	Present in Year 1 & 2
Onoclea sensibilis var. sensibilis	Sensitive Fern, Bead Fern	Onocleaceae	N	Present in Year 1 & 2
Oxalis dillenii	Southern Yellow Wood-sorrel	Oxalidaceae	N	Present in Year 2
Oxalis stricta	Common Yellow Wood-sorrel	Oxalidaceae	N	Present in Year 1
Oxydendrum arboreum	Sourwood, Sorrel Tree	Ericaceae	N	Present in Year 1 & 2
Panicum dichotomiflorum var. dichotomiflorum	Spreading Panic Grass, Fall Panic Grass	Poaceae or Gramineae	N	Present in Year 1, In Seed Mix
Parathelypteris noveboracensis	New York Fern	Thelypteridaceae	N	Present in Year 1 & 2, Dominant in Year 1 & 2
Parthenocissus quinquefolia	Virginia-creeper	Vitaceae	N	Present in Year 1 & 2
Persicaria hydropiperoides	Mild Water-pepper	Polygonaceae	N	Present in Year 1 & 2

Persicaria longiseta	Long-bristled Smartweed, Bristly Lady's-Thumb	Polygonaceae	NNI	Present in Year 1 & 2
Persicaria perfoliata	Mile-a-minute Weed, Mile-a-minute Vine, Asiatic Tearthumb	Polygonaceae	NNI	Present in Year 2
Persicaria punctata	Dotted Smartweed	Polygonaceae	N	Present in Year 1
Persicaria sagittata	Arrow-leaf Tearthumb	Polygonaceae	N	Present in Year 1 & 2
Persicaria virginiana	Virginia Knotweed, Jumpseed	Polygonaceae	N	Present in Year 1 & 2
Phytolacca americana var. americana	Common Pokeweed	Phytolaccaceae	N	Present in Year 1 & 2
Pinus virginiana	Virginia Pine, Scrub Pine	Pinaceae	N	Present in Year 2
Potentilla indica	Indian-strawberry	Rosaceae	NNI	Present in Year 2
Prunus serotina var. serotina	Black Cherry, Wild Black Cherry	Rosaceae	N	Present in Year 1 & 2
Quercus alba	White Oak	Fagaceae	N	Present in Year 1 & 2
Robinia pseudoacacia	Black Locust	Fabaceae or Leguminosae	N	Present in Year 1 & 2
Rosa multiflora	Multiflora Rose	Rosaceae	NNI	Present in Year 1 & 2
Rubus pensilvanicus	Pennsylvania Blackberry	Rosaceae	N	Present in Year 1 & 2
Rudbeckia hirta	Black-eyed Susan	Asteraceae or Compositae	N	Present in Year 1
Rumex crispus ssp. crispus	Curly Dock	Polygonaceae	NNI	Present in Year 2
Scirpus atrovirens	Dark Green Bulrush	Cyperaceae	N	Present in Year 1
Setaria parviflora	Knotroot Bristlegrass, Knotroot Foxtail	Poaceae or Gramineae	N	Present in Year 1
Setaria pumila ssp. pumila	Yellow Bristlegrass, Yellow Foxtail	Poaceae or Gramineae	NNI	Present in Year 1

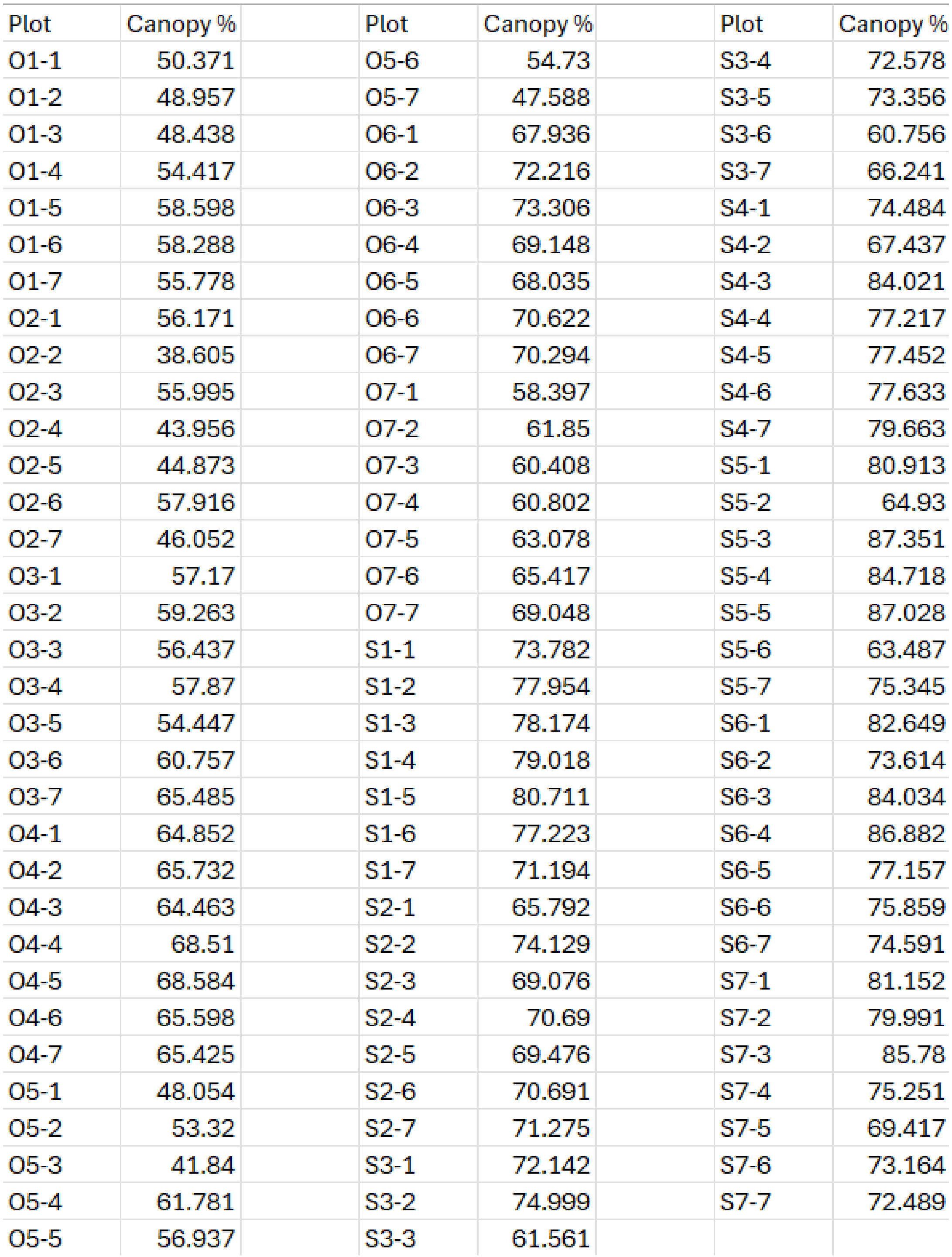
Smilax rotundifolia	Common Greenbrier, Bullbrier, Horsebrier	Smilacaceae	N	Present in Year 1 & 2
Solanum carolinense var. carolinense	Horse-nettle, Carolina Horse-nettle	Solanaceae	N	Present in Year 1
Solidago caesia var. caesia	Blue-stemmed Goldenrod, Wreath Goldenrod	Asteraceae or Compositae	N	Present in Year 2
Solidago canadensis var. canadensis	Canada Goldenrod	Asteraceae or Compositae	N	Present in Year 2
Solidago rugosa	Rough-stemmed Goldenrod, Wrinkle-leaf Goldenrod	Asteraceae or Compositae	N	Present in Year 1 & 2, In Seed Mix
Symphyotrichum racemosum var. racemosum	Small White Aster	Asteraceae or Compositae	N	Present in Year 2
Symphyotrichum undulatum	Wavy-leaved Aster	Asteraceae or Compositae	N	Present in Year 2
Teucrium canadense	Canada Germander, American Germander	Lamiaceae or Labiatae	N	Present in Year 2
Toxicodendron radicans var. radicans	Poison Ivy, Eastern Poison Ivy	Anacardiaceae	N	Present in Year 1 & 2
Ulmus americana	American Elm	Ulmaceae	N	Present in Year 1 & 2
Vaccinium pallidum	Early Lowbush Blueberry, Hillside Blueberry	Ericaceae	N	Present in Year 1
Verbesina alternifolia	Wingstem	Asteraceae or Compositae	N	Present in Year 1, In Seed Mix
Verbesina occidentalis	Yellow Crownbeard	Asteraceae or Compositae	N	Present in Year 2
Vernonia noveboracensis	New York Ironweed	Asteraceae or Compositae	N	Present in Year 2, In Seed Mix

Viola primulifolia	Primrose-leaved Violet	Violaceae	N	Present in Year 1 & 2
Viola sororia	Common Blue Violet, Confederate Violet	Violaceae	N	Present in Year 1 & 2
Vitis labrusca	Fox Grape	Vitaceae	N	Present in Year 1 & 2
Vitis rotundifolia var. rotundifolia	Muscadine Grape, Scuppernong	Vitaceae	N	Present in Year 1
Vitis vulpina	Frost Grape, Winter Grape, Chicken Grape	Vitaceae	N	Present in Year 1
Woodwardia areolata	Netted Chain Fern	Blechnaceae	N	Present in Year 1
Cyperaceae Species	Sedges	Cyperaceae	NA	Present in Year 1, Dominant in Year 1
Poceaceae Species	Grasses	Poceaceae	NA	Present in Year 1, Dominant in Year 1

* Status codes: N = Native; I = Introduced; NNI = Non-native Invasive; NA = Not Applicable (not enough information)

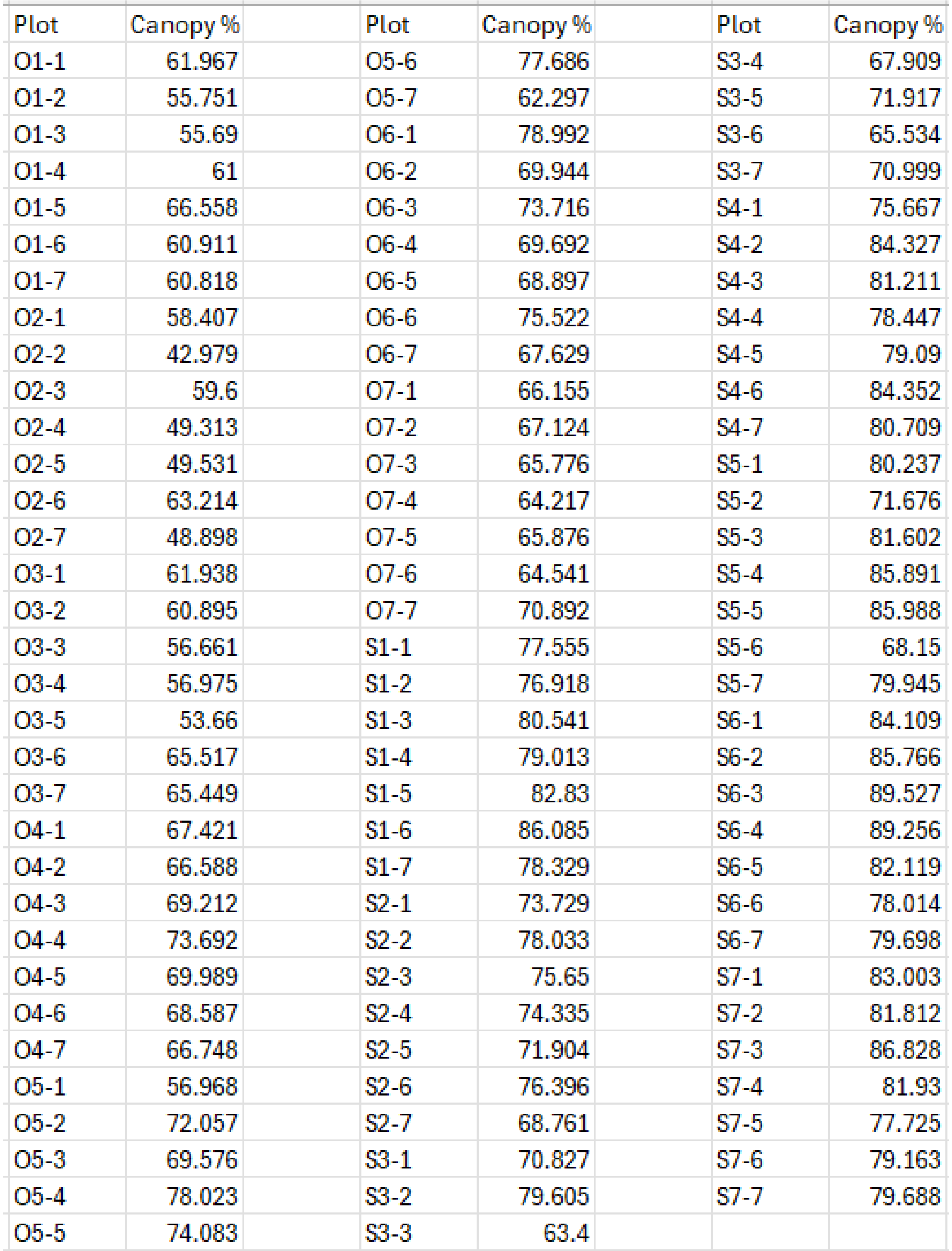
Figure C1. All recorded species in the plots from year one and two minus plots that we removed from the data set. Invasive species in red type.

Appendix D: Raw Data Tables



Plot	Canopy %
O1-1	50.371
O1-2	48.957
O1-3	48.438
O1-4	54.417
O1-5	58.598
O1-6	58.288
O1-7	55.778
O2-1	56.171
O2-2	38.605
O2-3	55.995
O2-4	43.956
O2-5	44.873
O2-6	57.916
O2-7	46.052
O3-1	57.17
O3-2	59.263
O3-3	56.437
O3-4	57.87
O3-5	54.447
O3-6	60.757
O3-7	65.485
O4-1	64.852
O4-2	65.732
O4-3	64.463
O4-4	68.51
O4-5	68.584
O4-6	65.598
O4-7	65.425
O5-1	48.054
O5-2	53.32
O5-3	41.84
O5-4	61.781
O5-5	56.937
O5-6	54.73
O5-7	47.588
O6-1	67.936
O6-2	72.216
O6-3	73.306
O6-4	69.148
O6-5	68.035
O6-6	70.622
O6-7	70.294
O7-1	58.397
O7-2	61.85
O7-3	60.408
O7-4	60.802
O7-5	63.078
O7-6	65.417
O7-7	69.048
S1-1	73.782
S1-2	77.954
S1-3	78.174
S1-4	79.018
S1-5	80.711
S1-6	77.223
S1-7	71.194
S2-1	65.792
S2-2	74.129
S2-3	69.076
S2-4	70.69
S2-5	69.476
S2-6	70.691
S2-7	71.275
S3-1	72.142
S3-2	74.999
S3-3	61.561
S3-4	72.578
S3-5	73.356
S3-6	60.756
S3-7	66.241
S4-1	74.484
S4-2	67.437
S4-3	84.021
S4-4	77.217
S4-5	77.452
S4-6	77.633
S4-7	79.663
S5-1	80.913
S5-2	64.93
S5-3	87.351
S5-4	84.718
S5-5	87.028
S5-6	63.487
S5-7	75.345
S6-1	82.649
S6-2	73.614
S6-3	84.034
S6-4	86.882
S6-5	77.157
S6-6	75.859
S6-7	74.591
S7-1	81.152
S7-2	79.991
S7-3	85.78
S7-4	75.251
S7-5	69.417
S7-6	73.164
S7-7	72.489

Table D1. Year one canopy %.



Plot	Canopy %
O1-1	61.967
O1-2	55.751
O1-3	55.69
O1-4	61
O1-5	66.558
O1-6	60.911
O1-7	60.818
O2-1	58.407
O2-2	42.979
O2-3	59.6
O2-4	49.313
O2-5	49.531
O2-6	63.214
O2-7	48.898
O3-1	61.938
O3-2	60.895
O3-3	56.661
O3-4	56.975
O3-5	53.66
O3-6	65.517
O3-7	65.449
O4-1	67.421
O4-2	66.588
O4-3	69.212
O4-4	73.692
O4-5	69.989
O4-6	68.587
O4-7	66.748
O5-1	56.968
O5-2	72.057
O5-3	69.576
O5-4	78.023
O5-5	74.083
O5-6	77.686
O5-7	62.297
O6-1	78.992
O6-2	69.944
O6-3	73.716
O6-4	69.692
O6-5	68.897
O6-6	75.522
O6-7	67.629
O7-1	66.155
O7-2	67.124
O7-3	65.776
O7-4	64.217
O7-5	65.876
O7-6	64.541
O7-7	70.892
S1-1	77.555
S1-2	76.918
S1-3	80.541
S1-4	79.013
S1-5	82.83
S1-6	86.085
S1-7	78.329
S2-1	73.729
S2-2	78.033
S2-3	75.65
S2-4	74.335
S2-5	71.904
S2-6	76.396
S2-7	68.761
S3-1	70.827
S3-2	79.605
S3-3	63.4
S3-4	67.909
S3-5	71.917
S3-6	65.534
S3-7	70.999
S4-1	75.667
S4-2	84.327
S4-3	81.211
S4-4	78.447
S4-5	79.09
S4-6	84.352
S4-7	80.709
S5-1	80.237
S5-2	71.676
S5-3	81.602
S5-4	85.891
S5-5	85.988
S5-6	68.15
S5-7	79.945
S6-1	84.109
S6-2	85.766
S6-3	89.527
S6-4	89.256
S6-5	82.119
S6-6	78.014
S6-7	79.698
S7-1	83.003
S7-2	81.812
S7-3	86.828
S7-4	81.93
S7-5	77.725
S7-6	79.163
S7-7	79.688

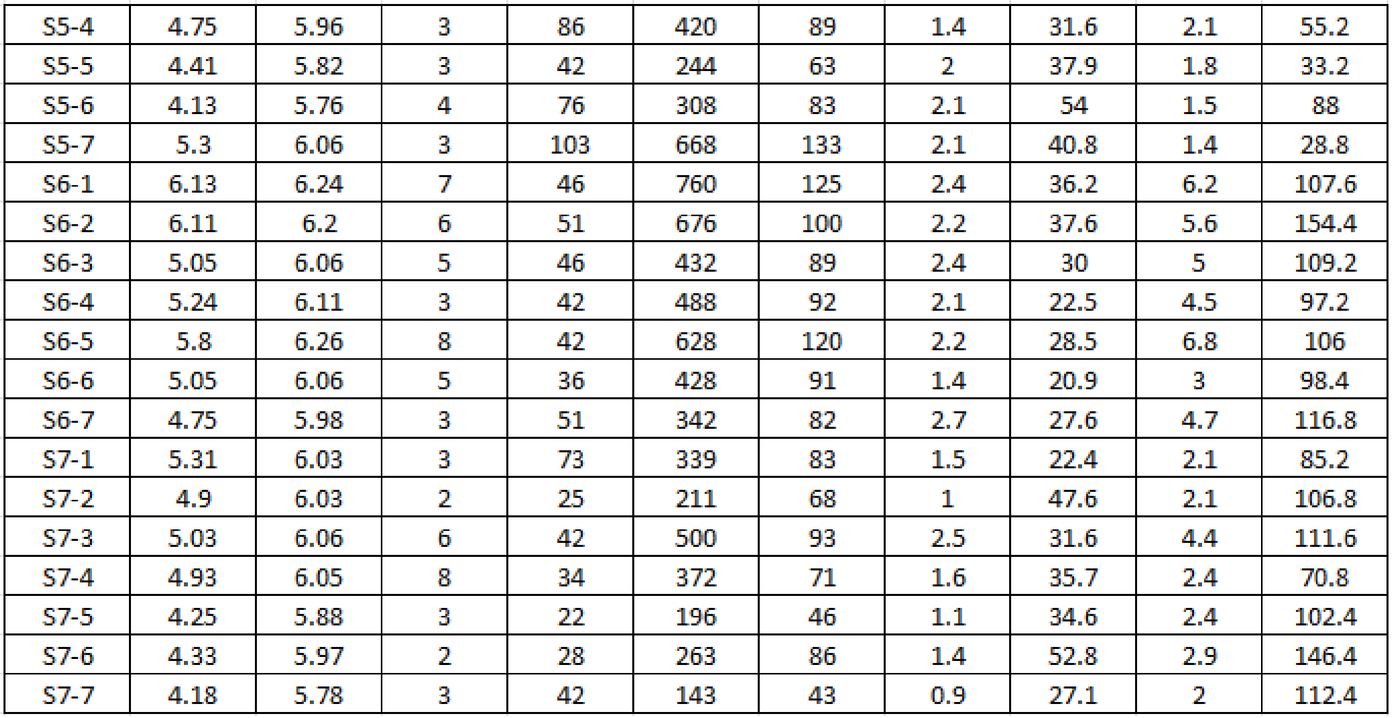
Table D2. Year two canopy %.

Plots	TCN	
	Total N %	Total C %
O1-1	0.10	1.88
O1-2	0.16	2.08
O1-3	0.09	1.17
O1-4	0.20	2.52
O1-5	0.19	2.46
O1-6	0.15	1.98
O1-7	0.11	1.45
O2-1	0.10	1.63
O2-2	0.17	3.23
O2-3	0.09	1.50
O2-4	0.05	1.08
O2-5	0.11	1.77
O2-6	0.09	1.52
O2-7	0.11	1.63
O3-1	0.11	1.86
O3-2	0.17	2.44
O3-3	0.10	1.51
O3-4	0.14	1.82
O3-5	0.12	1.57
O3-6	0.13	1.60
O3-7	0.09	1.13
O4-1	0.10	1.42
O4-2	0.15	1.85
O4-3	0.10	1.38
O4-4	0.13	1.69
O4-5	0.06	0.75
O4-6	0.10	1.25
O4-7	0.06	0.74
O5-1	0.20	2.41
O5-2	0.16	2.27
O5-3	0.13	1.55
O5-4	0.22	2.58
O5-5	0.14	1.76
O5-6	0.21	3.03
O5-7	0.14	1.63
O6-1	0.11	1.70
O6-2	0.15	2.84
O6-3	0.13	1.77
O6-4	0.11	1.59
O6-5	0.09	1.27
O6-6	0.13	1.74
O6-7	0.14	1.93
O7-1	0.12	1.87
O7-2	0.10	1.43
O7-3	0.15	2.43
O7-4	0.13	1.76
O7-5	0.14	1.59
O7-6	0.12	1.49
O7-7	0.11	1.48
S1-1	0.11	1.45
S1-2	0.13	1.78
S1-3	0.10	1.11
S1-4	0.10	1.23
S1-5	0.15	1.60
S1-6	0.11	1.21
S1-7	0.12	1.41
S2-1	0.16	2.76
S2-2	0.14	1.94
S2-3	0.12	1.47
S2-4	0.16	2.02
S2-5	0.14	1.72
S2-6	0.09	1.15
S2-7	0.13	1.72
S3-1	0.31	5.10
S3-2	0.23	3.60
S3-3	0.17	2.46
S3-4	0.12	1.67
S3-5	0.13	1.75
S3-6	0.15	1.89
S3-7	0.11	1.41
S4-1	0.11	1.62
S4-2	0.17	2.13
S4-3	0.12	1.48
S4-4	0.15	1.84
S4-5	0.14	1.58
S4-6	0.17	2.01
S4-7	0.12	1.34
S5-1	0.18	2.43
S5-2	0.19	2.29
S5-3	0.15	1.62
S5-4	0.21	2.36
S5-5	0.17	1.93
S5-6	0.21	2.33
S5-7	0.22	2.51
S6-1	0.18	2.06
S6-2	0.14	2.47
S6-3	0.13	1.61
S6-4	0.12	1.60
S6-5	0.09	1.19
S6-6	0.14	1.62
S6-7	0.17	1.98
S7-1	0.16	2.12
S7-2	0.13	1.58
S7-3	0.16	1.95
S7-4	0.13	1.62
S7-5	0.15	1.76
S7-6	0.12	1.39
S7-7	0.15	1.86

Table D3. Total nitrogen and carbon % in year one.

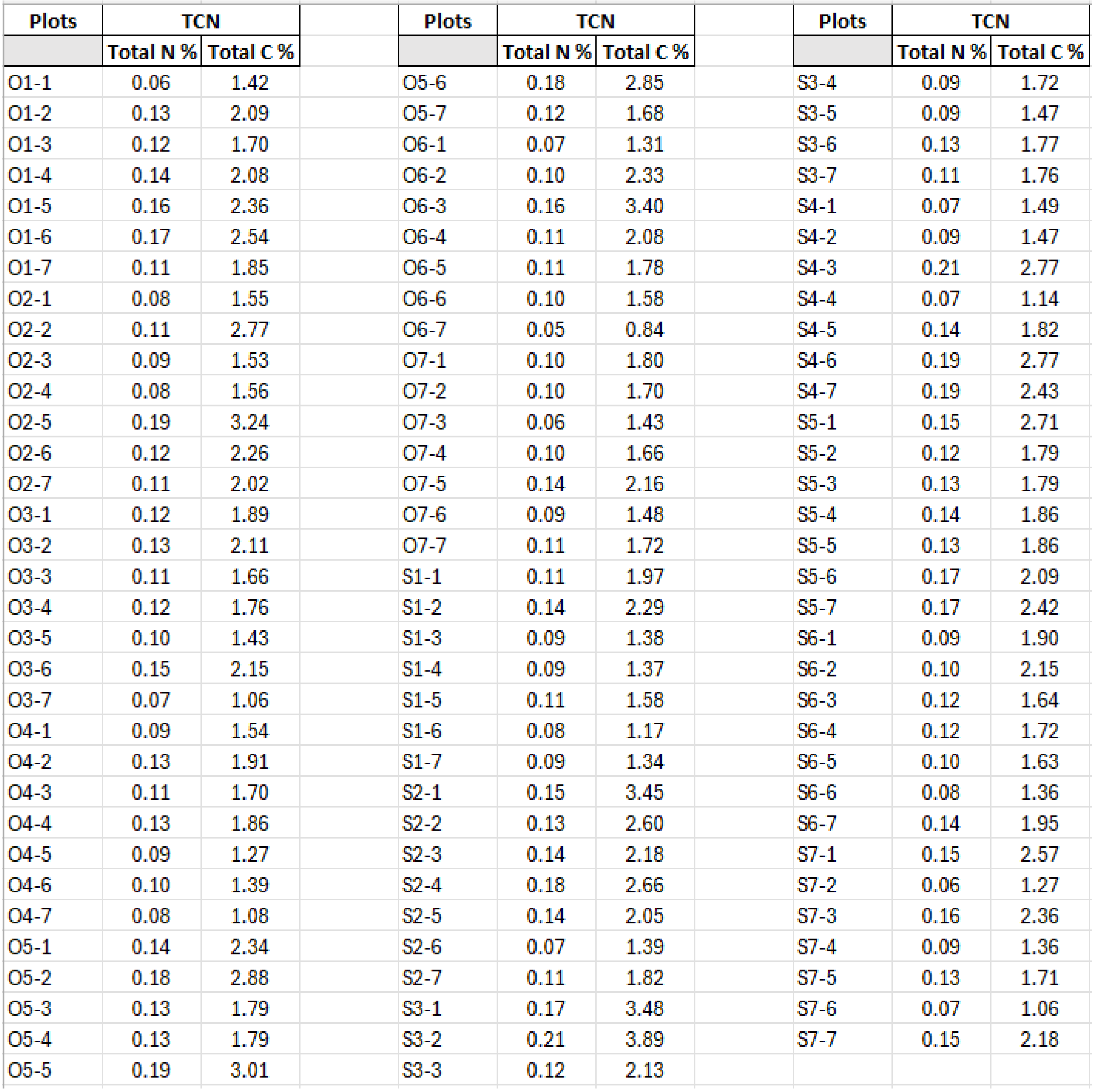
Plots	pH	BpH	P	K	Ca	Mg	Zn	Mn	Cu	Fe
	pH	BpH	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
O1-1	5.12	6.09	9	49	636	145	1.7	23.7	3	127.2
O1-2	4.05	5.74	5	41	263	45	1.7	61.6	1.6	60
O1-3	4.53	5.9	4	31	190	38	1.2	40.4	2.4	81.2
O1-4	4.25	5.72	4	76	341	53	1.4	39.3	1.2	33.9
O1-5	4.31	5.78	6	32	364	60	2.1	44.8	2.8	84
O1-6	4.51	6.2	5	43	236	55	2.3	54	2.8	108.8
O1-7	4.42	5.87	4	27	224	45	1.3	29	2.5	130.8
O2-1	5.06	6.03	4	60	480	72	1.8	16.7	2.3	154
O2-2	5.99	6.13	9	63	788	118	2.5	30.5	3.5	194.4
O2-3	5.06	5.95	3	38	317	55	1.3	13.3	1.9	136.4
O2-4	5.12	5.88	3	30	157	34	1	13.3	1.8	296.4
O2-5	4.96	5.96	5	42	520	82	1.9	17	2.6	209.6
O2-6	4.96	5.93	5	37	399	65	2	16.2	2	116
O2-7	5.35	6.12	8	43	644	111	2	17.5	2.6	106.4
O3-1	5.56	5.93	4	34	420	58	2.6	21.1	2.3	60.4
O3-2	5.14	6.2	6	51	381	76	0.3	14.2	0.5	13.5
O3-3	5.26	5.98	4	43	416	55	2.5	21.3	2.2	52
O3-4	5.29	6.07	5	40	608	69	3.7	32.3	3	46.4
O3-5	5.13	6.01	4	40	536	71	3.3	24.9	2.9	83.6
O3-6	5.24	6.07	5	43	564	66	4.2	26.5	2.9	49.2
O3-7	5.13	6.08	3	29	367	54	2.3	26.7	1.9	48
O4-1	5.1	6.02	2	27	231	32	1.1	22	1.3	73.2
O4-2	4.54	5.73	3	25	172	30	1.4	29.2	2.1	94
O4-3	4.57	5.98	3	29	204	32	1.3	24.5	1.1	77.2
O4-4	4.58	5.82	3	55	189	32	1	11.3	1	59.6
O4-5	4.64	6.02	4	32	224	38	1.1	13.1	1.6	68
O4-6	4.42	5.94	3	24	152	26	1.2	26	1.1	66.4
O4-7	4.47	5.95	2	16	194	34	0.9	13.1	1.1	44.8
O5-1	4.68	6.03	5	61	512	100	1.7	29.4	3.5	70.8
O5-2	5.45	6.2	5	49	684	103	3.4	22.7	2.9	58.8
O5-3	5.12	6.03	3	45	524	85	1.2	15.1	1.8	34.5
O5-4	5.19	6.14	7	63	764	108	3.5	21.6	4.1	52
O5-5	5.14	6.05	5	37	556	71	3.1	25.6	1.8	56
O5-6	5.43	6.11	9	44	1056	112	13.3	20.6	2.6	46
O5-7	4.58	5.88	3	27	282	49	1.8	15.9	1.9	50.4
O6-1	5.23	6.14	8	38	608	113	1.3	20.6	3	134.4
O6-2	6.24	6.23	7	55	776	123	1.3	30.8	3.1	175.2
O6-3	5.73	6.23	7	59	688	132	1.3	27.7	3	192.8
O6-4	5.45	6.15	6	64	536	105	1.5	25.1	2.9	152

O6-5	5.37	6.18	7	35	516	105	1.2	18.2	3.1	102
O6-6	5.06	6.13	6	41	504	102	1.6	22.9	4.4	172
O6-7	5.23	6.12	6	42	624	101	1.6	19.3	3.2	89.6
O7-1	5.7	6.19	7	38	664	133	1.5	26.8	4.4	148.8
O7-2	5.88	6.25	7	45	600	119	1.1	22.1	3.3	85.6
O7-3	5.87	6.17	6	55	616	110	1.7	28.4	4.2	151.6
O7-4	5.62	6.2	6	51	672	116	1.5	25	3.8	128
O7-5	5.1	6.1	3	57	436	98	1.2	17.9	2.9	98.4
O7-6	5.64	6.21	6	42	624	120	1.2	18.6	3.3	88
O7-7	5.64	6.24	6	49	664	125	1.3	19.4	3.7	90.4
S1-1	4.17	5.75	7	72	305	51	1.8	33.5	2	79.2
S1-2	4.29	5.76	6	44	369	65	1.6	27.8	2.1	78
S1-3	4.31	5.9	4	38	197	39	1.2	27.4	1.9	64
S1-4	4.67	5.95	4	32	357	65	1.1	23	2	67.2
S1-5	4.42	5.91	5	36	391	72	2	54.8	2.7	70.8
S1-6	4.32	5.9	4	25	276	53	1.7	35.9	1.8	62
S1-7	4.26	5.85	6	39	299	52	1.8	29.2	2	69.2
S2-1	5.02	5.91	4	79	400	92	2.2	32.2	1.2	52.8
S2-2	4.39	5.87	5	47	299	57	2.1	25.6	2	114.4
S2-3	4.62	5.97	4	42	246	51	1.2	16.2	1.9	79.2
S2-4	4.48	5.88	4	59	255	51	2.1	29.4	2.1	96.4
S2-5	4.49	5.94	3	59	378	87	1.8	29.5	0.9	46.4
S2-6	4.35	5.92	3	42	246	56	1.4	25.4	1.3	70.8
S2-7	4.45	5.82	3	61	212	51	1.1	21	1.1	85.2
S3-1	5.34	6.11	16	66	1296	131	15.6	45.2	3.5	39.8
S3-2	5.09	5.93	13	46	760	97	9.4	52	4.2	54.4
S3-3	5.1	6	8	32	636	66	5	30.7	2.3	64.8
S3-4	5.51	6.12	6	36	468	49	3.9	26.2	2.3	73.2
S3-5	4.88	5.99	6	41	424	52	3.1	30.1	1.9	106.8
S3-6	4.61	5.79	5	54	365	48	2.6	38.5	1.8	81.6
S3-7	4.72	5.97	4	35	305	43	2.2	23.8	1.6	70
S4-1	4.16	5.69	3	82	162	35	0.8	45.2	1.3	44.8
S4-2	3.98	5.53	4	66	181	40	1.2	65.6	1.5	47.2
S4-3	4.36	5.68	2	52	150	34	1	38.2	1.7	58
S4-4	4.15	5.73	4	80	319	55	1.4	74.8	1.2	45.6
S4-5	4.37	5.89	3	67	216	49	2.2	55.6	3.2	38
S4-6	3.96	5.61	5	62	255	50	1.7	95.6	1.3	51.2
S4-7	4.28	5.87	3	40	253	51	2.4	70	3	40.4
S5-1	5.16	5.98	3	71	404	96	1.8	27.4	3	64.8
S5-2	4.7	5.91	3	63	369	78	1.7	24.7	2.6	66
S5-3	4.68	5.97	3	67	375	83	1.8	34	3.8	67.2



S5-4	4.75	5.96	3	86	420	89	1.4	31.6	2.1	55.2
S5-5	4.41	5.82	3	42	244	63	2	37.9	1.8	33.2
S5-6	4.13	5.76	4	76	308	83	2.1	54	1.5	88
S5-7	5.3	6.06	3	103	668	133	2.1	40.8	1.4	28.8
S6-1	6.13	6.24	7	46	760	125	2.4	36.2	6.2	107.6
S6-2	6.11	6.2	6	51	676	100	2.2	37.6	5.6	154.4
S6-3	5.05	6.06	5	46	432	89	2.4	30	5	109.2
S6-4	5.24	6.11	3	42	488	92	2.1	22.5	4.5	97.2
S6-5	5.8	6.26	8	42	628	120	2.2	28.5	6.8	106
S6-6	5.05	6.06	5	36	428	91	1.4	20.9	3	98.4
S6-7	4.75	5.98	3	51	342	82	2.7	27.6	4.7	116.8
S7-1	5.31	6.03	3	73	339	83	1.5	22.4	2.1	85.2
S7-2	4.9	6.03	2	25	211	68	1	47.6	2.1	106.8
S7-3	5.03	6.06	6	42	500	93	2.5	31.6	4.4	111.6
S7-4	4.93	6.05	8	34	372	71	1.6	35.7	2.4	70.8
S7-5	4.25	5.88	3	22	196	46	1.1	34.6	2.4	102.4
S7-6	4.33	5.97	2	28	263	86	1.4	52.8	2.9	146.4
S7-7	4.18	5.78	3	42	143	43	0.9	27.1	2	112.4

Table D4. Soil characteristics for year one.

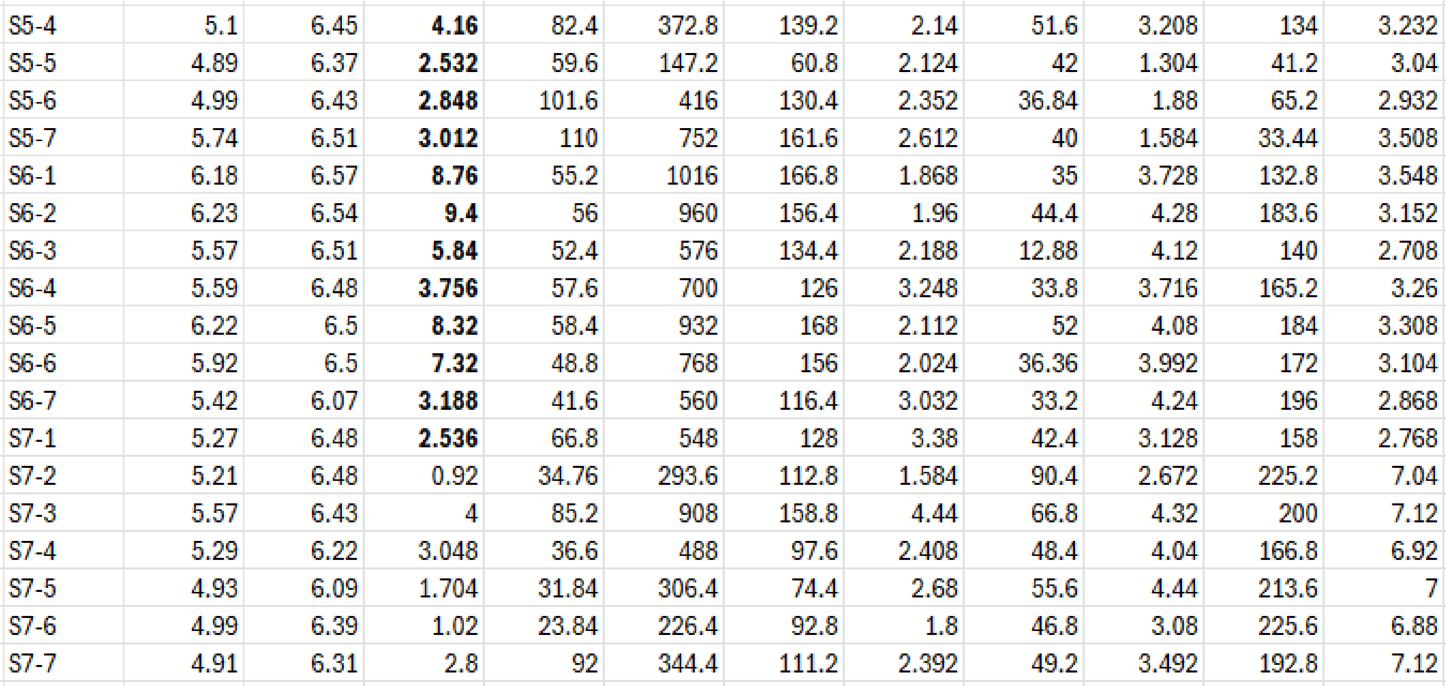


Plots	TCN	
	Total N %	Total C %
O1-1	0.06	1.42
O1-2	0.13	2.09
O1-3	0.12	1.70
O1-4	0.14	2.08
O1-5	0.16	2.36
O1-6	0.17	2.54
O1-7	0.11	1.85
O2-1	0.08	1.55
O2-2	0.11	2.77
O2-3	0.09	1.53
O2-4	0.08	1.56
O2-5	0.19	3.24
O2-6	0.12	2.26
O2-7	0.11	2.02
O3-1	0.12	1.89
O3-2	0.13	2.11
O3-3	0.11	1.66
O3-4	0.12	1.76
O3-5	0.10	1.43
O3-6	0.15	2.15
O3-7	0.07	1.06
O4-1	0.09	1.54
O4-2	0.13	1.91
O4-3	0.11	1.70
O4-4	0.13	1.86
O4-5	0.09	1.27
O4-6	0.10	1.39
O4-7	0.08	1.08
O5-1	0.14	2.34
O5-2	0.18	2.88
O5-3	0.13	1.79
O5-4	0.13	1.79
O5-5	0.19	3.01
O5-6	0.18	2.85
O5-7	0.12	1.68
O6-1	0.07	1.31
O6-2	0.10	2.33
O6-3	0.16	3.40
O6-4	0.11	2.08
O6-5	0.11	1.78
O6-6	0.10	1.58
O6-7	0.05	0.84
O7-1	0.10	1.80
O7-2	0.10	1.70
O7-3	0.06	1.43
O7-4	0.10	1.66
O7-5	0.14	2.16
O7-6	0.09	1.48
O7-7	0.11	1.72
S1-1	0.11	1.97
S1-2	0.14	2.29
S1-3	0.09	1.38
S1-4	0.09	1.37
S1-5	0.11	1.58
S1-6	0.08	1.17
S1-7	0.09	1.34
S2-1	0.15	3.45
S2-2	0.13	2.60
S2-3	0.14	2.18
S2-4	0.18	2.66
S2-5	0.14	2.05
S2-6	0.07	1.39
S2-7	0.11	1.82
S3-1	0.17	3.48
S3-2	0.21	3.89
S3-3	0.12	2.13
S3-4	0.09	1.72
S3-5	0.09	1.47
S3-6	0.13	1.77
S3-7	0.11	1.76
S4-1	0.07	1.49
S4-2	0.09	1.47
S4-3	0.21	2.77
S4-4	0.07	1.14
S4-5	0.14	1.82
S4-6	0.19	2.77
S4-7	0.19	2.43
S5-1	0.15	2.71
S5-2	0.12	1.79
S5-3	0.13	1.79
S5-4	0.14	1.86
S5-5	0.13	1.86
S5-6	0.17	2.09
S5-7	0.17	2.42
S6-1	0.09	1.90
S6-2	0.10	2.15
S6-3	0.12	1.64
S6-4	0.12	1.72
S6-5	0.10	1.63
S6-6	0.08	1.36
S6-7	0.14	1.95
S7-1	0.15	2.57
S7-2	0.06	1.27
S7-3	0.16	2.36
S7-4	0.09	1.36
S7-5	0.13	1.71
S7-6	0.07	1.06
S7-7	0.15	2.18

Figure D5. Total nitrogen and carbon % in year two.

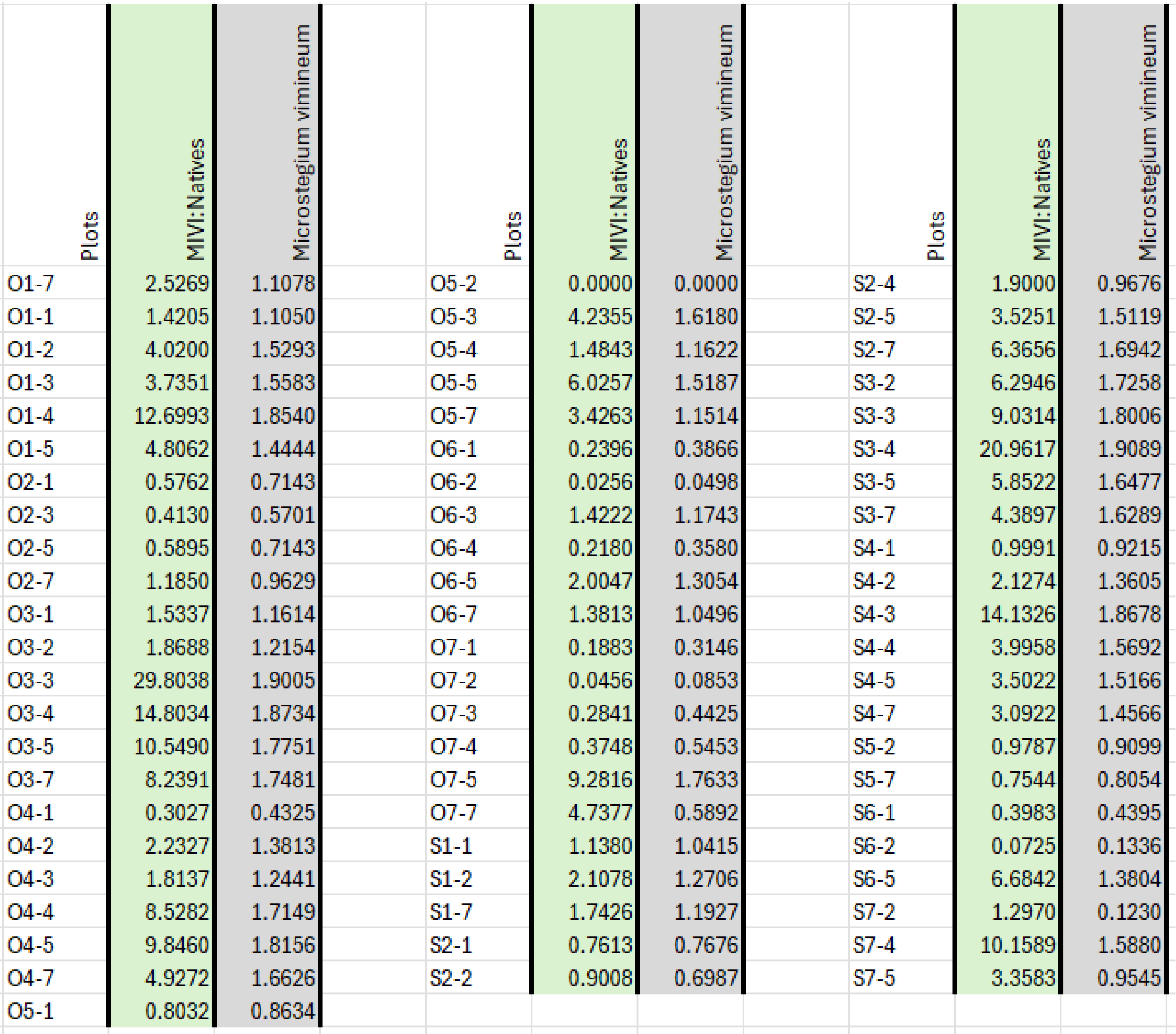
Plots	pH	BpH	P	K	Ca	Mg	Zn	Mn	Cu	Fe
	pH	BpH	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
O1-1	5.87	5.9	**8.56**	56.8	852	203.6	2.424	48.8	4.2	194.8
O1-2	4.76	5.65	**3.012**	45.6	330	63.6	2.472	62	2.436	84
O1-3	4.83	5.88	**4.48**	43.2	261.6	51.6	2.812	64.8	3.5	170
O1-4	4.82	6.13	**3.532**	56.4	376.8	59.6	2.496	42.8	1.968	42.4
O1-5	5.03	6.16	**6.4**	34.04	540	85.2	3.768	52.8	5.04	158.4
O1-6	5.1	6.11	**7.92**	84.4	548	114.8	6.12	57.2	3.924	144.8
O1-7	5.72	6	**6.88**	56.4	564	110.4	2.78	220	5.64	456
O2-1	5.27	5.85	**5.2**	59.2	540	98	2.804	38.88	2.656	143.2
O2-2	5.9	5.94	**10.12**	80.4	1188	194	4.04	126.4	6.44	373.2
O2-3	5.98	6.1	**2.864**	50.4	568	97.6	2.752	38.92	3.328	196.4
O2-4	5.4	5.93	**2.924**	29.76	285.6	53.6	1.604	32.48	2.724	265.2
O2-5	5.69	6.34	**6.32**	38.64	1276	155.2	4.4	58	4.24	201.6
O2-6	5.9	6.05	**5.16**	41.2	636	94.4	5.72	47.2	3.284	193.2
O2-7	6.01	6.33	**10.12**	48.8	1028	183.2	3.548	61.6	5.48	219.2
O3-1	5.94	6.05	**3.98**	62.8	600	84	4.52	61.2	3.388	74
O3-2	5.78	6.28	**4.52**	108.8	692	90.4	4	73.6	3.872	94.4
O3-3	5.9	6.16	**4.24**	56	752	86	5.08	62.8	4.28	118
O3-4	5.99	6.22	**4.96**	34.36	752	87.6	5.96	75.2	4.08	56.4
O3-5	6.01	5.99	**2.736**	41.6	544	78	3.672	66.4	3.252	80
O3-6	5.85	6.13	**7**	43.2	1068	112.8	9.28	64.8	5.36	85.6
O3-7	6	6.1	**3.06**	41.2	476	68.4	3.088	43.6	2.992	94.4
O4-1	4.86	6.13	**2.152**	35.6	252.8	39.16	1.508	44.8	2.048	141.2
O4-2	4.88	6.11	**3.748**	28.84	199.2	34.6	2.24	54.4	2.976	116
O4-3	5.14	6.09	**2.968**	46.4	277.6	50	2.24	46.8	2.18	183.2
O4-4	4.84	5.79	**4.4**	32.44	258	42.8	2.16	23.04	1.98	114.8
O4-5	5.02	6.38	**3.456**	38.96	336	57.6	2.276	29.36	2.704	131.6
O4-6	4.75	6.33	**2.496**	24	139.6	24.44	1.772	47.6	2.072	116
O4-7	5.01	6.38	**2.896**	23.56	288.4	50.4	1.74	19.88	1.764	56.4
O5-1	5.68	6.35	**4.8**	67.2	748	151.6	2.824	39.32	5.88	102.4
O5-2	5.93	6.32	**6.4**	88.4	1120	154.4	7.28	43.2	5.6	96.4
O5-3	5.74	6.4	**42**	125.6	868	88.4	4.16	6.6	4.16	24.88
O5-4	5.94	6.42	**4.88**	47.2	808	118.8	2.228	27.2	2.904	61.2
O5-5	5.8	6.14	**6.8**	70	916	154.8	3.964	35.88	4.92	72
O5-6	6.03	6.08	**5.96**	64.4	1108	137.2	7.56	42.4	3.18	85.2
O5-7	5.25	6.33	**10.96**	69.6	1608	174	20.88	42	4.16	50.8
O6-1	6.04	6.42	**3.504**	36.04	261.2	51.2	2.064	18.64	2.78	88.4
O6-2	6.13	6.44	**7.92**	28.12	752	168.8	2	50.8	4.56	230.8
O6-3	6.23	6.42	**8.48**	60.8	984	161.2	2.076	55.2	4.52	218.8
O6-4	5.97	6.42	**8.08**	100.8	1360	214	2.652	66.4	4.96	217.2

O6-5	6.06	6.42	**6.72**	73.2	928	142	2.536	65.6	4.72	271.6
O6-6	5.72	6.14	**7.68**	44	984	140.4	2.72	61.6	3.784	203.6
O6-7	5.75	6.17	**9.6**	69.6	724	143.6	2.812	48.8	5	269.2
O7-1	5.78	6.41	**6.28**	34.88	552	152.4	1.608	28.96	3.34	165.2
O7-2	6.08	6.37	**6.56**	40.8	716	140.8	1.992	54	4.64	271.6
O7-3	6.31	6.45	**7.32**	65.6	856	161.6	2.036	46	4.16	203.6
O7-4	6.17	6.34	**10.52**	35.84	924	182.8	1.816	72.4	4.84	205.6
O7-5	5.43	6.31	**5.52**	72.8	900	153.6	2.204	45.2	3.98	193.6
O7-6	5.94	6.38	**3.956**	96.4	508	132.4	2.732	40.8	3.948	200
O7-7	5.9	6.33	**5.84**	38.36	736	153.2	1.888	34.04	3.944	201.6
S1-1	5.18	6.09	**6.16**	46.8	800	148.4	2.308	47.2	5	269.2
S1-2	5.22	6.43	**5.64**	69.2	472	79.6	2.932	34.16	3.012	138
S1-3	5.11	6.42	**5.28**	69.2	712	105.2	2.808	62	2.928	169.2
S1-4	5.14	6.39	**4.04**	33.28	460	82.8	1.924	32.24	2.796	126.4
S1-5	5.21	6.41	**4.44**	47.2	480	88.8	2.748	69.2	3.196	109.2
S1-6	4.98	6.39	**3.748**	26.8	324.8	66.4	2.728	33.44	2.788	111.2
S1-7	5	6.42	**4.44**	40.8	369.2	68.4	2.488	35.88	2.652	117.2
S2-1	5.26	6.32	**5.32**	87.6	556	114.8	4.48	50.8	2.012	108.8
S2-2	5.16	6.37	**3.6**	69.2	380.4	83.2	4	43.2	2.924	211.2
S2-3	5.44	6.43	**5.6**	51.6	620	132	3.548	40.4	4.04	178.8
S2-4	4.99	6.4	**5.68**	82.8	452	97.2	4.2	52	2.752	122.4
S2-5	5.38	6.42	**4.12**	74.4	652	126.8	2.868	42.4	1.228	59.2
S2-6	5.21	6.39	**4.44**	65.2	324	80.8	2.372	25.76	1.948	118
S2-7	5.12	6.36	**3.296**	75.6	277.6	59.2	1.992	26.44	1.952	144.8
S3-1	6.18	6.43	**22.52**	56.8	1712	164	25.12	93.6	5.12	50.4
S3-2	5.84	6.36	**21.52**	88.8	1308	162	19.2	112.4	6.32	41.2
S3-3	5.88	6.13	**8.92**	72.8	1204	122.4	10.4	79.6	3.164	73.2
S3-4	5.91	6.08	**5.64**	38.2	476	56	5.28	89.6	3.276	177.2
S3-5	5.41	6.04	**2.164**	78.8	261.2	39.12	1.96	118.8	2.496	111.6
S3-6	5.42	5.97	**3.264**	38.6	408	56.4	3.088	107.2	2.748	93.6
S3-7	5.52	6.4	**3.856**	95.6	568	78.4	4.64	59.2	2.8	202
S4-1	4.78	6.35	**2.58**	53.6	134.8	33.48	1.348	43.2	1.344	45.2
S4-2	4.87	6.14	**2.04**	73.2	138.4	31.56	1.228	53.2	3.184	44.4
S4-3	4.77	6.23	**3.916**	66	282.8	63.6	3.544	58.8	1.948	41.2
S4-4	4.95	6.31	**2.816**	64.4	224.4	41.2	1.28	63.2	1.412	44.8
S4-5	4.89	6.33	**2.252**	44.8	260.8	56.4	3.016	85.6	1.692	42.8
S4-6	4.9	6.4	**3.992**	112.4	444	99.2	3.088	82.4	1.36	29.2
S4-7	4.9	6.27	**4.4**	62	416	92	4.76	90	1.82	33.04
S5-1	5.36	6.42	**2.912**	100.8	508	126	1.972	34.56	2.288	82.4
S5-2	5.31	6.46	**2.644**	56	416	89.2	1.712	37.56	2.756	87.6
S5-3	5.18	6.43	**2.72**	83.2	398	88	2.132	38.2	3.416	108.8



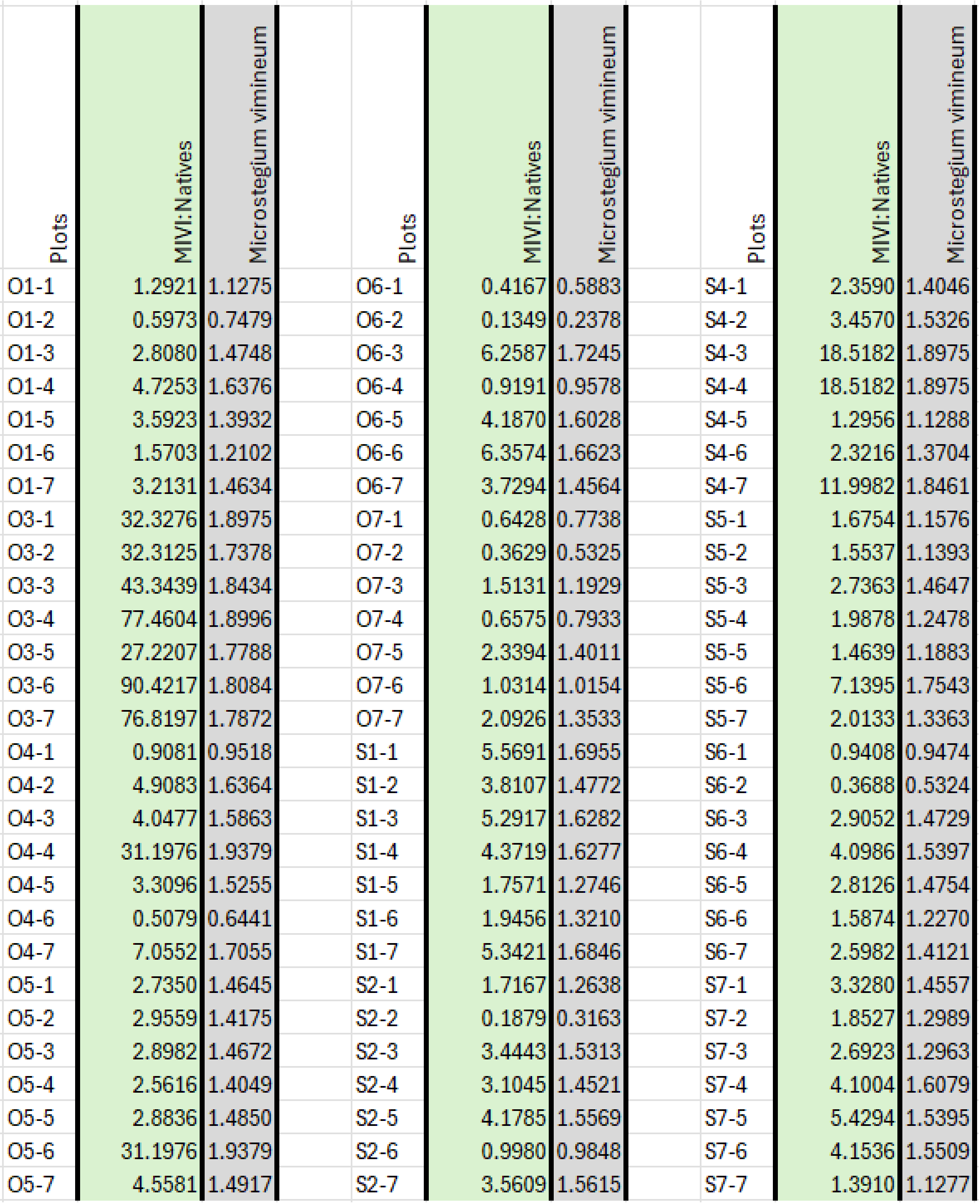
S5-4	5.1	6.45	**4.16**	82.4	372.8	139.2	2.14	51.6	3.208	134	3.232
S5-5	4.89	6.37	**2.532**	59.6	147.2	60.8	2.124	42	1.304	41.2	3.04
S5-6	4.99	6.43	**2.848**	101.6	416	130.4	2.352	36.84	1.88	65.2	2.932
S5-7	5.74	6.51	**3.012**	110	752	161.6	2.612	40	1.584	33.44	3.508
S6-1	6.18	6.57	**8.76**	55.2	1016	166.8	1.868	35	3.728	132.8	3.548
S6-2	6.23	6.54	**9.4**	56	960	156.4	1.96	44.4	4.28	183.6	3.152
S6-3	5.57	6.51	**5.84**	52.4	576	134.4	2.188	12.88	4.12	140	2.708
S6-4	5.59	6.48	**3.756**	57.6	700	126	3.248	33.8	3.716	165.2	3.26
S6-5	6.22	6.5	**8.32**	58.4	932	168	2.112	52	4.08	184	3.308
S6-6	5.92	6.5	**7.32**	48.8	768	156	2.024	36.36	3.992	172	3.104
S6-7	5.42	6.07	**3.188**	41.6	560	116.4	3.032	33.2	4.24	196	2.868
S7-1	5.27	6.48	**2.536**	66.8	548	128	3.38	42.4	3.128	158	2.768
S7-2	5.21	6.48	0.92	34.76	293.6	112.8	1.584	90.4	2.672	225.2	7.04
S7-3	5.57	6.43	4	85.2	908	158.8	4.44	66.8	4.32	200	7.12
S7-4	5.29	6.22	3.048	36.6	488	97.6	2.408	48.4	4.04	166.8	6.92
S7-5	4.93	6.09	1.704	31.84	306.4	74.4	2.68	55.6	4.44	213.6	7
S7-6	4.99	6.39	1.02	23.84	226.4	92.8	1.8	46.8	3.08	225.6	6.88
S7-7	4.91	6.31	2.8	92	344.4	111.2	2.392	49.2	3.492	192.8	7.12

Table D5. Soil characteristics for year two.



Plots	MIVI:Natives	Microstegium vimineum
O1-7	2.5269	1.1078
O1-1	1.4205	1.1050
O1-2	4.0200	1.5293
O1-3	3.7351	1.5583
O1-4	12.6993	1.8540
O1-5	4.8062	1.4444
O2-1	0.5762	0.7143
O2-3	0.4130	0.5701
O2-5	0.5895	0.7143
O2-7	1.1850	0.9629
O3-1	1.5337	1.1614
O3-2	1.8688	1.2154
O3-3	29.8038	1.9005
O3-4	14.8034	1.8734
O3-5	10.5490	1.7751
O3-7	8.2391	1.7481
O4-1	0.3027	0.4325
O4-2	2.2327	1.3813
O4-3	1.8137	1.2441
O4-4	8.5282	1.7149
O4-5	9.8460	1.8156
O4-7	4.9272	1.6626
O5-1	0.8032	0.8634
O5-2	0.0000	0.0000
O5-3	4.2355	1.6180
O5-4	1.4843	1.1622
O5-5	6.0257	1.5187
O5-7	3.4263	1.1514
O6-1	0.2396	0.3866
O6-2	0.0256	0.0498
O6-3	1.4222	1.1743
O6-4	0.2180	0.3580
O6-5	2.0047	1.3054
O6-7	1.3813	1.0496
O7-1	0.1883	0.3146
O7-2	0.0456	0.0853
O7-3	0.2841	0.4425
O7-4	0.3748	0.5453
O7-5	9.2816	1.7633
O7-7	4.7377	0.5892
S1-1	1.1380	1.0415
S1-2	2.1078	1.2706
S1-7	1.7426	1.1927
S2-1	0.7613	0.7676
S2-2	0.9008	0.6987
S2-4	1.9000	0.9676
S2-5	3.5251	1.5119
S2-7	6.3656	1.6942
S3-2	6.2946	1.7258
S3-3	9.0314	1.8006
S3-4	20.9617	1.9089
S3-5	5.8522	1.6477
S3-7	4.3897	1.6289
S4-1	0.9991	0.9215
S4-2	2.1274	1.3605
S4-3	14.1326	1.8678
S4-4	3.9958	1.5692
S4-5	3.5022	1.5166
S4-7	3.0922	1.4566
S5-2	0.9787	0.9099
S5-7	0.7544	0.8054
S6-1	0.3983	0.4395
S6-2	0.0725	0.1336
S6-5	6.6842	1.3804
S7-2	1.2970	0.1230
S7-4	10.1589	1.5880
S7-5	3.3583	0.9545

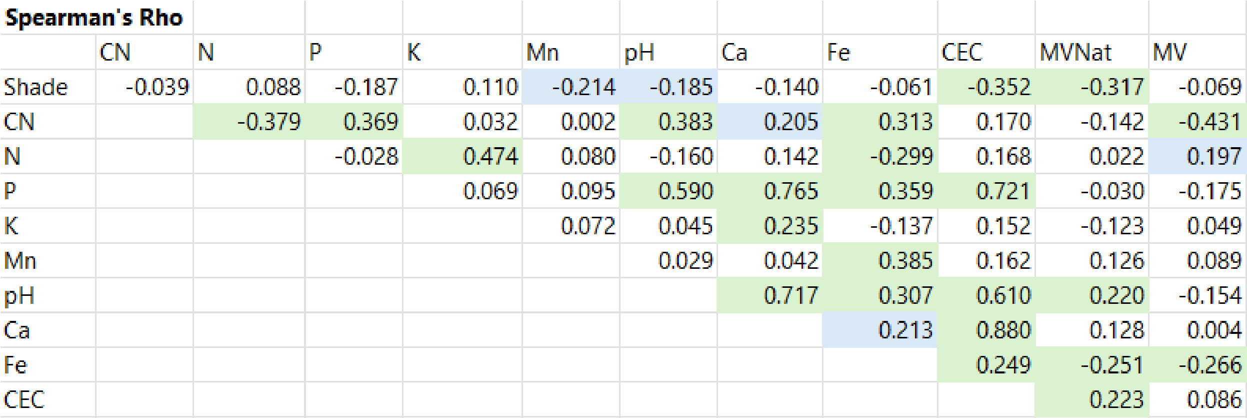
Table D6. Importance value of *M. vimineum* and *M. vimineum* to native species ratio in each plot for year one 1 (minus outliers).



Plots	MIVI:Natives	Microstegium vimineum	Plots	MIVI:Natives	Microstegium vimineum	Plots	MIVI:Natives	Microstegium vimineum
O1-1	1.2921	1.1275	O6-1	0.4167	0.5883	S4-1	2.3590	1.4046
O1-2	0.5973	0.7479	O6-2	0.1349	0.2378	S4-2	3.4570	1.5326
O1-3	2.8080	1.4748	O6-3	6.2587	1.7245	S4-3	18.5182	1.8975
O1-4	4.7253	1.6376	O6-4	0.9191	0.9578	S4-4	18.5182	1.8975
O1-5	3.5923	1.3932	O6-5	4.1870	1.6028	S4-5	1.2956	1.1288
O1-6	1.5703	1.2102	O6-6	6.3574	1.6623	S4-6	2.3216	1.3704
O1-7	3.2131	1.4634	O6-7	3.7294	1.4564	S4-7	11.9982	1.8461
O3-1	32.3276	1.8975	O7-1	0.6428	0.7738	S5-1	1.6754	1.1576
O3-2	32.3125	1.7378	O7-2	0.3629	0.5325	S5-2	1.5537	1.1393
O3-3	43.3439	1.8434	O7-3	1.5131	1.1929	S5-3	2.7363	1.4647
O3-4	77.4604	1.8996	O7-4	0.6575	0.7933	S5-4	1.9878	1.2478
O3-5	27.2207	1.7788	O7-5	2.3394	1.4011	S5-5	1.4639	1.1883
O3-6	90.4217	1.8084	O7-6	1.0314	1.0154	S5-6	7.1395	1.7543
O3-7	76.8197	1.7872	O7-7	2.0926	1.3533	S5-7	2.0133	1.3363
O4-1	0.9081	0.9518	S1-1	5.5691	1.6955	S6-1	0.9408	0.9474
O4-2	4.9083	1.6364	S1-2	3.8107	1.4772	S6-2	0.3688	0.5324
O4-3	4.0477	1.5863	S1-3	5.2917	1.6282	S6-3	2.9052	1.4729
O4-4	31.1976	1.9379	S1-4	4.3719	1.6277	S6-4	4.0986	1.5397
O4-5	3.3096	1.5255	S1-5	1.7571	1.2746	S6-5	2.8126	1.4754
O4-6	0.5079	0.6441	S1-6	1.9456	1.3210	S6-6	1.5874	1.2270
O4-7	7.0552	1.7055	S1-7	5.3421	1.6846	S6-7	2.5982	1.4121
O5-1	2.7350	1.4645	S2-1	1.7167	1.2638	S7-1	3.3280	1.4557
O5-2	2.9559	1.4175	S2-2	0.1879	0.3163	S7-2	1.8527	1.2989
O5-3	2.8982	1.4672	S2-3	3.4443	1.5313	S7-3	2.6923	1.2963
O5-4	2.5616	1.4049	S2-4	3.1045	1.4521	S7-4	4.1004	1.6079
O5-5	2.8836	1.4850	S2-5	4.1785	1.5569	S7-5	5.4294	1.5395
O5-6	31.1976	1.9379	S2-6	0.9980	0.9848	S7-6	4.1536	1.5509
O5-7	4.5581	1.4917	S2-7	3.5609	1.5615	S7-7	1.3910	1.1277

Table D7. Importance value of *M. vimineum* and *M. vimineum* to native species ratio in each plot for year two (minus outliers).

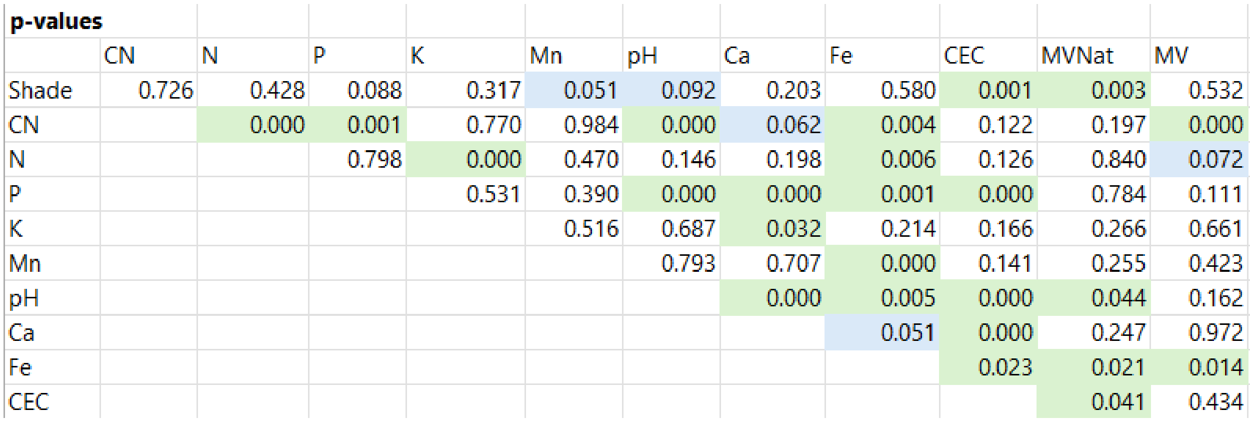
Appendix E: Statistical Output



Spearman's Rho

	CN	N	P	K	Mn	pH	Ca	Fe	CEC	MVNat	MV
Shade	-0.039	0.088	-0.187	0.110	-0.214	-0.185	-0.140	-0.061	-0.352	-0.317	-0.069
CN		-0.379	0.369	0.032	0.002	0.383	0.205	0.313	0.170	-0.142	-0.431
N			-0.028	0.474	0.080	-0.160	0.142	-0.299	0.168	0.022	0.197
P				0.069	0.095	0.590	0.765	0.359	0.721	-0.030	-0.175
K					0.072	0.045	0.235	-0.137	0.152	-0.123	0.049
Mn						0.029	0.042	0.385	0.162	0.126	0.089
pH							0.717	0.307	0.610	0.220	-0.154
Ca								0.213	0.880	0.128	0.004
Fe									0.249	-0.251	-0.266
CEC										0.223	0.086

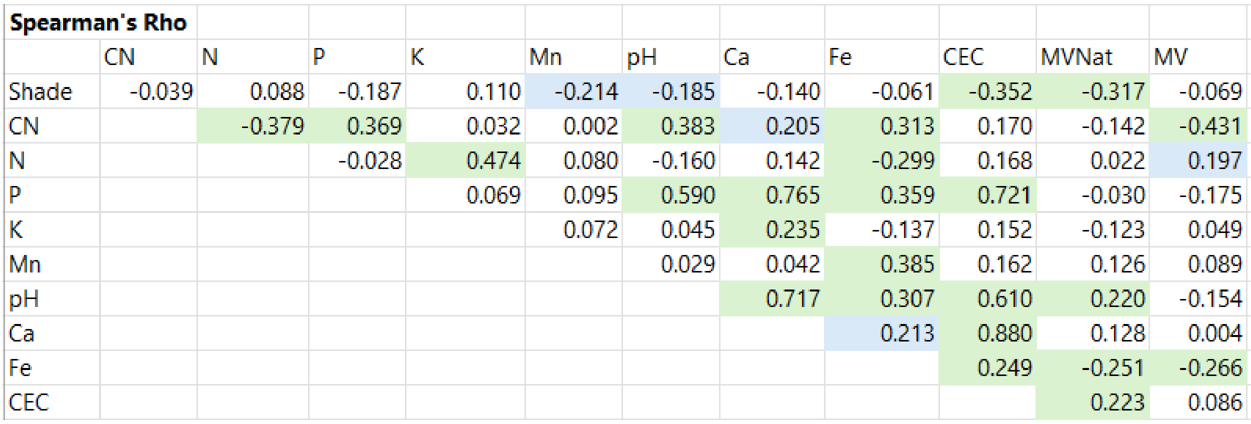
Table E1. Spearman’s Rho values for all the different soil values and *M. vimineum IV and M. vimineum* IV to native’s ratio for the first year of data. Green values represent a significance value of p<0.05 and blue represents p<0.1.



p-values

	CN	N	P	K	Mn	pH	Ca	Fe	CEC	MVNat	MV
Shade	0.726	0.428	0.088	0.317	0.051	0.092	0.203	0.580	0.001	0.003	0.532
CN		0.000	0.001	0.770	0.984	0.000	0.062	0.004	0.122	0.197	0.000
N			0.798	0.000	0.470	0.146	0.198	0.006	0.126	0.840	0.072
P				0.531	0.390	0.000	0.000	0.001	0.000	0.784	0.111
K					0.516	0.687	0.032	0.214	0.166	0.266	0.661
Mn						0.793	0.707	0.000	0.141	0.255	0.423
pH							0.000	0.005	0.000	0.044	0.162
Ca								0.051	0.000	0.247	0.972
Fe									0.023	0.021	0.014
CEC										0.041	0.434

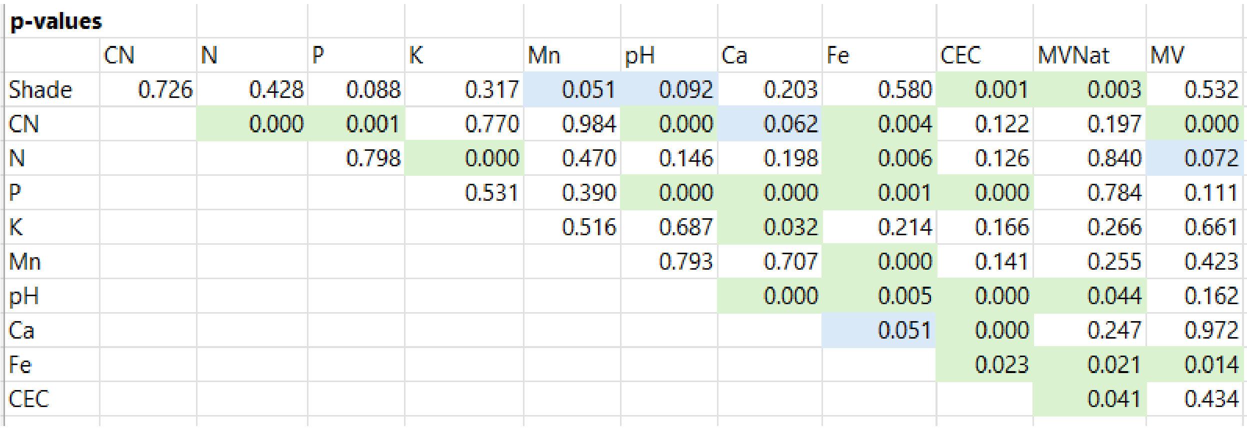
Table E2. Spearman’s p-values for all the different soil values and *M. vimineum IV and M. vimineum* IV to native’s ratio for the first year of data. Green values represent a significance value of p<0.05 and blue represents p<0.1.



Spearman's Rho

	CN	N	P	K	Mn	pH	Ca	Fe	CEC	MVNat	MV
Shade	-0.039	0.088	-0.187	0.110	-0.214	-0.185	-0.140	-0.061	-0.352	-0.317	-0.069
CN		-0.379	0.369	0.032	0.002	0.383	0.205	0.313	0.170	-0.142	-0.431
N			-0.028	0.474	0.080	-0.160	0.142	-0.299	0.168	0.022	0.197
P				0.069	0.095	0.590	0.765	0.359	0.721	-0.030	-0.175
K					0.072	0.045	0.235	-0.137	0.152	-0.123	0.049
Mn						0.029	0.042	0.385	0.162	0.126	0.089
pH							0.717	0.307	0.610	0.220	-0.154
Ca								0.213	0.880	0.128	0.004
Fe									0.249	-0.251	-0.266
CEC										0.223	0.086

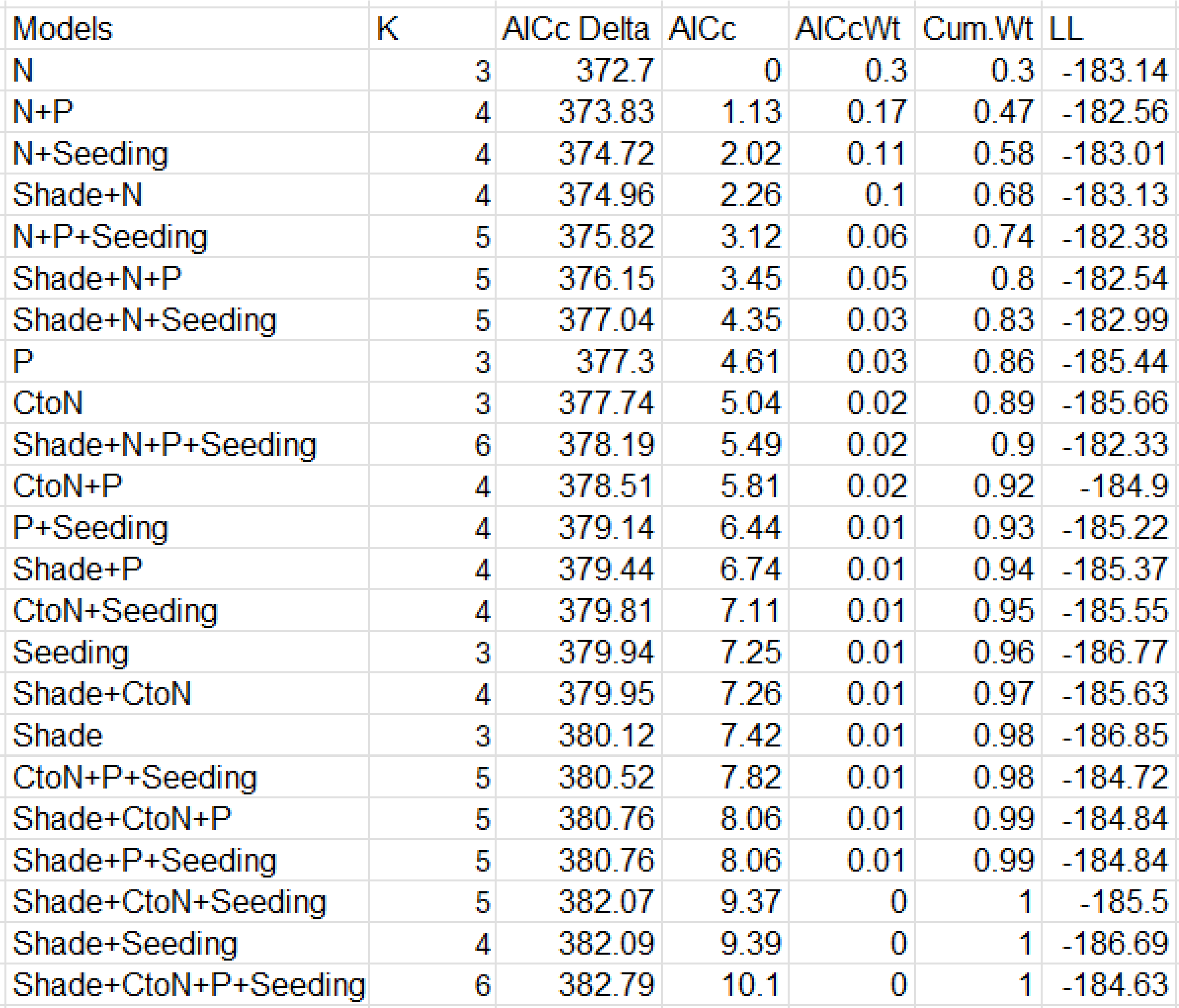
Table E3. Spearman's Rho values for all the different soil values and *M. vimineum IV and M. vimineum* IV to native's ratio for the second year of data. Green values represent a significance value of $p<0.05$ and blue represents $p<0.1$.



p-values

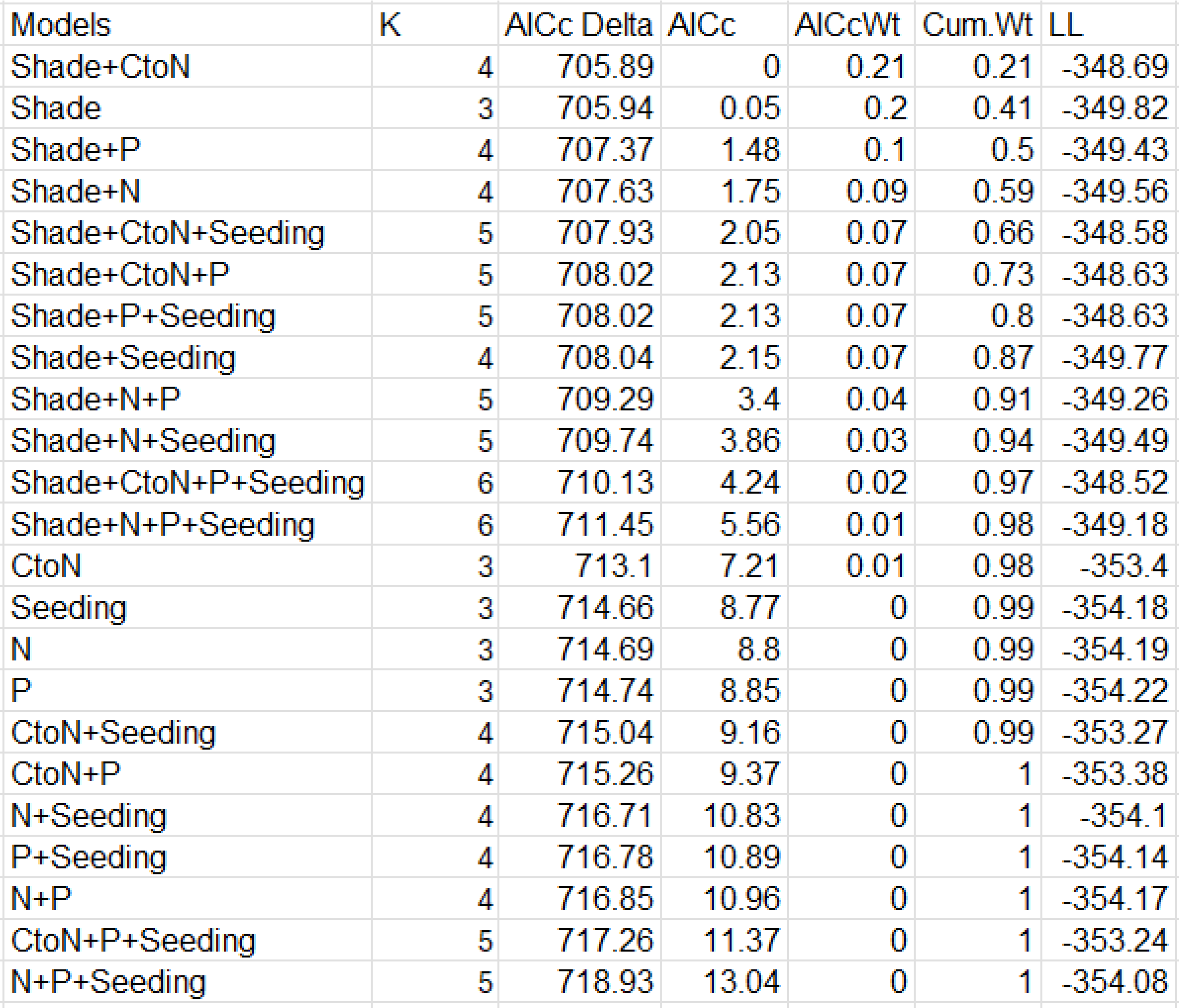
	CN	N	P	K	Mn	pH	Ca	Fe	CEC	MVNat	MV
Shade	0.726	0.428	0.088	0.317	0.051	0.092	0.203	0.580	0.001	0.003	0.532
CN		0.000	0.001	0.770	0.984	0.000	0.062	0.004	0.122	0.197	0.000
N			0.798	0.000	0.470	0.146	0.198	0.006	0.126	0.840	0.072
P				0.531	0.390	0.000	0.000	0.001	0.000	0.784	0.111
K					0.516	0.687	0.032	0.214	0.166	0.266	0.661
Mn						0.793	0.707	0.000	0.141	0.255	0.423
pH							0.000	0.005	0.000	0.044	0.162
Ca								0.051	0.000	0.247	0.972
Fe									0.023	0.021	0.014
CEC										0.041	0.434

Table E4. Spearman's p-values for all the different soil values and *M. vimineum IV and M. vimineum* IV to native's ratio for the second year of data. Green values represent a significance value of $p<0.05$ and blue represents $p<0.1$.



Models	K	AICc Delta	AICc	AICcWt	Cum.Wt	LL
N	3	372.7	0	0.3	0.3	-183.14
N+P	4	373.83	1.13	0.17	0.47	-182.56
N+Seeding	4	374.72	2.02	0.11	0.58	-183.01
Shade+N	4	374.96	2.26	0.1	0.68	-183.13
N+P+Seeding	5	375.82	3.12	0.06	0.74	-182.38
Shade+N+P	5	376.15	3.45	0.05	0.8	-182.54
Shade+N+Seeding	5	377.04	4.35	0.03	0.83	-182.99
P	3	377.3	4.61	0.03	0.86	-185.44
CtoN	3	377.74	5.04	0.02	0.89	-185.66
Shade+N+P+Seeding	6	378.19	5.49	0.02	0.9	-182.33
CtoN+P	4	378.51	5.81	0.02	0.92	-184.9
P+Seeding	4	379.14	6.44	0.01	0.93	-185.22
Shade+P	4	379.44	6.74	0.01	0.94	-185.37
CtoN+Seeding	4	379.81	7.11	0.01	0.95	-185.55
Seeding	3	379.94	7.25	0.01	0.96	-186.77
Shade+CtoN	4	379.95	7.26	0.01	0.97	-185.63
Shade	3	380.12	7.42	0.01	0.98	-186.85
CtoN+P+Seeding	5	380.52	7.82	0.01	0.98	-184.72
Shade+CtoN+P	5	380.76	8.06	0.01	0.99	-184.84
Shade+P+Seeding	5	380.76	8.06	0.01	0.99	-184.84
Shade+CtoN+Seeding	5	382.07	9.37	0	1	-185.5
Shade+Seeding	4	382.09	9.39	0	1	-186.69
Shade+CtoN+P+Seeding	6	382.79	10.1	0	1	-184.63

Table E5. AIC output for year one.



Models	K	AICc Delta	AICc	AICcWt	Cum.Wt	LL
Shade+CtoN	4	705.89	0	0.21	0.21	-348.69
Shade	3	705.94	0.05	0.2	0.41	-349.82
Shade+P	4	707.37	1.48	0.1	0.5	-349.43
Shade+N	4	707.63	1.75	0.09	0.59	-349.56
Shade+CtoN+Seeding	5	707.93	2.05	0.07	0.66	-348.58
Shade+CtoN+P	5	708.02	2.13	0.07	0.73	-348.63
Shade+P+Seeding	5	708.02	2.13	0.07	0.8	-348.63
Shade+Seeding	4	708.04	2.15	0.07	0.87	-349.77
Shade+N+P	5	709.29	3.4	0.04	0.91	-349.26
Shade+N+Seeding	5	709.74	3.86	0.03	0.94	-349.49
Shade+CtoN+P+Seeding	6	710.13	4.24	0.02	0.97	-348.52
Shade+N+P+Seeding	6	711.45	5.56	0.01	0.98	-349.18
CtoN	3	713.1	7.21	0.01	0.98	-353.4
Seeding	3	714.66	8.77	0	0.99	-354.18
N	3	714.69	8.8	0	0.99	-354.19
P	3	714.74	8.85	0	0.99	-354.22
CtoN+Seeding	4	715.04	9.16	0	0.99	-353.27
CtoN+P	4	715.26	9.37	0	1	-353.38
N+Seeding	4	716.71	10.83	0	1	-354.1
P+Seeding	4	716.78	10.89	0	1	-354.14
N+P	4	716.85	10.96	0	1	-354.17
CtoN+P+Seeding	5	717.26	11.37	0	1	-353.24
N+P+Seeding	5	718.93	13.04	0	1	-354.08

Table E6. AIC output for year two.

Bibliography

Adams, S. N. & Engelhardt, K. A. M. Diversity declines in Microstegium vimineum (Japanese stiltgrass) patches. *Biological Conservation* **142**, 1003–1010 (2009).

Alpert, P., Bone, E. & Holzapfel, C. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution and Systematics* **3**, 52–66 (2000).

Ammar, H. A., Abouelghar, G. E. & Abd El-ghany, E. Assessment of cytotoxicity and genotoxicity of acetamiprid (CETAM®) and Fluazifop-P-butyl (Fusilade Max®) pesticides using the Allium cepa test. **11**, 1–22 (2022).

Andreotti, G. *et al.* Agricultural Pesticide Use and Pancreatic Cancer Risk in the Agricultural Health Study Cohort. *International journal of cancer. Journal international du cancer* **124**, 2495 (2009).

Annett, R., Habibi, H.R. and Hontela, A., 2014. Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. *Journal of Applied Toxicology*, *34*(5), pp.458-479.

Averett, J. M., Klips, R. A., Nave, L. E., Frey, S. D. & Curtis, P. S. Effects of Soil Carbon Amendment on Nitrogen Availability and Plant Growth in an Experimental Tallgrass Prairie Restoration. *Restoration Ecology* **12**, 568–574 (2004).

Badani, H., Djadouni, F. & Haddad, F. Z. Effects of the herbicide glyphosate [n-(phosphonomethyl) glycine] on biodiversity and organisms in the soil. *EUROPEAN JOURNAL OF ENVIRONMENTAL SCIENCES* **13**, 5–14 (2023).

Baer, S. G., Blair, J. M., Collins, S. L. & Knapp, A. K. Soil Resources Regulate Productivity and Diversity in Newly Established Tallgrass Prairie. *Ecology* **84**, 724–735 (2003).

Barden, L. S. Invasion of Microstegium vimineum (Poaceae), An Exotic, Annual, Shade-Tolerant, C4 Grass, into a North Carolina Floodplain. *The American Midland Naturalist* **118**, 40–45 (1987).

Barringer, K. & Pannaman, L. Vascular Plants of the Fairview Lake Watershed, Sussex County, New Jersey. *The Journal of the Torrey Botanical Society* **130**, 47–54 (2003).

Beam, C. L., Siegert, C. M., Granger, J. J. & Iglay, R. B. Evaluation of Landowner Accessible Control Methods for Japanese Stiltgrass (Microstegium vimineum). *US Department of Agriculture Forest Service, Southern Research Station* **Vol. 268**, 100–106 (2022).

Beauchamp, V. B., Swan, C. M., Szlavecz, K. & Hu, J. Riparian community structure and soil properties of restored urban streams. *Ecohydrology* **8**, 880–895 (2015).

Beckschäfer, P. 2015: Hemispherical_2.0 – Batch processing hemispherical and canopy photographs with ImageJ – User Manual. Chair of Forest Inventory and Remote Sensing, GeorgAugust-Universität Göttingen, Germany. www.uni-goettingen.de/en/75936.html

Bergdolt, F. S., Prehumus, C. A. & Barham, J. B. *An Evaluation of Wetland Mitigation Site Compliance at the Washington State Department of Transportation.* (2005).

Brooks, R. P. & Gebo, N. Wetlands Restoration and Mitigation. in *Mid-Atlantic Freshwater Wetlands: Advances in Wetlands Science, Management, Policy, and Practice* 421–440 (Springer Science & Business Media, New York, NY, 2013).

Chapin, F. S., Matson, P. A. & Mooney, H. A. *Principles of Terrestrial Ecosystem Ecology*. (Springer, New York, NY, 2002). doi:10.1007/b97397.

Clair, E. *et al.* Effects of Roundup® and Glyphosate on Three Food Microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus. *Curr Microbiol* **64**, 486–491 (2012).

Clout, M.N. and Williams, P.A. eds. 2009. *Invasive Species Management: A Handbook of Principles and Techniques.* Oxford University Press.

Cohen, O. *et al.* Controlling the seed bank of the invasive plant Acacia saligna: comparison of the efficacy of prescribed burning, soil solarization, and their combination. *Biol Invasions* **20**, 2875–2887 (2018).

Costanza, R. *et al.* The Value of the World's Ecosystem Services and Natural Capital.

Craine, J. M. *Resource Strategies of Wild Plants*. (Princeton University Press, 2009).

Craine, J., Wedin, D., Chapin III, F. S. & Reich, P. Relationship between the structure of root systems and resource use for 11 North American grassland plants. *Plant Ecology* **165**, (2003).

Cristol, D.A., Brasso, R.L., Condon, A.M., Fovargue, R.E., Friedman, S.L., Hallinger, K.K., Monroe, A.P. and White, A.E., 2008. The movement of aquatic mercury through terrestrial food webs. *Science*, *320*(5874), pp.335-335.

Davis, J. G. & Whiting, D. Choosing a Soil Amendment. *Colorado State Univeristy* https://extension.colostate.edu/topic-areas/yard-garden/choosing-a-soil-amendment/ (2000).

Davis, M. A. Impacts of Invasion. in *Invasion Biology* 101–131 (OUP Oxford, 2009).

DeBerry, D. A. & Hunter, D. M. Drivers of Plant Invasion in Stream Restoration. *Forests* **15**, 964 (2024).

DeBerry, D. A. & Perry, J. E. Using the floristic quality concept to assess created and natural wetlands: Ecological and management implications. *Ecological Indicators* **53**, 247–257 (2015).

DeMeester, J. E. Feedbacks of nitrogen cycling and invasion with the non-native plant, Microstegium vimineum, in riparian wetlands. (Duke University, Durham, NC, 2009).

DeMeester, J. E. & deB. Richter, D. Restoring restoration: removal of the invasive plant Microstegium vimineum from a North Carolina wetland. *Biol Invasions* **12**, 781–793 (2010).

Diggins, T.P. A 300-year successional sequence in an Eastern United States riparian hardwood forest 1. *J. Torrey Bot. Soc.* 2013, *140*, 65–88.

Ehrenfeld, J. G., Kourtev, P. & Huang, W. Changes in Soil Functions Following Invasions of Exotic Understory Plants in Deciduous Forests. *Ecological Applications* **11**, 1287–1300 (2001).

EPA. Glyphosate. https://www.epa.gov/ingredients-used-pesticide-products/glyphosate (2014).

Fairbrothers, D. E. & Gray, J. R. Microstegium vimineum (Trin.) A. Camus (Gramineae) in the United States. *Bulletin of the Torrey Botanical Club* **99**, 97–100 (1972).

Flory, S. L. Management of Microstegium vimineum Invasions and Recovery of Resident Plant Communities. *Restoration Ecology* **18**, 103–112 (2010).

Flory, S. L. & Lewis, J. Nonchemical Methods for Managing Japanese Stiltgrass (Microstegium vimineum). *Invasive Plant Science and Management* **2**, 301–308 (2009).

Fluegge, K. & Fluegge, K. Glyphosate Use Predicts Healthcare Utilization for ADHD in the Healthcare Cost and Utilization Project net (HCUPnet): A Two-Way Fixed-Effects Analysis. *Pol. J. Environ. Stud.* **25**, 1489–1503 (2016).

Fortes, C. *et al.* Occupational Exposure to Pesticides With Occupational Sun Exposure Increases the Risk for Cutaneous Melanoma. *Journal of Occupational and Environmental Medicine* **58**, 370 (2016).

Fryer. Microstegium vimineum. *Fire Effects Information System* https://www.fs.usda.gov/database/feis/plants/graminoid/micvim/all.html (2011).

Gibson, D. J., Shupert, L. A. & Liu, X. Do No Harm: Efficacy of a Single Herbicide Application to Control an Invasive Shrub While Minimizing Collateral Damage to Native Species. *Plants* **8**, 426 (2019).

Gibson, D. J., Spyreas, G. & Benedict, J. Life History of Microstegium vimineum (Poaceae), an Invasive Grass in Southern Illinois. *The Journal of the Torrey Botanical Society* **129**, 207–219 (2002).

Gilliam, F. S. Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. *Journal of Ecology* **94**, 1176–1191 (2006).

Glasgow, L. S. & Matlack, G. R. The effects of prescribed burning and canopy openness on establishment of two non-native plant species in a deciduous forest, southeast Ohio, USA. *Forest Ecology and Management* **238**, 319–329 (2007).

Gordon, Brad A., Olivia Dorothy, and Christian F. Lenhart. Nutrient retention in ecologically functional floodplains: A review. *Water* 12.10 (2020): 2762.

Grime, J. P. *Plant Strategies, Vegetation Processes, and Ecosystem Properties,*. (John Wiley and Sons, Chichester, UK, 2001).

Guynn Jr, D. C., Guynn, S. T., Wigley, T. B. & Miller, D. A. Herbicides and forest biodiversity—what do we know and where do we go from here? *Wildlife Society Bulletin* **32**, 1085–1092 (2004).

Hefferman, K. Virginia Invasive Plants Species List. (2024).

Hobbs R J, Huenneke LF. 1992. Disturbance, diversity, and invasion: implications for conservation. ‘ Conservation Biology 6:324-337.

Homyak, P. M., Yanai, R. D., Burns, D. A., Briggs, R. D. & Germain, R. H. Nitrogen immobilization by wood-chip application: Protecting water quality in a northern hardwood forest. *Forest Ecology and Management* **255**, 2589–2601 (2008).

Hunter, D. M. & DeBerry, D. A. Environmental Drivers of Plant Invasion in Wetland Mitigation. *Wetlands* **43**, 81 (2023).

Huston, M. A. Management strategies for plant invasions: manipulating productivity, disturbance, and competition. *Diversity and Distributions* **10**, 167–178 (2004).

Judge, C. A., Neal, J. C. & Derr, J. F. Response of Japanese Stiltgrass (Microstegium vimineum) to Application Timing, Rate, and Frequency of Postemergence Herbicides1. *wete* **19**, 912–917 (2005).

Kettenring, K. M. & Adams, C. R. Lessons learned from invasive plant control experiments: a systematic review and meta-analysis. *Journal of Applied Ecology* **48**, 970–979 (2011).

Knight, T. M., Dunn, J. L., Smith, L. A., Davis, J. & Kalisz, S. Deer Facilitate Invasive Plant Success in a Pennsylvania Forest Understory. *naar* **29**, 110–116 (2009).

Lawrence, B., Lishawa, S., Rodriguez, Y. & Tuchman, N. Herbicide management of invasive cattail (Typha × glauca) increases porewater nutrient concentrations. *Wetlands Ecology and Management* **24**, (2016).

LeBauer, David S., and Kathleen K. Treseder. "Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed." *Ecology* 89.2 (2008): 371-379.

Leicht, S. A., Silander, J. A. & Greenwood, K. Assessing the Competitive Ability of Japanese Stilt Grass, Microstegium vimineum (Trin.) A. Camus. *The Journal of the Torrey Botanical Society* **132**, 573–580 (2005).

Leigh, C., Stubbington, R., Sheldon, F. & Boulton, A. J. Hyporheic invertebrates as bioindicators of ecological health in temporary rivers: A meta-analysis. *Ecological Indicators* **32**, 62–73 (2013).

Lewis, K. A., Tzilivakis, J., Warner, D. J. & Green, A. An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal* **22**, 1050–1064 (2016).

Lichtenthaler, H. K. The stress concept in plants: an introduction. *Ann N Y Acad Sci* **851**, 187–198 (1998).

Lu, K., Wu, H., Xue, Z., Lu, X. & Batzer, D. P. Development of a multi-metric index based on aquatic invertebrates to assess floodplain wetland condition. *Hydrobiologia* **827**, 141–153 (2019).

MacArthur, R.H., Wilson, E.O. *The Theory of Island Biogeography*. Princeton, N.J.: Princeton University Press. (1967).

Maguire RO, Heckendorn SE. 2019. Laboratory procedures: Virginia Tech Soil Testing Laboratory. Publication 452-881, SPES-91P, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Manning, S. and Miller, J. 2011. Manual, mechanical, and cultural control methods and tools. In Invasive Plant Management Issues and Challenges in the United States: 2011 Overview (pp. 231-244). American Chemical Society.

Marion, G. M., Miller, P. C., Kummerow, J. & Oechel, W. C. Competition for nitrogen in a tussock tundra ecosystem. *Plant Soil* **66**, 317–327 (1982).

Marks, C. O., Nislow, K. H. & Magilligan, F. J. Quantifying flooding regime in floodplain forests to guide river restoration. *Elem. Sci. Anth.* **2**, 000031 (2014).

Marks, C.O.; Yellen, B.C.; Nislow, K.H. Species distributions on successional and flooding gradients in Connecticut River floodplain forests. *Northeast. Nat.* 2021, *28*, 577–602.

Marshall, J. M. & Buckley, D. S. Influence of Microstegium vimineum Presence on Insect Abundance in Hardwood Forests. *sena* **8**, 515–526 (2009).

Marshall, J. M., Buckley, D. S. & Franklin, J. A. Competitive Interaction between Microstegium vimineum and First-Year Seedlings of Three Central Hardwoods. *The Journal of the Torrey Botanical Society* **136**, 342–349 (2009).

Mason, T. J. & French, K. Management regimes for a plant invader differentially impact resident communities. *Biological Conservation* **136**, 246–259 (2007).

Masters, R. A. & Sheley, R. Invited Synthesis Paper: Principles and practices for managing rangeland invasive plants. *Journal Of Range Management* **54**, (2001).

Mau-Crimmins, T. M. Effects of removing Cynodon dactylon from a recently abandoned agricultural field. *Weed Research* **47**, 212–221 (2007).

Mesnage, R., Defarge, N., Spiroux De Vendômois, J. & Séralini, G. E. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. *Food and Chemical Toxicology* **84**, 133–153 (2015).

Miller, N. P. & Matlack, G. R. BIODIVERSITY RESEARCH: Population expansion in an invasive grass, Microstegium vimineum: a test of the channelled diffusion model. *Diversity and Distributions* **16**, 816–826 (2010).

Mohanty, S. K. & Dash, R. N. Chemistry of waterlogged soils. in *Wetlands ecology and management* 10–17 (National Institute of Ecology and International Scientific Publications, New Delhi, India, 1982).

Morrison, J., Lubchansky, H., Mauck, K., McCartney, K.-M. & Dunn, B. Ecological comparison of two co-invasive species in eastern deciduous forests: Alliaria petiolata and Microstegium vimineum 1. *Journal of The Torrey Botanical Society - J TORREY BOT SOC* **134**, 1–17 (2007).

Moyer, S. & Brewer, S. Competitive Responses and Effects of the Invasive Grass Microstegium vimineum during Oak Woodland Restoration. *Natural Areas Journal* **38**, 139–147 (2018).

Mueller-Dombois, D. & Ellenberg, H. Vegetation types: a consideration of available methods and their suitability for various purposes. (1974).

Nitzsche, P. & Rector. Japanese Stiltgrass Control in the Home Lawn and Landscape. https://njaes.rutgers.edu/fs1237/.

Oswalt, C. M., Oswalt, S. N. & Clatterbuck, W. K. Effects of Microstegium Vimineum (Trin.) A. Camus on native woody species density and diversity in a productive mixed-hardwood forest in Tennessee. *Forest Ecology and Management* **242**, 727–732 (2007).

Pauchard, A. & Shea, K. Integrating the Study of Non-native Plant Invasions across Spatial Scales. *Biol Invasions* **8**, 399–413 (2006).

Perry, L. G., Blumenthal, D. M., Monaco, T. A., Paschke, M. W. & Redente, E. F. Immobilizing nitrogen to control plant invasion. *Oecologia* **163**, 13–24 (2010).

Perry, L. G., Galatowitsch, S. M. & Rosen, C. J. Competitive control of invasive vegetation: a native wetland sedge suppresses Phalaris arundinacea in carbon-enriched soil. *Journal of Applied Ecology* **41**, 151–162 (2004).

Phinney, A. Native, non-native and invasive species... what’s the difference? *Lilly Center for Lakes & Streams* https://lakes.grace.edu/native-non-native-invasive-species/ (2021).

Pimentel, D. Environmental and economic costs associated with alien invasive species in the United States. in *Biological invasions: Economic and environmental costs of alien plant, animal, and microbe species* 411–430 (Taylor & Fracis Group, London, England, 2011).

Pimentel, D. *et al.* Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment* **84**, 1–20 (2001).

Ponnamperuma, F. N. The Chemistry of Submerged Soils. in *Advances in Agronomy* (ed. Brady, N. C.) vol. 24 29–96 (Academic Press, 1972).

Powell, K. I., Chase, J. M. & Knight, T. M. A synthesis of plant invasion effects on biodiversity across spatial scales. *American Journal of Botany* **98**, 539–548 (2011).

Ranjan, V., Sen, P., Kumar, D. & Sarsawat, A. A review on dump slope stabilization by revegetation with reference to indigenous plant. *Ecol Process* **4**, 14 (2015).

Relyea, R. The Impact of Insecticides and Herbicides on the Biodiversity and Productivity of Aquatic Communities. *Ecological Applications* **15**, 618–627 (2005).

Ross, K., Ehrenfeld, J. & Patel, M. The effects of nitrogen addition on the growth of two exotic and two native forest understory plants. *Biological Invasions* **13**, 2203–2216 (2011).

Schimel, J. P. & Bennett, J. Nitrogen Mineralization: Challenges of a Changing Paradigm. *Ecology* **85**, 591–602 (2004).

Seebens, H. *et al.* No saturation in the accumulation of alien species worldwide. *Nat Commun* **8**, 14435 (2017).

Séralini, G.-E. *et al.* Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerantgenetically modified maize. *Environmental Sciences Europe* **26**, 14 (2014).

Shields, F.D., Copeland, R.R., Klingeman, P.C., Doyle, M.W., Simon, A. Design for stream restoration. J. Hydraul. Eng. **2003**, 129,
575–584.

Sheley, R. L. & Half, M. L. Enhancing Native Forb Establishment and Persistence Using a Rich Seed Mixture. *Restoration Ecology* **14**, 627–635 (2006).

Spencer, D.R.; Perry, J.E.; Silberhorn, G.M. Early secondary succession in bottomland hardwood forests of Southeastern Virginia. *Environ. Manag.* 2001, *27*, 559–570.

Tanaka, H. Pollination of some Gramineae. 2. *Journal of Japanese Botany* **50**, 25–31 (1976).

Templeton, S., Grover, A., Jackson, D. & Wurzbacher, S. Japanese Stiltgrass. *PennState Extension* https://extension.psu.edu/japanese-stiltgrass (2020).

Van Bruggen, A. H. C. *et al.* Environmental and health effects of the herbicide glyphosate. *Science of The Total Environment* **616–617**, 255–268 (2018).

Verdonschot, P. F. M. & Verdonschot, R. C. M. The role of stream restoration in enhancing ecosystem services. *Hydrobiologia* **850**, 2537–2562 (2023).

Warren II, R. J., Bahn, V., Kramer, T. D., Tang, Y. & Bradford, M. A. Performance and reproduction of an exotic invader across temperate forest gradients. *Ecosphere* **2**, art14 (2011).

Weakley, A. S. *et al.* Studies in the Vascular Flora of the Southeastern United States: Vi. *Journal of the*

Botanical Research Institute of Texas **14**, 199–239 (2020).

Webster, C. R., Rock, J. H., Froese, R. E. & Jenkins, M. A. Drought–herbivory interaction disrupts competitive displacement of native plants by Microstegium vimineum, 10-year results. *Oecologia* **157**, 497–508 (2008).

Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A. & Losos, E. Quantifying Threats to Imperiled Species in the United States. *BioScience* **48**, 607–615 (1998).

Williams, G. L. & Brewer, J. S. Naturally diverse plant communities do not resist invasion by the strong competitor, Microstegium vimineum. *American Journal of Botany* **111**, e16362 (2024).

Wilson, S. D. & Gerry, A. K. Strategies for Mixed-Grass Prairie Restoration: Herbicide, Tilling, and Nitrogen Manipulation. *Restoration Ecology* **3**, 290–298 (1995).

Winter, K., Schmitt, M. R. & Edwards, G. E. Microstegium vimineum, a shade adapted C4 grass. *Plant Science Letters* **24**, 311–318 (1982).

Yannelli FA, Karrer G, Hall R, Kollmann J, Heger T. Seed density is more effective than multi-trait limiting similarity in controlling grassland resistance against plant invasions in mesocosms. Applied Vegetation Science. 2018 Jul;21(3):411-8.

Zabaloy, M. C., Gómez, E., Garland, J. L. & Gómez, M. A. Assessment of microbial community function and structure in soil microcosms exposed to glyphosate. *Applied Soil Ecology* **61**, 333–339 (2012).

Zhan, H., Feng, Y., Fan, X. & Chen, S. Recent advances in glyphosate biodegradation. *Appl Microbiol Biotechnol* **102**, 5033–5043 (2018).

Zimdahl, R. L., Catizone, P. & Butcher, A. C. Degradation of Pendimethalin in Soil. *Weed Science* **32**, 408–412 (1984).

Zink Ret, T. A. & Allen, M. F. The Effects of Organic Amendments on the Restoration of a Disturbed Coastal Sage Scrub Habitat. *Restoration Ecology* **6**, 52–58 (1998).

Ziska, L. H., Tomecek, M. B., Valerio, M. & Thompson, J. P. Evidence for recent evolution in an invasive species, icrostegium vimineum, Japanese stiltgrass. *Weed Research* **55**, 260–267 (2015).

Glyphosate, Hard Water and Nephrotoxic Metals: Are They the Culprits Behind the Epidemic of Chronic Kidney Disease of Unknown Etiology in Sri Lanka? https://www.mdpi.com/1660-4601/11/2/2125.

IARC Monographs Volume 112: evaluation of.

Nitrogen & Phosphorus. *Chesapeake Bay Foundation* https://www.cbf.org/issues/agriculture/nitrogen-phosphorus.html.

Northern Virginia Stream Restoration Bank in Reston. *Wetland Studies and Solutions, Inc.* https://wssi-fieldnotes.com/nvsrb.

Pendimethalin 440 | 4Farmers. https://4farmers.com.au/products/herbicides/pendimethalin-440/.

Virginia Aquatic Resources Trust Fund. *The Nature Conservancy* https://www.nature.org/en-us/about-us/where-we-work/united-states/virginia/stories-in-virginia/virginia-aquatic-resources-trust-fund/.

Clean Water Act. *United States Code* vol. Title 331344 (1972).

Executive Order 13112 - Invasive Species. *Executive Order* vol. 13112 (1999).

Soil resources regulate productivity in newly established tallgrass prairie | Request PDF. *ResearchGate* https://www.researchgate.net/publication/228522257_Soil_resources_regulate_productivity_in_newly_established_tallgrass_prairie (2024).

Vita

Robert A. Sullivan was born in Hagerstown, MD in 1998 and was raised in Leesburg, VA. He graduated High School from Loudoun School for Advanced Studies as valedictorian and a member of the National Honor Society in 2016. Robert received his B.S. in Biology with a minor in Environmental Science & Policy from the College of William & Mary in 2021 and was the treasurer of the Botany Club and bass player for the Appalachia Music Ensemble. In 2022 he worked as an Environmental Field Scientist for VHB, Inc. and entered the Biology Master’s Program at William & Mary that fall. He won a student scholarship from the Virginia Association of Wetland Professionals in 2023 and is currently a member of the board for that organization.