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***MICROSTEGIUM VIMINEUM* INVASION IN CENTRAL PENNSYLVANIAN SLOPE,  
SEEP WETLANDS: SITE COMPARISONS, SEED BANK INVESTIGATION AND  
WATER AS A VECTOR FOR DISPERSAL**

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by  
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## ABSTRACT

The invasive grass, *Microstegium vimineum*, was only recently documented in slope, seep wetlands of the Centre County region of Central Pennsylvania. *M. vimineum* can replace other vegetation within three to five years of arrival and increased dominance of *M. vimineum* has been associated with decreased plant diversity. Currently, there are no studies that address *M. vimineum* in slope, seep wetlands yet it is rapidly and extensively invading these wetlands. Slope, seep wetlands within the forests of the Ridge and Valley Province of Central Pennsylvania are considered to have an exceptional variety of native flora and fauna. Therefore, the abundance of *M. vimineum* in these habitats is alarming. Since *M. vimineum* it is able to germinate, grow and reproduce in a wide range of environmental conditions it is vital that we understand more about what site characteristics in slope, seep wetlands support *M. vimineum* growth, describe aboveground and seed bank floral diversity in wetlands with and without *M. vimineum*, and examine how seed could be transported into these wetlands. For this research study slope, seep wetlands with and without *M. vimineum* were compared from June 2008 to September 2008 from two different locales, called 'AgRidge' and 'I99' within the Centre County region of Central Pennsylvania. Each locale contained 3 sites with *M. vimineum* and 3 sites without *M. vimineum*.

Results of a principle components analysis using environmental data of sites from the 'AgRidge' locale only revealed significant positive correlations between photosynthetically active radiation ( $\tau=0.600$ ) and the presence of *M. vimineum*, which supports the findings of others. *M. vimineum* can grow extensively in high light environments, including wetland habitats. There were no significant correlations between measured environmental variables and the presence of *M. vimineum* at the I99 locale, thus provoking questions related to propagule pressure. Future habitat studies like this one require an additional examination of propagule pressures, like site proximity to source populations of *M. vimineum*, in order to fully determine what suite of characteristics are correlated with the presence of *M. vimineum*.

The aboveground plant community in sites without *M. vimineum* was dominated by forbs whereas the sites with *M. vimineum* were not dominated by a single plant functional group. Results of the seed bank study from sites with and without *M. vimineum* indicated that greater percentages of *M. vimineum* seed germinated from seed banks of sites where the species was present aboveground, particularly from soil samples taken at a depth of 0-10 cm when compared to 10-20 cm soil depths. The 0-10 cm and 10-20 cm seed bank plant composition were very

similar to each other but different from the aboveground composition as confirmed by the multiple-response permutation procedures ( $p=0.0001$ ). Analysis of the seed bank compositions indicated that *M. vimineum* sites were dominated by sedges at the AgRidge locale and grasses at the I99 locale. Non- *M. vimineum* sites were dominated by forbs at the AgRidge and a combination of forbs and grasses at I99. It is very possible that different sampling times and the germination method used in the greenhouse were responsible for differences between the aboveground and seed bank compositions. Therefore, further studies of plant diversity in slope, seep wetland sites with and without *M. vimineum* should include a more detailed analysis of flora sampled several times over one growing season in order to compare aboveground and seed bank compositions. Additional studies are also needed to determine if sites with *M. vimineum* actually contain lower percentages of native flora and if an abundance of forbs renders some sites more inhabitable for invasive species.

The results of a seed dispersal study determined that *M. vimineum* seed travels via water and highlights the need for future landscape-scale dispersal studies closely examining propagule pressure and water conduits. In order to address control of *M. vimineum* in this region the mechanisms behind range expansion must be better understood. Therefore, source populations of *M. vimineum*, landscape contours, and water conduits need to be mapped to assist with the identification of currently unknown populations. By considering these variables in addition to environmental variables present on site which promote growth, it may be possible to locate sites at risk for invasion. Then, these areas can be monitored and populations eradicated earlier.

Overall, this research provides foundational descriptions of *M. vimineum* in slope, seep wetlands of the Centre County region of Central Pennsylvania and sets the stage to address further questions about habitat suitability, seed dispersal, and control of *M. vimineum*.

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## **DEDICATION**

I would like to dedicate this manuscript to my mother and father who let me play in the muck as a little kid by the Indian River in Florida and in Emerald Isle, North Carolina, enjoying the wetlands before I knew what wetlands were.

## Chapter 1

# Introduction

## Background

*Microstegium vimineum* (Trin.) A. Camus is an annual grass of Asiatic origin first collected in 1919 by a creek bank in Knoxville, Tennessee and documented by G.G. Ainslee (Fairbrothers and Gray 1972). It is known by many common names including Japanese stiltgrass, Nepal *Microstegium*, Chinese packing grass, wire grass, annual jewgrass, bamboo grass, flexible sesagrass, Japanese grass, Mary's grass, and Nepalese browntop (Redman 1995; Cole and Weltzin 2004; Judge et al. 2005a). *Microstegium vimineum*, hereafter referred to as *Microstegium*, is native to China, Korea, Japan, Nepal, India and Pakistan and its arrival in the United States was the likely result of its use as a packing material for fine china (Tu 2000). It has since invaded the entire Southeast, the Northeastern states of Connecticut, Maryland, Massachusetts, New Jersey, New York, Pennsylvania, and Rhode Island and the Midwestern states of Ohio, Indiana, Illinois, and Missouri. Populations are also established in Texas, the District of Columbia, and Puerto Rico (United States Department of Agriculture 2008). *Microstegium* grows in a variety of habitats including lawns, thickets, fields, and forests, wet areas like wetlands, riparian zones, stream or river banks, and floodplains, and areas of disturbance such as roadsides, ditches, train tracks, logging roads, and utility passages (Fairbrothers and Gray 1972; Hunt and Zaremba 1992; Redman 1995).

## Morphological Characteristics

*Microstegium* is a grass that typically grows in patches (Hunt and Zaremba 1992) and has leaves with shiny mid-ribs and stems with hairy nodes that can reach a height of up to fifty centimeters. It produces adventitious roots at its nodes (stilts) supporting the plant (Hunt and Zaremba 1992; Gibson et al. 2002; Cheplick 2006). As a grass, it has equal glumes on each spikelet and seed can be awned or awnless (Fairbrothers and Gray 1972). *Microstegium* is often misidentified in the field and confused with the non-invasive grass, *Leersia virginica*, commonly

referred to as white grass, which has no glumes and is awnless (Mehrhoff 2000). In addition, the shiny midrib is often difficult to visualize or not present in young plants, flowering plants, and plants found in sunny habitats (Redman 1995). Another unique morphological characteristic between *Microstegium* and other annual grasses is the presence of purple, red, or yellow stems and leaves before dieback (Mehrhoff 2000).

## **Reproductive Strategies**

*Microstegium* has the ability to produce cleistogamous or chasmogamous flowers (Williams 1998; Ehrenfeld 1999; Gibson 2002; Cheplick 2006). This is an advantageous life strategy for an invasive plant because cleistogamy confers a high degree of genetic similarity among progeny which could be beneficial if plants are locally acclimated (Cheplick 2008). On the contrary, chasmogamy allows for genetic variation and this could be advantageous in a heterogeneous environment. One defining characteristic between *Microstegium* and other grasses is the timing of flowering and seed set. *Microstegium* typically flowers in August or September and between 100 and 1000 seeds can be produced per plant (Barden 1987; Mehrhoff 2000; Gibson et al. 2002). In Tennessee, seed dispersal has occurred as late as November and December (Cole and Weltzin 2005) but may occur earlier in colder climates.

*Microstegium* is thought to create seed banks (Barden 1987; Gibson et al. 2002; Judge 2008) but persistence in the soil is still under speculation (Williams 1998; Cole and Weltzin 2005). Seeds appear to persist in the soil for longer in southern latitudes compared to northern latitudes (D. Mortensen, The Pennsylvania State University, personal communication). Gibson et al. (2002) states that *Microstegium* may have a transient seed bank or a persistent seed bank (where seeds remain in the soil for up to five years). In a study examining *Microstegium* control it was noted that viable seed was still present in the seed bank after attempts were made to prevent new seed bank recruitment for three consecutive years (Judge 2008). In contrast, a laboratory germination experiment on seed viability demonstrated that all viable seeds germinated during one season which suggests that *Microstegium* may not create a persistent seed bank (Williams 1998).

*Microstegium* has no defined seed dispersal strategy and utilizes a variety of mechanisms to distribute seeds. More *Microstegium* seed dispersal studies are needed because very few have thoroughly examined dispersal distances and underlying mechanisms (Cole and Weltzin 2004;

2005). Therefore, seed dispersal is noted mostly in the literature by personal observations. As mentioned previously, *Microstegium* seeds can be awned or awnless. Awned seeds have a hook like appendage that allows them to cling to animal fur, human clothing, and shoes (Fairbrothers and Gray 1972). Wind transport and simple seed drop due to gravity, often called seed rain, is possible (Gibson et al. 2002). Vehicular transport of *Microstegium* seed is thought to contribute tremendously to dispersal because plants are commonly found along roadways (Mehrhoff 2000; Peskin 2005). Road graders, logging machinery and trains can transport seed (Peskin 2005; Christen and Matlack 2009). Anecdotal accounts of seed travel by water exist and seed have been observed floating in wetlands during flood periods (Mehrhoff 2000; Christen and Matlack 2009).

### **Habitat Suitability**

While *Microstegium* is widely distributed it remains absent from a number of habitats that seem suitable (Redman 1995; Christen and Matlack 2009) suggesting its distribution remains limited by propagule pressure. However, many authors suggest that there are other environmental or habitat factors that may better explain both the presence or absence of *Microstegium* and the extent of patch size in particular habitats (Cole and Weltzin 2005; Marshall and Buckley 2008). The effects of soil moisture and light on *Microstegium* growth are two prominent factors studied recently. Other researchers have examined how carbon dioxide, interspecific competition, soil properties, and disturbance affect growth.

### **Light**

Traditionally, a  $C_4$  grass is expected to exhibit greater growth and productivity in high light conditions because more energy is required by these species to fix carbon (Taiz and Zeiger 2006).  $C_4$  plants are often considered more evolutionarily advanced compared to  $C_3$  plants because they are adapted to arid environments and warmer climates (Kennedy and Laetsch 1974; Taiz and Zeiger 2006). It has been suggested that the development of  $C_4$  photosynthesis in plants was a response to decreased atmospheric  $CO_2$  (Taiz and Zeiger 2006).

One of the first studies concerning light and *Microstegium* growth was conducted by Winter et al. (1982) and concluded that *Microstegium* was a species adapted to low light due to its

ability to produce dry matter (although reduced) at 5% of full sunlight. In addition, dry matter production under the light treatments of 100%, 63% and 18% was not significantly different. Photosynthetic saturation of experimental plants exposed to either 100% or 5% of full sunlight occurred at  $500 \mu\text{E m}^{-2}\text{s}^{-1}$ . Apparently, the  $\text{C}_4$  pathway was not a disadvantage to *Microstegium* because dry matter production was similar under both low and high light conditions (Winter et al. 1982). Horton and Neufeld (1998) concluded that even though *Microstegium* may grow in low light environments it can effectively utilize sunflecks. Evidence of this was demonstrated by stomatal tracking mechanisms in *Microstegium* which respond to different light availabilities as well as short induction periods (time span between low light operating conditions and achievement of maximum photosynthetic rate during sunflecks) (Horton and Neufeld 1998). A more recent greenhouse study by Claridge and Franklin (2002) revealed that biomass was greater for plants receiving greater light. Christen and Matlack (2009) recorded a significantly greater number of *Microstegium* stems and leaves per plant in Ohio roadside sites with greater sunlight (open canopy) compared to roadsides with less light.

### Soil Water Content

Since *Microstegium* can be found in wet habitats like wetlands, riparian zones, and stream banks it is likely that soil moisture plays a critical role in growth and patch size. Christen and Matlack (2009) observed that hardy growth occurred in moist soils like those of swales. Barden (1987) found that 20-60 centimeters of running water during one flood event was enough to decrease *Microstegium* cover by approximately 25% from one year to the next. However, it was also noted that the population rebounded three years after the flood to cover a greater area compared to pre-flood conditions. Abundance of *Microstegium* appeared to be connected to flood intensity where *Microstegium* thrived in areas greatly disturbed by the flood. In contrast, Gibson et al. (2002) noted that floods have the capacity to reduce if not temporarily eliminate, plants. One *Microstegium* study conducted at the Oak Ridge Tennessee Free Air  $\text{CO}_2$  Enrichment (FACE) site examined the extent that soil moisture and  $\text{CO}_2$  levels influenced *Microstegium* biomass and cover for two consecutive years. Soil moisture (measured by volumetric water content; %) was thought to play an important role during the year when *Microstegium* aboveground biomass and percent cover were greater because soil remained wetter for a longer duration (Belote et al. 2003).



## Soil pH

There are sporadic records of soil pH under *Microstegium* stands. Barden (1987) noted a mean pH of  $5.2 \pm 0.06$  for alluvial soil from the Piedmont of North Carolina where *Microstegium* grew in field plots and noted that plants began to die off in areas with increased soil pH. Redman (1995) noted a pH range of 4.8-5.8 in Maryland and Washington, D.C. while Cole and Weltzin (2004) found a pH range of 4.4-6.5 in Tennessee. In contrast to Barden (1987), Cole and Weltzin (2004) noticed that as pH increased within the range of 4.4-6.5, *Microstegium* was nearly twice as likely to be present in that habitat. The authors suggested that more studies are needed to determine if *Microstegium* is capable of altering soil pH (Cole and Weltzin 2004). A more extensive study by Ehrenfeld et al. (2001) found *Microstegium* growing in soil with a pH range from 4.5-5.5 in New Jersey. This was one of the first experiments to examine the likelihood that *Microstegium* may alter its soil environment. Ehrenfeld et al. (2001) states this could be possible if the increased nitrogen concentrations found in *Microstegium* roots were the result of increased soil nitrification and thus, an increase in soil pH. However, since this was a field study, it was not possible to determine whether the presence of *Microstegium* was completely responsible for increased soil pH (Ehrenfeld et al. 2001).

## Disturbance

Disturbance is now considered a crucial factor contributing to *Microstegium* distribution and patch size (Barden 1987; Cole and Weltzin 2004; Oswalt et al. 2007; Marshall and Buckley 2008; Baiser et al. 2008; Christen and Matlack 2009). Barden (1987) noted that luxuriant stands of *Microstegium* could be found growing in a sewer line passage that was mowed yearly. *Microstegium* biomass increased in a study where the disturbance consisted of canopy removal (Oswalt et al. 2007). Marshall and Buckley (2008) conducted a study to determine if mineral soil disturbance and litter removal influenced the growth of individual *Microstegium* plants. Interestingly, they found no significant differences between disturbance treatments and growth of individuals. Instead, disturbance in this study encouraged seed spread.

Very few studies have examined the extent to which disturbance caused by other organisms influence *Microstegium* growth or seed dispersal (Cole and Weltzin 2005). Disturbance of the subcanopy by white tailed deer, *Odocoileus virginianus*, in New Jersey was

documented by Baiser et al. (2008) and thought to contribute to *Microstegium* expansion. Few animals, if any, are known to eat this species (US Department of Agriculture 2007). Deer also do not eat *Microstegium* but can create canopy openness through selective grazing which can encourage understory growth (Baiser et al. 2008).

### **Interspecific Competition**

Competition between *Microstegium* and other plant species is a relatively new area of ecological focus. Since light is a key factor affecting the growth of this species, one study examined how light reduction by other plants affected *Microstegium*. Cole and Weltzin (2005) determined that allelopathy by *Asimina triloba*, the pawpaw tree, was not a factor influencing *Microstegium* growth in a greenhouse experiment but that the lower light conditions under the canopy of the pawpaw were responsible for the absence of *Microstegium*. *Microstegium* is considered to be a better competitor among *Lolium perenne* ssp. *Multiflorum*, also known as Italian ryegrass, and *Muhlenbergia mexicana*, or Mexican muhly. In a greenhouse study using these two species, *Microstegium* grew taller much earlier than the other species. This suggested that *Microstegium* may be capable of utilizing available resources (particularly light) before other species which could be problematic if seeds continue to be dispersed to open areas and seed banks become established (Leicht et al. 2005).

### **Invasive Characteristics of *Microstegium***

There are many reasons why a plant may be a successful, weedy invader. Certain life history characteristics are considered to be advantageous and may allow plants to easily reproduce in a habitat with an optimal combination of resources, also referred to as “hit[ting] the weed jackpot” (Baker 1974). *Microstegium*, in particular, has many weedy characteristics including its ability to produce a large number of seeds which are distributed by nonspecific means, to self-pollinate (cleistogamy) or cross-pollinate (chasmogamy) (Williams 1998; Ehrenfeld 1999; Gibson et al. 2002; Cheplick 2006), and to grow in habitats with varying light (Winter et al. 1982; Horton and Neufeld 1998). This species gets a head start by germinating before many other herbaceous plants, often in March or April depending on the climate of the

region. In Central North Carolina, Northern Virginia, and Central Pennsylvania, *Microstegium* is typically present in lawns or large patches and plant diversity in these areas often appears low (Romanello, personal observation). There is still speculation about *Microstegium*'s ability to preclude other plant species by altering soil pH (Ehrenfeld et al. 2001). At this point, it has not been determined whether the growth of *Microstegium* alone prevents the establishment of other plant species. This species has been found growing in areas of greater soil water contents including wetlands and areas of comparatively lower soil water contents including fields, forests, and rocky roadsides (Fairbrothers and Gray 1972; Redman 1995). Although *Microstegium* is commonly found in open habitats with high light, this grass has also been found in areas of lower light (Horton and Neufeld 1998). Soil texture and composition under *Microstegium* stands is also variable and includes loams (silty, sandy, clay) and those soils derived from limestone, marble, siltstone, shale, or sandstone (Redman 1995; Cole and Weltzin 2004; Christen and Matlack 2009). *Microstegium* exhibits growth tolerance in a wide soil pH range. Evidence of this is demonstrated by its ability to grow in more alkaline soil (Cole and Weltzin 2004; Peskin 2005) and in areas with more acidic soil like wetlands (Redman 1995). Because of *Microstegium*'s ability to persist in many different habitats and tolerate a wide variety of environmental conditions, the species fits Baker's (1974) concept of a "general purpose genotype"

## Control and Management

Comparative studies of different control and management strategies have been conducted for *Microstegium* including herbicides used before plants emerge (PRE) and after plants emerge (POST), hand removal, and cutting (both selective and non-selective). Again, *Microstegium* persistence in the seed banks is speculated (Barden 1987; Williams 1998; Gibson et al. 2002; Judge 2008). Therefore, the best current strategy is controlling plants before seed set (Tu 2000; Judge et al. 2005b) and often includes a combination of the aforementioned strategies. The herbicide Rodeo®, which contains glyphosphate, has been used in wetlands to control *Microstegium* growth (Tu 2000). However, there are no studies that formally illustrate its possible negative effects on other wetland plant species. In habitats other than wetlands, Fenoxaprop-P, Imazapic, and Sethoxydim have demonstrated effective control (Tu 2000; Judge et al. 2005b; Peskin 2005). A later study by Judge et al. (2008) highly recommended that management techniques be applied for at least three consecutive growing seasons because seeds are thought to

persist in the seed bank from three to five years (Gibson et al. 2002). Again *Microstegium* persistence in the soil may be shorter in northern latitudes (D. Mortensen, The Pennsylvania State University, personal communication).

### **Wetland Invasibility**

There are many reasons why an ecosystem may be susceptible to plant invasion. Davis et al. (2000) asserts that fluctuating resource availability, varying environmental conditions, and disturbance increase invasibility. Wetlands are particularly susceptible to colonization by invasive species because they often exhibit all three of these characteristics and can act as fragmented or sink landscapes due to their pulse-like nature (Sakai et al. 2001; Zedler and Kercher 2004). Davis et al. (2000) states that, “A plant community becomes more susceptible to invasion whenever there is an increase in the amount of unused resources.” In this theory, if resource use by the current vegetation declines or if there is a greater supply than demand for resources then there is an increase in the amount of unused resources. Successful invasive plants can take advantage of changes in resource availability. For example, the alteration of soil nutrient cycling in a wetland after a disturbance event such as a flood can render the environment susceptible to invasion. Flood scouring encourages seed germination of many species considered weeds (Zedler and Kercher 2004). In addition, hydrologic disturbances can change the temporal variation in wet and dry cycles which can then affect soil organic matter content and alter plant species composition (Campbell et al. 2002; Brooks et al. 2005).

Slope wetlands are a type of hydrogeomorphic (HGM) class defined by Brinson (1993) which are primarily fed by groundwater in addition to overland flow. They are prevalent in the Ridge and Valley Province of Central Pennsylvania and can be found at the base of a slope or where discontinuities in the slope occur (Cole et al. 1997). There is evidence that slope wetlands may be a crucial part of an existing landscape and used as corridors or temporary habitat for some salamanders during wet periods (Semlitsch 2000). Herbaceous wetland plants uniquely adapted to nutrient and water pulses from the surrounding environment are also found in slope wetlands. The more recent presence of *Microstegium* in these slope, seep wetlands is alarming considering floral and faunal diversity could be at stake. *Microstegium* dominance has been associated with decreased plant diversity (Belote et al. 2003). *Microstegium* is also a superior plant competitor compared to *Lonicera japonica*, *Muhlenbergia Mexicana*, and *Lolium perenne* ssp. *multiflorum*

(Belote and Weltzin 2006; Leicht 2005), known to replace other vegetation within three to five years of arrival (Tennessee Exotic Pest Plant Council 2007), and thought to be a threat to forest tree regeneration (Oswalt et al. 2007).

## **Objectives and Hypotheses**

*Microstegium* is classified as an invasive species in Pennsylvania but it is not considered noxious at this point (Pennsylvania Department of Conservation and Natural Resources 2009). Due to its more recent occurrence in slope, seep wetlands of the Centre County region of Central Pennsylvania (C.A. Cole, The Pennsylvania State University, personal observation), further research is needed to describe these habitats so that future management plans include an assessment of the impact of *Microstegium* on wetland habitat integrity and the role of wetlands in *Microstegium* seed dispersal. For Chapters 2 and 3, two locales were chosen and six sites were selected per locale where three sites had *Microstegium* stems present and three sites did not have *Microstegium* stems present. The locales were: a) “AgRidge”: downslope from Kepler Road on Tussey Mountain accessed via Gate G on PA 45 at Penn State’s Russell E. Larson Agricultural Research Center and b) “I99”; downslope from Interstate-99 on Bald Eagle Mountain accessed via US 220 S, 8-16 km south of Port Matilda, PA at Pennsylvania Game Commission Land, parcel #278. At this point it is thought that the origin of *Microstegium* seed in AgRidge sites arrived via the upslope Kepler road and via the newly constructed Interstate-99 into I99 sites. Chapter 2 includes 12 site comparisons of slope, seep wetlands with and without *Microstegium* and Chapter 3 examines the seed bank composition of the same sites from Chapter 2. Chapter 4 includes a study of seed dispersal via water where seed traps were created and placed in front of drainage pipes located downslope of the newly constructed Interstate-99. Chapter 5 contains research summaries, recommendations for future studies, and final concluding thoughts. The objectives and hypotheses for Chapters 2, 3, and 4 were as follows:

### **Chapter 2: Site Comparisons of Slope, Seep Wetlands with and without *Microstegium* in Central Pennsylvania**

The objective of this study was to determine what characteristics, both biotic and abiotic, define slope, seep sites with and without *Microstegium*. It was expected that a unique suite of characteristics would be correlated with the presence of the invasive.

### **Chapter 3: Seed Bank Analyses of Slope, Seep Wetlands with and without *Microstegium* in Central Pennsylvania**

The objective of this study was to sample the seed banks of all sites from Chapter 2 through soil sampling at two depths, 0-10 cm and 10-20 cm, to determine the percentage of *Microstegium* seed present in the seed bank. It was anticipated that greater *Microstegium* percent cover at some sites would be reflected by greater percentages of *Microstegium* present in the seed bank. In addition, the seed bank composition was classified by plant functional groups. It was expected that sites with *Microstegium* would be dominated by different functional groups when compared to sites without *Microstegium*.

### **Chapter 4: Seed Trap Study: Water as a Vector for Dispersal**

The objective of this study was to determine if *Microstegium* seed travels via water runoff through the use of seed traps held in place at the base of randomly selected drain pipes downslope from Interstate-99. It was expected that *Microstegium* seed would be present in seed traps after a rain event greater than 0.5 mm.

## Literature Cited

- Baiser, B., Lockwood, J. L., La Puma, D. and M. F. J. Aronson. 2008. A perfect storm: two ecosystem engineers interact to degrade deciduous forests of New Jersey. *Biological Invasions* 10:785-795.
- Baker, H. G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics* 5:1-24.
- Barden, L. S. 1987. Invasion of *Microstegium vimineum*, an exotic, annual, shade-tolerant C<sub>4</sub> grass, into a North Carolina floodplain. *American Midland Naturalist* 118:40-45.
- Belote, R. T., J. F. Weltzin, and R. J. Norby. 2003. Response of an understory plant community to elevated [CO<sub>2</sub>] depends on differential responses of dominant invasive species and is mediated by soil water availability. *New Phytologist* 161:827-835.
- Belote, R. T. and J. F. Weltzin. 2006. Interactions between two co-dominant, invasive plants in the understory of a temperate deciduous forest. *Biological Invasions* 8:1629-1641.
- Brinson, M. M. 1993. A hydrogeomorphic classification for wetlands. WRP-DE-4, Army Corps of Engineers Wetlands Research Program Technical Report.
- Brooks, R. P., D. H. Wardrop, C. A. Cole, and D. A. Campbell. 2005. Are we purveyors of wetland homogeneity? A model of degradation and restoration to improve wetland mitigation performance. *Ecological Engineering* 24:331-340.
- Campbell, D. A., C. A. Cole, and R. P. Brooks. 2002. A comparison of created and natural wetlands in Pennsylvania, USA. *Wetlands Ecology and Management* 10:41-49.

- Cheplick, G. P. 2006. A modular approach to biomass allocation in an invasive annual (*Microstegium vimineum*; Poaceae). *American Journal of Botany* 93:539-545.
- Cheplick, G. P. 2008. Growth trajectories and size-dependent reproduction in the highly invasive grass *Microstegium vimineum*. *Biological Invasions* 10:761-770.
- Christen, D. C. and G. R. Matlack. 2009. The habitat and conduit functions of roads in the spread of three invasive plant species. *Biological Invasions* 11:453-465.
- Claridge, K. and S. B. Franklin. 2002. Compensation and plasticity in an invasive plant species. *Biological Invasions* 4:339-347.
- Cole, C. A., R. P. Brooks, and D. H. Wardrop. 1997. Wetland hydrology as a function of hydrogeomorphic (HGM) subclass. *Wetlands* 17:456-467.
- Cole, P. G. and J. F. Weltzin. 2004. Environmental correlates of the distribution and abundance of *Microstegium vimineum*, in east Tennessee. *Southeastern Naturalist* 3:545-562.
- Cole, P. G. and J. F. Weltzin. 2005. Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. *Biological Invasions* 7:477-488.
- Davis, M. A., P. Grime, and K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invisibility. *The Journal of Ecology* 88:528-534.
- Ehrenfeld, J. G. 1999. A rhizomatous, perennial form of *Microstegium vimineum* (Trin.) A. Camus in New Jersey. *Journal of the Torrey Botanical Society* 126:352-358.
- Ehrenfeld, J. G. 2001. Changes in soil functions following invasions of exotic understory plants in deciduous forests. *Ecological Applications* 11:1287-1300.



- Fairbrothers, D. E. and J. R. Gray. 1972. *Microstegium vimineum* (Trin.) A. Camus (Gramineae) in the United States. Bulletin of the Torrey Botanical Club 99:97-100.
- Gibson, D. J., G. Spyreas, and J. Benedict. 2002. Life history of *Microstegium vimineum* (Poaceae), an invasive grass in southern Illinois. Journal of the Torrey Botanical Society 129:207-219.
- Horton, J. L. and H. S. Neufeld. 1998. Photosynthetic responses of *Microstegium vimineum* (Trin.) A. Camus, a shade-tolerant, C<sub>4</sub> grass, to variable light environments. Oecologia 114:11-19.
- Hunt, D. M. and R. E. Zaremba. 1992. The northeastern spread of *Microstegium vimineum* (Poaceae) into New York and adjacent states. Rhodora 94:167-170.
- Judge, C. A., J. C. Neal, and J. F. Derr. 2005a. Preemergence and postemergence control of Japanese Stiltgrass (*Microstegium vimineum*). Weed Technology 19:183-189.
- Judge, C. A., J. C. Neal, and J. F. Derr. 2005b. Response of Japanese Stiltgrass (*Microstegium vimineum*) to application timing, rate, and frequency of postemergence herbicides. Weed Technology 19:912-917.
- Judge, C. A., J. C. Neal, and T. H. Shear. 2008. Japanese stiltgrass (*Microstegium vimineum*) management for restoration of native plant communities. Invasive Plant Science and Management 1:111-119.
- Kennedy, R. A., and W. M. Laetsch. 1974. A plant species intermediate in C<sub>3</sub>/C<sub>4</sub> type photosynthesis. Science 184: 1087-1089.
- Leicht, S. S., J. A. Silander Jr., and K. Greenwood. 2005. Assessing the competitive ability of Japanese stiltgrass, *Microstegium vimineum* (Trin.) A. Camus. Journal of the Torrey Botanical Society 132:573-580.

- Marshall, J. M. and D. S. Buckley. 2008. Influence of litter removal and mineral soil disturbance on the spread of an invasive grass in a central hardwood forest. *Biological Invasions* 10:531-558.
- Mehrhoff, L. J. 2000. Perennial *Microstegium vimineum* (Poaceae): an apparent misidentification? *Journal of the Torrey Botanical Society* 127:251-254.
- Oswalt, C. M., S. N. Oswalt, and W. K. Clatterbuck. 2007. 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.
- Pennsylvania Department of Conservation and Natural Resources. Invasive Plants in Pennsylvania. Harrisburg, PA 17105-8767 USA  
(<http://www.dcnr.state.pa.us/forestry/wildplant/invasive.aspx>, 30 March 2009)
- Peskin, N. 2005. Habitat suitability of Japanese stiltgrass *Microstegium vimineum* in an Appalachian forest. M.S. thesis. The Pennsylvania State University, University Park, PA.
- Redman, D. E. 1995. Distribution and habitat types for Nepal *Microstegium* [*Microstegium vimineum* (Trin.) Camus] in Maryland and the District of Columbia. *Castanea* 60:270-275.
- Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics* 32:305-332.
- Semlitsch, R. D. 2000. Principles for management of aquatic-breeding amphibians. *The Journal of Wildlife Management* 64:615-631.

- Taiz L., and E. Zeiger. 2006. Plant Physiology, Fourth Edition. Sinauer Associates, Inc., Publishers, Sunderland, MA. 764 pp.
- Tennessee Exotic Pest Plant Council. Tennessee Exotic Plant Management Manual. 2007. (<http://www.tneppc.org/>, 12 December 2007).
- Tu, M. 2000. Elemental stewardship abstract for *Microstegium vimineum*. The Nature Conservancy, Arlington, VA  
(<http://tncweeds.ucdavis.edu/esadocs/documents/micrvim.html>, 12 December 2007).
- US Department of Agriculture, Natural Resources Conservation Service. 2007. National Plant Data Center, Baton Rouge, LA 70874-4490 USA. The PLANTS Database (<http://plants.usda.gov>, 12 December 2007).
- Williams, L. D. 1998. Factors affecting growth and reproduction in the invasive grass *Microstegium vimineum*. M.S. thesis, Appalachian State University, Boone, NC.
- Winter, K., M. R. Schmitt, and G. E. Edwards. 1982. *Microstegium vimineum*, a shade adapted C<sub>4</sub> grass. Plant Science Letters 24:311-318.
- Zedler, J. B. and S. Kercher. 2007. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. Critical Reviews in Plant Sciences 23: 431-452.

## Chapter 2

## Site Comparisons of Slope, Seep Wetlands with and without *Microstegium vimineum* in Central Pennsylvania

### Abstract

Very little is known about the invasive grass, *M. vimineum* in slope, seep wetlands of the Centre County region of Central Pennsylvania because its presence was only recently documented. To gain a better understanding of *M. vimineum* in slope, seep wetlands, sites with and without the invasive were compared with an overall goal of determining if a unique suite of site characteristics would be correlated with the presence of the invasive. Environmental and biotic variables were collected over one growing season (June 2008-September 2008) from sites with and without *M. vimineum* at two locales, 'AgRidge' and 'I99'. A principle components analysis of AgRidge sites only revealed positive correlations between the presence of *M. vimineum* and high light (photosynthetically active radiation;  $\tau=0.600$ ), greater distance from the road ( $\tau=0.552$ ), greater soil silt content ( $\tau=0.552$ ), and more alkaline soils ( $\tau=0.138$ ). Percent soil organic matter ( $\tau=-1.000$ ) was negatively correlated with the presence of *M. vimineum* at AgRidge sites. Available water level data from 3 sites with *M. vimineum* demonstrated similar median depth to the water table (range, 27.1 cm to 28.1 cm) and mean saturated depth to the water table (range, 22.8 cm to 24.9 cm). When plant composition by functional groups was compared between sites with and without *M. vimineum*, forbs dominated the communities of sites without *M. vimineum*. This study reflects that while significant positive correlations were found for some sites when comparing the presence of *M. vimineum* to specific environmental variables, its absence in seemingly suitable habitats indicates that it is likely dispersal limited.

## Introduction

*Microstegium vimineum* (Trin.) A. Camus, commonly known as Japanese stiltgrass and hereafter referred to as *Microstegium*, is a prominent invasive grass throughout the eastern United States, much of the Northeast, and a few Midwestern states (United States Department of Agriculture 2008). *Microstegium* grows in a variety of habitats including lawns, thickets, fields, and forests, wet areas like wetlands, riparian zones, stream or river banks, and floodplains, and areas of disturbance such as roadsides, ditches, train tracks, logging roads, and utility passages (Fairbrothers and Gray 1972; Hunt and Zaremba 1992; Redman 1995). This species exhibits phenotypic plasticity and has many characteristics of an ‘ideal weed’ (Baker 1974). *Microstegium* can grow under a broad range of irradiance levels (Winter et al. 1982; Horton and Neufeld 1998), produces many seeds that are dispersed by non-specific means (Gibson et al. 2002), and can undergo self-pollination or cross-pollination (Williams 1998; Ehrenfeld 1999; Gibson et al. 2002; Cheplick 2006). There is also evidence that increased soil moisture promotes greater biomass production (Belote et al. 2003; Romanello and Touchette, unpublished data). *Microstegium* dominance has been inversely correlated with decreased plant diversity (Belote 2003). This grass is often a superior competitor (Belote and Weltzin 2006; Leicht 2005), known to replace other vegetation within three to five years of arrival (Tennessee Exotic Pest Plant Council 2007), and thought to be a threat to forest tree regeneration (Oswalt et al. 2007).

There are many reasons why an ecosystem may be susceptible to plant invasion. Davis et al. (2000) asserts that areas with fluctuating resource availability, varying environmental conditions, and those influenced by disturbance have increased invasibility. Wetlands are particularly susceptible to colonization by invasive species because they often exhibit all three of these characteristics and can act as fragmented or sink landscapes due to their pulse-like nature (Sakai et al. 2001; Zedler and Kercher 2004). In a theory proposed by Davis et al. (2000), an increase in the amount of unused resources in a given environment occurs if resource usage by the current vegetation declines or if there is a greater supply than demand for resources. Successful invasive plants can take advantage of changes in resource availability. For example, the alteration of soil nutrient cycling in a wetland after a flood can render the environment susceptible to invasion. Flood scouring can also encourage seed germination of many species considered weeds (Zedler and Kercher 2004). In addition, hydrologic disturbances can change the temporal variation in wet and dry cycles which can then affect soil organic matter content and alter plant species composition (Campbell et al. 2002; Brooks et al. 2005).

Slope wetlands are a type of hydrogeomorphic (HGM) class defined by Brinson (1993) that are fed predominately by groundwater but also by overland water flow. They are prevalent in the Ridge and Valley Province of Central Pennsylvania and are found at the base of slopes or where discontinuities in slopes occur (Cole et al. 1997). There is evidence that slope wetlands may be a crucial part of an existing landscape and used as corridors or temporary habitats by some salamanders during wet periods (Semlitsch 2000). Slope wetlands in the Centre County region of Central Pennsylvania also seem to be areas of greater plant diversity when compared to the forests which surround them (Romanello, personal observation).

This is the first study to examine *Microstegium* in slope, seep wetlands. Previously, the presence of *Microstegium* in wetlands was only accounted for anecdotally in the literature and no information was given about the class of wetland the invasive was found in. The presence of *Microstegium* in slope wetlands of central Pennsylvania's Bald Eagle Ridge and Tussey Mountain is a new occurrence (C.A. Cole, The Pennsylvania State University, personal observation). It is thought that the construction of Interstate-99 may have played a role in dispersal of seeds from the road and eventually into slope, seep wetlands within the I99 locale. The origins of *Microstegium* seed in AgRidge sites could have arrived via the upslope Kepler road. Due to the susceptibility of wetlands to plant invaders and the ability of wetlands to act as landscape sinks, a pilot study of *Microstegium* in slope, seep wetlands of Central Pennsylvania was needed to determine if a unique suite of characteristics, both biotic (including aboveground plant family composition and percent *Microstegium* cover) and abiotic (environmental data), distinguishes slope, seep sites with and without *Microstegium*.

## Materials and Methods

### Site Selection

Six slope, seep wetland sites were selected in early May 2008 from two locales, a) downslope from Kepler Road on Mount Nittany accessed via Gate G on PA 45 at Penn State's Russell E. Larson Agricultural Research Center and b) downslope from Interstate-99 on Bald Eagle Mountain accessed via US 220 S, 8-16 km south of Port Matilda, PA at Pennsylvania Game Commission Land, parcel #278. Locale 'a' is hereafter referred to as 'AgRidge' and locale 'b' as 'I99' (Figure 2.1). Within the six sites selected at each locale, three sites had *Microstegium*

stems (referred to as '*Microstegium* sites') and three sites had no observable *Microstegium* stems (referred to as 'non-*Microstegium* sites'). All selected sites were surrounded by forested habitat; I99 sites were located on actively managed forested game land covered predominately with deciduous trees while AgRidge sites were located on unmanaged forested land covered with both conifers and deciduous trees. At each locale, all sites were within a 1-7 kilometer walking distance of each other because no roadways were present for vehicular access to individual sites. However, all sites were in close proximity to a roadway with AgRidge sites between 824 to 1050 meters downslope from Kepler Road (Figure 2.2) and I99 sites between 333 to 735 meters downslope of Interstate-99 (Figure 2.3). In addition, I99 sites were also 0.5-1 kilometers downslope from an active logging road located along the northern-most perimeter of the game land parcel. Attempts were made to select sites of similar area but access problems including fencing, impenetrable understory brush (*Rosa multiflora* and *Berberis* sp.), and stream crossing problems prevented this in some cases. A list of all twelve sites is included in Table 2.1. Aerial photographs of site locations are in Appendix A. The distance between the upslope road and downslope sites was estimated using ArcGIS™.

### Data Collection

A sampling grid was established at each site with 4 meters between each sampling point. In order to keep all points within the confines of the slope, seep wetland, all sites did not have the same number of sampling points. Before site comparisons were made, the following data were collected from each site: aboveground plant family composition (%), *Microstegium* cover (%), photosynthetically active radiation (PAR;  $\mu\text{mol}$ ), soil water content (%), soil pH, soil phosphorus content (ppm), soil zinc content (ppm), soil copper content (ppm), soil sulfur content (ppm), soil nitrogen content (%), soil carbon content (%), soil organic matter content (%), soil particle size analysis (% sand, silt, clay), soil type and depth to water (cm).

Aboveground plant family composition was determined using 5, 1 m<sup>2</sup> quadrats at 5 randomly selected sampling points from the sampling grid at each site during July 2008. Given that not all plants were flowering during the month of July when sampling occurred, it was difficult to identify plants to the species taxonomic level. A visually estimated percentage of plants from each family were identified from each quadrat in the field with the aid of Newcomb's Wildflower Guide (1977). Any family representing 5% or greater coverage was documented within each quadrat (Hoeltje and Cole 2007). Care was taken to account for plants of various

heights. Thus, it was possible for quadrats to have greater than 100% total coverage. Plants that could not be identified by family in the field were documented, collected, and pressed to obtain assistance with identification. Composition was also later categorized into the following plant functional groups: grass, sedge, rush, forb, fern, moss and woody in order to determine if *Microstegium* and non-*Microstegium* sites were dominated by different functional groups.

Percent *Microstegium* cover was determined by creating regular square and rectangular shapes around patches to calculate the perimeter of each patch using a meter tape. To obtain the percent *Microstegium* cover, the area of the entire site was divided by the total area of all *Microstegium* patches.

Photosynthetically active radiation (PAR;  $\mu\text{mol}$ ) readings were taken at each site from ten randomly selected points from the sampling grid. Data were collected twice a month at regular intervals throughout the growing season (June through September 2008). The same points were used throughout the study and data were collected at random times on both sunny and cloudy days. A LI-COR Model LI-189 Quantum/Radiometer/Photometer was hand-held horizontally approximately 1 to 1.5 meters above the ground (waist level) at an arms-length away. Precautions were taken by the individual holding the meter to avoid creating a shadow over the sensor and interfering with the PAR readings. To be consistent, one reading was recorded after waiting 10 seconds at each point. Median PAR values were calculated for each collection period.

Soil water content samples were obtained twice a month from June through September 2008 from the same ten points selected for the PAR readings. Soil plugs, inserted approximately 1-3 cm below the surface of the soil were collected and taken back to the lab to weigh. Both wet and dry mass (oven dried at 60°C) were recorded using a precision balance (AdamLab AFP/L Series; New Milford, CT, USA). The mass of water per mass of the wet soil was determined by the following equation:

$$\text{Soil water content (\%)} = [\text{Wet soil (g)} - \text{dry soil (g)} / \text{Wet soil (g)}] * 100$$

Median percent soil water content was calculated for the entire study period for each site.

Soil samples were tested by the Penn State Agricultural Analytical Services Laboratory to determine soil pH, soil nutrient contents (phosphorus, zinc, copper, sulfur, and nitrogen) as well as soil organic matter using procedures tailored for the soils of this region (The Pennsylvania State University 2008). Percent organic carbon was estimated by dividing the percent organic matter by 2 (Mitsch and Gosselink 2000). Twenty randomly selected points were used to collect



soil samples from a depth of 0-20 cm (approximately 1000 cubic cm per sample). Samples were taken from the field during late May and early June 2008 using a bulb corer (volume approximately 300 cubic cm) with a spring loaded plastic handle that permitted easy removal of the soil sample. A small portion of these samples was saved for a seed bank study described in Chapter 3 and the remainder were randomly mixed together to form three subsamples to submit for soil testing. Sub-samples were stored in sealed bags in a dark location as recommended by the Penn State Agricultural Analytical Services Laboratory until submitted for testing during October 2008. Median values for each soil test were calculated for each site.

Soil particle size analysis was conducted using the hydrometer (Bouyoucous) method adapted from Gee and Bauder (1979). Fifty grams of air dried soil from each of three sub-samples was used to obtain a median percent sand, silt, and clay value for each site. A textural triangle (Brady and Weil 2008) was used to verify soil type.

A water level recorder (RDS Ecotone or WL40) was installed in each slope, seep wetland site during June 2008 and programmed to record depth to water (cm) every 3 hours. Hydrographs were created using all readings collected from June through October 2008. Median depth to water and average depth to saturation were also calculated. In addition, the number of times the depth to water was in a different zone, either dry ( $< 30$  cm depth to water), saturated (0 - 30 cm depth to water) or inundated ( $> 0$  cm depth to water) was tallied and a percentage was calculated.

### **Data Analysis**

To determine if a unique suite of characteristics distinguished *Microstegium* and non-*Microstegium* sites, Kruskal-Wallis tests were performed (Minitab 14 Student Statistical Software®, Minitab Inc., State College, PA). This test was used to conduct a non-parametric one-way analysis of variance with medians by ranks. Median values for environmental variables from all sites were used because of small sample sizes. All p-values less than or equal to 0.05 were considered significant.

Shannon-Weiner Index and evenness values were calculated using aboveground family compositions collected in July 2008 for all sites. A Shannon-Weiner Index value 'H' encompassed both the number of families in the community and the proportions of each family represented at each site. An 'H' value of 4.6 is indicative of greater diversity. Evenness values were also reported. The standard range for evenness values is from 0 to 1 where a value of 1 indicates equal numbers within each family are present.

Multiple response permutation procedures (MRPP) tested for differences in environmental or biotic variables in *Microstegium* and non-*Microstegium* sites (PC-ORD Version 5, MjM software™, Gleneden Beach, OR). For this test, a p value less than 0.05 indicated that a multivariate compositional difference was evident (J. Peck, The Pennsylvania State University, personal communication).

Principal Components Analyses (PCA) were conducted using PC-ORD to assess the relationship between environmental variables and presence or absence of *Microstegium* in low-dimension, multivariate space. Any axis with a p-value less than 0.05 was considered significant and reported with the percent variance explained by the axis. Non-parametric Kendal correlations (tau) based on ranks were also recorded for significant axes.

## Results

### Kruskal-Wallis Tests

Median environmental data were compared for all sites (both locales) using Kruskal-Wallis tests to determine if there were significant differences between *Microstegium* and non-*Microstegium* sites. There were no significant differences between *Microstegium* and non-*Microstegium* sites for the median soil nutrient concentrations of copper, sulfur, zinc and phosphorus (Figures 2.4, 2.5, 2.6, 2.7). Soil pH also was not significantly different between *Microstegium* and non-*Microstegium* sites (Figure 2.8) but the median pH range for *Microstegium* sites was quite large (4.4-6.0). There were no significant trends between sites where *Microstegium* was present or absent for median soil water content (Figure 2.9). Median percent soil organic matter, nitrogen and estimated carbon were generally greater in all I99 sites when compared to all AgRidge sites but, as a whole, *Microstegium* and non-*Microstegium* sites were not significantly different from each other (Figures 2.10, 2.11, 2.12, respectively).

Generally, *Microstegium* infestations were likely to be found in sites exposed to higher irradiance. Median photosynthetically active radiation (PAR) for all collection periods was often greater in *Microstegium* sites from both locales. Occasionally non-*Microstegium* sites would have greater PAR than *Microstegium* sites (Figure 2.13 and 2.14). Kruskal-Wallis tests for all sites from both locales indicated that median PAR was significantly greater in *Microstegium* sites for 2

out of 8 total collection periods (“Wk 2 June”,  $H=5.03$ ,  $DF=1$ ,  $p=0.025$  and “Wk 2 August”,  $H=5.03$ ,  $DF=1$ ,  $p=0.025$ ). When median PAR was analyzed for each collection period at the AgRidge locale only, 4 out of 8 collection periods were significantly greater in *Microstegium* versus non-*Microstegium* sites (“Wk 2 June”,  $H=3.86$ ,  $DF=1$ ,  $p=0.050$ ; “Wk 1 Aug”,  $H=3.86$ ,  $DF=1$ ,  $p=0.050$ ; “Wk 2 Aug”,  $H=3.86$ ,  $DF=1$ ,  $p=0.050$ ; “Wk 2 Sept”,  $H=3.86$ ,  $DF=1$ ,  $p=0.050$ ). In addition, the median PAR range for AgRidge sites with *Microstegium* was much greater compared to I99 sites with *Microstegium* (33-1798  $\mu\text{mol}$  and 8-967  $\mu\text{mol}$ , respectively).

### **Aboveground Plant Community and Percent *Microstegium* Cover**

Shannon-Weiner Index and evenness values were calculated from aboveground plant family composition data (noted in Appendix B). The  $H$  and evenness values were not different among AgRidge sites (Figures 2.15 and 2.16). However, 2 out of 3 *Microstegium* sites at I99 had lower  $H$  values compared to non-*Microstegium* sites (Figure 2.17). There were no observable trends for I99 evenness values between *Microstegium* and non-*Microstegium* sites (Figure 2.18).

The percent of plants from each family were further placed into a plant functional group in order to obtain a better comparison of aboveground compositions (Appendix D). All non-*Microstegium* sites were dominated by forbs (Figure 2.19 and 2.20). The grass functional group was not always dominant in sites where *Microstegium* was present. Percent *Microstegium* cover was variable within each site (Table 2.1).

### **Hydrology**

Hydrographs were created from water level data of two sites at the I99 locale (one non-*Microstegium* site, NG2, Figure 2.21 and one *Microstegium* site, GR2, Figure 2.22) and two sites at the AgRidge locale (*Microstegium* sites Bearsite and Mvland, Figures 2.23 and 2.24, respectively). It is important to note that the bottom of the wells which held the water level recorders did not extend beyond the root zone (below -30 cm) in many cases. Due to the rocky substrate present in these wetlands, the resulting hydrographs may or may not reflect the presence of water below -30 cm. Therefore, depth of water below -30 cm can be assumed for time periods when data hovers around -30 cm but the exact depth of water below that point is unknown. In addition, hardware malfunctions did not permit the transference of data from some recorders and

other recorders were destroyed by bears. Attempts were made to recover as much data as possible. Median depth to water, average depth to saturated water, and percent of time periods soil water was in a different zone (either dry, saturated, or inundated) was recorded in Tables 2.3 and 2.4. Generally, all three *Microstegium* sites (2 from the AgRidge locale and 1 from the I99 locale) had very similar average depth of water in the saturated zone and ranged from 22.82 cm to 24.93 cm while the one non-*Microstegium* site had an average depth of water in the saturated zone of 13.45 cm. Median depth to water was also similar for these same sites and ranged from 27.124 cm to 28.132 cm, contrasting with the non-*Microstegium* site median depth to water of 11.741 cm.

### **Particle Size Analysis and Soil Type**

Slope, seep wetlands are fed mostly by groundwater and the water table in these systems can be relatively high. The slope, seep wetlands used for this study typically contained shallow soils with poor drainage. Gleying was evident in some soil samples indicating anoxic conditions. Other samples appeared to contain large amounts of iron due to their dark red coloration. It was very common to encounter large rocks below the soil. Sites in this study dominated by grasses and sedges, particularly those sites with *Microstegium*, had a thick layer of decaying plant material (about 1 cm in depth) leftover from previous growing seasons (Romanello, personal observation). The bedrock of the sites consisted of a combination of shale and limestone. Most of the sites in this study had a loam soil type which is composed of similar proportions of sand, silt, and clay (Brady and Weil 2008). There was only one site at the AgRidge locale and three sites at the I99 locale which were classified as having silt loam soil types. Generally, silt content must be quite high to classify a soil as a silt loam (Brady and Weil 2008). Percentages are present in Tables 2.4 and 2.5.

### **Multiple-response Permutation Procedures and Indicator Species Analysis**

The first MRPP analysis included all sites from both the AgRidge and I99 locales. An environmental distance matrix was created and contained all environmental data. A second distance matrix included a coding variable to differentiate *Microstegium* and non-*Microstegium* sites. The resulting p-value of 0.0818 indicated that there were no significant positive associations

between specific environmental variables and the presence or absence of *Microstegium* at any sites.

The second MRPP analysis utilized a distance matrix with aboveground plant family composition data and a second distance matrix with a coding variable to differentiate *Microstegium* and non-*Microstegium* sites. Again, an insignificant p-value of 0.234 indicated that there were no positive correlations between the aboveground plant family community and the presence of *Microstegium* at any sites.

### Principle Components Analyses

An environmental distance matrix containing all environmental data and a coding variable to differentiate *Microstegium* from non-*Microstegium* sites was used to conduct a principle components analysis (PCA) for twelve sites. Axis 1 was significant ( $p=0.001$ ) and explained 40.073% of the variance. Axis 1 positive Kendall correlations were: distance to the upslope road ( $\tau=0.840$ ), percent sand ( $\tau=0.424$ ), and light ( $\tau=0.333$ ). For Axis 1, the highest negative correlations were evident for soil organic matter ( $\tau=-0.870$ ) and carbon ( $\tau=-0.870$ ). A scatter-plot was created using PC-ORD by overlaying the matrix containing the coding variable and the environmental matrix. The resulting output confirmed that the distance between AgRidge sites and I99 sites was high which was visually evident by the clustering of sites by locale on opposite ends of the axis (Figure 2.25). Thus, it was determined that locale was a very strong gradient and a PCA was run for each locale separately. The PCA of only the AgRidge sites resulted in 1 significant axis ( $p=0.039$ ) which explained 44.898% of the variance (Figure 2.26). Kendall correlations were positive for light ( $\tau=0.600$ ), percent silt ( $\tau=0.552$ ), distance to the road ( $\tau=0.552$ ) and pH ( $\tau=0.138$ ). Kendall correlations were negative for all other environmental variables with soil organic matter and carbon concentrations highly negatively correlated with the presence of *Microstegium* ( $\tau=-1.000$  and  $-1.000$ , respectively). The PCA for I99 did not result in any significant axes.

## Discussion

### Environmental Variables

The ability of *Microstegium* to grow in high light environments was demonstrated previously (Claridge and Franklin 2002; Christen and Matlack 2009) and is evident in this study by a high positive correlation ( $\tau=0.600$ ) between greater photosynthetically active radiation and presence of the invasive for sites at the AgRidge locale. Light availability can influence many other environmental conditions including, but not limited to, soil organic matter content, nutrient availability, and soil water content. Soil organic matter content is often lower in high light environments (Brady and Weil 2008) because aerobic microbes readily breakdown plant matter. This may explain why soil organic matter content was highly negatively correlated ( $\tau=-1.00$ ) with the presence of *Microstegium* in higher light environments at the AgRidge locale. Conversely, very little organic matter decomposition may be found in low light environments because water evaporation, transpiration by plants, and aerobic activity are comparatively lower (Brady and Weil 2008). Lower soil cation exchange capacities and nutrient availability are associated with lower soil organic matter contents which would explain the negative correlations between the presence of *Microstegium* and a majority of the soil nutrient variables at the AgRidge locale.

Several studies have noted large populations of *Microstegium* in moist soils or have asserted that increasing soil water content is a critical factor influencing expansive *Microstegium* growth (Belote et al. 2003; Christen and Matlack 2009). Previous research in a controlled greenhouse demonstrated significantly greater phytomass production for *Microstegium* plants grown under moderate soil water content (20-35%) when compared to low (5-15%) and saturated soil water contents ( $\geq 65\%$ ) (Romanello and Touchette, unpublished data). The mean soil water contents for *Microstegium* sites at I99 for this study was 42.08% and 41.62% at the AgRidge. Thus, it was anticipated that there would be a positive correlation between greater soil water content and the presence of *Microstegium*. However, the principle components analysis for all sites from both locales resulted in a negative correlation ( $\tau=-0.121$ ) between the presence of *Microstegium* and greater soil water content for Axis 1. The principle components analysis of AgRidge sites only also demonstrated a negative correlation ( $\tau=-0.467$ ) between soil water content and the presence of *Microstegium*. It is likely that the number of sites was insufficient to

resolve such affects. Soil water content may still be an important factor associated with the presence of *Microstegium* depending on habitat and resource availability but this study alone indicates that it may not be as important as high photosynthetically active radiation. There are currently no published studies on the effect of soil water content between 40 and 60% on *Microstegium* growth and patch size. Therefore, this is the first documentation of this species ability to persist at this soil water content.

Comparisons of hydrographs at different sites, irrespective of the number of sampling points per recorder, have been conducted in many wetland studies (Cole et al. 1997; Hoeltje 2005; Cole et al. 2008). Even though depth to water data were not always available for the dry zone (below ~30 cm), the available hydrographs indicated that water was present within the root zone (0- ~30 cm) frequently for some of the *Microstegium* sites. Continuously available soil water coupled with high light makes these habitats suitable to support *Microstegium* growth. From these factors alone, one would expect *Microstegium* patch size to be quite large and represent a greater proportion of the aboveground plant composition. *Microstegium* percent cover was indeed greater than 60% of the total site area for some sites but *Microstegium* cover at other sites represented a small proportion of the total site area. This further indicates that either the sample size for this study was inadequate or that other mechanisms such as plant competition or seed dispersal limitations influence *Microstegium* cover.

### **Biotic Variables**

One objective of this research was to determine if any biotic influences were strongly associated with the presence of *Microstegium*. It was anticipated that the aboveground plant family compositions would be different between *Microstegium* and non-*Microstegium* sites. However, the presence of *Microstegium* was not significantly associated ( $p=0.234$ ) with aboveground plant family composition. This could have been due to small sample sizes or it could indicate that all sites were truly very similar. From this study and analysis alone, an association between plant family composition and the presence of *Microstegium* could not be determined.

Shannon-Weiner Index and evenness values did not provide any extensive information to differentiate *Microstegium* from non-*Microstegium* sites. Typically, the Shannon-Weiner Index is used to assess species diversity and this index may not be a reliable indicator of diversity for this study because it is possible for sites to have high family diversity but low species diversity. Or, it

is possible that data on species diversity may be more important than data on family diversity. The use of this metric for families also does not allow transferability among other studies which examine species diversity. A better index of vegetative diversity to use for future research would be the Floristic Quality Assessment Index (FQAI) based on plant species because it is less subjective and ranks species based on their tendency to occur in specific locations. For example, Lopez and Fennessy (2002) conducted a FQAI on depressional wetlands and determined that wetlands considered to be disturbed had lower FQAI values and were comprised of species present in more cultivated settings. The completion of a FQAI over several seasons at each site looking specifically at species present in disturbed versus undisturbed sites could provide more information to differentiate *Microstegium* sites from non-*Microstegium* sites.

Analysis of sites with and without *Microstegium* using plant functional groups indicated that all sites without *Microstegium* were dominated by forbs. Grasses, sedges, and forbs predominated in *Microstegium* sites. Grasses only dominated in 4 out of 6 *Microstegium* sites (both locales) which could be a product of the percent *Microstegium* cover. Further studies are needed to determine if forb dominated slope, seep wetlands contain greater abundances of native species and if these wetlands have greater floral diversity. Greater floral diversity may indicate that these wetlands are less invasible. Additional greenhouse studies could also examine if *Microstegium* productivity (including biomass, growth rates, leaf area, number of stems, number of inflorescences, and number of seeds) is affected when other plant species occupy overlapping space and compete for the same resources.

## Conclusions

This study examined both environmental and biotic variables of slope, seep wetlands to determine if there was a unique suite of characteristics which differentiated *Microstegium* from non-*Microstegium* sites. Even though site sample size was small, the defining characteristic between *Microstegium* and non-*Microstegium* sites at the AgRidge was the significant positive correlation between the presence of *Microstegium* and high photosynthetically active radiation. Aboveground plant family composition did not reveal any differences between *Microstegium* and non-*Microstegium* sites. However, when plants were categorized by functional group, non-*Microstegium* sites were all dominated by forbs thus provoking questions about the role of floral composition and habitat invasibility. Overall, studies which examine environmental habitat



suitability differences are one important part of understanding why the invasive *Microstegium* is able to persist. In order to fully address control and management in the future it is necessary to examine seed dispersal constraints as well. While some sites may be suitable for *Microstegium* populations based on environmental or biotic variables, the presence of *Microstegium* is likely limited by seed dispersal. Since disturbance also encourages *Microstegium* seed dispersal and growth it would be useful to determine if correlations exist between historical land-use, such as logging or farming, and the locations of current *Microstegium* populations.

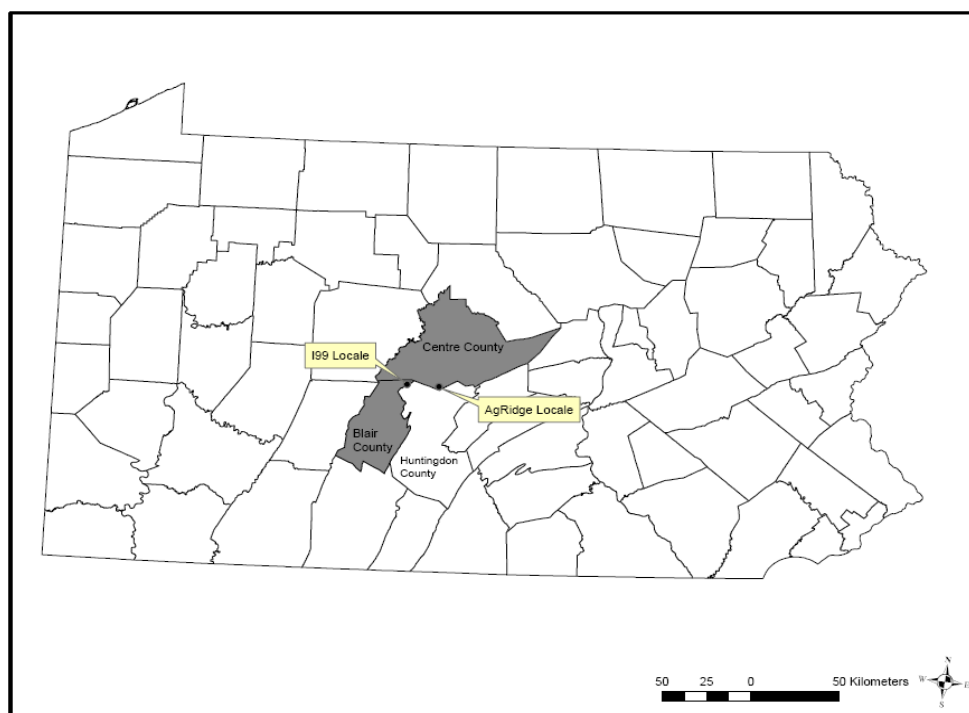


Figure 2.1: AgRidge and I99 locales in Centre and Blair counties, Pennsylvania, respectively.

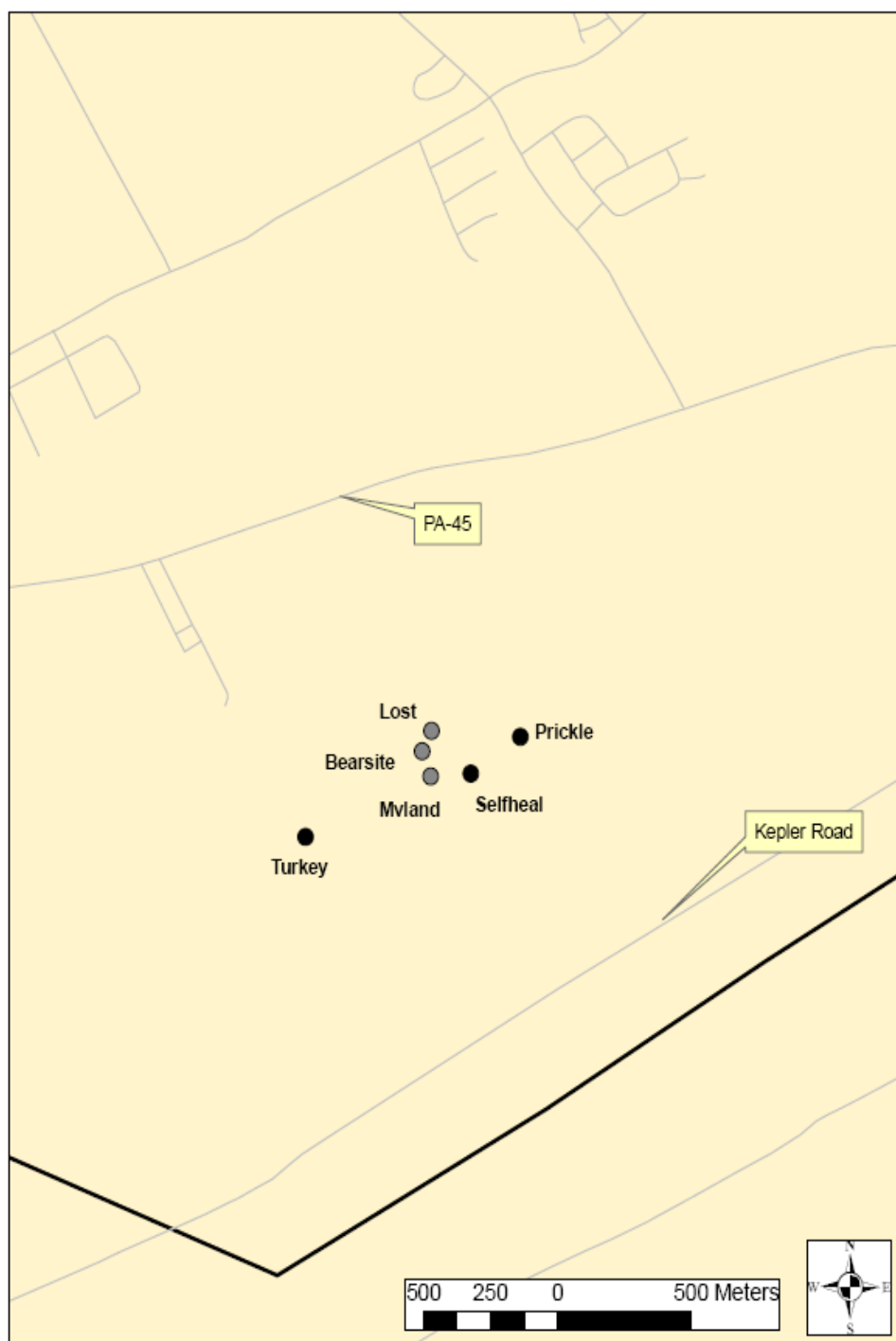


Figure 2.2: Locations of AgRidge sites and upslope Kepler Road. Darkest line represents the Centre county boundary. Gray dots represent *Microstegium* sites and black dots represent non-*Microstegium* sites.

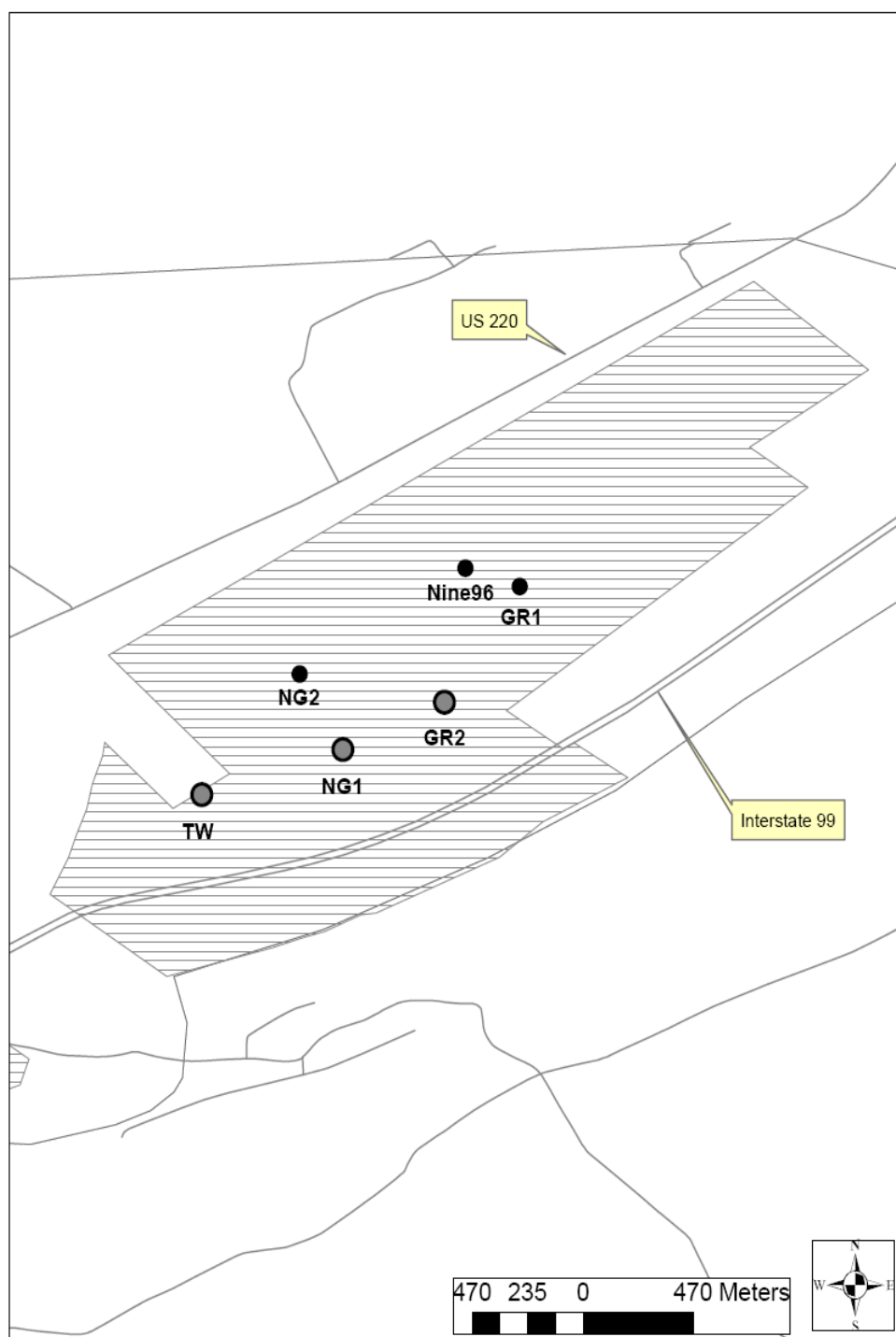


Figure 2.3: Locations of I99 sites and upslope Interstate-99. Gray dots represent *Microstegium* sites and black dots represent non-*Microstegium* sites. Hatched area represents Pennsylvania Game Commission Land, parcel #278.

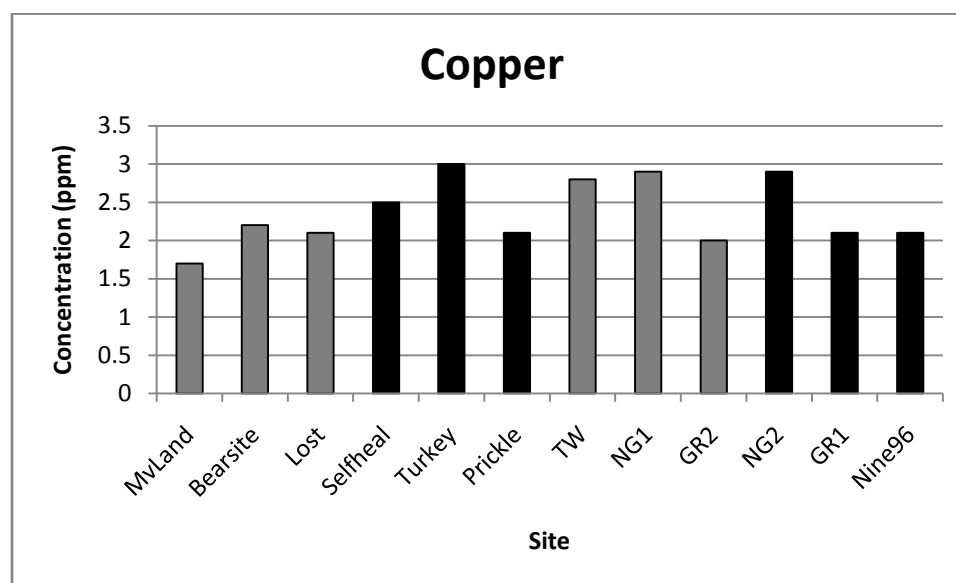


Figure 2.4: Median soil copper content (ppm) for all twelve sites from both the AgRidge and I99 locales. Concentrations ranged from 1.7-2.9 ppm in *Microstegium* sites (gray) and 2.1-3.0 ppm in non-*Microstegium* sites (black).

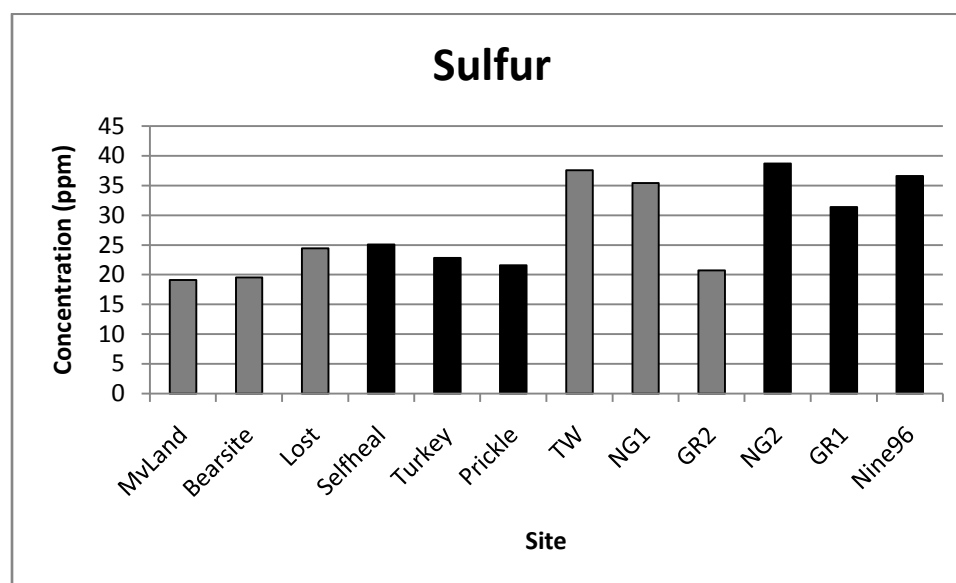


Figure 2.5: Median soil sulfur content (ppm) for all twelve sites from both the AgRidge and I99 locales. Concentrations ranged from 19.1-37.5 ppm in *Microstegium* sites (gray) and 21.6-38.7 ppm in non-*Microstegium* sites (black).

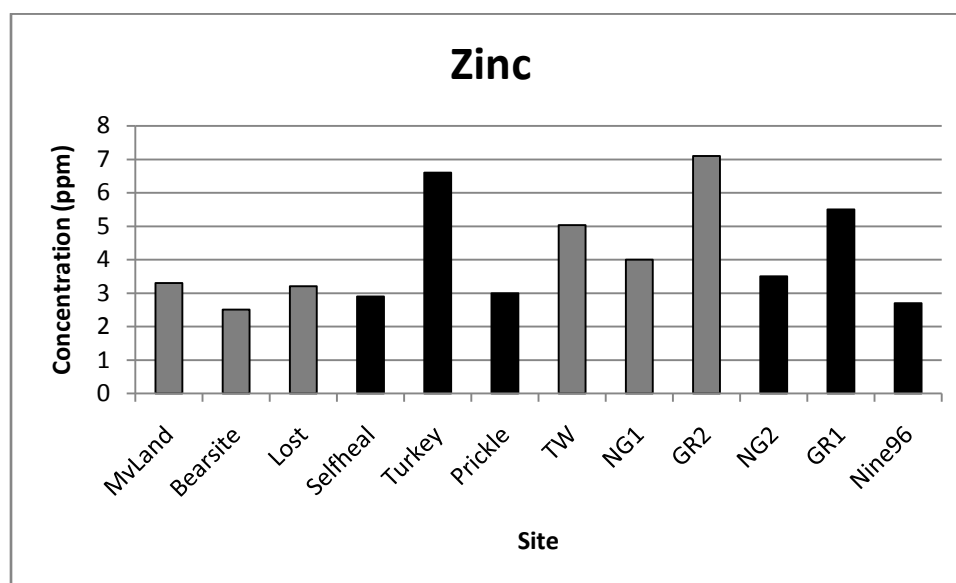


Figure 2.6: Median soil zinc content (ppm) for all twelve sites from both the AgRidge and I99 locales. Concentrations ranged from 2.5-7.1 ppm in *Microstegium* sites (gray) and 5.4-10.1 ppm in non-*Microstegium* sites (black).

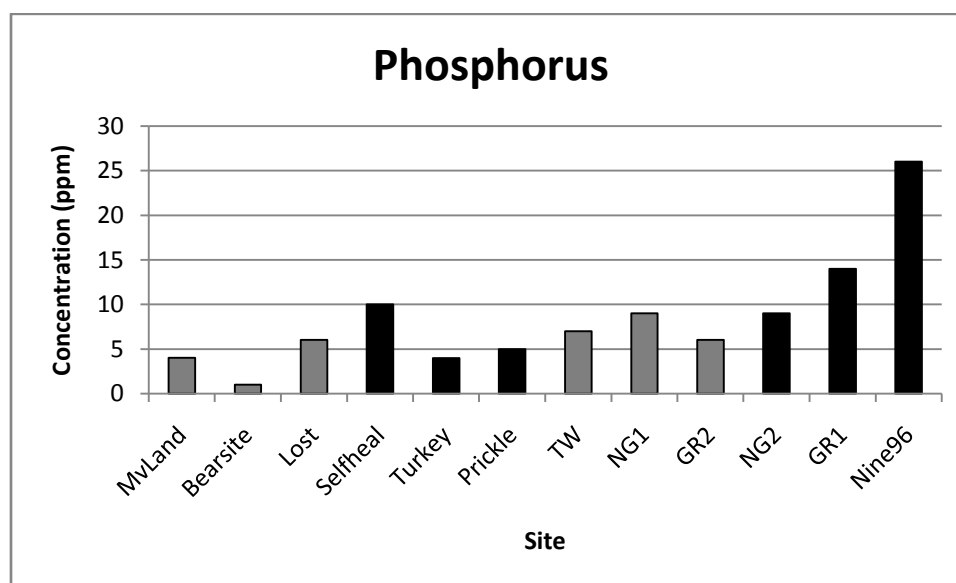


Figure 2.7: Median soil phosphorus content (ppm) for all twelve sites from both the AgRidge and I99 locales. Concentrations ranged from 1.0-9.0 ppm in *Microstegium* sites (gray) and 4.0-26.0 ppm in non-*Microstegium* sites (black).

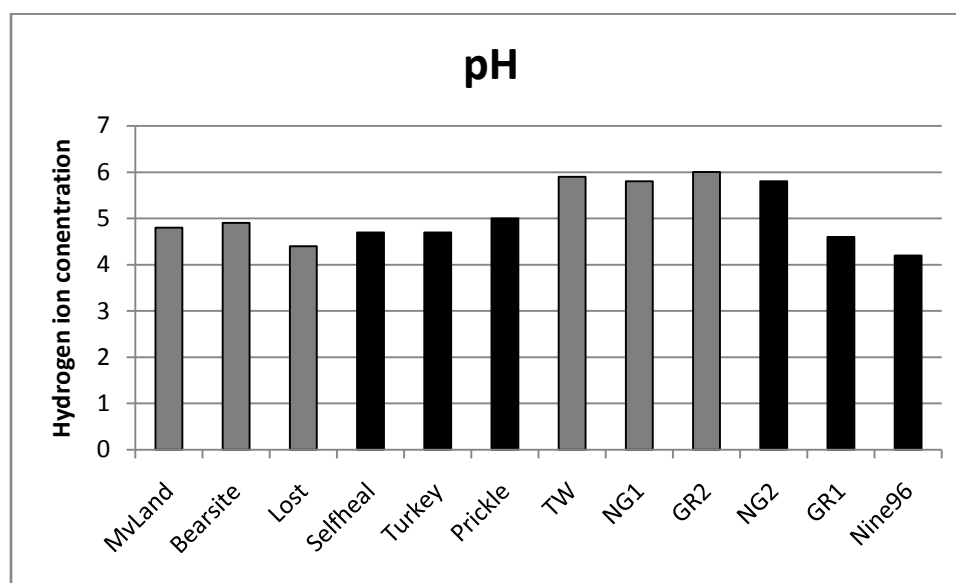


Figure 2.8: Median soil pH for all twelve sites from both the AgRidge and I99 locales.  $H^+$  concentrations ranged from 4.4-6.0 in *Microstegium* sites (gray) and 4.2-5.8 in non-*Microstegium* sites (black).

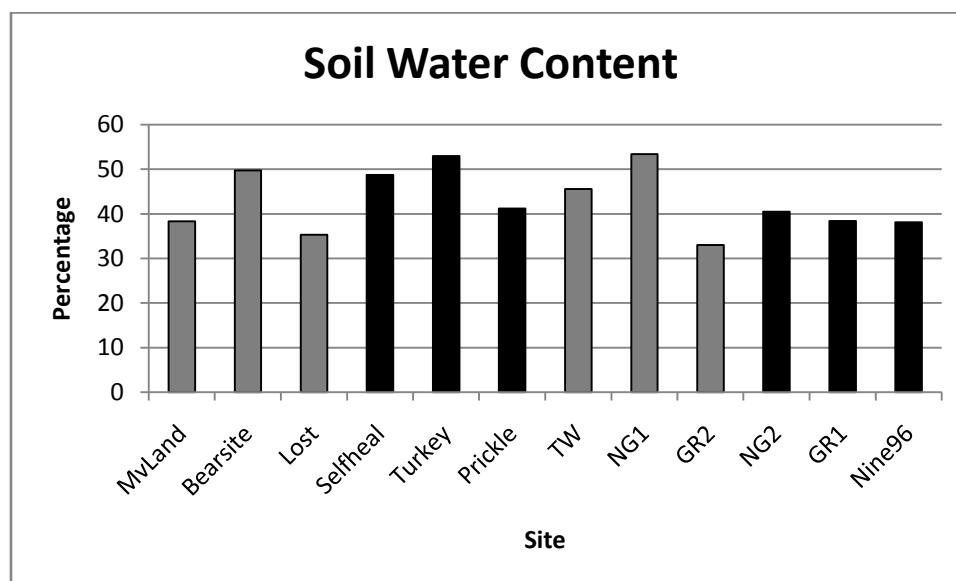


Figure 2.9: Median soil water content (%) for all twelve sites from both the AgRidge and I99 locales. Soil water content ranged from 33.0-53.3% in *Microstegium* sites (gray) and 38.1-52.9% in non-*Microstegium* sites (black).

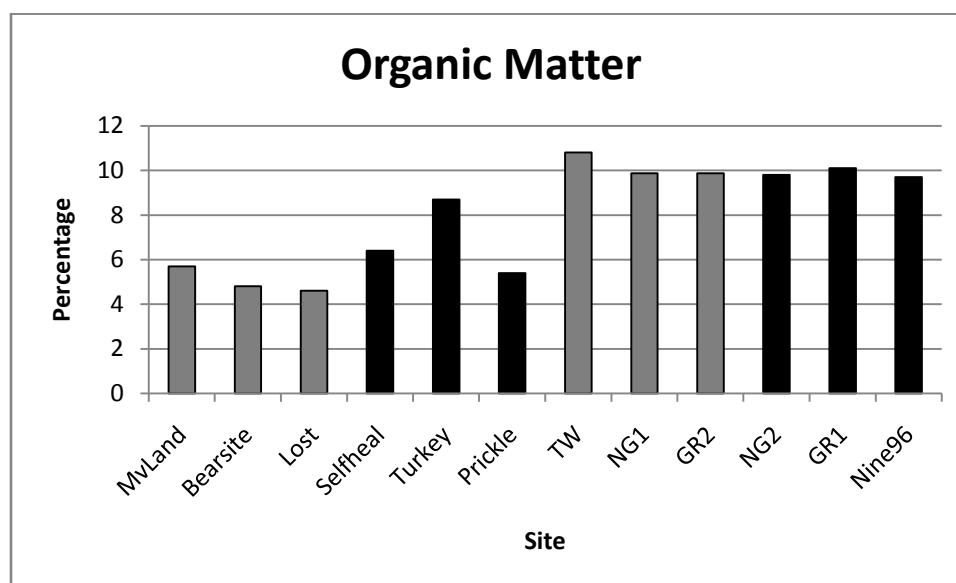


Figure 2.10: Median percent soil organic matter content for all twelve sites from both the AgRidge and I99 locales. Soil organic matter content ranged from 4.6-10.8% in *Microstegium* sites (gray) and 5.4-10.1% in non-*Microstegium* sites (black).

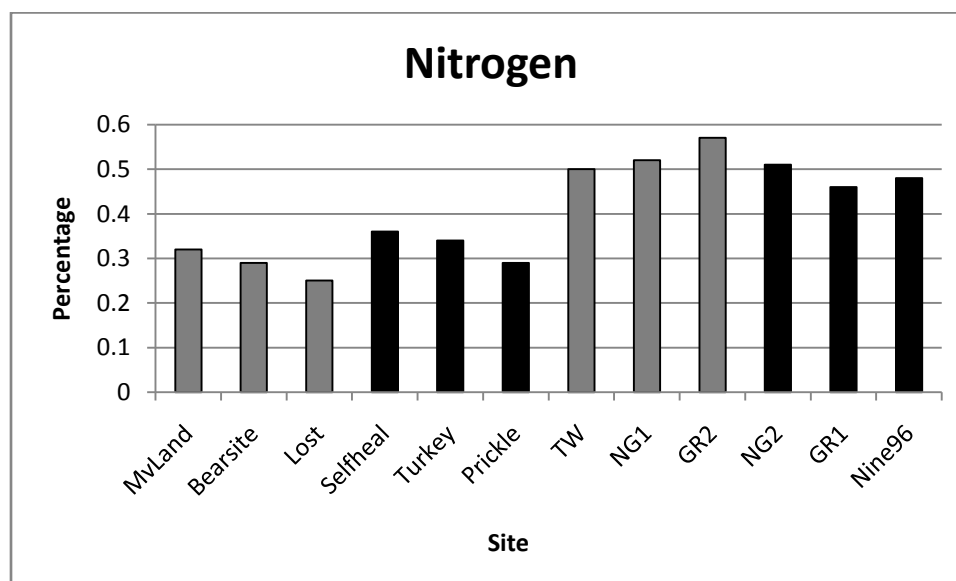


Figure 2.11: Median soil nitrogen content (%) for all twelve sites from both the AgRidge and I99 locales. Soil nitrogen content ranged from 0.25-0.57% in *Microstegium* sites (gray) and 0.29-0.51% in non-*Microstegium* sites (black).



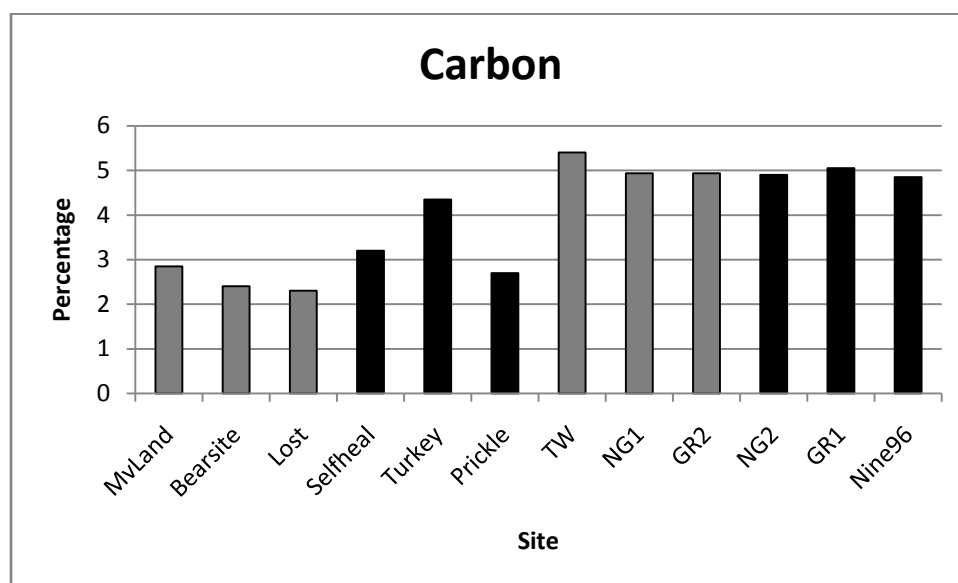


Figure 2.12: Estimated soil carbon content (%) from median soil organic matter content (%) for all twelve sites from both the AgRidge and I99 locales. Estimated soil carbon content ranged from 2.3-5.4% in *Microstegium* sites (gray) and 2.7-5.1% in non-*Microstegium* sites (black).

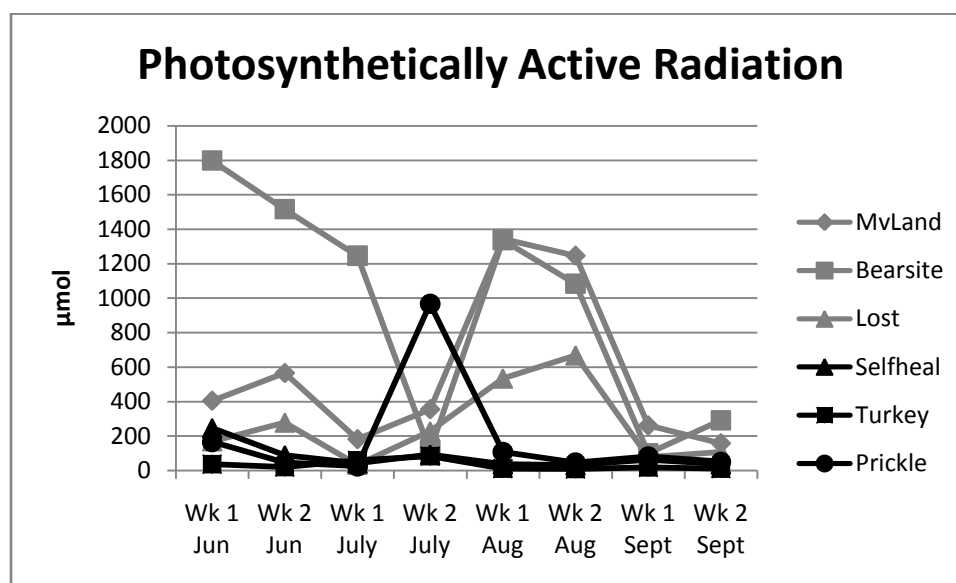


Figure 2.13: Median photosynthetically active radiation for the AgRidge locale ranged from 33-1798  $\mu\text{mol}$ . *Microstegium* sites are in gray while non-*Microstegium* sites are in black.

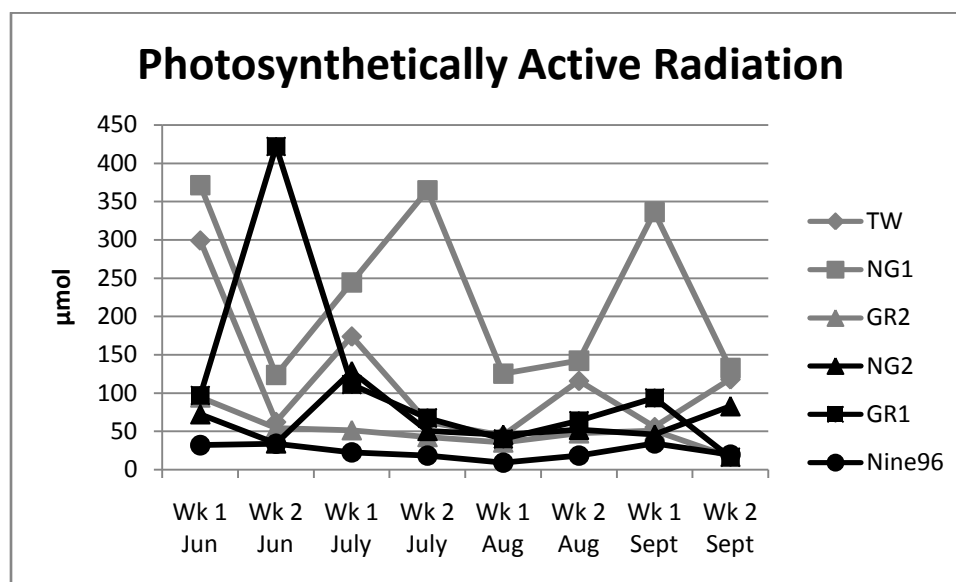


Figure 2.14: Median photosynthetically active radiation for the I99 locale ranged from 8-967  $\mu\text{mol}$ . *Microstegium* sites are in gray while non-*Microstegium* sites are in black.

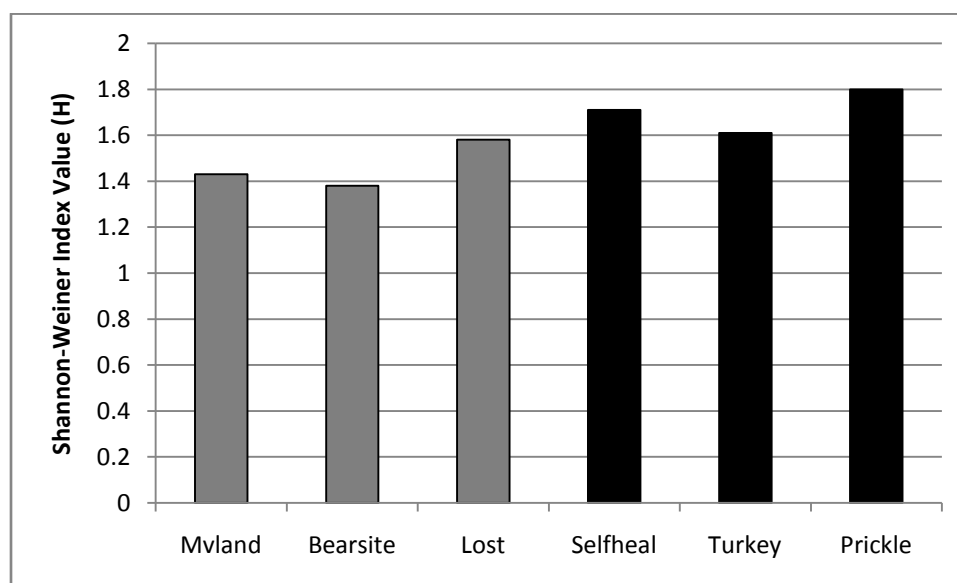


Figure 2.15: Shannon-Weiner Index values for AgRidge sites. *Microstegium* sites are in gray while non-*Microstegium* sites are in black.

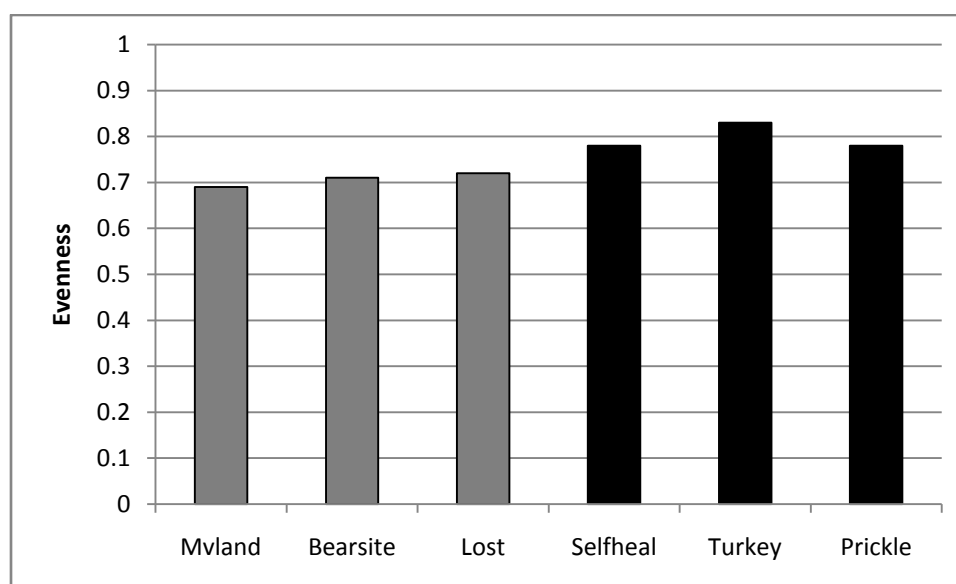


Figure 2.16: Evenness values for AgRidge sites. *Microstegium* sites are in gray while non-*Microstegium* sites are in black.

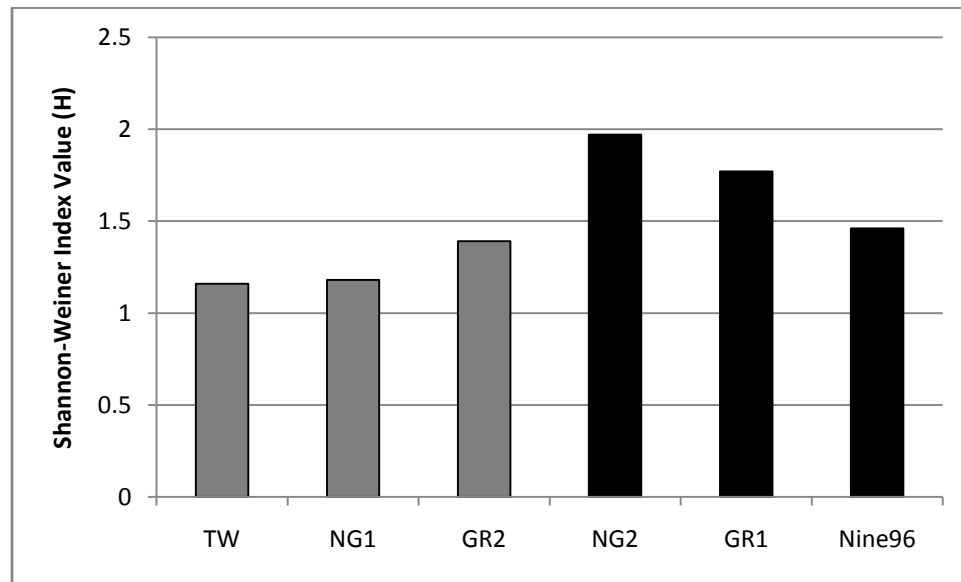


Figure 2.17: Shannon-Weiner Index values for I99 sites. *Microstegium* sites are in gray while non-*Microstegium* sites are in black.

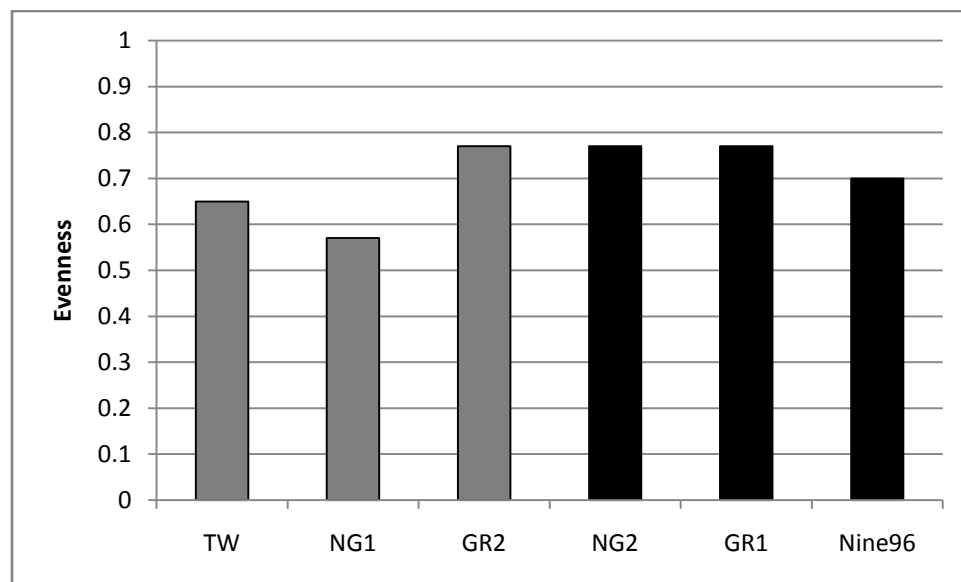


Figure 2.18: Evenness values for I99 sites. *Microstegium* sites are in gray while non-*Microstegium* sites are in black.

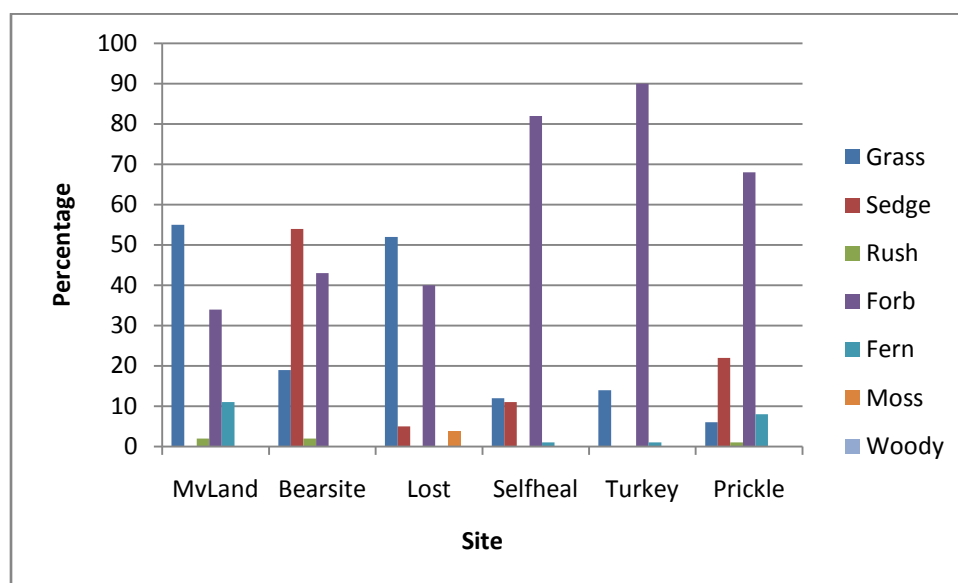


Figure 2.19: Plant functional groups from the aboveground community for all sites at the AgRidge locale. *Microstegium* was present in sites Mvland, Bearsite, and Lost. *Microstegium* was absent in sites Selfheal, Turkey and Prickle.

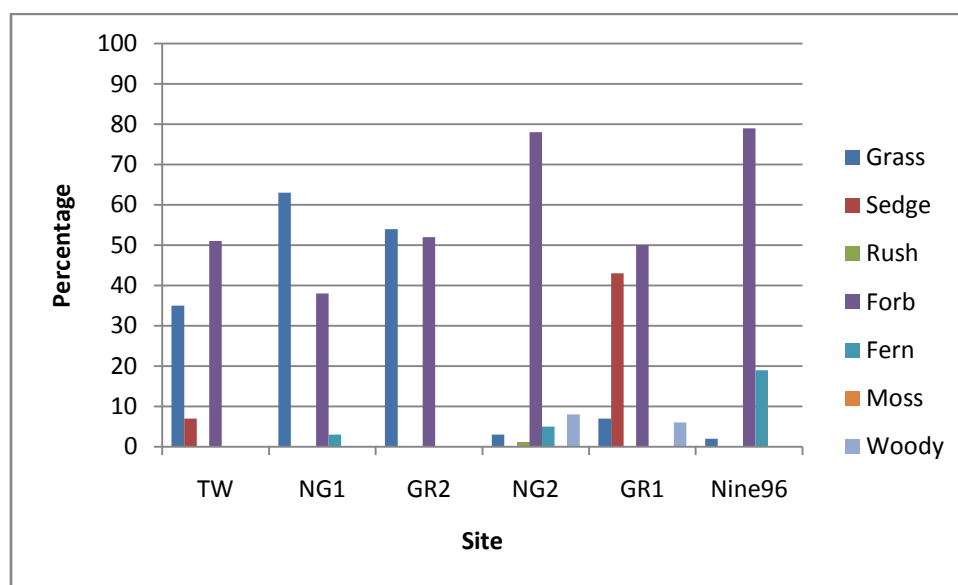


Figure 2.20: Plant functional groups from the aboveground community for all sites at the I99 locale. *Microstegium* was present in sites TW, NG1, and GR2. *Microstegium* was absent in sites NG2, GR1, and Nine96.

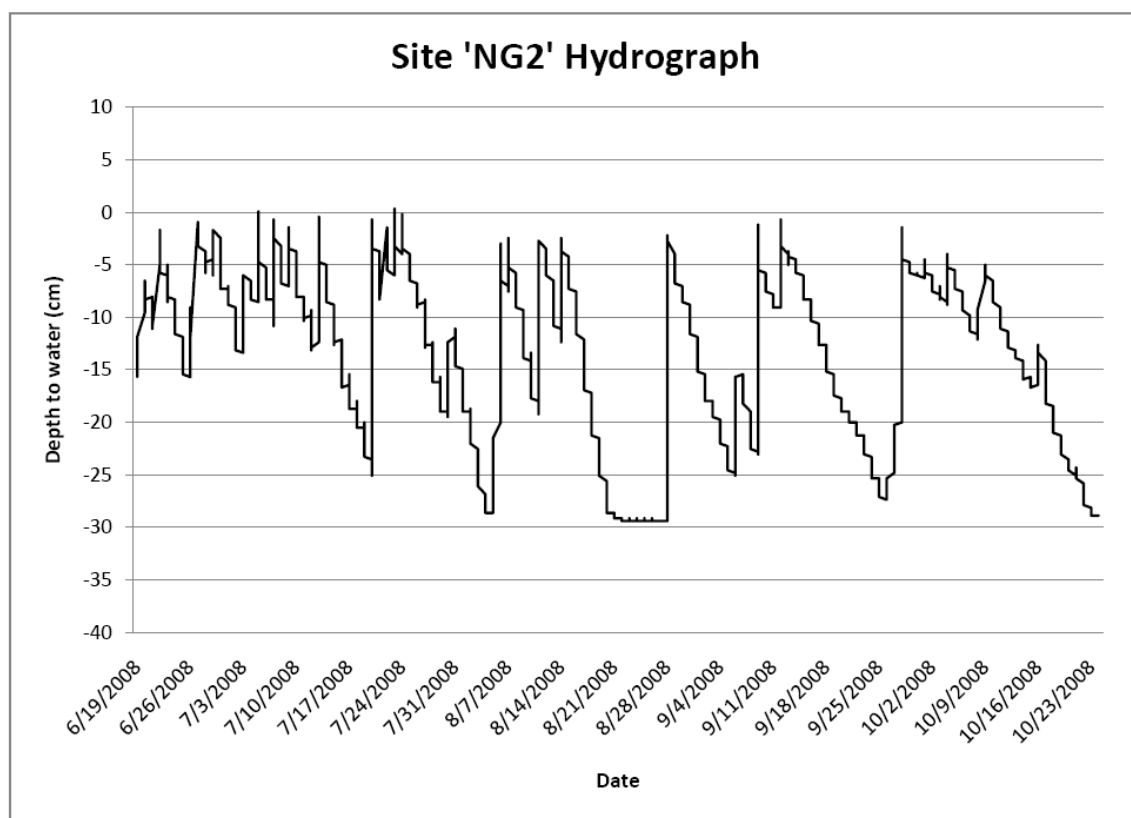


Figure 2.21: Hydrograph for non-*Microstegium* site (NG2) at I99 locale from June 19, 2008 to October 23, 2008. Depth to water (cm) was recorded every 3 hours (n=1015).

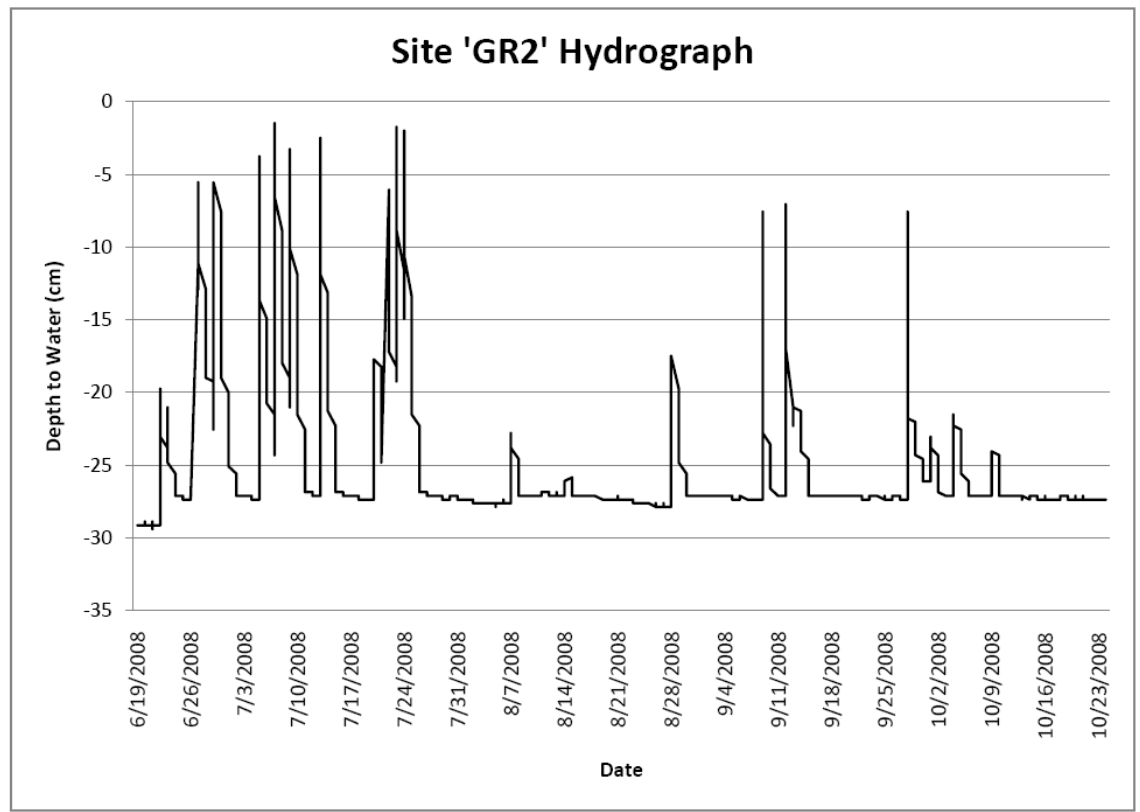


Figure 2.22: Hydrograph for *Microstegium* site (GR2) at I99 locale from June 19, 2008 to October 23, 2008. Depth to water (cm) was recorded every 3 hours (n=1015).

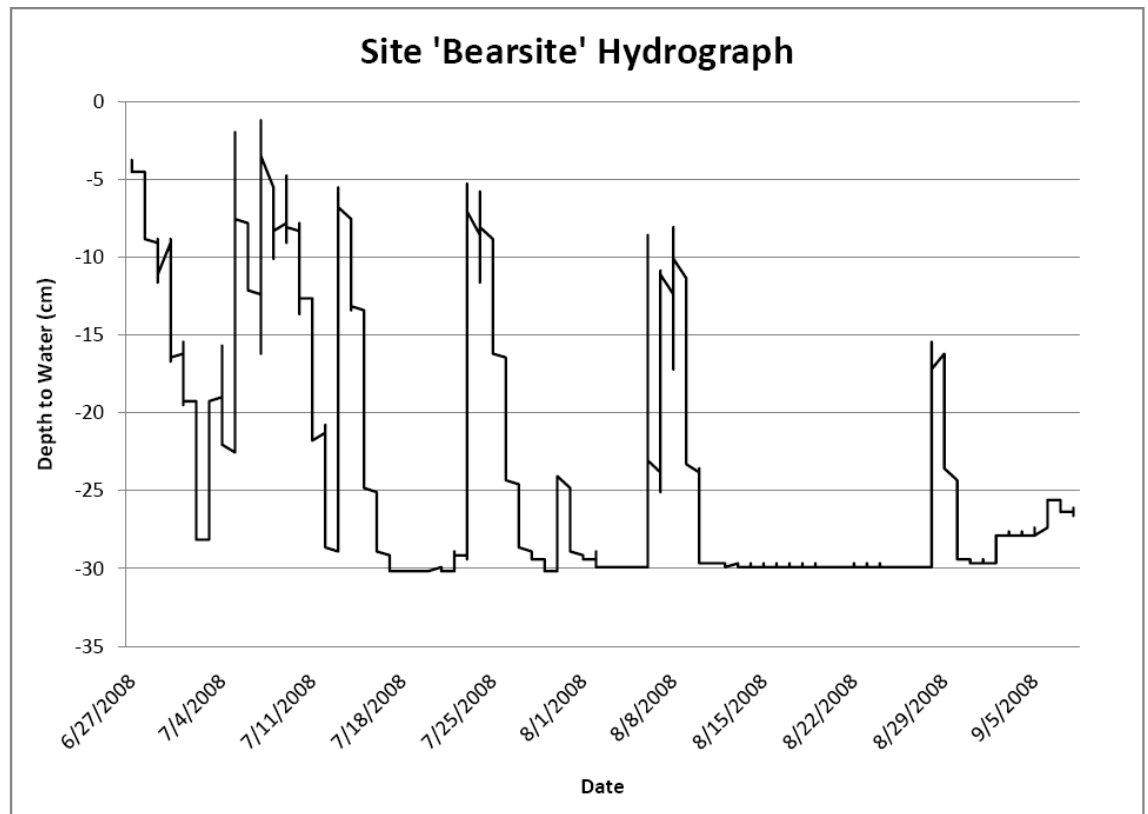


Figure 2.23: Hydrograph for *Microstegium* site (Bearsite) from AgRidge locale from June 27, 2008 to September 9, 2008. Depth to water (cm) was recorded every 3 hours (n=592).



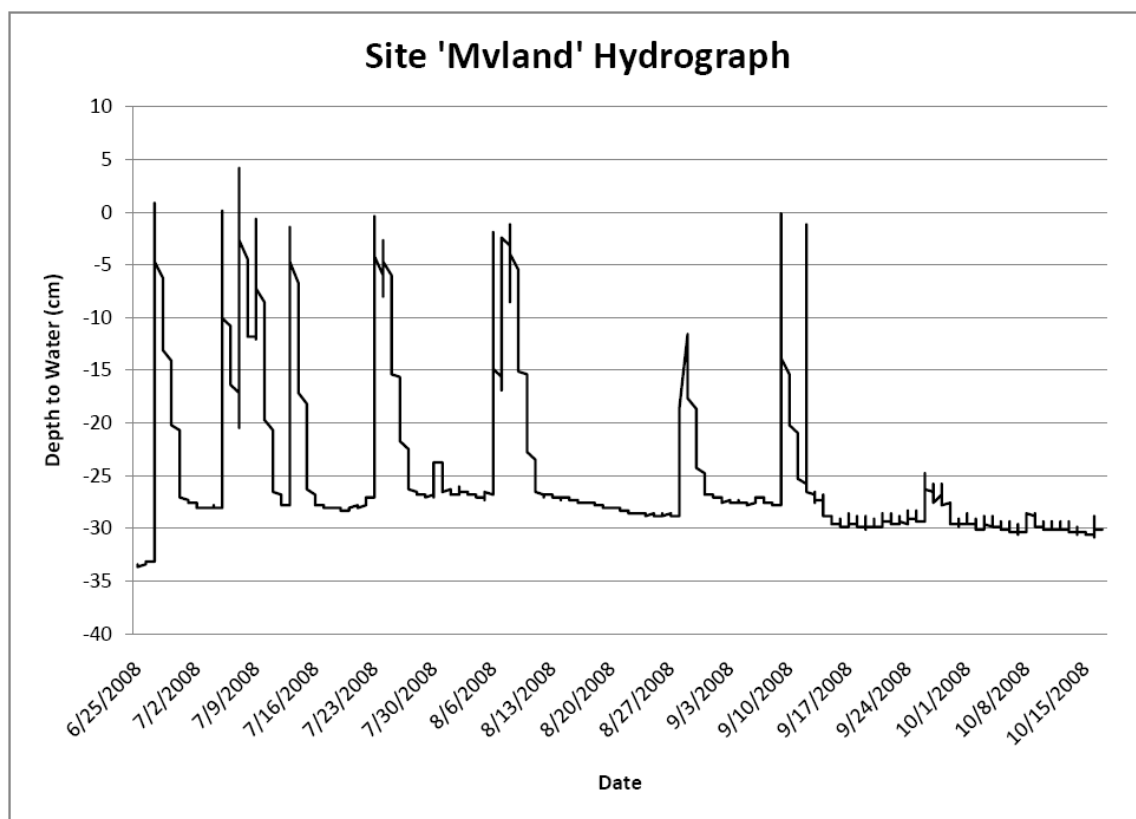


Figure 2.24: Hydrograph for *Microstegium* site (Mvland) at AgRidge locale from June 25, 2008 to October 15, 2008. Depth to water (cm) was recorded every 3 hours (n=911).

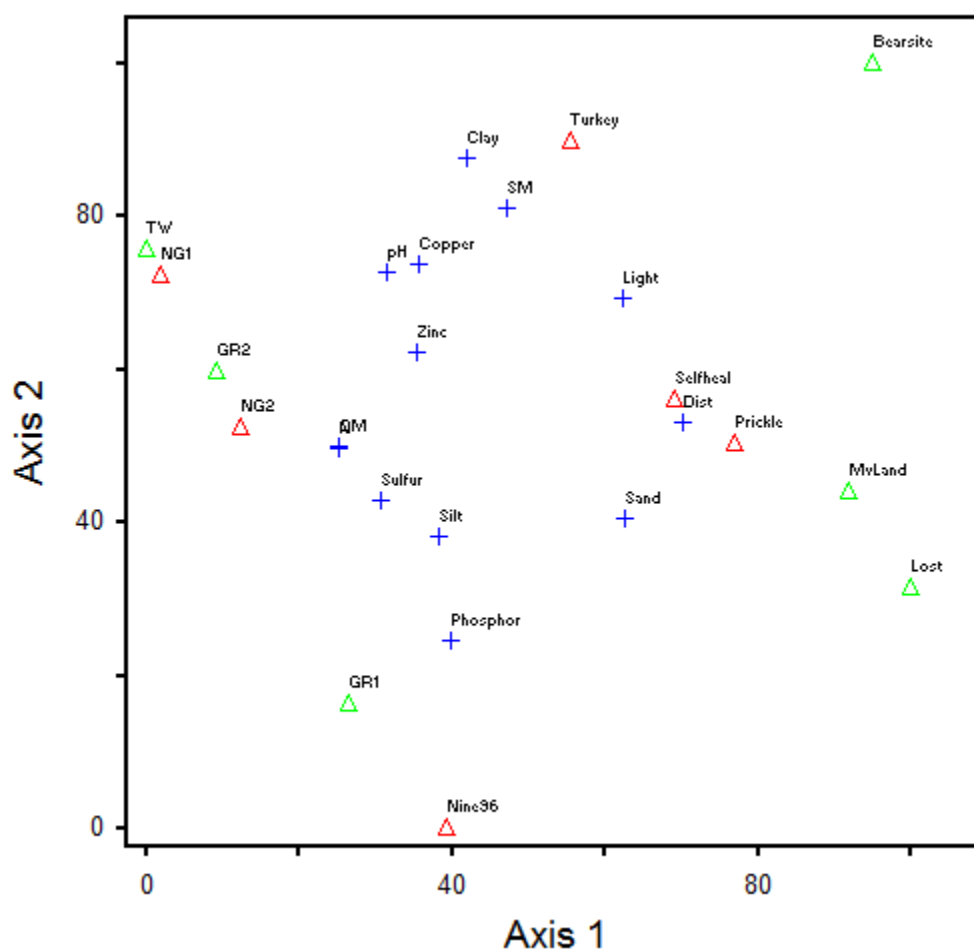


Figure 2.25: Principle components analysis using environmental data and coding variable (*Microstegium* versus non-*Microstegium* sites) for all sites from both locales. Green triangles indicate *Microstegium* sites, red triangles indicate non-*Microstegium* sites, and blue plus symbols indicate environmental variables. Axis 1 ( $p=0.001$ ) explained 40.073% of the variance.

#### Key to Environmental Data Abbreviations

<b>C</b>	Soil carbon (ppm)	<b>Phosphor</b>	Soil phosphorus (ppm)
<b>Clay</b>	Percent clay in soil	<b>pH</b>	pH
<b>Copper</b>	Soil copper (ppm)	<b>Sand</b>	Percent sand in soil
<b>Dist</b>	Distance to road (m)	<b>Silt</b>	Percent silt in soil
<b>Light</b>	PAR ( $\mu\text{mol}$ )	<b>SM</b>	Soil water content (%)
<b>N</b>	Soil nitrogen (%)	<b>S</b>	Sulfur (ppm)
<b>OM</b>	Soil organic matter (%)	<b>Zinc</b>	Zinc (ppm)

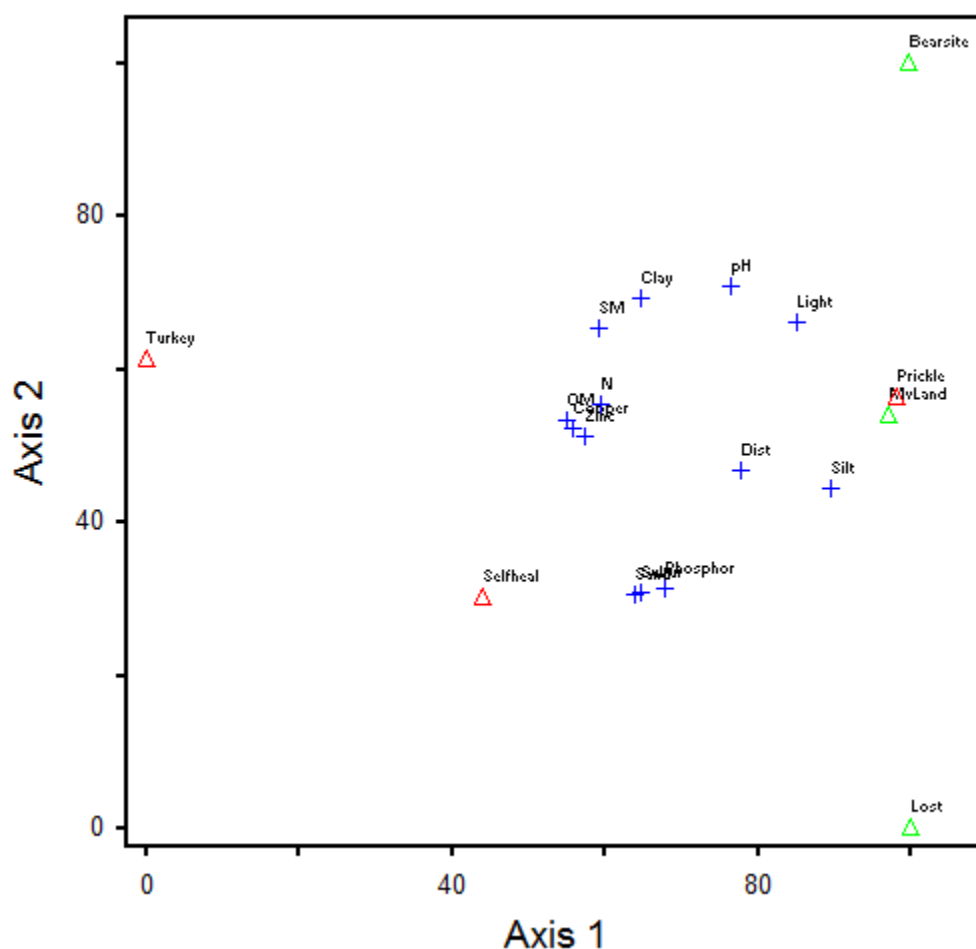


Figure 2.26: Principle components analysis using environmental data and coding variable (*Microstegium* versus non-*Microstegium* sites) for all sites from AgRidge locale only. Green triangles indicate *Microstegium* sites, red triangles indicate non-*Microstegium* sites, and blue plus symbols indicate environmental variables. Axis 1 ( $p=0.039$ ) explained 44.898% of the variance.

#### Key to Environmental Data Abbreviations

<b>C</b>	Soil carbon (ppm)	<b>Phosphor</b>	Soil phosphorus (ppm)
<b>Clay</b>	Percent clay in soil	<b>pH</b>	pH
<b>Copper</b>	Soil copper (ppm)	<b>Sand</b>	Percent sand in soil
<b>Dist</b>	Distance to road (m)	<b>Silt</b>	Percent silt in soil
<b>Light</b>	PAR ( $\mu\text{mol}$ )	<b>SM</b>	Soil water content (%)
<b>N</b>	Soil nitrogen (%)	<b>S</b>	Sulfur (ppm)
<b>OM</b>	Soil organic matter (%)	<b>Zinc</b>	Zinc (ppm)

Table 2.1: Descriptions of the twelve study sites including longitude, latitude, site area (m<sup>2</sup>), distance from upslope road, *Microstegium* cover (expressed as the percent of the total site area covered by the invasive) and presence (shaded gray) or absence (not shaded) of *Microstegium*. Area for AgRidge sites ranged from 160-320 m<sup>2</sup>. Area for I99 sites ranged from 288-672 m<sup>2</sup>. Upslope distance from AgRidge sites to Kepler Road ranged from 824-1050 m. Upslope distance from I99 sites to Interstate-99 ranged from 333-735 m.

Locale	Site Name	Longitude	Latitude	Site Area (m <sup>2</sup> )	Distance from Upslope Road (m)	<i>Microstegium</i> Cover (%)
AgRidge	Mvland	40°42'25.7"	77°56'17.3"	256	880	73%
	Bearsite	40°42'28.5"	77°56'18.8"	320	976	5%
	Lost	40°42'30.8"	77°56'17.4"	320	1050	10%
	Selfheal	40°42'26.2"	77°56'10.9"	208	824	0%
	Turkey	40°42'18.5"	77°56'36.9"	192	943	0%
	Prickle	40°42'30.5"	77°56'03.2"	160	880	0%
I99	TW	40°43'26.6"	78°09'17.2"	672	333	62%
	NG1	40°43'32.3"	78°08'57.8"	640	365	98%
	GR2	40°43'38.0"	78°08'43.8"	320	344	73%
	NG2	40°43'41.9"	78°08'03.7"	640	693	0%
	GR1	40°43'53.0"	78°08'33.4"	512	581	0%
	Nine96	40°43'55.3"	78°08'40.9"	288	735	0%

Table 2.2: Water level data for two *Microstegium* sites (shaded gray) at the AgRidge locale.

Locale: AgRidge							
Site	n (3 hr time intervals)	n (days)	Median depth to water (cm)	% Dry ( < 30 cm)	% Saturated ( 0 - 30 cm)	% Inundated ( > 0 cm)	Average Depth of Saturated (cm)
Bearsite	592	74	-28.132	0%	57%	43%	-22.82
Mvland	911	113.88	-27.568	12%	46%	42%	-24.28

Table 2.3: Water level data for one *Microstegium* site (shaded gray) and one non-*Microstegium* site (not shaded) at the I99 locale.

Locale: I99							
Site	n (3 hr time intervals)	n (days)	Median depth to water (cm)	% Dry ( < 30 cm)	% Saturated ( 0 - 30 cm)	% Inundated ( > 0 cm)	Average Depth of Saturated (cm)
GR2	1015	126.88	-27.124	0%	100%	0%	-24.93
NG2	1015	126.88	-11.741	0%	100%	0%	-13.45

Table 2.4: Soil particle size analysis for AgRidge sites with corresponding soil types. *Microstegium* sites are shaded in gray and non-*Microstegium* sites are not shaded.

	Site					
	Mvland	Bearsite	Lost	Selfheal	Turkey	Prickle
% Sand	42.79	36.69	47.22	44.65	45.67	37.22
% Silt	44.31	41.75	45.34	41.75	33.34	50
% Clay	9.40	19.4	9.27	13.76	17.37	11.42
Soil type	Loam	Loam	Loam	Loam	Loam	Silt loam

Table 2.5: Soil particle size analysis for I99 sites with corresponding soil types. *Microstegium* sites are shaded in gray and non-*Microstegium* sites are not shaded.

	Site					
	TW	NG1	GR2	NG2	GR1	Nine96
% Sand	36.80	32.15	34.59	36.25	38.26	45.0
% Silt	44.04	53.17	47.43	50.0	53.5	44.25
% Clay	17.83	14.66	16.39	13.75	8.34	10.98
Soil type	Loam	Silt loam	Loam	Silt loam	Silt loam	Loam

## Literature Cited

- Baker, H. G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics* 5:1-24.
- Barden, L. S. 1987. Invasion of *Microstegium vimineum*, an exotic, annual, shade-tolerant C<sub>4</sub> grass, into a North Carolina floodplain. *American Midland Naturalist* 118:40-45.
- Belote, R. T., J. F. Weltzin, and R. J. Norby. 2003. Response of an understory plant community to elevated [CO<sub>2</sub>] depends on differential responses of dominant invasive species and is mediated by soil water availability. *New Phytologist* 161:827-835.
- Belote, R. T. and J. F. Weltzin. 2006. Interactions between two co-dominant, invasive plants in the understory of a temperate deciduous forest. *Biological Invasions* 8:1629-1641.
- Brady, N. C. and R. R. Weil. 2008. The nature and properties of soils, Fourteenth Edition. Pearson Prentice Hall, Upper Saddle River, NJ, 965 pp.
- Brinson, M. M. 1993. A hydrogeomorphic classification for wetlands. WRP-DE-4, Army Corps of Engineers Wetlands Research Program Technical Report.
- Brooks, R. P., D. H. Wardrop, C. A. Cole, and D. A. Campbell. 2005. Are we purveyors of wetland homogeneity? A model of degradation and restoration to improve wetland mitigation performance. *Ecological Engineering* 24:331-340.
- Campbell, D. A., Cole, C. A., and R. P. Brooks. 2002. A comparison of created and natural wetlands in Pennsylvania, USA. *Wetlands Ecology and Management* 10:41-49.

- Cheplick, G. P. 2006. A modular approach to biomass allocation in an invasive annual (*Microstegium vimineum*; Poaceae). *American Journal of Botany* 93:539-545.
- Christen, D. C. and G. R. Matlack. 2009. The habitat and conduit functions of roads in the spread of three invasive plant species. *Biological Invasions* 11:453-465.
- Claridge, K. and S. B. Franklin. 2002. Compensation and plasticity in an invasive plant species. *Biological Invasions* 4:339-347.
- Cole, C. A., R. P. Brooks, and D. H. Wardrop. 1997. Wetland hydrology as a function of hydrogeomorphic (HGM) subclass. *Wetlands* 17:456-467.
- Cole, C. A., C. P. Cirimo, D. H. Wardrop, R. P. Brooks, and J. Peterson-Smith. 2008. Transferability of an HGM wetland classification scheme to a longitudinal gradient of the central Appalachian mountains: initial hydrological results. *Wetlands* 28:439-449.
- Cole, P. G. and J. F. Weltzin. 2005. Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. *Biological Invasions* 7:477-488.
- Davis, M. A., P. Grime, and K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invisibility. *The Journal of Ecology* 88:528-534.
- Ehrenfeld, J. G. 1999. A rhizomatous, perennial form of *Microstegium vimineum* (Trin.) A. Camus in New Jersey. *Journal of the Torrey Botanical Society* 126:352-358.
- Fairbrothers, D. E. and J. R. Gray. 1972. *Microstegium vimineum* (Trin.) A. Camus (Gramineae) in the United States. *Bulletin of the Torrey Botanical Club* 99:97-100.



- Gee, G. W. and J. W. Bauder. 1979. Particle size analysis by hydrometer: a simplified method for routine textural analysis and a sensitivity test of measurement parameters. *Soil Science Society of America Journal* 43:1004-1007.
- Gibson, D. J., G. Spyreas, and J. Benedict. 2002. Life history of *Microstegium vimineum* (Poaceae), an invasive grass in southern Illinois. *Journal of the Torrey Botanical Society* 129:207-219.
- Hoeltje, S. M. 2005. Functional assessment of created and natural wetlands in central Pennsylvania. M.S. thesis, The Pennsylvania State University, University Park, PA.
- Hoeltje, S. M. and C.A. Cole. 2007. Losing function through wetland mitigation in central Pennsylvania, USA. *Environmental Management* 39:385-402.
- Horton, J. L. and H. S. Neufeld. 1998. Photosynthetic responses of *Microstegium vimineum* (Trin.) A. Camus, a shade-tolerant, C<sub>4</sub> grass, to variable light environments. *Oecologia* 114:11-19.
- Hunt, D. M. and R. E. Zaremba. 1992. The northeastern spread of *Microstegium vimineum* (Poaceae) into New York and adjacent states. *Rhodora* 94:167-170.
- Leicht, S. S., J. A. Silander Jr., and K. Greenwood. 2005. Assessing the competitive ability of japanese stiltgrass, *Microstegium vimineum* (Trin.) A. Camus. *Journal of the Torrey Botanical Society* 132:573-580.
- Lopez, R. D. and M. S. Fennessy. 2002. Testing the floristic quality assessment index as an indicator of wetland condition. *Ecological Applications* 12:487-492.
- Mitsch, W. J. and J. G. Gosselink. 2000. *Wetlands*, Third Edition. John Wiley & Sons, Inc., New York, NY. 920 pp.

- Newcomb, L. 1977. Newcomb's Wildflower Guide. Little, Brown and Company, Boston, MA, 490 pp.
- Oswalt, C. M., S. N. Oswalt, and W. K. Clatterbuck. 2007. 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.
- Pennsylvania State University. Agricultural Analytical Services Lab. 2008. University Park, PA, 16802, USA. (<http://www.aasl.psu.edu/>, 12 December 2008).
- Redman, D. E. 1995. Distribution and habitat types for Nepal *Microstegium* [*Microstegium vimineum* (Trin.) Camus] in Maryland and the District of Columbia. *Castanea* 60:270-275.
- Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics* 32:305-332.
- Semlitsch, R. D. 2000. Principles for management of aquatic-breeding amphibians. *The Journal of Wildlife Management* 64:615-631.
- Tennessee Exotic Pest Plant Council. Tennessee Exotic Plant Management Manual. 2007. (<http://www.tneppc.org/>, 12 December 2007).
- US Department of Agriculture, Natural Resources Conservation Service. 2007. National Plant Data Center, Baton Rouge, LA 70874-4490 USA. The PLANTS Database (<http://plants.usda.gov>, 12 December 2007).
- Williams, L. D. 1998. Factors affecting growth and reproduction in the invasive grass *Microstegium vimineum*. M.S. thesis, Appalachian State University, Boone, NC.

Winter, K., M. R. Schmitt, and G. E. Edwards. 1982. *Microstegium vimineum*, a shade adapted C<sub>4</sub> grass. Plant Science Letters 24:311-318.

Zedler, J. B. and S. Kercher. 2007. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. Critical Reviews in Plant Sciences 23: 431-452.

## Chapter 3

## **Seed Bank Analyses of Slope, Seep Wetlands with and without *Microstegium vimineum* in Central Pennsylvania**

### **Abstract**

The invasive grass, *Microstegium vimineum*, commonly known as Japanese stiltgrass, is a relative newcomer to many slope, seep wetlands in the Centre County region of Central Pennsylvania. Since *M. vimineum* is an annual, seed banks are crucial to secure future populations of this species. Currently, there are no studies which have examined the seed banks of slope, seep wetlands with and without *M. vimineum*. The goal of this study was to examine the plant composition at each site using the seed germination method from soils sampled at two depths (0-10 cm and 10-20 cm) to determine whether the proportion of *Microstegium* seed present in the seed bank was reflective of the percent *Microstegium* cover in the field. Generally, greater percentages of *Microstegium* seed were present in the 0-10 cm seed bank samples for sites where it was present aboveground. However, these percentages seemed low compared to the percent *Microstegium* cover present in the field. This may have been due to differences in environmental factors (e.g. light, soil water content, or nutrients) between field and greenhouse growing conditions or due to the fact that seed bank sampling took place too late in the season (late May 2008) when many of the seeds were already germinating. Multiple-response permutation procedures indicated that plant family composition from both seed bank depths was similar ( $p=0.9991$ ) but aboveground composition and seed bank composition combined were significantly different ( $p=0.0012$ ) which may also be the result of different sampling times (July 2008 and May 2008, respectively). When the seed bank composition was classified by plant functional groups, *Microstegium* sites were dominated by sedges or grasses (with the exception of one site dominated by forbs). Conversely, over half of the non-*Microstegium* sites were dominated by forbs. Future seed bank germination studies testing the effect of specific environmental factors on germination from seed bank samples of slope, seep wetlands with and without *Microstegium* could elucidate optimal environmental suitability (determined by greater biomass or seed produced) and demonstrate the effect of plant competition on productivity.

## Introduction

*Microstegium vimineum* (Trin.) A. Camus, commonly known as Japanese stiltgrass and hereafter referred to as *Microstegium*, is a prominent invasive grass throughout the eastern United States, much of the Northeast, and a few Midwestern states (United States Department of Agriculture 2008). *Microstegium* grows in a variety of habitats including lawns, thickets, fields, and forests, wet areas like wetlands, riparian zones, stream or river banks, and floodplains, and areas of disturbance such as roadsides, ditches, train tracks, logging roads, and utility passages (Fairbrothers and Gray 1972; Hunt and Zaremba 1992; Redman 1995). This species exhibits phenotypic plasticity and has many characteristics of an ‘ideal weed’ (Baker 1974). *Microstegium* can grow under a broad range of irradiance levels (Winter et al. 1982; Horton and Neufeld 1998), produces many seeds that are dispersed by non-specific means (Gibson et al. 2002), and can undergo self-pollination or cross-pollination (Williams 1998; Ehrenfeld 1999; Gibson et al. 2002; Cheplick 2006). There is also evidence that increased soil moisture promotes greater biomass production (Belote et al. 2003; Romanello and Touchette, unpublished data). *Microstegium* dominance has been inversely correlated with decreased plant diversity (Belote 2003). This grass is often a superior competitor (Belote and Weltzin 2006; Leicht 2005), known to replace other vegetation within three to five years of arrival (Tennessee Exotic Pest Plant Council 2007), and thought to be a threat to forest tree regeneration (Oswalt et al. 2007).

As an annual, *Microstegium* relies on seed to secure future progeny. Most of the literature states that this species does create a seed bank (Barden 1987; Gibson et al. 2002; Judge 2008) but persistence of seeds in the soil is still under speculation (Cole and Weltzin 2005). Gibson et al. (2002) states that *Microstegium* can have a transient (short-term) seed bank or a persistent seed bank where seeds remain in the soil for up to five years. In a study examining *Microstegium* control, it was noted that viable seed were still present in the seed bank after attempts were made to prevent new seed bank recruitment for three consecutive years (Judge 2008).

In order to eventually develop better methods to control *Microstegium*, more information about the seed quantities present the seed bank are needed. To date, there are no studies that have looked at *Microstegium* seed in slope, seep wetland seed banks. One objective of this study was to examine the seed bank of the sites used in Chapter 2 through soil sampling at two depths, 0-10 cm (UPPER) and 10-20 cm (LOWER), to determine the percent of *Microstegium* seed present in the seed bank at each site and compare the percent germinated to the percent *Microstegium* cover in the field. In addition, seed bank composition (by family and by plant functional group) was

compared to determine if there were compositional differences between *Microstegium* and non-*Microstegium* sites. Greater percentages of *Microstegium* were expected to germinate from the UPPER soil sample depth of sites with greater percent *Microstegium* cover and it was anticipated that *Microstegium* sites would be dominated by different plant functional groups when compared to non-*Microstegium* sites.

## Materials and Methods

Seed banks were sampled from the same six slope, seep wetland sites that were selected in early May 2008 from Chapter 2: a) downslope from Kepler Road on Mount Nittany accessed via Gate G on PA 45 at Penn State's Russell E. Larson Agricultural Research Center and b) downslope from Interstate-99 on Bald Eagle Mountain accessed via US 220 S, 8-16 km south of Port Matilda, PA at Pennsylvania Game Commission Land, parcel #278. Locale 'a' is hereafter referred to as 'AgRidge' and locale 'b' as 'I99' (Figure 2.1). Within the six sites selected at each locale, three sites had *Microstegium* stems (referred to as '*Microstegium* sites') and three sites had no observable *Microstegium* stems (referred to as 'non-*Microstegium* sites'). All selected sites were surrounded by forested habitat; I99 sites were located on actively managed forested game land covered predominately with deciduous trees while AgRidge sites were located on unmanaged forested land covered with conifers and deciduous trees. All sites were within a 1-7 kilometer walking distance of each other because no roadways were present for vehicular access to individual sites. However, all sites were in close proximity to a roadway with AgRidge sites between 824 to 1050 meters downslope from Kepler Road (Figure 2.2) and I99 sites between 333 to 735 meters downslope of Interstate-99 (Figure 2.3). In addition, I99 sites were 0.5-1 kilometers downslope from an active logging road located along the northern-most perimeter of the game land parcel. Attempts were made to select sites of similar area but access problems including fencing, impenetrable understory brush (*Rosa multiflora* and *Berberis* sp.), and stream crossing problems prevented this in some cases. The distance of each site from the upslope road was estimated using ArcGIS™. A list of all twelve sites is included in Table 2.1.

The same sampling grid established in Chapter 2 was used to collect seed bank samples. Twenty points were selected from a random number table for soil sample collection. A spring-loaded bulb corer (volume capacity approximately 300 cubic cm) was used to obtain two soil samples (taken side-by-side or no further than 5-10 cm apart from each other) from the upper soil

(“UPPER”) located just below the organic layer to approximately 10 cm in depth and two soil samples from the lower soil (“LOWER”) located 10 cm to 20 cm in depth. Between 500 and 700 cubic cm of soil was brought back to the greenhouse in sealed plastic bags from each of the UPPER and LOWER sample depths. Samples were mixed within each bag to break up soil aggregates before potting. Each pot (Kord Regal Standard 10 cm round x 9 cm deep) contained 50% all purpose potting soil (50% organic compost materials, 35% peat humus, and 15% sand/perlite/other) and 50% seed bank sample which resulted in 500-600 cubic cm of soil per pot. There were a total of 80 pots for each site; 2 UPPER and 2 LOWER sample pots per collection point which means there were 40 total pots from the UPPER depth and 40 total pots from the LOWER depth. The two sample pots from each depth were pooled for data analysis to form one sample for the UPPER soil and one sample for the LOWER soil. Thus, each pooled sample contained about 1000 cubic cm of soil. Control pots were created for each site to ensure that seedling emergence occurred due to the seed bank samples, not from contaminated potting soil. Pots were clustered by site on benches (2.4 m x 1.5 m) in the greenhouse and randomly aligned within each site for overhead, automatic misting. Pots were misted for 15 minutes twice a day (once in the morning and once in the evening) to keep soil moist and similar to field conditions. Greenhouse temperatures and photoperiod were moderated by outdoor environmental conditions. Care was taken to prevent over-heating through the use of blowers and ventilation.

The germination method is widely accepted for seed bank studies including those from wetlands (Poiani and Johnson 1988; Baldwin and DeRico 1999; Peterson and Baldwin 2004) and has been used in a study involving *Microstegium* (Gibson et al. 2002). For this research, plants were grown until they could be identified to the plant family taxonomic level and then carefully removed. Plants were not identified to the species taxonomic level because potting space to allow plants to reach flowering stages was limited. The numbers of plants in each family from each pot were counted starting in early June 2008. Plants that were difficult to identify to the family taxonomic level were photographed and pressed in order to obtain assistance with identification. Counting ceased in early July 2008 after no new seeds germinated. The percentage of plants per family out of the the total plants which germinated was calculated per site.

Aboveground plant family composition from the study in Chapter 2 was determined using 5, 1 m<sup>2</sup> quadrats at 5 randomly selected sampling points from the sampling grid during July 2008. Given that not all plants were flowering during the month of July when sampling occurred, it was difficult to identify plants by species. Therefore, composition was categorized by plant family. A visually estimated percentage of plants from each family were identified from each

quadrat in the field with the aid of Newcomb's Wildflower Guide (1977). Any family representing 5% or greater coverage was documented within each quadrat (Hoeltje and Cole 2007). Care was taken to account for plants of various heights. Thus, it was possible for quadrats to have greater than 100% total coverage. Plants that could not be identified by family in the field were documented, collected, and pressed to obtain assistance with identification. Plant composition was also categorized into the following plant functional groups: grass, sedge, rush, forb, fern, moss and woody to determine if *Microstegium* and non-*Microstegium* sites were dominated by different functional groups (Figures 2.19 and 2.20).

### **Data Analysis**

Non-parametric Mantel tests were used to compare the seed bank composition at both depths to aboveground composition and environmental variables collected in Chapter 2. Two matrices were used for each of the four Mantel tests (PC-ORD Version 5, MjM software™, Gleneden Beach, OR). The Mantel tests conducted included: 1) LOWER seed bank composition and environmental variables 2) UPPER seed bank composition and environmental variables 3) LOWER seed bank composition and aboveground plant composition, and 4) UPPER seed bank composition and aboveground plant composition. For each Mantel test, a significant p value (less than or equal to 0.05) was considered significant and indicated similarity between the two matrices (J. Peck, The Pennsylvania State University, personal communication).

Non-parametric multiple response permutation procedures (MRPP) for UPPER, LOWER and aboveground plant family compositions were used to determine if a significant difference existed among the compositions (PC-ORD Version 5, MjM software™, Gleneden Beach, OR). For this test, a p value less than 0.05 indicated that a multivariate compositional difference was evident (J. Peck, The Pennsylvania State University, personal communication). When a significant compositional difference was present, an indicator species analysis was conducted to determine which families were likely responsible for the differences. Plant families with significant p-values less than or equal to 0.05 and corresponding indicator values were reported. Indicator values ranged from 0-100 where 0 represented no indication or inability to identify a specific family with a specific location (UPPER, LOWER or aboveground) and 100 represented perfect indication or complete ability to identify a specific family with a specific location (UPPER, LOWER, and aboveground).



Shannon-Weiner index and evenness values for all sites were calculated based on the number of plants present within each family. A Shannon-Weiner Index value 'H' encompassed both the number of families in the community and the proportions of each family represented at each site. An 'H' value of 4.6 is indicative of greater diversity. Evenness values were also reported. The standard range for evenness values is from 0 to 1 where a value of 1 indicates equal numbers within each family are present.

## **Results**

### **Mantel Tests**

Mantel tests were used to determine if positive associations existed among the plant composition which germinated from each seed bank depth and the environmental variables present at each site. There were no significant associations between environmental variables at each site and the composition of the seed banks at either depth (UPPER,  $p=0.1879$ ; LOWER,  $p=0.1717$ ). Significant p-values were reported from Mantel tests comparing aboveground herbs with either the UPPER ( $p=0.0465$ ) or LOWER ( $p=0.0498$ ) seed bank composition. This indicated similarity between the UPPER seed bank and aboveground family composition and similarity between the LOWER seed bank and aboveground family composition.

### **Multiple-Response Permutation Procedures (MRPP) and Indicator Species Analyses (ISA)**

To determine if the UPPER and LOWER seed bank compositions were different from each other, a MRPP was calculated for all sites from both locales. The p-value from this analysis was 0.9982, thus demonstrating compositional similarity in depth. In addition, an MRPP was performed for all sites from both locales using the UPPER, LOWER and aboveground compositions and resulted in a significant p-value of 0.0001 which indicated that the aboveground, UPPER and LOWER seed bank compositions were different from each other in some way. An Indicator Species Analysis of the UPPER, LOWER, and aboveground communities revealed significant p-values and indicator values (IV) for the following families: Asteraceae (IV=92,  $p=0.002$ ), Violaceae (IV=60,  $p=0.0072$ ), Rosaceae (IV=65,  $p=0.0022$ ),

Chenopodiaceae (IV=47,  $p=0.0220$ ), Brassicaceae (IV=44,  $p=0.0110$ ), Onocleaceae (IV=41,  $p=0.0072$ ), and Balsaminaceae (IV=39,  $p=0.0416$ ).

### Shannon-Weiner Index (H) and Evenness

#### AgRidge

The numbers of plants present in each family were recorded to calculate a percentage (Appendix C) and to obtain Shannon-Weiner Index and evenness values. H values and evenness values were not very different between *Microstegium* and non-*Microstegium* sites (Figures 3.1 and 3.2). Generally, the UPPER and LOWER seed bank H values for each site were also not different from each other with the exception of two sites, Lost (a *Microstegium* site) and Turkey (a non-*Microstegium* site). In this case, the LOWER seed bank for site Lost had a greater H value, indicating greater diversity than its corresponding UPPER seed bank. The reverse was true for site Turkey where the UPPER seed bank had a greater H value indicating greater diversity when compared to the LOWER seed bank. There were differences for UPPER and LOWER seed bank evenness values for two sites with *Microstegium*, Bearsite and Lost. For site Lost, the UPPER seed bank was more even than the LOWER and the opposite was true for site Bearsite.

#### I99

The numbers of plants present in each family were recorded to calculate a percentage (Appendix C) and to obtain Shannon-Weiner Index and evenness values. Again, there was no clear pattern between *Microstegium* and non-*Microstegium* sites for values of H or evenness values (Figures 3.3 and 3.4). Within-site and among site H values for the UPPER and LOWER seed banks were not different for sites TW, NG1, GR2, and NG2. Sites GR1 and Nine96 did not demonstrate any within-site significance for the UPPER and LOWER seed banks. However, the UPPER seed bank from these two sites was different from sites TW, NG1, GR2, and NG2. Within-site UPPER and LOWER seed bank evenness values were very similar for all sites. Site GR1 demonstrated different UPPER and LOWER seed bank evenness values when compared to the five other sites.

### Seed Bank Plant Composition by Functional Group

The percent of plants from each family were further placed into a plant functional group in order to obtain a better comparison of UPPER and LOWER compositions (Appendix E). For *Microstegium* sites at the AgRidge locale (Figures 3.5 and 3.6), sedges dominated in all sites for the UPPER and LOWER seed bank with the exception of one site (Lost) which had a greater percentage of forbs than sedges in the LOWER seed bank. Non-*Microstegium* sites at the AgRidge were all dominated by forbs. *Microstegium* sites at the I99 locale were dominated by grasses and non-*Microstegium* sites at I99 were either dominated by forbs or grasses (Figures 3.7 and 3.8).

### Percent *Microstegium* in the Seed Bank and *Microstegium* Cover

The percentages of *Microstegium* stems that germinated (# of *Microstegium* stems/total number of stems which germinated from all families) at both depths, UPPER and LOWER, are reported in Table 3.1. *Microstegium* cover per site is reported in Table 2.1. *Microstegium* was present in the UPPER seed bank for all sites where it was present aboveground. It was present in the LOWER seed bank for all sites where it was present aboveground with the exception of site Lost at the AgRidge locale. Interestingly, *Microstegium* seed also germinated from sites where it was not present aboveground in the I99 locale including site NG2 (present in LOWER seed bank) and site Nine96 (present in the UPPER seed bank). The percent of *Microstegium* present in the seed bank from all *Microstegium* sites ranged from 1.84-25.13% in the UPPER seed bank and 0.81-21.52% in the LOWER seed bank.

### Discussion

Generally, greater percentages of *Microstegium* seed germinated from the UPPER seed bank samples of sites where *Microstegium* plants were present aboveground. When comparing the percent *Microstegium* cover in many sites (sometimes exceeding 60% of the total site area) from Chapter 2, the percentages of *Microstegium* evident from the seed bank study are low particularly since the number of stems per surface area is typically quite high in the field (Romanello, personal observation). There are a few reasons that might explain why the resulting

percentage of *Microstegium* seed which germinated in the greenhouse was low. First, greater number of stems is not indicative of greater numbers of seed. Also, *Microstegium* can germinate in April in Central Pennsylvania and it is very likely that the seed bank was sampled too late in the season (late May 2008). There is also a possibility that the misting regime used in the greenhouse during this study was not suitable for *Microstegium* germination although unlikely since growth occurs in varying soil water contents (Romanello and Touchette, unpublished data). If a similar study were conducted again in Central Pennsylvania, the seed bank must be sampled immediately after snow-melt in the spring. Sampling the seed bank after seed drop in autumn is also possible. However, seed need to be overwintered (Cheplick 2008).

Since the germination requirements for many plants remain unknown, the watering regime in the greenhouse may have been unsuitable for other species which could have affected the overall UPPER and LOWER seed bank compositions. There are several accounts from seed bank identification studies which indicate that different methods produce significantly different species compositions. For example, Brown (1992) found that seedling emergence and extraction methods did not produce comparable results. Ter Heerdt et al. (1999) concluded that different soil water treatments produced significantly different compositions. It was evident from this study that the UPPER and LOWER seed bank sample compositions were similar to one another, as expected. However, when the UPPER, LOWER, and aboveground plant family compositions were compared, a significant difference ( $p=0.0012$ ) was evident. One probable explanation for this may be that the conditions in the greenhouse spurred the germination of hardy, weed seedlings which do not require such narrowly defined germination requirements. It is also possible that the different sampling time for the seed bank and aboveground composition (late May 2008 and July 2008, respectively) affected the overall compositions. The large contribution of the Brassicaceae and Chenopodiaceae family to the greenhouse composition would be expected from soils sampled during early spring since many of these species are clearly evident in the field during this time. The large contribution of ferns (Onocleaceae) to the field composition and not the seed bank composition is also somewhat intuitive considering that the high light and low humidity conditions in the greenhouse were probably not conducive for fern growth. Past research indicates that aboveground vegetation is not necessarily reflective of seed bank composition in wetlands (Leck 1989). Nevertheless, it is not possible at this point to determine that the seedling germination method used in this study was completely unsuitable. A combination of seed extraction and seed emergence methods would have to be employed to determine whether the greenhouse method was suitable.

Examination of the UPPER and LOWER seed bank communities by functional group revealed similarities between the compositions at both depths. Since all I99 sites had *Microstegium* cover greater than 50%, grasses dominated, as expected. *Microstegium* cover at AgRidge sites was 73%, 5%, and 10% (sites MvLand, Bearsite, and Lost, respectively) which was more variable compared to cover at the I99 sites which was 62%, 98%, and 73% (sites TW, NG1, and GR2, respectively). Greater percentages of sedges in the *Microstegium* AgRidge sites could be contributed to low percentages of grasses. Non-*Microstegium* sites at the AgRidge were all dominated by forbs. Future studies should determine if there is a relationship between grass/sedge dominated communities versus forb dominated communities and whether previous land use (such as logging) is associated with specific floral communities.

It was surprising that *Microstegium* seed was noted in the seed bank of two sites (one UPPER seed bank and one LOWER seed bank) at the I99 locale which did not have *Microstegium* present aboveground. The presence of *Microstegium* seed in the LOWER seed bank of one I99 site ('NG2') could have been due to contamination because it seems highly unlikely for a site without *Microstegium* to contain seed at a depth of 10-20 cm. The presence of *Microstegium* in the UPPER seed bank at another I99 site ('Nine96') could also be the result of contamination but is doubtful since no other sites were sampled or potted that day and clean digging tools were used. The only factor that differed during that day compared to the sampling of other sites on different days was the weather; site Nine96 was sampled in the rain. Since site Nine96 was also in close proximity to an intermittent stream, seed travel via water into this site is a possibility.

Downward vertical movement of seed in a soil profile to a depth of 0-10 cm can occur by earthworms, small burrowing animals, or insects. Seeds located at a depth of 10-20 cm are less likely to be moved by organisms since oxygen availability in the soil is lower (or anoxic) at that depth. Sampling a seed bank at a soil depth of 10-20 cm would be unreasonable in many habitats because it is unlikely that these seeds would have the opportunity to germinate. Seed viability at these depths is also unknown. However, slope, seep wetlands are prone to disturbance and surface scouring due to overland flow and erosion. Flood scouring is thought to promote the growth of weedy seeds in wetlands (Zedler and Kercher 2004). Water levels can also interact to determine the herbaceous community (Mitsch and Gosselink 2000). A small percentage of viable seed from a LOWER seed bank sample may be important to note in this case particularly since other studies have documented that flood scouring encourages *Microstegium* growth (Barden 1987). Simply because seed is present does not mean it is viable or will germinate. However, considering that

slope, seep wetlands are prone to scouring, it is possible for disturbance events to bring seed closer to the surface of the soil to germinate.

The presence of *Microstegium* seed in the soil at the 10-20 cm depth could be due to compaction over time or percolation through the soil as other plant roots decay and leave crevices for seeds (Leck 1989). The I99 locale had greater percentages present in the LOWER seed bank for *Microstegium* sites compared to the LOWER seed banks of the AgRidge locale. This may indicate that *Microstegium* has been present in the I99 locale longer compared to the AgRidge locale. It is possible that the active logging that occurs at the I99 locale has contributed to the compaction of seeds over time. When this study was conducted, logging took place from late June 2008 to the end of October 2008. During this time period, heavy machinery was operated in the forest interior and logs were dragged through the forest. In a few open canopy sites, tire tracks were noted. For this study it is unlikely that the logging operations interfered with data collection. However, future studies on *Microstegium* or other invasive plants in this area should consider the impact of logging operations on seed dispersal.

## Conclusions

Slope, seep wetlands are thought to be areas of high native plant diversity yet their floral composition, both aboveground and in seed banks is severely understudied. Based on the UPPER, LOWER, and aboveground analysis of plant functional groups, forbs dominated in over half of the non-*Microstegium* sites. Results from this study indicated that *Microstegium* seed germinated from seed bank samples of sites where it was present aboveground and generally in greater percentages from the 0-10 cm depth. The percentage of *Microstegium* that germinated from seed bank samples seemed low in comparison to percent *Microstegium* cover in the field. It is possible that the timing of seed bank sampling was likely responsible for this outcome. However, the low percentage of *Microstegium* seed in the seed bank (particularly in the LOWER samples) could also indicate that *Microstegium* was a recent arrival to these sites and that not enough time has lapsed to form a persistent seed bank. Future research should examine native plant composition in slope, seep wetland seed banks to determine whether specific plant community structures are associated with less invasibility. Additional greenhouse seed bank studies could also determine whether floral composition in these wetlands is determined by the number of seeds in the soil or whether environmental conditions (such as soil moisture or light) have a greater effect on

composition. This pilot seed bank study provokes many questions about the factors which contribute to plant community structure in *Microstegium* and non-*Microstegium* slope, seep wetlands and provides an opportunity to expand upon habitat invasibility and floristic composition.

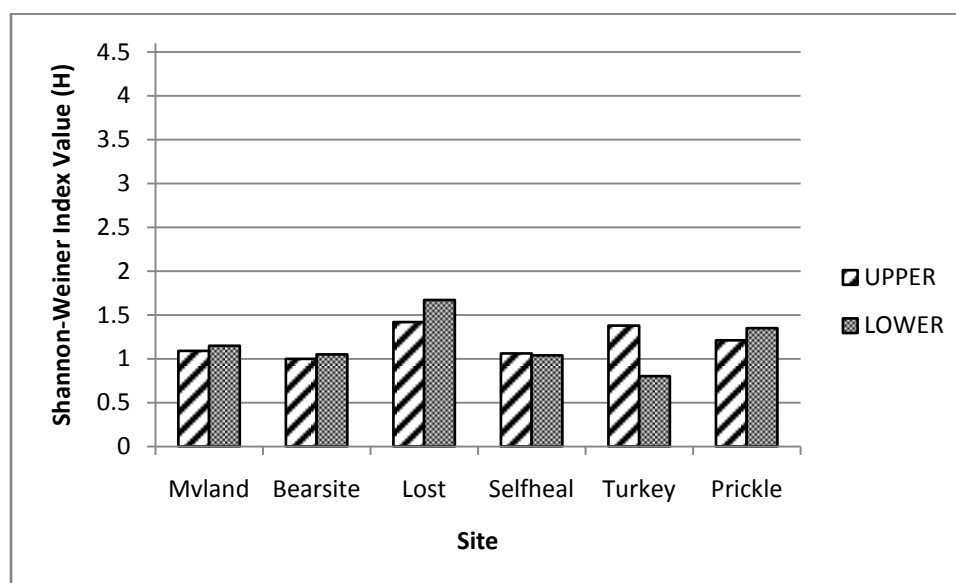


Figure 3.1: Shannon-Weiner Index values for UPPER and LOWER seed banks from the AgRidge locale. *Microstegium* was present in sites Mvland, Bearsite, and Lost. *Microstegium* was absent in sites Selfheal, Turkey and Prickle.

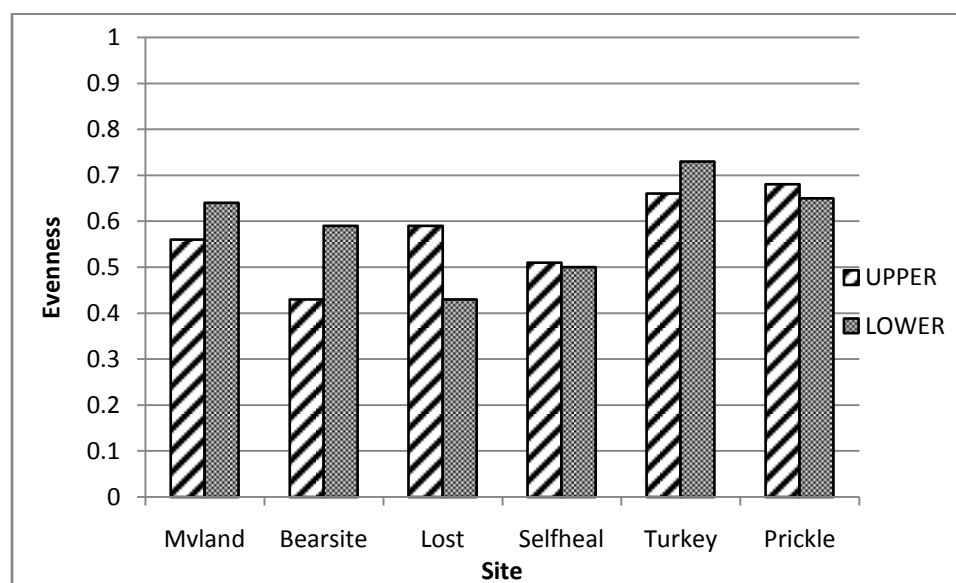


Figure 3.2: Evenness values for UPPER and LOWER seed banks from the AgRidge locale. *Microstegium* was present in sites Mvland, Bearsite, and Lost. *Microstegium* was absent in sites Selfheal, Turkey and Prickle.



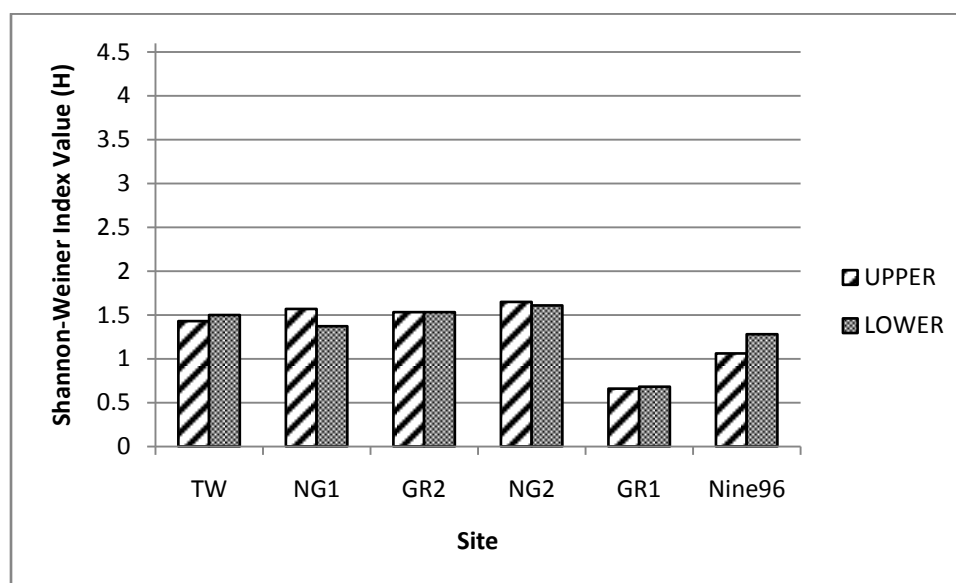


Figure 3.3: Shannon-Weiner Index values for UPPER and LOWER seed banks from the I99 locale. *Microstegium* was present in sites TW, NG1, and GR2. *Microstegium* was absent in sites NG2, GR1, and Nine96.

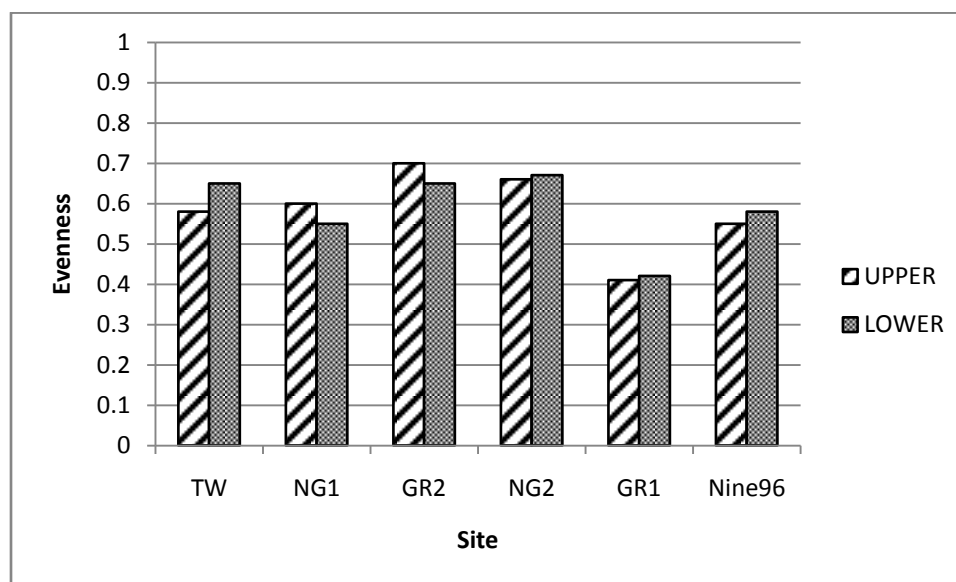


Figure 3.4: Evenness values for UPPER and LOWER seed banks from the I99 locale. *Microstegium* was present in sites TW, NG1, and GR2. *Microstegium* was absent in sites NG2, GR1, and Nine96.

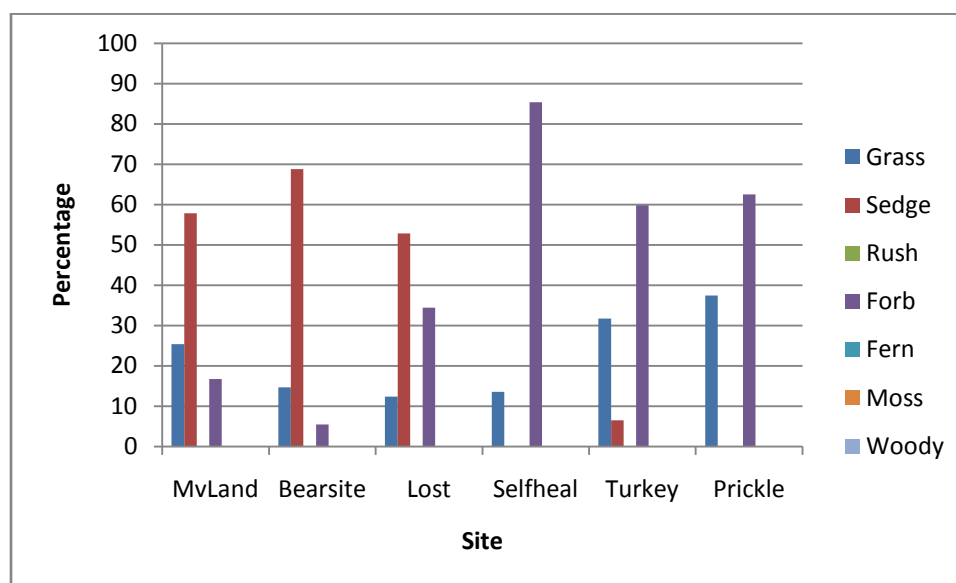


Figure 3.5: Plant functional groups from the UPPER seed bank community for all sites at the AgRidge locale. *Microstegium* was present in sites Mvland, Bearsite, and Lost. *Microstegium* was absent in sites Selfheal, Turkey and Prickle.

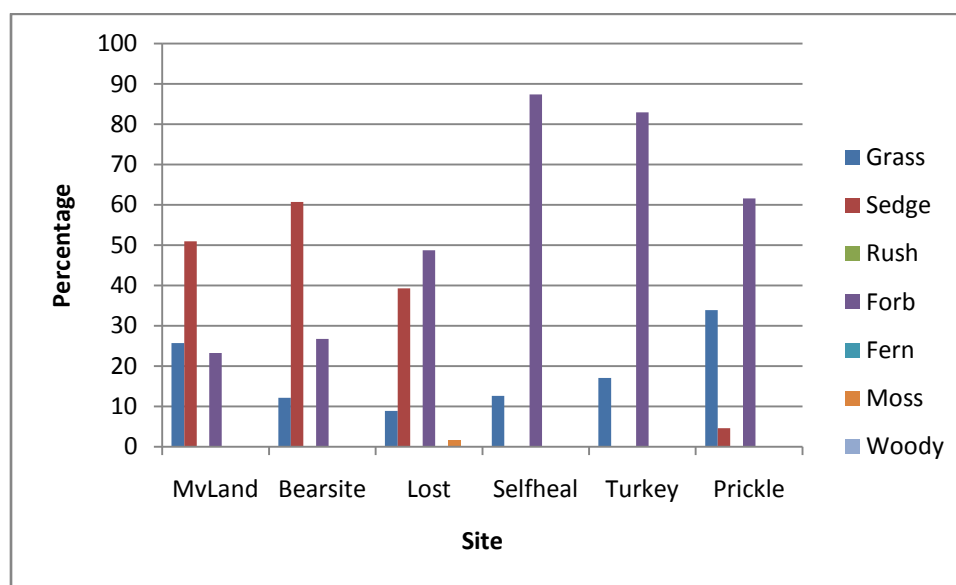


Figure 3.6: Plant functional groups from the LOWER seed bank community for all sites at the AgRidge locale. *Microstegium* was present in sites Mvland, Bearsite, and Lost. *Microstegium* was absent in sites Selfheal, Turkey and Prickle.

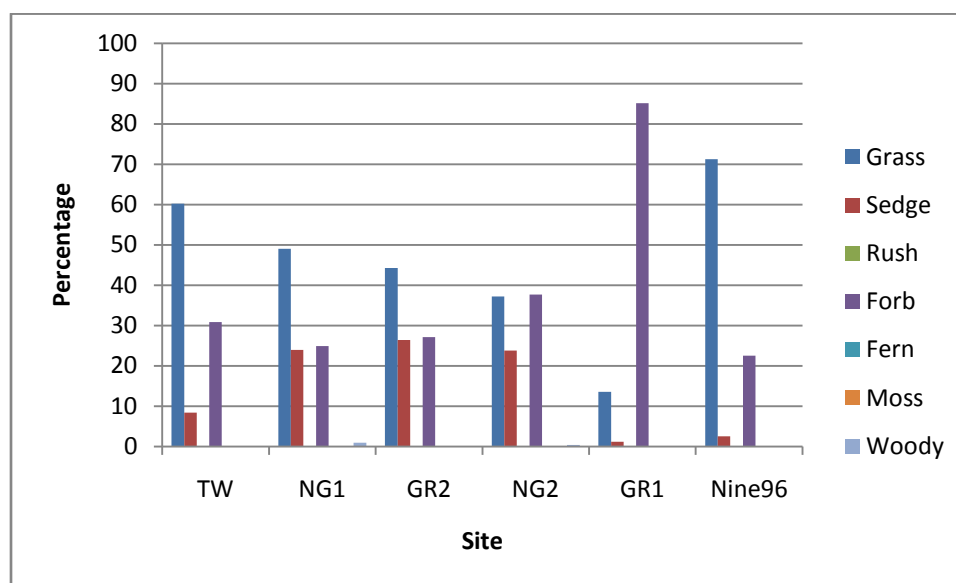


Figure 3.7: Plant functional groups from the UPPER seed bank community for all sites at the I99 locale. *Microstegium* was present in sites TW, NG1, and GR2. *Microstegium* was absent in sites NG2, GR1, and Nine96.

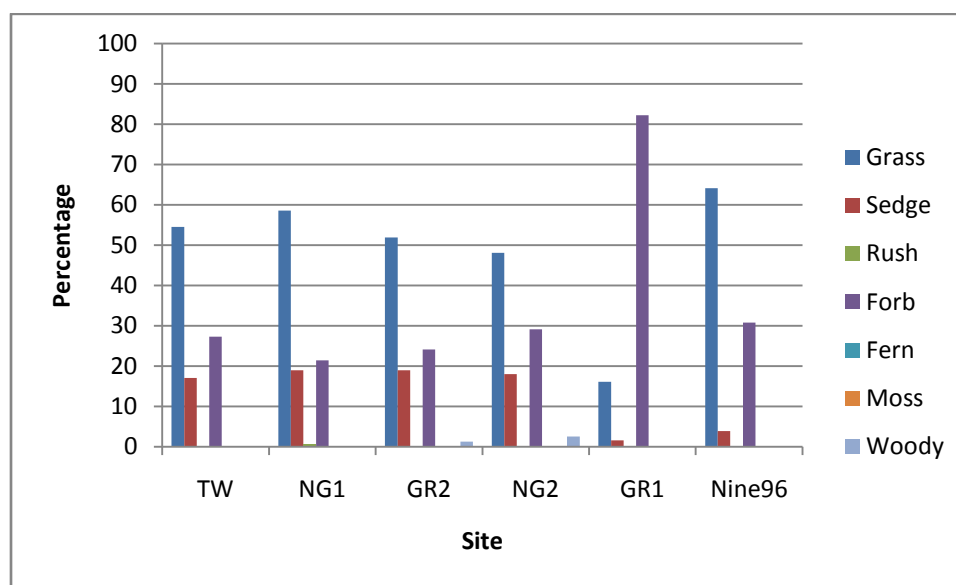


Figure 3.8: Plant functional groups from the LOWER seed bank community for all sites at the I99 locale. *Microstegium* was present in sites TW, NG1, and GR2. *Microstegium* was absent in sites NG2, GR1, and Nine96.

Table 3.1: Percent of *Microstegium* plants that germinated from UPPER (0-10 cm) and LOWER (10-20 cm) seed bank samples from all sites at both AgRidge and I99 locales. *Microstegium* sites are shaded in gray and non-*Microstegium* sites are not shaded.

Locale	Site	UPPER seed bank (%)	LOWER seed bank (%)
AgRidge	Mvland	8.93	5.94
	Bearsite	1.86	0.81
	Lost	1.84	0
	Selfheal	0	0
	Turkey	0	0
	Prickle	0	0
I99	TW	25.13	20.45
	NG1	7.14	2.86
	GR2	15.0	21.52
	NG2	0	1.27
	GR1	0	0
	Nine96	6.25	0

## Literature Cited

- Baker, H. G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics* 5:1-24.
- Baldwin, A. H. and E. F. Derico. 1999. The seed bank of a restored tidal freshwater marsh in Washington, DC. *Urban Ecosystems* 3:5-20.
- Barden, L. S. 1987. Invasion of *Microstegium vimineum*, an exotic, annual, shade-tolerant C<sub>4</sub> grass, into a North Carolina floodplain. *American Midland Naturalist* 118:40-45.
- Belote, R. T., J. F. Weltzin, and R. J. Norby. 2003. Response of an understory plant community to elevated [CO<sub>2</sub>] depends on differential responses of dominant invasive species and is mediated by soil water availability. *New Phytologist* 161:827-835.
- Belote, R. T. and J. F. Weltzin. 2006. Interactions between two co-dominant, invasive plants in the understory of a temperate deciduous forest. *Biological Invasions* 8:1629-1641.
- Brown, D. 1992. Estimating the composition of a forest seed bank: a comparison of the seed extraction and seedling emergence methods. *Canadian Journal of Botany* 70:1603-1612.
- Cheplick, G. P. 2006. A modular approach to biomass allocation in an invasive annual (*Microstegium vimineum*; Poaceae). *American Journal of Botany* 93: 539-545.
- Cheplick, G. P. 2008. Growth trajectories and size-dependent reproduction in the highly invasive grass *Microstegium vimineum*. *Biological Invasions* 10:761-770.
- Cole, P. G. and J. F. Weltzin. 2005. Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. *Biological Invasions* 7:477-488.

- Ehrenfeld, J. G. 1999. A rhizomatous, perennial form of *Microstegium vimineum* (Trin.) A. Camus in New Jersey. *Journal of the Torrey Botanical Society* 126:352-358.
- Fairbrothers, D. E. and J. R. Gray. 1972. *Microstegium vimineum* (Trin.) A. Camus (Gramineae) in the United States. *Bulletin of the Torrey Botanical Club* 99:97-100.
- Gibson, D. J., G. Spyreas, and J. Benedict. 2002. Life history of *Microstegium vimineum* (Poaceae), an invasive grass in southern Illinois. *Journal of the Torrey Botanical Society* 129:207-219.
- Hoeltje, S. M. and C. A. Cole. 2007. Losing function through wetland mitigation in central Pennsylvania, USA. *Environmental Management* 39:385-402.
- Horton, J. L. and H. S. Neufeld. 1998. Photosynthetic responses of *Microstegium vimineum* (Trin.) A. Camus, a shade-tolerant, C<sub>4</sub> grass, to variable light environments. *Oecologia* 114:11-19.
- Hunt, D. M. and R. E. Zaremba. 1992. The northeastern spread of *Microstegium vimineum* (Poaceae) into New York and adjacent states. *Rhodora* 94:167-170.
- Judge, C. A., J. C. Neal, and T. H. Shear. 2008. Japanese stiltgrass (*Microstegium vimineum*) management for restoration of native plant communities. *Invasive Plant Science and Management* 1:111-119.
- Leck, M. A. 1989. Wetland Seed Banks. Pages 283-305 *In* M. A. Leck, V. T. Parker and R. L. Simpson, editors. *Ecology of Soil Seed Banks*. Academic Press, Inc., New York.
- Leicht, S. S., J. A. Silander Jr., and K. Greenwood. 2005. Assessing the competitive ability of japanese stiltgrass, *Microstegium vimineum* (Trin.) A. Camus. *Journal of the Torrey Botanical Society* 132:573-580.

- Mitsch, W. J. and J. G. Gosselink. 2000. Wetlands, Third Edition. John Wiley & Sons, Inc., New York, NY. 920 pp.
- Newcomb, L. 1977. Newcomb's Wildflower Guide. Little, Brown and Company, Boston, MA, 490 pp.
- Oswalt, C. M., S. N. Oswalt, and W. K. Clatterbuck. 2007. 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.
- Peterson, J. E. and A. H. Baldwin. 2004. Seedling emergence from seed banks of tidal freshwater wetlands: response to inundation and sedimentation. Aquatic Botany 78:243-254.
- Poiani, K. A. and W. C. Johnson. 1988. Evaluation of the emergence method in estimating seed bank composition of prairie wetlands. Aquatic Botany 32:91-97.
- Redman, D. E. 1995. Distribution and habitat types for Nepal *Microstegium* [*Microstegium vimineum* (Trin.) Camus] in Maryland and the District of Columbia. Castanea 60:270-275.
- Tennessee Exotic Pest Plant Council. Tennessee Exotic Plant Management Manual. 2007. (<http://www.tneppc.org/>, 12 December 2007).
- Ter Heerdt, G. N. J., A. Schutter, and J. P. Bakker. 1999. The effect of water supply on seed-bank analysis using the seedling-emergence method. Functional ecology 13:428-430.
- US Department of Agriculture, Natural Resources Conservation Service. 2007. National Plant Data Center, Baton Rouge, LA 70874-4490 USA. The PLANTS Database (<http://plants.usda.gov>, 12 December 2007).

- Williams, L. D. 1998. Factors affecting growth and reproduction in the invasive grass *Microstegium vimineum*. M.S. thesis, Appalachian State University, Boone, NC.
- Winter, K., M. R. Schmitt, and G. E. Edwards. 1982. *Microstegium vimineum*, a shade adapted C<sub>4</sub> grass. Plant Science Letters 24:311-318.
- Zedler, J. B. and S. Kercher. 2007. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. Critical Reviews in Plant Sciences 23: 431-452.



## Chapter 4

## **Seed Trap Study: Water as a Vector for *Microstegium vimineum* Seed Dispersal**

### **Abstract**

The invasive grass, *Microstegium vimineum*, has recently been found growing extensively in many slope, seep wetlands of the Centre County region of Central Pennsylvania. At this time is not possible to determine exactly how or when seed arrived into these wetlands but it is speculated that water run-off traveling downslope from the newly constructed Port Matilda, PA portion of Interstate-99 (I-99) may have transported seed. For this study, seed traps (n=11) were used to collect water outflow after a rain event greater than 0.5 mm from drain pipes located along a logging road downslope from I-99. The results of this presence/absence study conducted from September 24 through November 3, 2008, determined that *Microstegium* seed travels via water. Seed were present at least once over all collection periods in 55% of traps. From this study alone, patterns among the presence of *M. vimineum* seed in traps and the distance from I-99, presence of nearby aboveground *M. vimineum* (0.5 m or less from end of pipes), and the amount of rainfall were not evident. Future studies of *M. vimineum* seed dispersal via water would be improved through an analysis of factors influencing propagule pressure including locations of upslope source populations of *M. vimineum* and pathways of water flow downslope from I99.

## Introduction

*Microstegium vimineum* (Trin.) A. Camus, commonly known as Japanese stiltgrass and hereafter referred to as *Microstegium*, is a prominent invasive grass throughout the eastern United States, much of the Northeast, and a few Midwestern states (United States Department of Agriculture 2008). *Microstegium* grows in a variety of habitats including lawns, thickets, fields, and forests, wet areas like wetlands, riparian zones, stream or river banks, and floodplains, and areas of disturbance such as roadsides, ditches, train tracks, logging roads, and utility passages (Fairbrothers and Gray 1972; Hunt and Zaremba 1992; Redman 1995). This species exhibits phenotypic plasticity and has many characteristics of an ‘ideal weed’ (Baker 1974). *Microstegium* can grow under a broad range of irradiance levels (Winter et al. 1982; Horton and Neufeld 1998), produces many seeds that are dispersed by non-specific means (Gibson et al. 2002), and can undergo self-pollination or cross-pollination (Williams 1998; Ehrenfeld 1999; Gibson et al. 2002; Cheplick 2006). There is also evidence that increased soil moisture promotes greater biomass production (Belote et al. 2003; Romanello and Touchette, unpublished data). *Microstegium* dominance has been inversely correlated with decreased plant diversity (Belote 2003). This grass is often a superior competitor (Belote and Weltzin 2006; Leicht 2005), known to replace other vegetation within three to five years of arrival (Tennessee Exotic Pest Plant Council 2007), and thought to be a threat to forest tree regeneration (Oswalt et al. 2007).

The mechanisms at work behind the spread of *Microstegium* seeds remain relatively unknown and most accounts of seed dispersal are anecdotal. *Microstegium* has not been found growing in many suitable habitats where one would expect to find it (Redman 1995; Christen and Matlack 2009) and limited seed dispersal could be one reason why this is evident. Very few studies have examined the extent in which organisms spread *Microstegium* seed (Cole and Weltzin 2005). Ground disturbance of the subcanopy by white tailed deer, *Odocoileus virginianus*, in New Jersey was documented by Baiser et al. (2008) and thought to contribute to *Microstegium* growth. Few animals, if any, are known to eat this species (US Department of Agriculture 2008). Deer also do not eat *Microstegium* but can create canopy openness when other plants in the vicinity of *Microstegium* stands are eaten (Baiser et al. 2008).

Disturbance is now considered a crucial factor which contributes to *Microstegium* distribution and patch size (Barden 1987; Cole and Weltzin 2004; Peskin 2005; Oswalt et al. 2007; Christen and Matlack 2009; Marshall and Buckley 2008; Baiser et al. 2008). There are many different circumstances which could be classified as a disturbance such as flood events,

mowing, or road grading. There are numerous personal observations and anecdotal accounts from field studies indicating that seed dispersal of *Microstegium* was encouraged by disturbance. Barden (1987) noted that luxuriant stands of *Microstegium* could be found growing in sewer line passages which were mowed yearly. *Microstegium* biomass increased in one disturbance field study when canopy cover decreased (Oswalt et al. 2007). Marshall and Buckley (2008) conducted a study to determine if mineral soil disturbance and litter removal influenced the growth of individual *Microstegium* plants. Interestingly, they found no significant differences between disturbance treatments and growth of individuals. Instead, disturbance in this study encouraged seed spread. One reason slope, seep wetlands may be susceptible to *Microstegium* colonization is because their hydrology, the driving force behind the functions of the wetland, is often pulsing. In fact, the cyclical wet and dry cycles occurring in slope, seep wetlands could even be considered a disturbance. Wetlands are also considered sink landscapes prone to invasion because disturbances can encourage the germination of plants considered weeds (Zedler and Kercher 2004).

In the Centre County region of Central Pennsylvania, located within the Ridge and Valley Physiographic Province, *Microstegium* is absent from many suitable locations. However, it has recently been found in slope, seep wetlands of this area (C.A. Cole, The Pennsylvania State University, personal observation) exhibiting its typical weedy behavior and growing as tall as 90 to 100 cm (Romanello, personal observation) which is considerably taller than other reports in the literature (Ehrenfeld 1999, Belote et al. 2003). Due to the topography of the region, seep wetlands could easily receive *Microstegium* seed via water run-off. In addition, water from these wetlands continues to flow downslope and occasionally underground, thereby passively transporting seed.

Seed dispersal studies are needed for the development of better management practices (Cole and Weltzin 2004). *Microstegium* seed is thought to travel via water into wetlands (Mehrhoff 2000; Christen and Matlack 2009) and via roadways (Mehrhoff 2000; Peskin 2005; Christen and Matlack 2009). The unique landscape of the Ridge and Valley Province of Central Pennsylvania and the existence of seep, slope wetlands within this landscape present a unique opportunity to study seed dispersal through water run-off from upslope. Seed dispersal by water was addressed in this study through the use of hand-made seed traps located at downslope drain pipes from the newly constructed Interstate-99 (I-99). The objective of the study was to determine whether *Microstegium* seeds travel via water. It was hypothesized that *Microstegium* seed will be present in seed traps after a rain event.

## Materials and Methods

Direct access to drain pipes immediately downslope from I-99 was not possible due to restricted access, therefore, pipes underneath an active, one-lane logging road downslope of I99, accessed via Pennsylvania Game Commission Land, parcel #278 (Figure 4.1), were deemed suitable due to ease of accessibility and close proximity to the newly constructed highway. Eleven drainage pipes (0.5 m in diameter) were randomly selected from a total of 14 possible drain pipes. Drain pipes were located between 362 to 736 meters from I-99 (Figure 4.1; Table 4.1). All drain pipes extended just beyond the width of the one-lane logging road. Of the eleven drainage pipes selected, *Microstegium* was present aboveground on both sides of five drain pipes no further than 0.5 meters away from the end of these pipes.

Seed traps (Figure 4.2) were created using 10 cm diameter FLEX-Drain™ (Cleveland Tubing, Inc., Cleveland, TN) expandable black plastic drain pipe and Sediment Shield™ (NDS™, Lindsay, CA) sediment socks. Each trap consisted of a 61 cm pipe with a 91 cm sediment sock attached to one end. Sediment socks were held in place about halfway along the trap with rubber bands. Sediment socks were ideal for this study because the fabric was perforated which allowed water to pass but was still capable of snagging *Microstegium* seed, thus preventing it from leaving the trap. About 45 cm of sock was permitted to hang off of the trap in order to allow water to dissipate during high rain events. The sediment sock was tied in a knot at the loose end. A trap of this size was suitable for this study because it did not completely block water, leaves, organisms, and other debris from exiting the drainage pipes.

Since most of the eleven drain pipes used in this study were nearly flush with the ground, one trap was placed against the lip of each pipe and covered with nearby rocks to both secure and camouflage traps (Figure 4.3). Each trap was left in the field until a rain event (0.5 mm or greater) occurred. After a rain event, all traps were collected and replaced immediately by another clean trap. In order to determine if *Microstegium* seed were present in each trap, sediment socks were carefully removed and rinsed in a basin. Larger debris was removed by hand and all smaller sediments (including seeds) were sieved using a #35, 500 µm, 0.0197 inch opening USA standard macroinvertebrate sieve (Newark Wire Cloth Company, Clifton, NJ). The presence or absence of *Microstegium* seed was noted for each trap. Seeds collected in each trap were compared to *Microstegium* seed collected in the Fall of 2007 from plants growing downslope from the logging road using a dissecting microscope to verify correct identification.

## Results

The distance of each drainpipe (and thus seed trap) from I-99 was estimated using ArcGIS™ (Table 4.1). At the end of the seed trap study, the presence of *Microstegium* seed in each trap for each collection period was totaled (Table 4.2). For all collection periods and all traps, *Microstegium* seed was present 31% of the time. The percent of traps containing *Microstegium* seed at least once during all five collection periods was 55%. When *Microstegium* plants were present aboveground 0.5 meters or less from both ends of the selected drain pipes (n=5), *Microstegium* seed was present in traps at least once 60% of the time and present 48% of the time when all collection periods were totaled. When *Microstegium* plants were not present aboveground 0.5 meters or less from both ends of selected drain pipes (n=6), *Microstegium* seed was present in these traps at least once 50% of the time and present 17% of the time when all collection periods were totaled.

## Discussion

The results of this study determined that *Microstegium* seed travels via water and confirms the anecdotal accounts of others (Mehrhoff 2000; Christen and Matlack 2009). Although the purpose of this study was simply to determine the presence or absence of *Microstegium* seed in seed traps after rainfall events, it was suspected that more traps would contain seed when greater rainfall events occurred due to greater water velocity and downward flow. Interestingly, there is not a clear connection for the amount of rainfall between collection periods and the presence of *Microstegium* seed found in the seed traps from this study alone. Populations of *Microstegium* upslope from the drain pipes should be documented as well because upslope water conduits could funnel *Microstegium* seed through these pipes. Future studies examining these variables and the quantity of *Microstegium* seed transported per rain event would greatly enhance our understanding of *Microstegium* seed dispersal. There was also not a clear relationship between distance of each seed trap from I-99 and the presence of *Microstegium* seed because seed was present in traps that were close to I-99 and in those that were further from I-99. Since the location of the drain pipes were not directly off of the newly constructed I-99 it cannot be confirmed that seed were traveling into the surrounding forest directly from the highway.

Therefore, seed origin could be from I-99 or some location downslope of I-99. It is the distance of the upslope source population from the receiving end of each pipe that must be determined to specifically examine correlations between this distance and the presence and/or abundance of *Microstegium* seed in each trap. It was originally suspected that *Microstegium* seed would not be present in traps when *Microstegium* were not present aboveground in close proximity (0.5 meters or less) to pipes. The results indicate that the absence of *Microstegium* plants close to the pipe did not necessarily indicate that seed would be absent from the trap. This illustrates, again, that there were other factors such as water velocity, landscape contours and pipe proximity to *Microstegium* source populations that influenced the outcomes of this study.

It has been confirmed that roadsides transport *Microstegium* seed (Mehrhoff 2000; Peskin 2005; Christen and Matlack 2009) but the actual distance traveled per seed is likely low unless aided by another force such as water movement (Peskin 2005). Slope, seep wetlands are greatly influenced by seepage from belowground and overland flow. Therefore, it is possible for these wetlands to receive seed from upslope. Some seed may remain on site to germinate and establish future populations. It is also possible for these wetlands to export seed. In the future, a landscape-scale dispersal study examining transport pathways of the seed via water would greatly enhance our understanding of the connections between slope, seep wetlands and *Microstegium* seed dispersal.

## Conclusions

This research provides an important insight pertaining to *Microstegium* seed dispersal; it was experimentally determined through this seed trap study that *Microstegium* seed travels via water. Future *Microstegium* via water dispersal studies must address propagule pressure including factors such as landscape contours and water conduits. It is also important to understand how the locations of upslope source populations of *Microstegium* feed downslope populations of *Microstegium*. The percent *Microstegium* cover and seed produced at upslope populations may in fact determine the percent cover in downslope populations. Overall, this research presents an opportunity to expand upon seed dispersal via water mechanisms and illustrates the need to develop a more comprehensive study before information can be appropriately used by land managers seeking to prevent or control *Microstegium*.

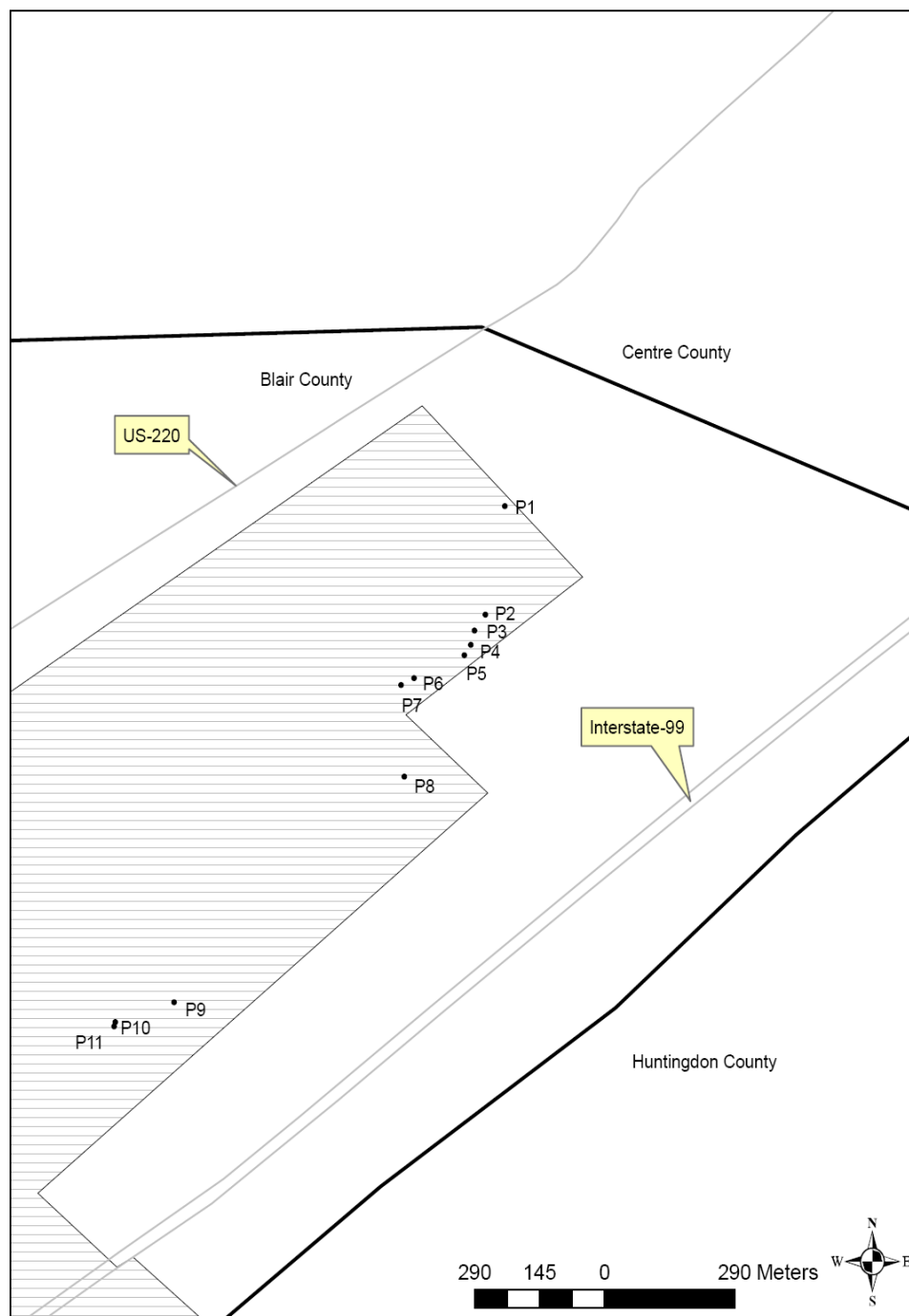


Figure 4.1: Location of each drain pipe and distance in meters from the newly constructed I-99. Drain pipe numbers also correspond with seed trap number. Darkest lines represent county boundaries. Hatched area represents Pennsylvania Game Commission Land, parcel #278.



Figure 4.2: Each seed trap consisted of 10 cm of plastic pipe and 91 cm of drain sock secured with 2 rubber bands. Approximately 45 cm of sock was permitted to hang off of the trap and was tied at the loose end.



Figure 4.3: Drain pipe (0.5 meter diameter) with a seed trap flush against the lip and disguised with rocks.



Table 4.1: Drainpipe distance from I-99 ranged from 362-736 m. Pipe number corresponds with seed trap number.

	Pipe #										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Distance from I99 (m)	736	583	573	554	540	572	577	421	362	401	406

Table 4.2: Seed collected via water through the use of seed traps (n=11) over five collection periods from September 24 through November 3, 2008. Total rainfall for the study period was estimated at 59.2 mm.

		Trap Number										
Collection Period	Rainfall (mm)*	1	2	3	4	5†	6†	7	8	9†	10†	11†
1	16.4		X			X					X	
2	2.0				X	X	X					
3	0.5				X	X					X	
4	37.3		X			X	X				X	
5	3.0					X	X	X			X	
% of Time Present		0%	40%	0%	40%	100%	60%	20%	0%	0%	80%	0%

\* Cumulative rainfall estimated from Port Matilda, PA between collection periods starting when the trap was placed in the field until it was removed and replaced by another

X *Microstegium* seed present in trap

† *Microstegium* present aboveground 0.5 meters or less from both ends of the drain pipe

## Literature Cited

- Baiser, B., J. L. Lockwood, D. La Puma, and M. F. J. Aronson. 2008. A perfect storm: two ecosystem engineers interact to degrade deciduous forests of New Jersey. *Biological Invasions* 10:785-795.
- Baker, H. G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics* 5:1-24.
- Barden, L. S. 1987. Invasion of *Microstegium vimineum*, an exotic, annual, shade-tolerant C<sub>4</sub> grass, into a North Carolina floodplain. *American Midland Naturalist* 118:40-45.
- Belote, R. T., J. F. Weltzin, and R. J. Norby. 2003. Response of an understory plant community to elevated [CO<sub>2</sub>] depends on differential responses of dominant invasive species and is mediated by soil water availability. *New Phytologist* 161:827-835.
- Belote, R. T. and J. F. Weltzin. 2006. Interactions between two co-dominant, invasive plants in the understory of a temperate deciduous forest. *Biological Invasions* 8:1629-1641.
- Cheplick, G. P. 2006. A modular approach to biomass allocation in an invasive annual (*Microstegium vimineum*; Poaceae). *American Journal of Botany* 93:539-545.
- Christen, D. C. and G. R. Matlack. 2009. The habitat and conduit functions of roads in the spread of three invasive plant species. *Biological Invasions* 11:453-465.
- Cole, P. G. and J. F. Weltzin. 2004. Environmental correlates of the distribution and abundance of *Microstegium vimineum*, in east Tennessee. *Southeastern Naturalist* 3:545-562.

- Cole, P. G. and J. F. Weltzin. 2005. Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. *Biological Invasions* 7:477-488.
- Ehrenfeld, J. G. 1999. A rhizomatous, perennial form of *Microstegium vimineum* (Trin.) A. Camus in New Jersey. *Journal of the Torrey Botanical Society* 126:352-358.
- Fairbrothers, D. E. and J. R. Gray. 1972. *Microstegium vimineum* (Trin.) A. Camus (Gramineae) in the United States. *Bulletin of the Torrey Botanical Club* 99:97-100.
- Gibson, D. J., G. Spyreas, and J. Benedict. 2002. Life history of *Microstegium vimineum* (Poaceae), an invasive grass in southern Illinois. *Journal of the Torrey Botanical Society* 129:207-219.
- Horton, J. L. and H. S. Neufeld. 1998. Photosynthetic responses of *Microstegium vimineum* (Trin.) A. Camus, a shade-tolerant, C<sub>4</sub> grass, to variable light environments. *Oecologia* 114:11-19.
- Hunt, D. M. and R. E. Zaremba. 1992. The northeastern spread of *Microstegium vimineum* (Poaceae) into New York and adjacent states. *Rhodora* 94:167-170.
- Leicht, S. S., J. A. Silander Jr., and K. Greenwood. 2005. Assessing the competitive ability of japanese stiltgrass, *Microstegium vimineum* (Trin.) A. Camus. *Journal of the Torrey Botanical Society* 132:573-580.
- Marshall, J. M. and D. S. Buckley. 2008. Influence of litter removal and mineral soil disturbance on the spread of an invasive grass in a central hardwood forest. *Biological Invasions* 10:531-558.
- Mehrhoff, L. J. 2000. Perennial *Microstegium vimineum* (Poaceae): an apparent misidentification? *Journal of the Torrey Botanical Society* 127:251-254.

- Oswalt, C. M., S. N. Oswalt, and W. K. Clatterbuck. 2007. 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.
- Peskin, N. 2005. Habitat suitability of Japanese stiltgrass *Microstegium vimineum* in an Appalachian forest. M.S. thesis. The Pennsylvania State University, University Park, PA.
- Redman, D. E. 1995. Distribution and habitat types for Nepal *Microstegium* [*Microstegium vimineum* (Trin.) Camus] in Maryland and the District of Columbia. *Castanea* 60:270-275.
- Tennessee Exotic Pest Plant Council. Tennessee Exotic Plant Management Manual. 2007. (<http://www.tneppc.org/>, 12 December 2007).
- US Department of Agriculture, Natural Resources Conservation Service. 2007. National Plant Data Center, Baton Rouge, LA 70874-4490 USA. The PLANTS Database (<http://plants.usda.gov>, 12 December 2007).
- Williams, L. D. 1998. Factors affecting growth and reproduction in the invasive grass *Microstegium vimineum*. M.S. thesis, Appalachian State University, Boone, NC.
- Winter, K., M. R. Schmitt, and G. E. Edwards. 1982. *Microstegium vimineum*, a shade adapted C<sub>4</sub> grass. *Plant Science Letters* 24:311-318.
- Zedler, J. B. and S. Kercher. 2007. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. *Critical Reviews in Plant Sciences* 23: 431-452.

## Chapter 5

## Epilogue

### Summary

Since the presence of *Microstegium* in slope, seep wetlands of the Centre County region of Central Pennsylvania is relatively new, the goal of the pilot study in Chapter 2 was to characterize sites to determine if a unique suite of environmental and biotic characteristics were present in *Microstegium* sites compared to non-*Microstegium* sites. This study revealed that greater light (photosynthetically active radiation) was of overriding importance in *Microstegium* sites at the AgRidge locale. This agrees with the research of others indicating that *Microstegium* growth is promoted in high light environments (Cole and Weltzin 2004; Claridge and Franklin 2002; Christen and Matlack 2009). Another important trend evident in all non-*Microstegium* sites at both locales was the dominance of the forb functional group in the aboveground compositions. Future studies should investigate whether non-*Microstegium* sites have lower proportions of invasives and higher proportions of native forbs. In addition, previous land-use history may in fact dictate the floral community structure in these wetlands and provide information about the successional stage of the wetlands used in this study. It is quite possible that all sites used in this study are suitable for *Microstegium* growth. Dispersal limitations need to be addressed in order to provide a more detailed analysis of why the invasive is present in some slope, seep wetlands and not in others.

From Chapter 3, comparison of the UPPER and LOWER seed bank plant functional groups indicated that forbs dominated over half of the non-*Microstegium* sites. Results from this study also demonstrated that *Microstegium* seed germinated from seed bank samples of sites where it was present aboveground and generally in greater percentages from the 0-10 cm depth. The percentage of *Microstegium* that germinated from seed bank samples seemed low in comparison to percent *Microstegium* cover in the field. It is possible that the timing of seed bank sampling was likely responsible for this outcome. However, the low percentage of *Microstegium* seed in the seed bank could also indicate that *Microstegium* recently arrived to these sites and that not enough time has lapsed to form a persistent seed bank. Slope, seep wetlands are thought to be

areas of high native plant diversity yet their floral composition, both aboveground and in seed banks is severely understudied. Future research should examine native plant composition in slope, seep wetland seed banks to determine whether specific plant community structures are associated with less invasibility. Additional greenhouse seed bank studies could also determine whether floral composition in these wetlands is determined by the number of seeds in the soil or whether environmental conditions (such as soil moisture or light) have a greater effect on composition.

Previous seed dispersal studies have noted that roadways can transport *Microstegium* seed (Mehrhoff 2000; Peskin 2005) but have suggested that there must be other mechanisms used for range expansion (Christen and Matlack 2009). The seed trap study in Chapter 4 experimentally determined that water is a vector for *Microstegium* seed dispersal. Future *Microstegium* seed transport via water studies must address propagule pressure including factors such as landscape contours and water conduits. It is also important to understand how the locations of upslope source populations of *Microstegium* feed downslope populations of *Microstegium*. The percent *Microstegium* cover and seed produced in upslope populations may in fact determine the percent cover in downslope populations. Overall, this research presents an opportunity to expand upon seed dispersal via water mechanisms and illustrates the need to develop a more comprehensive study before information can be appropriately used by land managers seeking to prevent or control *Microstegium*.

## **Recommendations for Future Studies**

The three studies presented in this manuscript (site comparisons, seed bank analyses, and water as a vector for dispersal) were foundational to our understanding of the relatively new presence of *Microstegium* in slope, seep wetlands. Overall, this research provides a starting point for the development of more extensive studies pertaining to invasion, habitat suitability, and dispersal processes. My recommendations and implications for future studies are described below.

*Microstegium* demonstrates plasticity and has many advantageous weedy characteristics. However, this does not explain why it is present in some locations and absent in seemingly suitable habitats. The results of the seed dispersal study indicated that *Microstegium* seed travels via water. However, *Microstegium* seed is also likely transported by other organisms but only

anecdotal accounts exist. Civitello et al. (2008) noted *Microtus* activity in sites used for their study on tick abundance and *Microstegium* growth. Baiser et al. (2008) noted that deer were involved indirectly by encouraging *Microstegium* growth through selective browsing. The extent of involvement of small burrowing organisms (earthworms, rodents, etc.), birds, and large mammals in *Microstegium* seed dispersal is unknown. Both awned and awnless *Microstegium* seeds can be produced. Awned seeds cling to animal fur but this has yet to be studied with *Microstegium*. Flattened patches of *Microstegium* were noted in many of the sites used during this study suggesting that deer may seek shelter and sleep in these habitats (Romanello, personal observation). It is possible that organisms transport seed which may result in the seemingly isolated, small *Microstegium* patches often found far from source populations. In the future, a field enclosure study could examine the role of burrowing animals or deer in *Microstegium* seed dispersal.

Land-use should be an integral component in future *Microstegium* studies. Previous logging and farming in the Centre County region of Central Pennsylvania undoubtedly affected floristic community structure. In fact, what we see in slope, seep wetlands today is likely the result of previous land-use. For example, sites at the I99 locale were located on actively managed forest land. The slope, seep wetlands from this locale appeared to be in an ‘early successional’ stage whereby hardy invasive plants easily fill unoccupied soils. Evidence of this was demonstrated by the fact that most sites had very few hardwood trees and relatively open canopies (as confirmed by high photosynthetically active radiation). Another possible indicator of early succession was the presence of other invasive plants such as *Rosa multiflora* and *Berberis* spp. at many sites. A few intriguing questions arise when land-use, succession and floristic composition are considered: Is it possible to eliminate *Microstegium* considering current land-use trends (like road construction) and modes of seed dispersal? How would the floral composition change if *Microstegium* was eliminated from slope, seep wetlands? Would the space previously occupied by *Microstegium* become overrun by another invasive colonizer? A combination of ecological models and field experiments could address these questions in the future.

There are very few studies which have examined the effects of plant competition on *Microstegium* growth. One experiment by Cole and Weltzin (2005) determined that lower light conditions from pawpaw trees negatively influenced *Microstegium* growth. More frequently, questions arise about the effect of *Microstegium* on native plant community structure. In this case, *Microstegium* is thought to replace native plants within three to five years of arrival (Tennessee Exotic Pest Plant Council 2007). *Microstegium* growth and expansion is also considered a threat

to woody regeneration (Oswalt et al. 2007). Since there was a significant difference in this study between the aboveground plant family composition and the seed bank composition and evidence of forb domination in many non-*Microstegium* sites, it is possible that plant competition may be a factor which influences germination. There are a few questions which arise from this topic: Is this species more likely to grow and expand in early succession areas unoccupied by many other plants when compared to late successional areas? Will *Microstegium* seed germinate if it lands in an area already overrun by other invasive plant species? Does the stage of succession and corresponding plant composition of the area influence *Microstegium* patch size? Greenhouse and field studies on the effects of plant competition on *Microstegium* growth would aide in predicting which environments may be more easily inhabitable.

### **Final Thoughts**

There are still many unanswered questions about *Microstegium* and its presence or absence in specific habitats. Many *Microstegium* field studies have focused on this species response to a specific variable like light, pH, or CO<sub>2</sub> but none of these studies provide a solid explanation for why this species is present in some locations and not in others. Thus, the predictive power for assessing areas at risk for *Microstegium* invasion is low using these studies alone. Even though dense *Microstegium* growth was documented in many slope, seep wetlands, this research cannot assess the potential for *Microstegium* to become a pesky, problematic invasive in Central Pennsylvania because we have yet to gather information on propagule pressure and constraints on seed dispersal.

It is important to remember that *Microstegium* exhibits plasticity and seems to have what Baker (1974) refers to as a “general purpose genotype” which has no doubt contributed to its success as an invader in some respects but only when the environment in which it invades is also considered. The extent of *Microstegium* expansion varies in different areas of the United States and is considerably more problematic in warmer climates with milder winters in states such as Tennessee, North Carolina and Virginia (Romanello, personal observation). In Central Pennsylvania, *Microstegium* grows prolifically in some isolated locations like slope, seep wetlands and some roadsides but overall appears to be cold-weather averse when compared to its expansion in warmer states. It seems reasonable, in warmer states, for there to be concern about *Microstegium*’s ability to suppress woody regeneration (Oswalt et al. 2007) or replace native



vegetation 3 to 5 years after arrival (Tennessee Exotic Pest Plant Council 2007) particularly if there are negative consequences associated with permitting *Microstegium* to prevail. In Pennsylvania, *Microstegium* is considered an invasive but yet to be classified as noxious. Therefore, we have the opportunity to get a head start in Pennsylvania by closely monitoring current populations and by using landscape scale studies to examine both short and long-range dispersal of *Microstegium* seed by water. It is very likely that many slope, seep wetlands are vulnerable to *Microstegium* invasion. Closer examination of the arrangement of slope, seep wetlands within the Ridge and Valley Province of Pennsylvania and the water conduits which connect them may reveal source populations of *Microstegium*, pathways for seed dispersal and eventually provide the information needed for the development of management plans.

## Literature Cited

- Baiser, B., J. L. Lockwood, D. La Puma, and M. F. J. Aronson. 2008. A perfect storm: two ecosystem engineers interact to degrade deciduous forests of New Jersey. *Biological Invasions* 10:785-795.
- Baker, H. G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics* 5:1-24.
- Christen, D. C. and G. R. Matlack. 2009. The habitat and conduit functions of roads in the spread of three invasive plant species. *Biological Invasions* 11:453-465.
- Civitello, D. J., S. L. Flory, and K. Clay. 2008. Exotic grass invasion reduces survival of *Amblyomma americanum* and *Dermacentro variabilis* Ticks (Acari: Ixodidae). *Journal of Medical Entomology* 45:867-872.
- Claridge, K. and S. B. Franklin. Compensation and plasticity in an invasive plant species. *Biological Invasions* 4:339-347.
- Cole, P. G. and J. F. Weltzin. 2004. Environmental correlates of the distribution and abundance of *Microstegium vimineum*, in east Tennessee. *Southeastern Naturalist* 3:545-562.
- Cole, P. G. and J. F. Weltzin. 2005. Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. *Biological Invasions* 7:477-488.
- Mehrhoff, L. J. 2000. Perennial *Microstegium vimineum* (Poaceae): an apparent misidentification? *Journal of the Torrey Botanical Society* 127:251-254.
- Oswalt, C. M., S. N. Oswalt, and W. K. Clatterbuck. 2007. 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.

Peskin, N. 2005. Habitat suitability of Japanese stiltgrass *Microstegium vimineum* in an Appalachian forest. M.S. thesis. The Pennsylvania State University, University Park, PA.

Tennessee Exotic Pest Plant Council. Tennessee Exotic Plant Management Manual. 2007. (<http://www.tneppc.org/>, 12 December 2007).

## Appendix A Aerial Photos and Site Locations

### AgRidge Locale



Gray dots represent *Microstegium* sites and black dots represent non-*Microstegium* sites.



## I99 Locale



Gray dots represent *Microstegium* sites and black dots represent non-*Microstegium* sites.

## Appendix B

## Aboveground Plant Family Composition\*

<u>Site</u>	<u>Plant Family</u>																								
	Acer <sup>†</sup>	Aster	Bals	Bet	Blech	Clus	Cyper	Dryop	Equis	Faba	Junc	Lily	Mint	Onagr	Onocl	Oxal	Poa	Polyg	Primul	Rose	Rubia	Solon	Sphag	Urtic	Viola
MvLand	0	19	0	0	5	0	0	0	0	0	2	0	2	0	6	0	55	0	0	2	0	0	0	0	11
Bearsite	0	32	0	0	0	1	54	0	0	0	2	0	0	0	0	0	19	4	0	0	0	0	0	0	6
Lost	0	12	1	0	0	0	5	0	0	0	0	0	2	0	0	0	52	4	0	7	0	0	4	0	14
Selfheal	0	37	3	0	0	0	11	0	0	0	0	0	6	0	1	0	12	4	0	0	2	0	0	0	30
Turkey	0	13	16	0	0	0	0	1	0	0	0	0	3	0	0	0	14	39	0	0	0	0	0	0	19
Prickle	0	12	38	0	0	0	22	0	0	0	1	0	2	0	8	0	6	13	0	1	2	0	0	0	0
TW	0	45	0	0	0	0	7	0	0	0	0	0	2	0	0	0	35	0	0	3	0	0	0	0	1
NG1	0	25	0	0	0	0	0	0	0	0	0	0	0	0	3	1	63	1	0	6	3	0	0	0	2
GR2	0	8	0	0	0	0	0	0	0	4	0	0	0	0	0	0	54	0	0	17	4	0	0	0	19
NG2	0	39	8	8	4	0	0	0	1	0	0	0	1	0	1	2	3	0	3	6	5	0	0	0	14
GR1	6	3	0	0	0	0	43	0	0	0	0	0	0	0	0	9	7	6	0	2	2	3	0	25	0
Nine96	0	0	0	0	19	0	0	0	0	0	0	1	0	2	0	14	2	0	0	49	14	0	0	0	1

\*Values are visually estimated percents. Gray represents *Microstegium* sites.

† For full plant family names refer to Appendix F

## Appendix C

## UPPER Seed Bank Composition\*

Site	Plant Family																		
	Am ↑	Aster	Bals	Brass	Capri	Cheno	Cyper	Euphorb	Mint	Onocl	Oxal	Poa	Polyg	Rose	Rubia	Scroph	Urtic	Viola	
MvLand	1.43	0	0	0	0	13.57	57.86	0	0	0	0.36	25.36	0	0	0.71	0	0	0.71	
Bearsite	1.16	0	0	0.23	0	1.09	68.84	0	0	0	0.47	14.65	0.23	0	1.86	0	0.23	0.23	
Lost	3.68	0.003	0	0.53	0	20.26	52.89	0	0	0	3.95	12.37	0.26	0	0.53	0	0.79	4.47	
Selfheal	8.33	0.01	0	1.04	0	69.79	0	0	0	0	0	13.54	3.13	0	0	0	2.08	1.04	
Turkey	7.48	0.02	0	0	0	46.73	6.54	0	0	0	0.93	31.78	3.74	0	0	0	0.93	0	
Prickle	8.33	0	0	1.39	0	45.83	0	0	5.56	0	0	37.5	1.39	0	0	0	0	0	
TW	0	0.005	0	0	0.52	3.66	8.38	0	0.52	0	8.9	60.21	9.95	0.52	0	0.52	2.09	4.19	
NG1	0	0.009	0.92	0.23	0	1.38	23.96	0.46	5.07	0.23	6.22	49.08	5.07	0	1.61	0	4.15	0.69	
GR2	0	0.02	0	0	8.57	0	26.43	0	0	0	11.43	44.29	0	0	0.71	1.43	2.14	2.86	
NG2	0	0.009	0.43	0	0	0	23.81	0	3.03	0	23.38	37.23	0.87	1.73	3.03	0.87	1.3	3.46	
GR1	0	0	0	0	1.85	0	1.23	0	0	0	2.47	13.58	80.86	0	0	0	0	0	
Nine96	0	0.04	0	0	3.75	0	2.5	0	0	0	8.75	71.25	0	1.25	0	0	0	8.75	

\*Values represent germination percents. Gray represents *Microstegium* sites.

<sup>†</sup> For full plant family names refer to Appendix F

### LOWER Seed Bank Composition\*

Site	Plant Family																			
	Am †	Aster	Bals	Brass	Capri	Cheno	Cyper	Faba	Junc	Mint	Oxal	Poa	Polyg	Primul	Rose	Rubia	Scroph	Sphag	Urtic	Viola
MvLand	1.49	0	0	0.99	0	20.3	50.99	0	0	0	0	25.74	0.5	0	0	0	0	0	0	0
Bearsite	2.43	0	0	0.81	0	23.08	60.73	0.4	0	0	0	12.15	0	0	0	0	0	0	0	0
Lost	9.42	0.02	0	1.57	0	30.37	39.27	0	0	0	3.14	8.9	0.52	0	0.52	0.52	0	1.57	0	2.62
Selfheal	12.62	0	0	1.94	0	68.93	0	0	0	0.97	0.97	12.62	0.97	0	0	0.97	0	0	0	0
Turkey	12.2	0	0	0	0	70.73	0	0	0	0	0	17.07	0	0	0	0	0	0	0	0
Prickle	1.54	0	0	3.08	0	47.69	4.62	0	0	4.62	1.54	33.85	3.08	0	0	0	0	0	0	0
TW	0	0.01	0	0	4.55	1.14	17.05	0	0	0	10.22	54.55	0	0	4.55	0	1.14	0	2.27	3.41
NG1	0.36	0.004	0	0	0	1.07	18.93	0	0.71	7.5	5.36	58.57	4.29	0	0	0.36	0	0	1.43	1.07
GR2	2.53	0.04	1.27	0	5.06	0	18.99	1.27	0	0	5.06	51.9	1.27	1.27	0	3.8	0	0	2.53	1.27
NG2	0	0.01	2.53	1.27	0	1.27	18	0	0	0	15.19	48.1	2.53	0	2.53	1.27	0	0	0	5.06
GR1	0	0	0	0	0	1.61	1.61	0	0	0	0	16.13	79.03	0	0	0	1.61	0	0	0
Nine96	0	0.01	0	1.28	6.41	0	3.85	0	0	1.28	14.1	64.1	0	0	2.56	0	0	0	0	5.13

\*Values represent germination percents. Gray represents *Microstegium* sites.

† For full plant family names refer to Appendix F



## Appendix D

### Aboveground Plant Functional Groups\*

Site	Plant Functional Group						
	Grass	Sedge	Rush	Forb	Fern	Moss	Woody
MvLand	55	0	2	34	11	0	0
Bearsite	19	54	2	43	0	0	0
Lost	52	5	0	40	0	4	0
Selfheal	12	11	0	82	1	0	0
Turkey	14	0	0	90	1	0	0
Prickle	6	22	1	68	8	0	0
TW	35	7	0	51	0	0	0
NG1	63	0	0	38	3	0	0
GR2	54	0	0	52	0	0	0
NG2	3	0	1	78	5	0	8
GR1	7	43	0	50	0	0	6
Nine96	2	0	0	79	19	0	0

\*Values are visually estimated percents. Gray represents *Microstegium* sites. A list of families included in each functional group is located in Appendix F.

## Appendix E

### UPPER Seed Bank Plant Functional Groups\*

Site	Plant Functional Group						
	Grass	Sedge	Rush	Forb	Fern	Moss	Woody
MvLand	25.36	57.86	0	16.78	0	0	0
Bearsite	14.65	68.84	0	5.5	0	0	0
Lost	12.37	52.89	0	34.473	0	0	0
Selfheal	13.54	0	0	85.42	0	0	0
Turkey	31.78	6.54	0	59.83	0	0	0
Prickle	37.5	0	0	62.5	0	0	0
TW	60.21	8.38	0	30.875	0	0	0
NG1	49.08	23.96	0	24.889	0.23	0	0.92
GR2	44.29	26.43	0	27.16	0	0	0
NG2	37.23	23.81	0	37.679	0	0	0.43
GR1	13.58	1.23	0	85.18	0	0	0
Nine96	71.25	2.5	0	22.54	0	0	0

\*Values represent germination percents. Gray represents *Microstegium* sites. A list of families included in each functional group is located in Appendix F.

### LOWER Seed Bank Plant Functional Groups\*

Site	Plant Functional Group						
	Grass	Sedge	Rush	Forb	Fern	Moss	Woody
MvLand	25.74	50.99	0	23.28	0	0	0
Bearsite	12.15	60.73	0	26.72	0	0	0
Lost	8.9	39.27	0	48.7	0	1.57	0
Selfheal	12.62	0	0	87.37	0	0	0
Turkey	17.07	0	0	82.93	0	0	0
Prickle	33.85	4.62	0	61.55	0	0	0
TW	54.55	17.05	0	27.29	0	0	0
NG1	58.57	18.93	0.71	21.444	0	0	0
GR2	51.9	18.99	0	24.1	0	0	1.27
NG2	48.1	18	0	29.13	0	0	2.53
GR1	16.13	1.61	0	82.25	0	0	0
Nine96	64.1	3.85	0	30.77	0	0	0

\*Values represent germination percents. Gray represents *Microstegium* sites. A list of families included in each functional group is located in Appendix F.

## Appendix F

## Key to Plant Family Abbreviations and Functional Groups

<b><u>Abbreviation</u></b>	<b><u>Full Name</u></b>	<b><u>Functional Group</u></b>
<b>Acer</b>	Aceraceae	Woody
<b>Am</b>	Amaranthaceae	Forb
<b>Aster</b>	Asteraceae	Forb
<b>Bals</b>	Balsaminaceae	Forb
<b>Bet</b>	Betulaceae	Woody
<b>Blech</b>	Blechnaceae	Fern
<b>Brass</b>	Brassicaceae	Forb
<b>Capri</b>	Caprifoliaceae	Forb
<b>Cheno</b>	Chenopodiaceae	Forb
<b>Clus</b>	Clusiaceae	Forb
<b>Cyper</b>	Cyperaceae	Sedge
<b>Dryop</b>	Dryopteridaceae	Fern
<b>Equis</b>	Equisetaceae	Rush
<b>Euphorb</b>	Euphorbiaceae	Forb
<b>Faba</b>	Fabaceae	Forb
<b>Junc</b>	Juncaceae	Rush
<b>Lily</b>	Liliaceae	Forb
<b>Mint</b>	Lamiaceae	Forb
<b>Onagr</b>	Onagraceae	Forb
<b>Onocl</b>	Onocleaceae	Fern
<b>Oxal</b>	Oxalidaceae	Forb
<b>Poa</b>	Poaceae	Grass
<b>Polyg</b>	Polygonaceae	Forb
<b>Primul</b>	Primulaceae	Forb
<b>Rose</b>	Rosaceae	Forb
<b>Rubia</b>	Rubiaceae	Forb
<b>Scroph</b>	Scrophulariaceae	Forb
<b>Solon</b>	Solonaceae	Forb
<b>Sphag</b>	Sphagnaceae	Moss
<b>Urtic</b>	Urticaceae	Forb
<b>Viola</b>	Violaceae	Forb